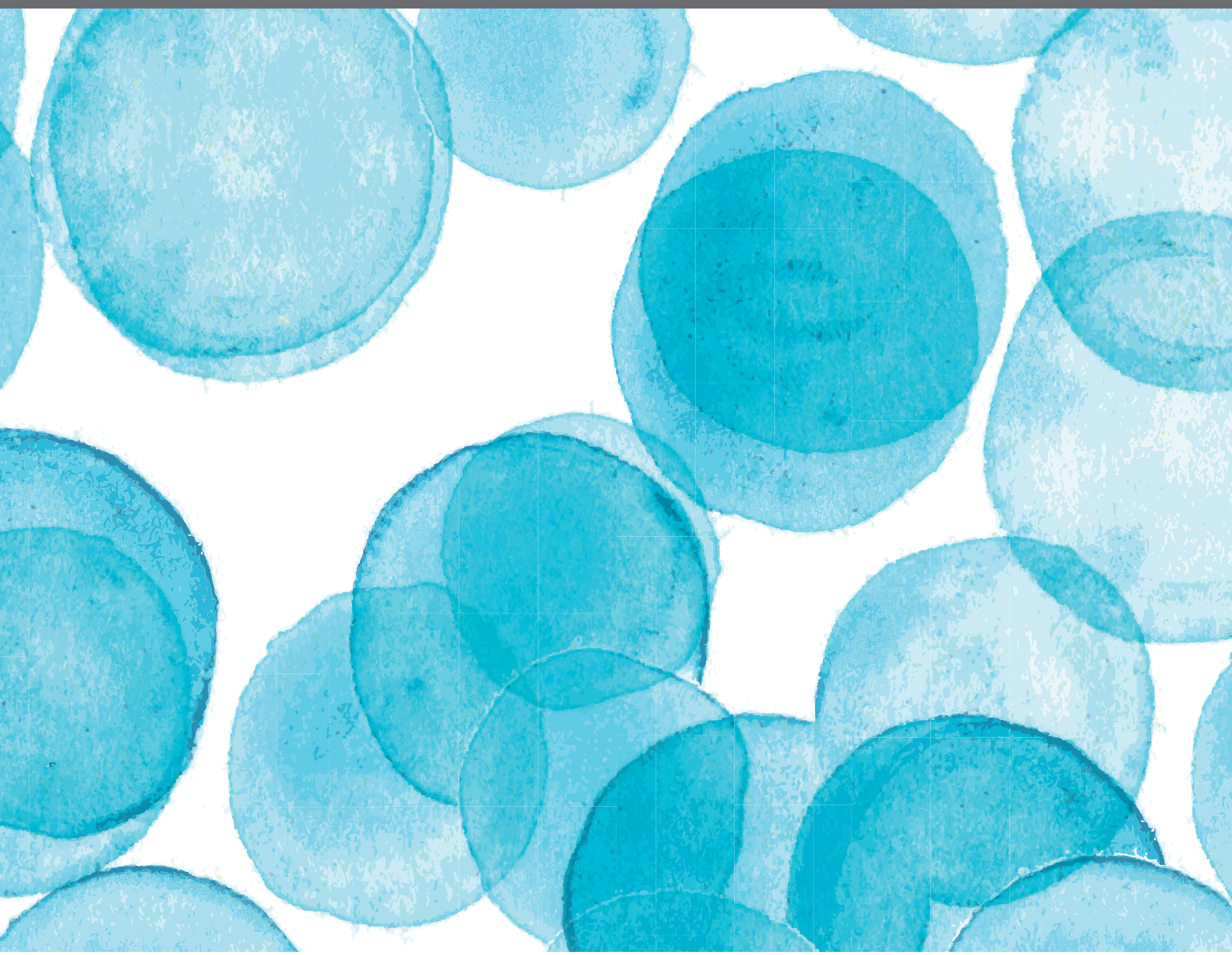
MICROBIAL SYSTEMS AS PARADIGMS OF SUCCESSFUL AND SUSTAINABLE INTERACTIONS

EDITED BY: Enrica Pessione, Nathalie Connil and Anna Luganini

PUBLISHED IN: Frontiers in Microbiology



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-054-3
DOI 10.3389/978-2-88974-054-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

MICROBIAL SYSTEMS AS PARADIGMS OF SUCCESSFUL AND SUSTAINABLE INTERACTIONS

Topic Editors:
Enrica Pessione, University of Turin, Italy
Nathalie Connil, Université de Rouen, France
Anna Luganini, University of Turin, Italy

Citation: Pessione, E., Connil, N., Luganini, A., eds. (2022). Microbial Systems as Paradigms Of Successful and Sustainable Interactions. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-054-3

Table of Contents

frontiers in Microbiology

EDITORIAL
published: 24 November 2021
doi: 10.3389/fmicb.2021.785106

Editorial: Microbial Systems as Paradigms of Successful and Sustainable Interactions

Nathalie Connil[1], *Anna Luganini*[2]* *and Enrica Pessione*[3]*

[1] Laboratory of Microbiology Signals and Microenvironment (LMSM EA 4312), University of Rouen, Evreux, France,
[2] Laboratory of Microbiology and Virology, Department of Life Sciences and Systems Biology, University of Torino, Turin, Italy,
[3] Laboratory of Microbial Applied Biochemistry and Proteomics, Department of Life Sciences and Systems Biology, University of Torino, Turin, Italy

Keywords: microbial ecosystems, biofilms, resilience, evolution, cooperation, cross-feeding, molecular signaling

Editorial on the Research Topic

Microbial Systems as Paradigms of Successful and Sustainable Interactions

The idea at the basis of the present Research Topic was to explore how evolution selected microbial behaviors to ensure the generation of more and more complex and sustainable lifestyles that help microbial systems in coping with scarcity and rapidly changing environments. The keywords were communication, interaction, cooperation, adaptation, energy earning and complexity. The rich web of interactions that characterize microbial life can represent a paradigm of the concept "the whole is more than the sum of its parts" and the interactive lifestyle is the best one ensuring survival and evolution. It is well-assessed that, besides vertical inheritance, signal exchange, horizontal gene transfer and epigenetic events account for the fast evolution and plasticity observed (Bapteste, 2014). Understanding these interspecies and inter-kingdom relationships, as well as those with the external environment unravels the underlying rule of these interactions: sense the enemy/constraint, cope and adjust to acquire novel lifestyle opportunities. Thus, observing microbial systems can inspire us offering old solutions to new emerging problems.

Bacteria have a long evolutionary history both as planktonic cells and as parts of microbial consortia such as biofilms. They can live in the external environment in abiotic habitats but they can also colonize different animal and vegetal districts to establish a symbiotic way of living with higher organisms. The evolutionary pressure has selected bacterial behaviors intended to facilitate both reciprocal and inter-kingdom interactions. All these relationships have the peculiarity to be cooperatively successful and sustainable (long-lasting).

In the present Research Topic, different aspects of bacteria lifestyles are explored. First, their reciprocal interactions (which include cooperation but also conflicts) that are made-up by sustainable behaviors like earning energy and resources, optimize rather than maximize, anticipate, sharing, and recycling. In this context direct connections (biofilm) but also soluble and vesicle-embedded signals are used to communicate (Pessione). A paradigmatic example are the interactions and the exchange of signals in a multispecies biofilm characterized by high metabolic heterogeneity. Generally, cross-feeding, ensuring removal of toxic metabolic products, allows enhanced growth rates, however, these biofilm-living communities can affect also the interaction with the human host (Joshi et al.). In addition, metabolite exchange (thiamine) occurring in solvent-degrading bacterial communities is reported as an example of syntrophy among species (Huang et al.). Secondarily, bacterial plasticity and ability to interact with different animal models such as mammals and crabs is analyzed. An interesting example is the study of lemur's gut microbiome. Correa et al. reported that bacterial alpha and beta diversity are affected by family

Edited by:
George Tsiamis,
University of Patras, Greece

Reviewed by:
Vassiliki Karapapa,
Municipality of Agrinio, Greece

***Correspondence:**
Enrica Pessione
enrica.pessione@unito.it
Anna Luganini
anna.luganini@unito.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: 28 September 2021
Accepted: 01 November 2021
Published: 24 November 2021

Citation:
Connil N, Luganini A and Pessione E (2021) Editorial: Microbial Systems as Paradigms of Successful and Sustainable Interactions. Front. Microbiol. 12:785106. doi: 10.3389/fmicb.2021.785106

group and sex. Furthermore, the contribution of gut bacteria to lemurs' evolution, by processing diet-acquired strategic molecules or ions that the animal up-takes directly from the ground is reported, suggesting an ecological value of soil as a nutrient supply. As far as the human host is concerned, symbiont bacteria can establish inter-kingdom communication by affecting brain signaling (bottom up control of the gut-brain axis) but also by responding to top-down signals, demonstrating that host and microbiota share the same molecular language, in agreement with the concept of economizing molecules by using multitasking signals (Boukerb et al.). Another crucial function of symbiont bacteria is to allow to crabs the transition from water world to terrestrial life permitting the utilization of novel food sources. This is achieved by means of cellulolytic symbiont bacteria ensuring the availability of free mono- and disaccharides promoting a carbohydrate based diet instead of protein-based meals for the host crab (Cannicci et al.). Interestingly, crabs living in different aquatic ecological niches host a different commensal microbiota displaying diversity especially in fatty acid profiles (Su et al.).

Apart from bacteria, other microorganisms such as yeasts can set up a successful lifestyle by interacting with insects: the well-established symbiosis between *Saccharomyces cerevisiae* and social wasps provides advantages to both partners favoring yeast mating (a not frequent event in natural environments) and modulating the immune system of insect host (Meriggi et al.).

An interesting example of community fitness is the one of lichens. They can colonize extreme environments (boreal forest, arctic tundra, lava fields and tide-flooded areas) and they are good bio-indicators for monitoring pollution by heavy metals or sulfur dioxide. However, some lichens prove to be resistant to sulfur dioxide because of their surface hydrophobicity (Hauck et al., 2008) and to metals due to oxalate production (Purvis, 2014). In this Research Topic, two articles dealing with lichens are included. The former (Grimm et al.), underlines the importance of all "omics" approaches and molecular imaging in deciphering the complex structure and physiology of these interacting communities. Metaproteome analyses confirm the partition of functions in lichen partnerships also highlighting the (still poorly explored) diversity of the lichen microbiota and the additional roles of commensal bacteria and viruses in the lichen ecosystem, and describes interesting aspects of the bacterial microbiota in supporting nutrient uptake, adaptability, stress tolerance and in coping with exogenous pathogenic bacteria. These lichen social systems, whose composition differs in different geographical sites, prove to be excellent inter-kingdom aggregations able to adapt to climate changes and displaying resilience to external disturbances underlining the concept "Unity is strength" (Grimm et al.). The second article (Nazem-Bokaee et al.) mainly explores the lichen symbiosis by network modeling using the tools of systems biology for analyzing the metabolic interplays and the underlying molecular mechanisms of lichen signaling pathways. This holistic approach, going beyond the genomic information, highlights the importance of deciphering the flux distribution in lichen metabolic pathways and the co-dependence between symbionts.

Finally, the role of viruses, in establishing successful interactions supporting human fitness and evolution is explored in two reviews of this Topic Issue. Endogenization of retroviruses is crucial in human evolution, for instance in the placenta formation but also when retroviruses exert a neuroprotective effect on the human brain. The article by Luganini and Gribaudo provides amazing examples of cooperation essential for survival that strongly support the idea that each infection could be an opportunity of evolution, mediated by mobile genetic elements. This is also the main thread that emerges when considering the complex and multilevel interaction between phages, bacteria and the animal host: like a Russian Doll model, bacteria can talk with their inner (phage) and outer (human) hosts. Phage infection can offer to bacteria weapons to cope with competing microbial species and with the host immune system. Phage populations in the human gut also help the host to select beneficial symbiont bacteria. In addition, bacteria can communicate with their animal host by means of multitasking moonlighting proteins and by using post-translational modifications that affect the host perception of the microbial commensal and that can alter host pathways and gene expression as well (Pessione).

Taken together all these data suggest that every interaction among microbes and between microbes and higher organisms can be an opportunity of evolution, generating a higher degree of complexity. Moreover, evolution seems to have selected all those behaviors that support collective resilience, adaptation and other winning strategies to cope with a crowded environment and to counteract fast changing conditions like those observable in this period of climate change and global warming. Nevertheless, it can be highlighted that this Research Topic is an incomplete work: other aspects for instance the symbioses in the vegetal world or the interactions occurring in the aquatic ecosystems can add other bricks to this fascinating world.

AUTHOR CONTRIBUTIONS

EP wrote the first draft of the manuscript. AL, NC, and EP contributed to manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from Ricerca Locale from the University of Turin (PESE_RILO 20_01 to EP and LUGA_RILO_21_01 to AL).

REFERENCES

Bapteste, E. (2014). The origins of microbial adaptations: how introgressive descent, egalitarian evolutionary transitions and expanded kin selection shape the network of life. *Front. Microbiol.* 5:83. doi: 10.3389/fmicb.2014.00083

Hauck, M., Jürgens, S. R., Brinkmann, M., and Herminghaus, S. (2008). Surface hydrophobicity causes SO_2 tolerance in lichens. *Ann. Bot.* 101, 531–539. doi: 10.1093/aob/mcm306

Purvis, O. W. (2014). Adaptation and interaction of saxicolous crustose lichens with metals. *Bot. Stud.* 55:23. doi: 10.1186/1999-3110-55-23

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Microbiology

MINI REVIEW
published: 28 May 2020
doi: 10.3389/fmicb.2020.01140

Retroviruses of the Human Virobiota: The Recycling of Viral Genes and the Resulting Advantages for Human Hosts During Evolution

Anna Luganini and Giorgio Gribaudo*

Laboratory of Microbiology and Virology, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

All humans are colonized by a vast diversity of microbes (bacteria, archaea, protozoa, yeast, and fungi; collectively referred to as the microbiota) and viruses (the virobiota). This latter group includes viruses infecting prokaryotic cells (bacteriophages), viruses infecting eukaryotic-host cells, and virus-derived genetic elements present in host chromosomes. Although these eukaryotic viruses are mostly known to be pathogens, they are also able to establish mutualistic relationships with humans. Little is known about the mutualistic aspects of viral infection. Nevertheless, it is clear that evolution of some animal virus-host interactions has led to benefits in the health of the hosts, as is the case with symbiogenesis and endogenization of retroviruses that has exerted a neuroprotective effect on the human brain, and an important role in the fetal development, thus on the evolution of host species. In this review, we summarize how retroviruses provide amazing examples of cooperative-evolution, i.e., successful exchange between viruses and host, and how, in some cases, the benefits have become essential for the hosts' survival.

Keywords: human endogenous retrovirus, symbiotic relationship, evolution, syncytin, beneficial functions

OPEN ACCESS

Edited by:
Stefanie Widder,
Medical University of Vienna, Austria

Reviewed by:
Philippe Colson,
IHU Mediterranee Infection, France
Irene Goerzer,
Medical University of Vienna, Austria

****Correspondence:***
Anna Luganini
anna.luganini@unito.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *17 January 2020*
Accepted: *05 May 2020*
Published: *28 May 2020*

Citation:
Luganini A and Gribaudo G (2020) Retroviruses of the Human Virobiota: The Recycling of Viral Genes and the Resulting Advantages for Human Hosts During Evolution. Front. Microbiol. 11:1140. doi: 10.3389/fmicb.2020.01140

INTRODUCTION

Recent metagenomics studies have revealed that, not only is the human body colonized by microorganisms belonging to all three biological domains (i.e., Eukarya, Archaea, and Bacteria), but that the human microbiota is also composed of many viruses (Handley et al., 2012; Duerkop and Hooper, 2013). As such, the term "microbiota" also comprises the term "virobiota," a huge community of viruses that science has only just begun to explore and whose physiological roles are still unknown (White et al., 2012; **Figure 1A**). The viruses present in the human virobiota are bacteriophages (infecting bacteria) and eukaryotic viruses, which replicate in animal cells or that infect plants associated with the host's diet. Thanks to the presence of this last category of viruses the virobiota is considered a dynamic community that varies based on what each of us eats and where we live (Freer et al., 2018). While we might consider some bacteriophages as stable residents of the human body, since they infect the bacteria that stably colonize the human host (Allen and Abedon, 2013), the situation is quite different with regard to eukaryotic viruses. In this latter case, metagenomic studies have found it very difficult to discriminate truly resident viruses associated with the host from viruses whose presence is due to acute (e.g., influenza virus), chronic (e.g., hepatitis B virus), or latent infections (e.g., herpesvirus) (Duerkop and Hooper, 2013).

According to the canonical definition, viruses are parasites associated with negative consequences; in other words, pathogenic viruses cause diseases in the human host, who either survives or dies as a consequence (e.g., influenza virus, rabies virus, and measles virus). Only recently, has it been shown that among the 220 viruses able to infect humans, only about 100 are pathogenic (Parker, 2016). The remainder is composed of apathogenic viruses that establish commensal or symbiotic interactions with host (**Figure 1A**). To the best of our knowledge, anelloviruses (AV) could provide the prototypical example of a human commensal virus and are also a major component of the virobiota. This virus family includes a group of single stranded DNA-viruses, newly discovered (in 1997), that are present in both the blood and tissues of most humans, where they replicate persistently without causing disease (Freer et al., 2018).

A key example of a symbiotic virus-host relationship, on the other hand, is provided by the human endogenous retroviruses (HERVs). A symbiotic interaction is a form of mutualism whereby both virus and the human host gain benefits from their relationship (**Figure 1A**). More specifically, HERVs are primary symbionts because the viruses, after infecting germ cells, are transmitted vertically from parent to child (Parker, 2016). Although some evidence exists indicating that HERVs could contribute to the insurgence of certain pathologies [e.g., multiple sclerosis (MS), carcinogenesis, or bipolar disorder; Antony et al., 2004; Galli et al., 2005], in this review, we will focus on the beneficial effects of HERVs on humans. HERVs in fact provide an excellent example of how a "negative" event, such as a viral infection, can have a positive impact on the host's biology, establishing a successful interaction with it.

HUMAN ENDOGENOUS RETROVIRUSES (HERVs)

Data provided by the Human Genome Project suggest that the most abundant repetitive class of human DNA sequences is made up of distinct families of transposable elements (about 46–47% of human genome), of which 2–3% correspond to DNA transposons and 42–43% to retroelements (Smit, 1999). Based on the presence or absence of a long terminal repeat (LTR), retroelements are then divided into two large groups: non-LTR retroelements (corresponding to 33% of the human genome) and including LINEs (long interspersed nuclear elements) and SINEs (short interspersed nuclear elements) (Larsson and Andersson, 1998), and LTR-containing viral retroelements (around 8% of the human genome) that consist of HERV and retrotransposons, which differ from each other by the presence/absence of *env* gene (**Figure 1B**; Nelson et al., 2004).

Human endogenous retroviruses represent therefore a group of transposable elements that were originally acquired through the exogenous infection of primate hosts by ancestor retroviruses over the course of primate evolution (Katzourakis et al., 2009; Hayward et al., 2015), i.e., between 5 and 70 million years ago (Aiewsakun and Katzourakis, 2015). In contrast with exogenous retroviruses that are horizontally transmitted between hosts and usually associated with disease, HERVs infections have affected both somatic cells and germ-lines. Hence, following an occasional process of endogenization and further fixation, through which viral DNA copies permanently integrated into the host's germ-line chromosomes, HERVs were vertically transmitted to progeny in a Mendelian fashion, determining their coevolution with the host genome (Katzourakis et al., 2009; Lee et al., 2013). As such, once endogenized, HERVs retain their similarities with the ancestral exogenous virus for an exceedingly long time (millions of years) since they incur point mutations at the same rate of the host, about 10^{-9} substitutions per site per year (s/n/y) instead of a virus's rate of 10^{-3} s/n/y (Hanada et al., 2004; Sanjuán, 2012). Ancestral HERVs integrated into the human genome around 35–45 million years ago, during the split between Old and New World monkeys (Bannert and Kurth, 2006). Only HERV-K viruses are the most recently integrated group (occurring ˜5 million years ago); they are exclusive to humans and contain the most complete proviral sequences.

Human endogenous retroviruses have a genomic organization similar to that of the exogenous retroviruses, composed of the viral genes *gag*, *pro-pol*, and *env,* flanked by two LTRs (**Figure 2A**). LTRs are formed during the reverse transcription of the viral RNA genome and regulate the viral genes' expression due to the presence of promoters, enhancers, and polyadenylation signals (Schön et al., 2009; Vargiu et al., 2016). The *gag* gene encodes three structural virion components: the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. The *pol* gene encodes two enzymes necessary for the viral life cycle: reverse transcriptase/integrase (*pol*) and protease (*pro*), which synthesize the complementary DNA from the viral RNA and enable proviral integration. The *env* gene encodes two envelope proteins: transmembrane protein (TM) and surface protein (SU) (Larsson and Andersson, 1998), which mediate virus entry into and egress from host cells.

Human endogenous retroviruses can sometimes harbor more than these four retroviral genes, like type I HERV-K, whose *env* gene gives rise to two splice variants: the accessory proteins Np9 and Rec (Armbruester et al., 2002; Grandi et al., 2017), or type II HERV-K with Sp and Rec (Ruggieri et al., 2009; Douville and Avindra, 2014). Between the 5′LTR and *gag*, a primer-binding site (PBS) is located, and a polypurine trait (PPT) lies between *env* and the 3′LTR. Both these sequences localized between the LTRs exert important functions: the cellular tRNA becomes bound to the PBS during the reverse transcription process, whereas the PPT is the primer for (+) strand DNA production. Moreover, the PBS sequence has traditionally been used for a systematic nomenclature of HERV, since HERV group names are generally identified using a letter that characterizes the human tRNA type that binds to the viral PBS sequences during the retrotranscription process of the viral genome (e.g., HERV-K for lysine, HERV-W for tryptophan, etc.). If the PBS sequence is not available, HERV groups are also named according to unconventional criteria, such as the name of a neighboring gene (HERV-ADP), a clone number (e.g., HERV-S71), or a particular amino acid motif (HERV-FRD) (Werner et al., 1990; Lyn et al., 1993; Seifarth et al., 1995). However, this characterization of the HERV groups at the genomic level is considered inadequate and incomplete

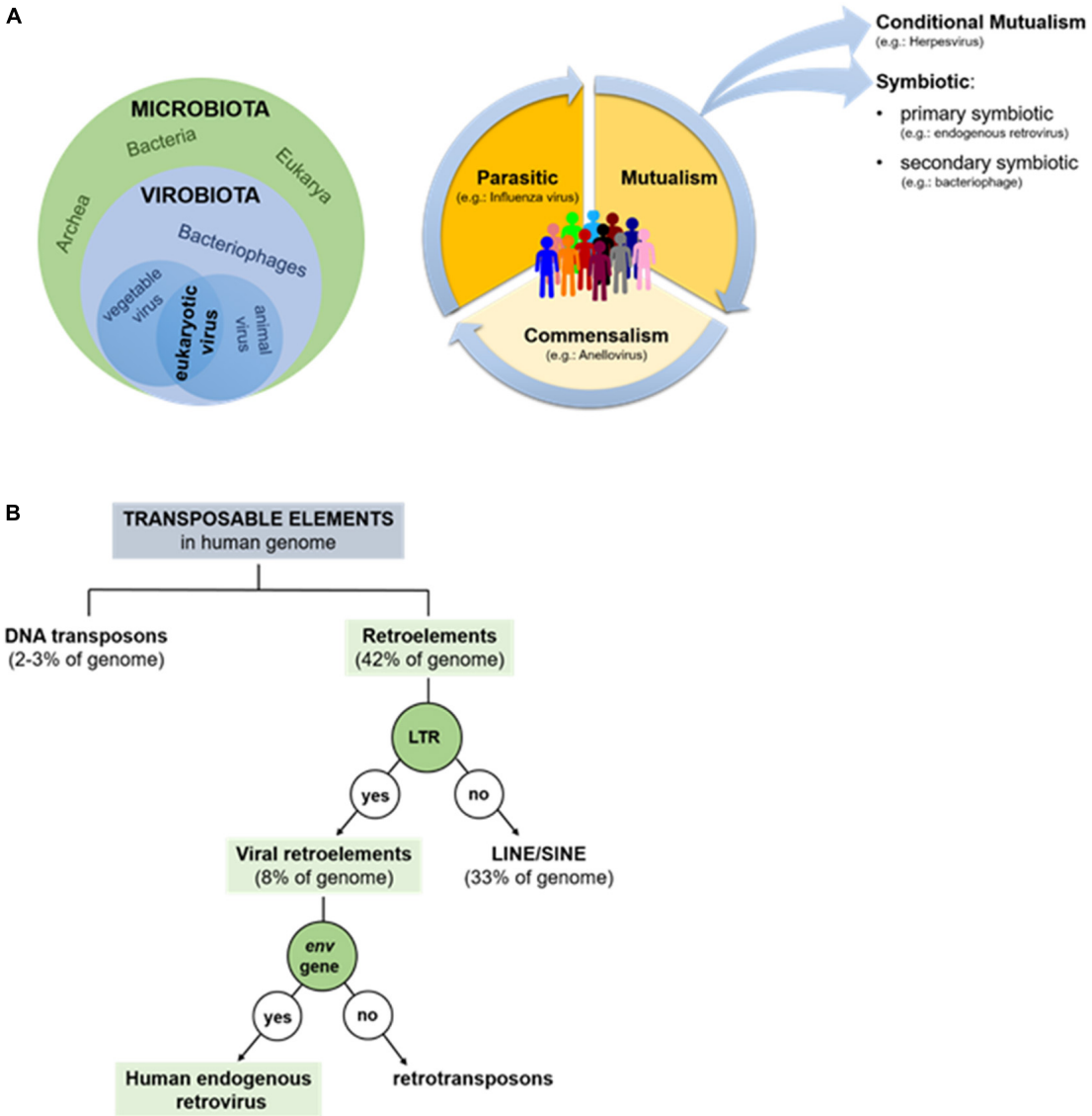
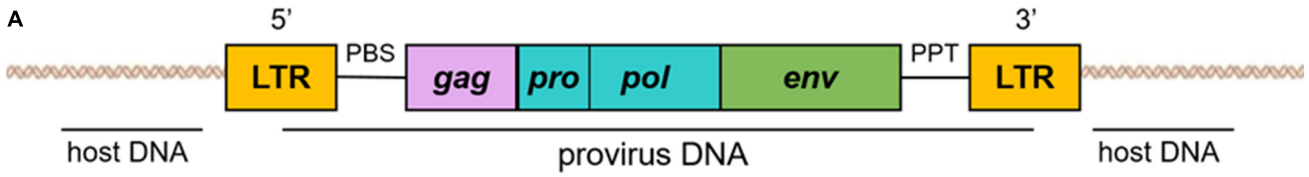


FIGURE 1 | (A) Human microbiota and virobiota. The green circle contains the components of the microbiota (Bacteria, Archea, and Eukaria), while the blue-light contains all the members of the virobiota (bacteriophages, eukaryotic viruses), which in turn is included in the microbiota (on left). Types of dynamic interaction between microorganisms and human-host: the community can shift from parasitism to mutualism and commensalism, favoring an improved state of health; on the contrary if microorganisms shift toward parasitism, they favor the disease (on right). **(B)** Repetitive mobile sequences present in human genome: almost half of genome is made up by transposable elements: DNA transposons (2–3%) and retroelements (42%). The former amplifies without RNA, the latter requires a reverse transcriptase.

because univocal guidelines for the naming of groups are missing (Grandi and Tramontano, 2018b). Recently, a new multi-step approach based on a phylogenetic study (considering the highly conserved *pol* gene) and univocal taxonomic rules allowed the splitting up of about 3200 HERV insertions, identified using the RetroTector program (ReTe) (Sperber et al., 2007), into 39 "canonical" groups plus other 31 "non-canonical" clades (Vargiu et al., 2016). Nonetheless, conventional classification also divides each HERV group into three classes according to sequence homology with exogenous retroviruses: class I (Gammaretrovirus- and Epsilonretrovirus-like: HERV-E, -F, -H, -I, -P, -R, -T, -W, and HERV-FRD, etc.); class II (Betaretrovirus-like: HERV-K); and class III (Spumaretrovirus-like: HERV-L) (Larsson and Andersson, 1998).

Hosts infected by HERVs can adopt various strategies to repress the viral replication and prevent the horizontal transmission of infections between individuals, such as viral genome silencing through epigenetic regulation mechanisms (DNA methylation) or the association of histones to viral DNA to limit its accessibility to the proteins involved in gene transcription (Grandi and Tramontano, 2018b). When viral DNA is integrated into the human host genome, it can undergo



B

Class	Retrovirus progenitor (viral gene)	Role and function in human host
class I (Gammaretrovirus)	HERV-W *(env)*	fusogenic activity placental morphogenesis fetomaternal tolerance
	HERV-FRD *(env)*	fusogenic activity placental morphogenesis fetomaternal tolerance
	HERV-R *(env)*	fetomaternal tolerance fetus and newborn protection against viral infections maturation and functional activity of steroid-dependent organs
class II (Betaretrovirus)	HERV-K *(env)*	neuroprotective function

FIGURE 2 | (A) Genomic organization of an endogenous retrovirus. The integrated double-stranded DNA is characterized by the long terminal repeats (LTRs) at its 5′ and 3′ ends. LTRs flank the retroviral *gag, pro/pol,* and *env* genes. **(B)** Schematization of the beneficial effects of HERVs to human host.

over time recombination events leading to the formation of a solitary LTR, or to insertions, deletions, and mutations, thus inactivating the ability of HERVs to produce infectious viruses. In this regard, one of the more ancient components of the cellular machinery that confer intrinsic immunity against mobile elements is represented by the APOBEC cytidine deaminases. Among these cellular enzymes, members of the APOBEC3 (A3) protein family induce G-to-A hypermutations in HERV genomes, thus leading to suppression of HERV replication and its mobility in favor of the host's genomic integrity (Ito et al., 2020).

However, as we will see, this is not always the case, because several HERVs have been co-opted into physiological functions of the host, with some members being actively transcribed and differentially expressed depending on host cell type and the physiological circumstances (**Figure 2B**).

HERV AND THE PLACENTA

In some cases, retroviral genes are recycled since they confer an advantage to the host: the most famous examples are syncytin-1 and syncytin-2. Syncytin-1 and -2 are functional glycoproteins that mediate cell-cell fusion between cytotrophoblasts and placental syncytiotrophoblasts localized at the fetomaternal interface, which plays a fundamental role in the exchange of nutrients, hormones, and gases between the mother and the fetus and is required for normal embryonic growth (Muyan and Boime, 1997; Handwerger and Freemark, 2000). Additionally, syncytiotrophoblasts keep the maternal immunosuppressive state under control, preventing fetal rejection (Munoz-Suano et al., 2001; Nakamura, 2009). It has been known for some years that the syncytin-1 sequence is homologous to that of the *env* gene of the complete HERV-W provirus (Perron et al., 2005); whereas syncytin-2 corresponds with the HERV-FRD-*env* gene.

It is probable that the syncytin proteins of retroviruses enable membrane fusion with host cells, facilitating viral infection. This function has been "commandeered" in placental mammals as it makes the placenta more invasive toward the maternal uterus, thus conveying an evolutionary advantage to the embryo. Similarly to other retroviral glycoproteins, syncytin-1 and syncytin-2 are synthesized as precursors that only become functional after their cleavage into two functional subunits: SU (involved in cellular receptor binding) and TM (involved in cell-cell fusion). Both proteins also harbor an immunosuppressive domain (ISD) that, as mentioned above, is required for generating the fetomaternal tolerance state during pregnancy, although the molecular mechanisms involved in the modulation of the immune response are not known (Lokossou et al., 2014).

High expression of HERV-R (class I retrovirus) also helps protect the fetus from the maternal immune response. In fact, its expression results in elevated concentrations of env protein that is potentially immunosuppressive. A direct correlation has been identified between the expression levels of fusogenic syncytin-1 and -2 and preclampsia (or gestosis) events, since both proteins levels were shown to be decreased in preclampsia following hypermethylation in the 3′LTR (Vargas et al., 2011). Considering this strong correlation, these HERV proteins could be used as potential markers for the early diagnosis of preclampsia.

In addition to this known function in placentation in mammals, a recent study has shown that syncytin also supports myogenesis, increasing muscle mass in male mice (Redelsperger et al., 2016). In fact, deletion of the syncytin B isoform led to a

20% reduction in muscle mass in knockout mice compared with animals with both syncytin A and syncytin B. This reduction was male-specific because not visible in female. As suggested by the authors of the study, syncytin B could play a decisive role in the muscular development of male mice, and its role may explain the muscle sexual dimorphism observed in all placental mammals (Redelsperger et al., 2016).

HERV AND BRAIN PROTECTION

A number of HERVs (e.g., -W, -K, -R, and -E) are expressed in human brain tissues (Antony et al., 2006, 2007), but a neuroprotective function was only ascribed to class II HERV-K *env* gene. In fact, deep transcriptomic sequencing analyses revealed significantly greater HERV-K expression in healthy fetal brain than all other HERVs (Bhat et al., 2014). Using an *in vitro* paradigm, the same study showed that transfection of the human neuronal cell line (SK-N-SH) with an HERV-K*env* expression plasmid enhanced the transcription of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) compared with cells transfected with a control construct (pGFP). Both these neurotrophins contribute toward the growth and survival of developing neurons and to the maintenance of neuronal function (Bhat et al., 2014). Indeed, it has been proposed that their depletion might lead to the development of CNS disorders (Allen et al., 2013). Bhat and colleagues also showed HERV-K *env* to confer neurotrophic effects on neuronal cells exposed to neurotoxins, as observed in murine NG108 neuronal cells transfected with pHERV-K*env* and treated with different neurotoxins, staurosporine and HIV-1 Vpr protein, in which the cellular viability reached 100 and 60%, respectively, in response to the treatment. These results substantiate a beneficial effect of HERV-K on neuronal cells by preventing neuronal injury mediated by these two neurotoxins (Bhat et al., 2014). A neuroprotective effect of HERV-K was also demonstrated *in vivo* by means of a neurobehavioral study performed with immunodeficient HIV-vpr/ RAG1^{-}/$^{-}$ mice able to express the neurotoxic Vpr protein. For this experiment, neuronal stem cells (NSCs) were transfected with pHERV-K *env* or pGFP, as control, and then implanted into the striatum of the animals. In line with the results of the *in vitro* experiment, NSCs cells expressing HERV-K env protein showed high levels of BDNF without any changes in neuronal morphology or density. Moreover, diminished tumor necrosis factor alpha (TNFα) expression (a well-known toxic factor) was observed together with reduced neuroinflammation. Striatal function was also evaluated by assessing the rotational behavior of the animals caused by a lesion resulting from the unilateral intrastriatal injection of amphetamine (Bhat et al., 2014). To summarize briefly, following the induced neurological injury, mice would exhibit ipsiversive movement toward the side of the lesion; in terms of their rotary behavior, the mice implanted with pHERV-K *env*-transfected cells exhibited reduced neurobehavioral deficits respect to mice implanted with the control construct. The results of this *in vivo* experiment strengthen the *in vitro* data obtained so far on the neuroprotective properties exerted by HERV-K *env*.

HERV AND HORMONE-DEPENDENT ORGANS

Another possible role of HERV *env*-encoded proteins could be their involvement in the maturation and functional activity of steroid-dependent organs. As in the case of the placenta, HERV-R expression is particularly high during fetal development and it is thought to be engaged in embryogenesis (Andersson et al., 2002). A previous study conducted on three developing fetuses (5–6 weeks) demonstrated an organ-specific expression of HERV-R, since high levels of HERV-R *env* mRNA in the secretary epithelium of the gut, the kidneys, the tongue, the heart, the liver, and the central nervous system, but not in the epidermidis were observed. However, the highest level of expression was demonstrated in the placenta and in the adrenal cortex (Andersson et al., 2002). In the latter case, env protein was also detected using immunofluorescence in a postpartum fetus. This expression may be controlled by the presence of androgens that regulate HERV-R expression, since this retrovirus contains androgen receptor (AR) sites in its 5′LTR (Kato et al., 1987). In fact, it is known that about half way through pregnancy the adrenal cortex secretes androgens necessary for the growth and development of the fetus (O'Rahilly, 1983), which, could, in turn, bind to the retroviral AR sites. Moreover, since high levels of HERV-R have also been reported in human adult adrenal cortex (Katsumata et al., 1998), we might suppose that *env* expression is constitutive and not limited to embryogenesis period. Although a role remains to be established in human adult, the most accredited hypothesis is that HERV-R could be involved in protecting the fetus and newborn against further retroviral infections through the mechanism of receptor interference. In fact, since the sebum makes up part of the vernix caseosa that covers the fetus's skin, it is possible that the presence of HERV-R env protein in this secretion is able to prevent a viral infection by blocking the corresponding receptor (Andersson et al., 1996). This hypothesis may be reasonable for female subjects, but a potential function of HERV-R *env* expression in steroid hormone-dependent male organs remains to be discovered.

HERV AND ANTIVIRAL ACTIVITY

During viral infections, host cells activate different restriction factors, coping with such infections. In the context of exogenous retrovirus infections, more than nine groups of cellular restriction factors have been reported to interfere with Human Immunodeficiency Virus type 1 (HIV-1) replication. They include the well-characterized APOBEC3G, SAMHD1, Tetherin/BST-2, and TRIM5α proteins (Johnson, 2013), and those more recently characterized such as MX-2, SERINC3/5, IFITMs, Schlafen 11, and MARCH2/8 (Goujon et al., 2013; Zhang et al., 2018). Relevant to this, endogenized HERVs can be potentially considered *per se* as restriction factors able to exert protective effects against exogenous retroviruses (Grandi and Tramontano, 2018a). Three possible mechanisms have been suggested by which HERV could promote resistance to exogenous retrovirus infections: (1) Occurrence of

complementary interactions between HERV mRNAs and homologous RNAs originated by exogenous retrovirus, with formation of dsRNA molecules that, in turn, can stimulate the Toll-Like Receptor 3 (TLR3) and thus an innate immune response (Zhou et al., 2010). (2) Aggregation of HERV and retroviral proteins, as observed in cells co-infected by both HIV-1 and HERV-K, in which gag proteins of both viruses colocalized at the plasma membrane and co-assembly into the same HIV-1 virions, thus inhibiting release of new HIV-1 infectious particles (Monde et al., 2017). (3) Superinfection interference, as that exerted by HERV pseudo-viral particles or HERV-derived proteins that block retrovirus entry through cellular-receptor interference. This was the case of the truncated HERV-F env protein (lacking the TM subunit) encoded by the *suppressyn* gene, that by binding the cell receptor ASCT2 could prevent the entry of several type D-retroviruses (Sugimoto et al., 2013).

Eventually, a negative effect of the full-length HERV-K 108 env protein was observed *in vitro* on the release of infectious HIV-1 virions production, albeit further studies are needed to elucidate the underlying molecular mechanism, it seems that it does not belong to any of the above described (Terry et al., 2017).

However, despite the above *in vitro* observations, there is not yet direct evidence that the 30 *env* genes found in the human genome confer resistance to retroviruses *in vivo* (Johnson, 2013).

CONCLUSION

Human endogenous retroviruses are an integral part of the human genome and result from ancestral infections by their exogenous progenitors of cells of the human germline, where they have undergone replication together with host genes. Theoretically, the presence of these viral sequences could be potentially harmful for the host because their integration could lead to mutations. However, HERVs were not eliminated during evolution, but rather they were maintained in the host-genome. If, in the past, HERVs were mistakenly considered as useless elements of the human genome (DNA junk), today some are recognized as conferring biological advantages. In fact, in some cases, HERV genomes have undergone a process of positive selection during evolution, being exploited by the hosts to benefit important physiological processes.

As we have summarized in this review, HERVs appear to play important roles in physiology, fetal development, and human evolution: if the accidental infection of a mammalian ancestor by an exogenous retrovirus had never occurred, the placenta and the mammals that produce it, including humans, would never have existed. These beneficial consequences can explain why HERVs have been "fixed" into the genome instead of being eliminated over the years. Nevertheless, the endogenization of retroviruses was not without consequences: during evolution, the majority of HERVs accumulated deletions and mutations that generally compromised their ability to replicate and produce proteins unless under specific conditions. This was the price to be paid for their survival. In this review, we have reported on current findings regarding the biological advantages that HERVs confer to humans. Indeed, endogenous retroviruses provide a perfect example of a symbiotic relationship between a virus and its host, yet a profound understanding of HERV biology that could lead to a better comprehension of their roles is still missing. However, some HERVs are also compatible with parasitic relationship. In fact, various studies have highlighted an association between the presence of HERVs, probably those that have integrated into human genome more recently, and some rare and incurable diseases, in particular autoimmune, neurodegenerative, and cancer diseases (Antony et al., 2004; Grandi and Tramontano, 2017, 2018a; Ibba et al., 2018). These findings are highly relevant because, if a clear evidence emerges that HERVs are explicitly involved in the onset of these pathologies, this would open up new treatment prospects, as they would no-longer be considered incurable diseases, but as pathologies involving one or more retroviruses against which antiretroviral therapies could be used or developed. Indeed, this knowledge would pave the way to important new therapeutic approaches for the treatment of such pathologies.

AUTHOR CONTRIBUTIONS

AL conceptualized and wrote the manuscript. GG revised the manuscript. All authors gave final approval of the version to be submitted and any revised version.

FUNDING

We would like to acknowledge support from the Italian Ministry for Universities and Scientific Research (Research Programs of Significant National Interest, PRIN 2017–2020, Grant No. 2017HWPZZZ_002) to AL.

REFERENCES

Aiewsakun, P., and Katzourakis, A. (2015). Endogenous viruses: connecting recent and ancient viral evolution. *Virology* 479-480, 26–37. doi: 10.1016/j.virol.2015.02.011

Allen, H. K., and Abedon, S. T. (2013). That's disturbing! An exploration of the bacteriophage biology of change. *Front. Microbiol.* 4:295. doi: 10.3389/fmicb.2013.00295

Allen, S. J., Watson, J. J., Shoemark, D. K., Barua, N. U., and Patel, N. K. (2013). GDNF, NGF and BDNF as therapeutic options for neurodegeneration. *Pharmacol. Ther.* 138, 155–175. doi: 10.1016/j.pharmthera.2013.01.004

Andersson, A. C., Merza, M., Venables, P., Pontén, F., Sundström, J., Cohen, M., et al. (1996). Elevated levels of the endogenous retrovirus ERV3 in human sebaceous glands. *J. Invest. Dermatol.* 106, 125–128. doi: 10.1111/1523-1747.ep12329612

Andersson, A. C., Venables, P. J. W., Tonjes, R. R., Scherer, J., Eriksson, L., and Larsson, E. (2002). Developmental expression of HERV-R (ERV3) and HERV-K in human tissue. *Virology* 297, 220–225. doi: 10.1006/viro.2002.1428

Antony, J. M., Izad, M., Bar-Or, A., Warren, K. G., Vodjgani, M., Mallet, F., et al. (2006). Quantitative analysis of human endogenous retrovirus-W env in neuroinflammatory diseases. *AIDS Res. Hum. Retroviruses* 22, 1253–1259. doi: 10.1089/aid.2006.22.1253

Antony, J. M., van Marle, G., Opii, W., Butterfield, D. A., Mallet, F., Yong, V. W., et al. (2004). Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination. *Nat. Neurosci.* 10, 1088–1095. doi: 10.1038/nn1319

Antony, J. M., Zhu, Y., Izad, M., Warren, K. G., Vodjgani, M., Mallet, F., et al. (2007). Comparative expression of human endogenous retrovirus-W genes in multiple sclerosis. *AIDS Res. Hum. Retroviruses* 23, 1251–1256. doi: 10.1089/aid.2006.0274

Armbruester, V., Sauter, M., Krautkraemer, E., Meese, E., Kleiman, A., Best, B., et al. (2002). A novel gene from the human endogenous retrovirus K expressed in transformed cells. *Clin. Cancer Res* 8, 1800–1807.

Bannert, N., and Kurth, R. (2006). The evolutionary dynamics of human endogenous retroviral families. *Annu. Rev. Genomics Hum. Genet.* 7, 149–173. doi: 10.1146/annurev.genom.7.080505.115700

Bhat, R. K., Rudnick, W., Antony, J. M., Maingat, F., Ellestad, K. K., Wheatley, B. M., et al. (2014). Human endogenous retrovirus-K(II) envelope induction protects neurons during HIV/AIDS. *PLoS One* 9:e97984. doi: 10.1371/journal.pone.0097984

Douville, R. N., and Avindra, N. (2014). Human endogenous retroviruses and the nervous system. *Handb. Clin. Neurol.* 123, 465–485. doi: 10.1016/B978-0-444-53488-0.00022-25

Duerkop, B. A., and Hooper, L. V. (2013). Resident viruses and their interactions with the immune system. *Nat. Immunol.* 14, 654–659. doi: 10.1038/ni.2614

Freer, G., Maggi, F., Pifferi, M., Di Cicco, M. E., Peroni, D. G., and Pistello, M. (2018). The virome and its major component, anellovirus, a convoluted system molding human immune defenses and possibly affecting the development of asthma and respiratory diseases in childhood. *Front. Microbiol.* 9:686. doi: 10.3389/fmicb.2018.00686

Galli, U. M., Sauter, M., Lecher, B., Maurer, S., Herbst, H., Roemer, K., et al. (2005). Human endogenous retrovirus rec interferes with germ cell development in mice and may cause carcinoma in situ, the predecessor lesion of germ cell tumors. *Oncogene* 24, 3223–3228. doi: 10.1038/sj.onc.1208543

Goujon, C., Moncorgé, O., Bauby, H., Doyle, T., Ward, C. C., Schaller, T., et al. (2013). Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection. *Nature* 502, 559–562. doi: 10.1038/nature12542

Grandi, N., Cadeddu, M., Pisano, M. P., Esposito, F., Blomberg, J., and Tramontano, E. (2017). Identification of a novel HERV-K(HML10): comprehensive characterization and comparative analysis in non-human primates provide insights about HML10 proviruses structure and diffusion. *Mob. DNA*. 8:15. doi: 10.1186/s13100-017-0099-7

Grandi, N., and Tramontano, E. (2017). Type W human endogenous retrovirus (HERV-W) integrations and their mobilization by L1 machinery: contribution to the human transcriptome and impact on the host physiopathology. *Viruses* 9:162. doi: 10.3390/v9070162

Grandi, N., and Tramontano, E. (2018a). HERV envelope proteins: physiological role and pathogenic potential in cancer and autoimmunity. *Front. Microbiol.* 9:462. doi: 10.3389/fmicb.2018.00462

Grandi, N., and Tramontano, E. (2018b). Human endogenous retroviruses are ancient acquired elements still shaping innate immune responses. *Front. Immunol.* 9:2039. doi: 10.3389/fimmu.2018.02039

Hanada, K., Suzuki, Y., and Gojobori, T. (2004). A large variation in the rates of synonymous substitution for RNA viruses and its relationship to a diversity of viral infection and transmission modes. *Mol. Biol. Evol.* 21, 1074–1080. doi: 10.1093/molbev/msh109

Handley, S. A., Thackray, L. B., Zhao, G., Presti, R., Miller, A. D., Droit, L., et al. (2012). Pathogenic simian immunodeficiency virus infection is associated with expansion of the enteric virome. *Cell* 151, 253–266. doi: 10.1016/j.cell.2012.09.024

Handwerger, S., and Freemark, M. (2000). The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. *J. Pediatr. Endocrinol. Metab.* 13, 343–356. doi: 10.1515/JPEM.2000.13.4.343

Hayward, A., Cornwallis, C. K., and Jern, P. (2015). Pan-vertebrate comparative genomics unmasks retrovirus macroevolution. *Proc. Natl. Acad. Sci. U.S.A.* 112, 464–469. doi: 10.1073/pnas.1414980112

Ibba, G., Piu, C., Uleri, E., Serra, C., and Dolei, A. (2018). Disruption by SaCas9 endonuclease of HERV-Kenv, a retroviral gene with oncogenic and neuropathogenic potential, inhibits molecules involved in cancer and amyotrophic lateral sclerosis. *Viruses* 10:412. doi: 10.3390/v10080412

Ito, J., Gifford, R. J., and Sato, K. (2020). Retroviruses drive the rapid evolution of mammalian APOBEC3 genes. *Proc. Natl. Acad. Sci. U.S.A.* 117, 610–618. doi: 10.1073/pnas.1914183116

Johnson, W. E. (2013). Rapid adversarial co-evolution of viruses and cellular restriction factors. *Curr. Top. Microbiol. Immunol.* 371, 123–151. doi: 10.1007/978-3-642-37765-5_5

Kato, N., Pfeifer-Ohlsson, S., Kato, M., Larsson, E., Rydnert, J., Ohlsson, R., et al. (1987). Tissue-specific expression of human provirus ERV3 mRNA in human placenta: two of the three ERV3 mRNAs contain human cellular sequences. *J Virol.* 61, 2182–2191.

Katsumata, K., Ikeda, H., Sato, M., Harada, H., Wakisaka, A., Shibata, M., et al. (1998). Tissue-specific high-level expression of human endogenous retrovirus-R in the human adrenal cortex. *Pathobiology* 66, 209–215. doi: 10.1159/000028025

Katzourakis, A., Gifford, R. J., Tristem, M., Gilbert, M. T. P., and Pybus, O. G. (2009). Macroevolution of complex retroviruses. *Science* 325:1512. doi: 10.1126/science.1174149

Larsson, E., and Andersson, G. (1998). Beneficial role of human endogenous retroviruses: facts and hypotheses. *Scand. J. Immunol.* 48, 329–338. doi: 10.1046/j.1365-3083.1998.00428.x

Lee, A., Nolan, A., Watson, J., and Tristem, M. (2013). Identification of an ancient endogenous retrovirus, predating the divergence of the placental mammals. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 368:20120503. doi: 10.1098/rstb.2012.0503

Lokossou, A. G., Toudic, C., and Barbeau, B. (2014). Implication of human endogenous retrovirus envelope proteins in placental functions. *Viruses* 6, 4609–4627. doi: 10.3390/v6114609

Lyn, D., Deaven, L. L., Istock, N. L., and Smulson, M. (1993). The polymorphic ADP-ribosyltransferase (NAD+) pseudogene 1 in humans interrupts an endogenous pol-like element on 13q34. *Genomics* 18, 206–211. doi: 10.1006/geno.1993.1456

Monde, K., Terasawa, H., Nakano, Y., Soheilian, F., Nagashima, K., Maeda, Y., et al. (2017). Molecular mechanisms by which HERV-K Gag interferes with HIV-1 Gag assembly and particle infectivity. *Retrovirology* 14:27. doi: 10.1186/s12977-017-0351-8

Munoz-Suano, A., Hamilton, A. B., and Betz, A. G. (2001). Gimme shelter: the immune system during pregnancy. *Immunol. Rev.* 241, 20–38. doi: 10.1111/j.1600-065X.2011.01002.x

Muyan, M., and Boime, I. (1997). Secretion of chorionic gonadotropin from human trophoblasts. *Placenta* 18, 237–241. doi: 10.1016/S0143-4004(97)80056-2

Nakamura, O. (2009). Children's immunology, what can we learn from animal studies (1): decidual cells induce specific immune system of feto-maternal interface. *J. Toxicol. Sci.* 34, S331–S339. doi: 10.2131/jts.34.sp331

Nelson, P. N., Hooley, P., Roden, D., Davari Ejtehadi, H., Rylance, P., Warren, P., et al. (2004). Human endogenous retroviruses: transposable elements with potential? *Clin. Exp. Immunol.* 138, 1–9. doi: 10.1111/j.1365-2249.2004.02592.x

O'Rahilly, R. (1983). The timing and sequence of events in the development of the human endocrine system during the embryonic period proper. *Anat. Embryol.* 166, 439–451. doi: 10.1007/bf00305929

Parker, M. T. (2016). An ecological framework of the human virome provides classification of current knowledge and identifies areas of forthcoming discovery. *Yale J Biol Med.* 89, 339–351.

Perron, H., Lazarini, F., Ruprecht, K., Péchoux-Longin, C., Seilhean, D., Sazdovitch, V., et al. (2005). Human endogenous retrovirus (HERV)-W ENV and GAG proteins: physiological expression in human brain and pathophysiological modulation in multiple sclerosis lesions. *J. Neurovirol.* 11, 23–33. doi: 10.1080/13550280590901741

Redelsperger, F., Raddi, N., Bacquin, A., Vernochet, C., Mariot, V., Gache, V., et al. (2016). Genetic evidence that captured retroviral envelope syncytins contribute to myoblast fusion and muscle sexual dimorphism in mice. *PLoS Genet.* 12:e1006289. doi: 10.1371/journal.pgen.1006289

Ruggieri, A., Maldener, E., Sauter, M., Mueller-Lantzsch, N., Meese, E., Fackler, O. T., et al. (2009). Human endogenous retrovirus HERV-K(HML-2) encodes a stable signal peptide with biological properties distinct from Rec. *Retrovirology* 6:17. doi: 10.1186/1742-4690-6-17

Sanjuán, R. (2012). From molecular genetics to phylodynamics: evolutionary relevance of mutation rates across viruses. *PLoS Pathog.* 8:e1002685. doi: 10.1371/journal.ppat.1002685

Schön, U., Diem, O., Leitner, L., Günzburg, W. H., Mager, D. L., Salmons, B., et al. (2009). Human endogenous retroviral long terminal repeat sequences as cell type-specific promoters in retroviral vectors. *J. Virol.* 83, 12643–12650. doi: 10.1128/JVI.00858-09

Seifarth, W., Skladny, H., Krieg-Schneider, F., Reichert, A., Hehlmann, R., and Leib-Mösch, C. (1995). Retrovirus-like particles released from the human breast cancer cell line T47-D display type B- and C-related endogenous retroviral sequences. *J. Virol.* 69, 6408–6416.

Smit, A. F. (1999). Interspersed repeats and other mementos of transposable elements in mammalian genomes. *Curr. Opin. Genet. Dev.* 9, 657–663. doi: 10.1016/S0959-437X(99)00031-3

Sperber, G., Airola, T., Jern, P., and Blomberg, J. (2007). Automated recognition of retroviral sequences in genomic data-RetroTector. *Nucleic Acids Res.* 35, 4964–4976. doi: 10.1261/rna.047803.114

Sugimoto, J., Sugimoto, M., Bernstein, H., Jinno, Y., and Schust, D. (2013). A novel human endogenous retroviral protein inhibits cell-cell fusion. *Sci. Rep.* 3:1462. doi: 10.1038/srep01462

Terry, S. N., Manganaro, L., Cuesta-Dominguez, A., Brinzevich, D., Simon, V., and Muldera, L. C. F. (2017). Expression of HERV-K108 envelope interferes with HIV-1 production. *Virology* 50, 52–59. doi: 10.1016/j.virol.2017.06.004

Vargas, A., Toufaily, C., LeBellego, F., Rassart, É, Lafond, J., and Barbeau, B. (2011). Reduced expression of both syncytin 1 and syncytin 2 correlates with severity of preeclampsia. *Reprod. Sci.* 18, 1085–1091. doi: 10.1177/1933719111404608

Vargiu, L., Rodriguez-Tomé, P., Sperber, G. O., Cadeddu, M., Grandi, N., Blikstad, V., et al. (2016). Classification and characterization of human endogenous retroviruses; mosaic forms are common. *Retrovirology* 13:7. doi: 10.1186/s12977-015-0232-y

Werner, T., Brack-Werner, R., Leib-Mösch, C., Backhaus, H., Erfle, V., and Hehlmann, R. (1990). S71 is a phylogenetically distinct human endogenous retroviral element with structural and sequence homology to simian sarcoma virus (SSV). *Virology* 174, 225–238. doi: 10.1016/0042-6822(90)90071-x

White, D. W., Suzanne Beard, R., and Barton, E. S. (2012). Immune modulation during latent herpesvirus infection. *Immunol. Rev.* 245, 189–208. doi: 10.1111/j.1600-065X.2011.01074.x

Zhang, Y., Lu, J., and Liu, X. (2018). MARCH2 is upregulated in HIV-1 infection and inhibits HIV-1 production through envelope protein translocation or degradation. *Virology* 518, 293–300. doi: 10.1016/j.virol.2018.02.003

Zhou, Y., Wang, X., Liu, M., Hu, Q., Song, L., Ye, L., et al. (2010). A critical function of toll-like receptor-3 in the induction of anti-human immunodeficiency virus activities in macrophages. *Immunology* 131, 40–49. doi: 10.1111/j.1365-2567.2010.03270.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

MINI REVIEW
published: 14 July 2020
doi: 10.3389/fmicb.2020.01629

Saccharomyces cerevisiae – Insects Association: Impacts, Biogeography, and Extent

*Niccolo' Meriggi[1], Monica Di Paola[1], Duccio Cavalieri[1] and Irene Stefanini[2]**

[1] Department of Biology, University of Florence, Florence, Italy, [2] Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy

Over the last few years, an increasing number of studies have reported the existence of an association between the budding yeast *Saccharomyces cerevisiae* and insects. The discovery of this relationship has called into question the hypothesis that *S. cerevisiae* is unable to survive in nature and that the presence of *S. cerevisiae* strains in natural specimens is the result of contamination from human-related environments. *S. cerevisiae* cells benefit from this association as they find in the insect intestine a shelter, but also a place where they can reproduce themselves through mating, the latter being an event otherwise rarely observed in natural environments. On the other hand, insects also take advantage in hosting *S. cerevisiae* as they rely on yeasts as nutriment to properly develop, to localize suitable food, and to enhance their immune system. Despite the relevance of this relationship on both yeast and insect ecology, we are still far from completely appreciating its extent and effects. It has been shown that other yeasts are able to colonize only one or a few insect species. Is it the same for *S. cerevisiae* cells or is this yeast able to associate with any insect? Similarly, is this association geographically or topographically limited in areas characterized by specific physical features? With this review, we recapitulate the nature of the *S. cerevisiae*-insect association, disclose its extent in terms of geographical distribution and species involved, and present *YeastFinder*, a cured online database providing a collection of information on this topic.

Keywords: *Saccharomyces cerevisiae*, insect, yeast-insect association, biogeography, *Saccharomyces cerevisiae* evolution, *Saccharomyces cerevisiae* yeast ecology

OPEN ACCESS

Edited by:
Nathalie Connil, Université de Rouen, France

Reviewed by:
Emilia Garcia-Moruno, Council for Agricultural and Economics Research (CREA), Italy
Agustin Aranda, University of Valencia, Spain
Flaviano S. Martins, Federal University of Minas Gerais, Brazil

****Correspondence:***
Irene Stefanini
irene.stefanini@unito.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *03 May 2020*
Accepted: *22 June 2020*
Published: *14 July 2020*

Citation:
Meriggi N, Di Paola M, Cavalieri D and Stefanini I (2020) Saccharomyces cerevisiae – Insects Association: Impacts, Biogeography, and Extent. Front. Microbiol. 11:1629. doi: 10.3389/fmicb.2020.01629

SACCHAROMYCES CEREVISIAE IN NATURAL ENVIRONMENTS

Saccharomyces cerevisiae is widely used in the industry for winemaking, brewery, and bakery, as animal and human food supplement or probiotic (Palma et al., 2015), and for biofuel, flavorings, pharmaceuticals, and enzymes production (e.g., invertases, lactases) (Parapouli et al., 2020). The physiology and genetics of this yeast have been studied in depth, as well as molecular mechanisms shared with other eukaryotes (Resnick and Cox, 2000). Despite this broad range of applications, the natural diffusion and evolution of this yeast remained unexplored until recently. The ability to overgrow other microorganisms in fermentable substrates and the widespread use of *S. cerevisiae* in the industry of fermented products lead to the hypothesis that this yeast has been domesticated

and is confined to human activities (Gallone et al., 2016). However, over the last few decades, this hypothesis was compromised by the isolation of strains from natural environments (e.g., soil, barks, and water) and by the observation of the existence of strains genetically different from those used in the industry (Liti, 2015). A new hypothesis was proposed: a neutral model in which *S. cerevisiae* is functionally adapted to a range of different environments (Goddard and Greig, 2015). Despite improving our understanding of the natural spread of the budding yeast, these new findings did not clarify (i) how the yeast can survive when nutrient sources are lacking, (ii) where it was before humans started using it to ferment food, and (iii) how can this not airborne microorganism move among different environmental sources. The identification of the association between the budding yeast and social wasps helped fill in several of the gaps in our knowledge on *S. cerevisiae* natural cycle. Previous studies have already shown some aspects of yeast-insect associations (e.g., Stefanini, 2018 and Blackwell, 2017). This review recapitulates the current knowledge exclusively on associations involving *S. cerevisiae* also addressing new aspects: the extent and geographical distribution.

SACCHAROMYCES CEREVISIAE-INSECTS ASSOCIATION: THE YEAST SIDE OF THE COIN

Social wasps and hornets are omnivorous insects that move around a broad foraging area and visit substrates that can be colonized by *S. cerevisiae* strains. Among the substrates visited by wasps, grape skin is the main source of environmental *S. cerevisiae* strains, even if this yeast represents only a minor component of the residing microbial communities. *Polistes dominula* (social wasps) and *Vespa crabro* (hornets) host in their intestines *S. cerevisiae* cells all year long, thus providing an environment in which yeasts can reside and survive in the seasonal period with less access to sugary sources (Stefanini et al., 2012). Insects can spread *S. cerevisiae* cells among environmental substrates and, also, share them within the colony, passing it among adults and to larvae (Stefanini et al., 2012). Thus, the capability of spreading yeast cells increases exponentially with the increase of the insect colony. This has pivotal importance for the diffusion of *S. cerevisiae* cells in the wild, especially considering that the increase in the demographic rate of the wasp colony occurs at the same time of grape ripening (Stefanini et al., 2012). Hence, the high frequency of *S. cerevisiae* cells isolated after the ripening period correlates these insects to the dispersion of yeasts in the vineyard (Stefanini et al., 2012). In addition to social wasps and hornets, other insects bear and spread *S. cerevisiae* cells, as proven in laboratory conditions for *Drosophila* spp. (Christiaens et al., 2014) and confirmed in the wild for bees. In fact, *S. cerevisiae* strains isolated from vineyard specimens are highly similar to strains isolated from bees caught in the same geographic region, suggesting that insects are responsible for the local dispersion of yeast cells (Goddard et al., 2010). Interestingly, the genetic and phenotypic diversity of *S. cerevisiae* does not affect the capability of different yeast strains to survive in the insect intestines (Dapporto et al., 2016; Ramazzotti et al., 2019) or to produce volatile metabolites attracting insects (Palanca et al., 2013). Hence, the whole genetic and phenotypic variability of *S. cerevisiae* can potentially attract and be vectored among natural specimens thanks to insects (Stefanini et al., 2012; Dapporto et al., 2016).

Saccharomyces cerevisiae mating is infrequent in nature, possibly because wild yeast cells are mainly diploid and hence need to face sporulation and germination to be able to mate with other strains, conditions that rarely occur in nature (Cubillos et al., 2009). Contrarily, yeast mating does occur within insect intestines (Stefanini et al., 2016; **Figure 1**). *S. cerevisiae* spores can survive in the intestinal tract of *Drosophila melanogaster*, and, by passing through the insect intestines, the sporal ascus is broken and hence the mating among yeast strains is facilitated (Reuter et al., 2007). The differences among chemical and physiological characteristics of different tracts of the insect intestine could offer a series of environmental changes (Engel and Moran, 2013) promoting yeast sporulation and germination, and hence mating, as shown by experiments carried out in the laboratory (Stefanini et al., 2016). Hence, the intestine not only promotes the yeast ascus break, but also diploid yeast cells sporulation and yeast spores germination, and thus allowing the mating among potentially any yeast strain and ploidy (Stefanini et al., 2016).

SACCHAROMYCES CEREVISIAE-INSECTS ASSOCIATION: THE INSECT SIDE OF THE COIN

The *S. cerevisiae*-insect association has beneficial effects not only on the yeast, but also on its counterpart: insects (**Figure 1**).

The capability to detect food is fundamental for insect survival to obtain the nourishment and find an environment suitable for oviposition. *S. cerevisiae* attracts various insect species including *D. melanogaster* (Becher et al., 2012), *Vespula germanica*, and *V. vulgaris* (Babcock et al., 2017) to food, which is otherwise less appealing. The main features making this yeast capable of enticing insects are the presence of functional mitochondria (Schiabor et al., 2014) and the capability of producing volatile compounds, such as isoamyl acetate and ethyl acetate (Christiaens et al., 2014), which are produced at high levels by yeasts isolated from wasp intestines (Dapporto et al., 2016). Overall, these findings show that the presence of fermenting *S. cerevisiae* cells is a strong cue used by insects to detect sugary substrates. It is fair to consider that, besides *S. cerevisiae*, other microorganisms present on sugary substrates, such as *Hanseniaspora* spp. and *Gluconobacter* spp. (Bueno et al., 2019), and vectored by insects (Palanca et al., 2013; Quan and Eisen, 2018) could produce aromas attracting insects. Interestingly, species-specific attractions have been observed. While *D. melanogaster* is attracted by *S. cerevisiae*, *D. simulans* is indifferent to this yeast species (Gunther et al., 2019). At the same time, *D. melanogaster* and the subgenus *Sophophora* are preferentially attracted to baits seeded with *Hanseniaspora uvarum* than to *S. cerevisiae* and forest-dwelling *Drosophila* species (e.g., *D. tripunctata* and the *guarani* group) are more

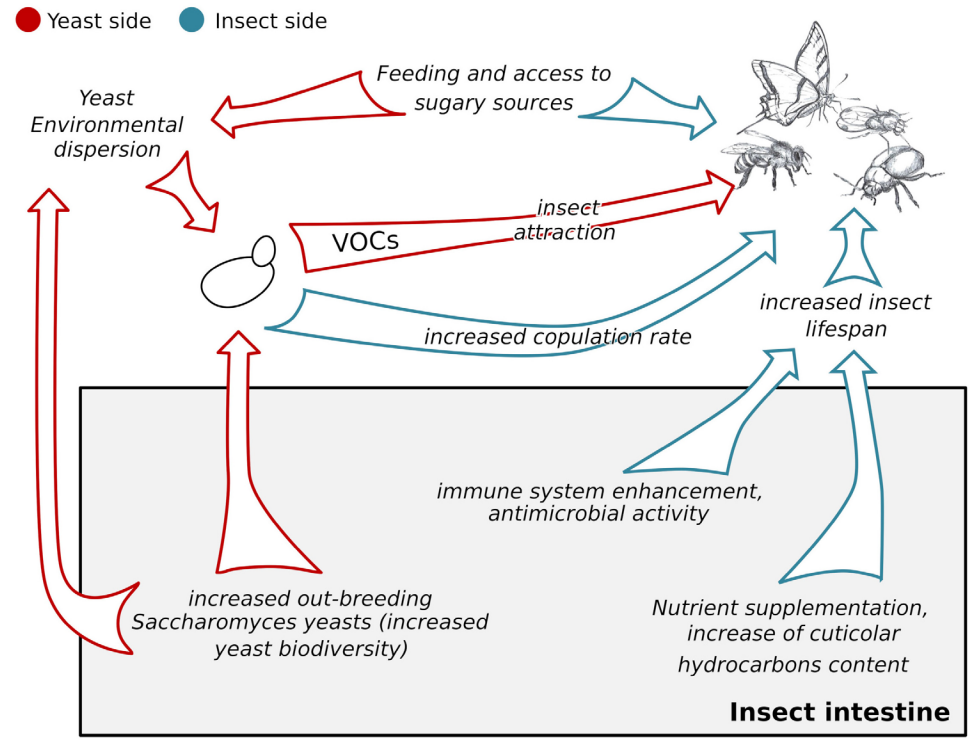


FIGURE 1 | Schematic representation of the impacts of the *S. cerevisiae*-insects association on the yeast and on the insect. Red arrows indicate the impacts of the association on yeasts, blue arrows indicate the impacts of the association on insects. The gray box indicates effects of the association occurring within the insect intestines, both on *S. cerevisiae* and on the insects. VOCs = Volatile Organic Compounds.

attracted by *S. cerevisiae* than by *H. uvarum* (Batista et al., 2017). The differential preferences of *Drosophila* spp. towards different yeast species may mirror what happens in the yeast–insect–morning glory ecosystem, where beetles and yeasts (mostly *Metschnikowia* spp. and *Candida* spp.) reciprocally influence the occupancy of associations-specific niches (Stefanini, 2018).

In the laboratory, insect rearing is based on the use of media providing the full range of nutrients to support larval development but also appealing to female adults and promoting egg deposition (Piper, 2017). To this aim, it is a common practice to use media including *S. cerevisiae* (Becher et al., 2012; Grangeteau et al., 2018). In fact, the yeast supports larval development mostly by providing nicotinic acid, vitamin B, pantothenic acid, inositol, choline, beta-alanine, and pimelic acid (Tatum, 1941). The presence of *S. cerevisiae* in the larval diet also defines the fitness and behavior of juvenile and adult insects (Grangeteau et al., 2018). Providing live *S. cerevisiae* cells to *Drosophila* larvae improves the copulation rate, increases the cuticular hydrocarbon content, extends the insect life, and makes adults preferring food supplemented with the yeast, compared to a diet based on yeast extracts or lacking the yeast (Grangeteau et al., 2018; Murgier et al., 2019). Transcriptional analyses carried out on *D. melanogaster* adults developed from germ-free larvae showed the over-expression of genes involved in several metabolic pathways, if the insect diet was supplemented with live *S. cerevisiae* compared to single or multiple bacterial species (Elya et al., 2016). Despite this effect being observed only in intestinal cells and not at the whole-body level, hence suggesting the impact of yeast on insects is only local, it has to be considered that over the developmental process genes expression is rapidly regulated by multiple factors. Thus, time-course transcriptional analyses would be required to appreciate at the molecular level the impact of *S. cerevisiae* on insect development. The impact of *S. cerevisiae* on development and related traits varies according to the yeast species. For instance, even if a substrate supplemented with the budding yeast improves the survival of adult mosquitoes (*Culex pipiens*) compared to substrates supplemented with other yeast species, it is not preferred for oviposition by gravid *C. pipiens* females (Díaz-Nieto et al., 2016).

As in bigger animals, even in insects the fate and impact of the encountered microorganisms are often determined by the host immune system. The insect immune system includes a cellular and a humoural component (Lemaitre and Hoffmann, 2007). Although several receptors on immune cells have been described as responsible for the recognition of microorganisms, their role in the response to *S. cerevisiae* and yeasts in general is still unclear (Lu and St Leger, 2016). Conversely, an entire pathway of the humoral response is responsible for the insect's reaction to yeasts: the Toll signaling pathway (Roh et al., 2009). When triggered by yeast cell wall β-glucans and proteases, this pathway induces the expression of Drosomycin, an antimicrobial

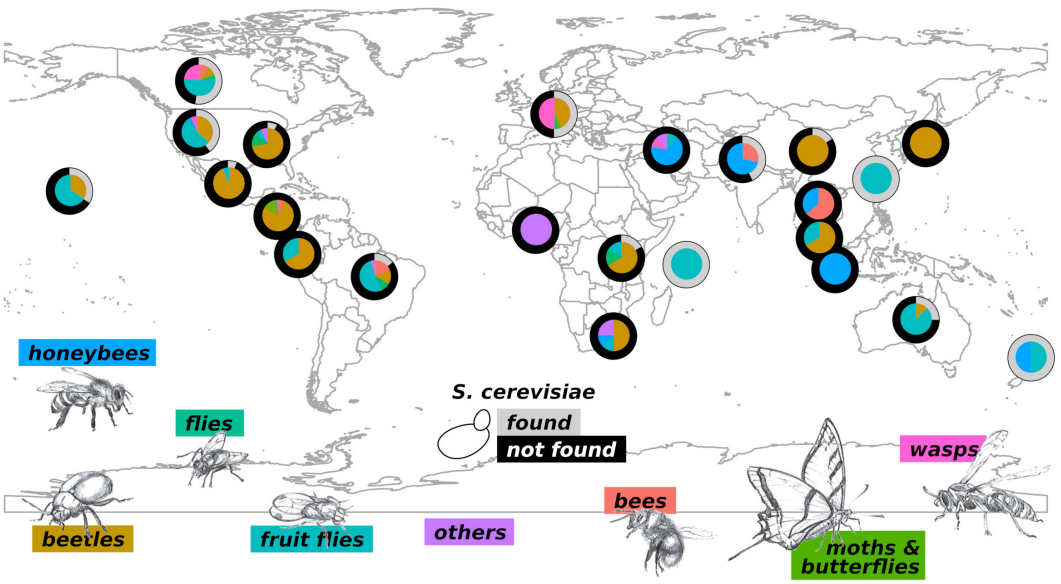


FIGURE 2 | Biogeography and extent of the *S. cerevisiae*-insect association. For each geographical location, the outer ring of the pie chart shows the percentages of insects in which *S. cerevisiae* has been (gray) or has not been (black) identified. The inner parts of pie charts show the types of insects investigated in the corresponding area and the relative proportions; colors are indicated for each insect group at the bottom of the figure. The group "others" refers to insects poorly represented: ants, lacewings, termites, mites, and mosquitoes.

agent (Gottar et al., 2006). Insects can also fight potential yeast pathogens through the Duox response pathway, which induces the production of not-specific antimicrobial reactive oxygen species (Hoang et al., 2015). To note, the vast majority of information on the insect immune response to *S. cerevisiae* has been obtained by using the *Drosophila* spp. model that presents perturbations in the Toll pathway (Alarco et al., 2004), making this insect susceptible to the budding yeast (Lionakis, 2011). Alternatively, the use of *Galleria mellonella*, naturally susceptible to *S. cerevisiae*, yielded fundamental information that could not be gathered with fruit flies. For instance, *G. mellonella* provided the first insights on the capability of a pre-exposure to *S. cerevisiae* cells or glucans to protect the insect against a subsequent infection with a lethal dose of *Candida albicans* (Bergin et al., 2006). This immune-enhancing elicited by *S. cerevisiae* has been recently confirmed in the social wasps *Polistes dominula*, which become more resistant to *Escherichia coli* infections upon pre-immunization with the yeast (Meriggi et al., 2019).

BIOGEOGRAPHY AND DIFFUSION

Insects can colonize habitats with extremely different characteristics and are considered, as defined by E.O. Wilson, "*the little things that run the world*" (Wilson, 1987). The large number of insect species makes it impossible to comprehensively analyze their biodiversity. Of the estimated 6 million species, only 1 million are known (Larsen et al., 2017). For a matter of clarity, we will report here information on insects broadly grouped. We have, however, created a detailed on-line database that can be browsed by the reader (YeastFinder,[1]). *S. cerevisiae* has been identified, through isolation or metabarcoding, in the intestine or on the body of several insects all over the World (**Figure 2**). Notably, studies carried out so far lack consistency in the methods adopted for yeast isolation, and this may greatly impact the capability of identifying *S. cerevisiae* associated with insects. However, in this review we will neglect the heterogeneity of the adopted methods, leaving the exploration of this topic to dedicated future studies. Interestingly, *S. cerevisiae* has not been found in insects caught in South Africa (SAf), Ecuador, Thailand, Indonesia, Nigeria (the only study on termites), Iran, Japan (J), Malaysia, and Central America (CA: Panama, Costa Rica, and Guatemala). It is worth to consider that the investigations carried out in Ecuador and Malaysia (Freitas et al., 2013), Thailand (Saksinchai et al., 2015), Indonesia (Basukriadi et al., 2010), Nigeria (Adelabu et al., 2019), and Iran (Siavoshi et al., 2018) are related to individual studies, hence the lack of identification of *S. cerevisiae* could be ascribed to the procedure adopted for yeast isolation. On the other hand, multiple studies failed in identifying *S. cerevisiae* in association with beetles, honeybees and mosquitoes, butterflies, mites, and moths collected in J (Toki et al., 2012; Ninomiya et al., 2013), SAf (de Vega et al., 2012; Steyn et al., 2016), and CA (Lachance et al., 2001a,b, 2006, Suh and Blackwell, 2006; Suh et al., 2006, 2007; Rivera et al., 2009; Urbina et al., 2013; Ravenscraft et al., 2018). The lack of identification of *S. cerevisiae* in insects of these areas could indicate an unusual situation that is worth to be further investigated. All the insects investigated in New Zealand, Taiwan, and Seychelles Islands bore *S. cerevisiae* (**Figure 2**). However, the number of cases studied

[1]www.stefaninilab.com/tools/

in these areas is low ($n = 6$), and hence this observation may be poorly representative of the real situation.

Multiple studies investigating various insects in other locations (shown in **Figure 2**) indicated an even geographical distribution of *S. cerevisiae*. The budding yeast has not been isolated from ants, lacewings, termites, mites, and mosquitoes (the group "others" in **Figure 2**), which were, however, poorly investigated (two species in different locations at most) (Carreiro et al., 1997; Lachance et al., 2003; Suh et al., 2005; Nguyen et al., 2006; Steyn et al., 2016; Siavoshi et al., 2018). Similarly, *S. cerevisiae* has not been found in butterflies ($n = 11$ species in different locations) and moths ($n = 4$) (Suh et al., 2006; Witzgall et al., 2012; Ravenscraft et al., 2018). Interestingly, only the 0.03% of beetles, which have been widely investigated ($n = 236$), bear *S. cerevisiae* (Kurtzman and Robnett, 1998; Lachance et al., 2001a,b, 2006; Six, 2003; Suh and Blackwell, 2004, Suh et al., 2005, 2006, 2007, 2013; Delalibera et al., 2005; Nguyen et al., 2006; Rosa et al., 2007; Rivera et al., 2009; de Vega et al., 2012; Hui et al., 2012; Toki et al., 2012; Freitas et al., 2013; Kaltenpoth and Steiger, 2013; Ninomiya et al., 2013; Urbina et al., 2013; Cline et al., 2014; Ren et al., 2014, 2015; Liu et al., 2016; Tanahashi and Hawes, 2016; Wang et al., 2016; Briones-Roblero et al., 2017; Chai et al., 2019). Similarly, bees only accidentally bear *S. cerevisiae*, with only 1 occurrence over 21 reported cases (Sandhu and Waraich, 1985; Lachance et al., 2003; Rosa et al., 2003; Daniel et al., 2013; Charron et al., 2014; Saksinchai et al., 2015). Conversely, *S. cerevisiae* has been found in a large portion of investigated flies, fruit flies, honey-bees, and wasps (29, 57, 20, and 71%, respectively) (Phaff and Knapp, 1956; Batra et al., 1973; Sandhu and Waraich, 1985; Morais et al., 1993, 1994; Rosa et al., 1994; Lachance et al., 1995, 2003, 2006; Suh et al., 2005; Nguyen et al., 2006, 2007; Basukriadi et al., 2010; Goddard et al., 2010; Chandler et al., 2012; de Vega et al., 2012; Hamby et al., 2012; Stefanini et al., 2012; Freitas et al., 2013; Buser et al., 2014; Charron et al., 2014; Lam and Howell, 2015; Saksinchai et al., 2015; Batista et al., 2017; Deutscher et al., 2017; Jimenez et al., 2017; Piper et al., 2017; Quan and Eisen, 2018; Siavoshi et al., 2018; dos Santos et al., 2019; Meriggi et al., 2019; Park et al., 2019). A few possible scenarios could explain the higher occurrence of *S. cerevisiae* in these groups of insects: (i) they are more prone to visit human-related environments, such as wineries and vineyards, that are likely to host higher amounts of *S. cerevisiae* cells, (ii) they are more attracted by substrates inhabited by the budding yeast compared to other insects, (iii) diet and physical-chemical intestine conditions facilitate the housing of *S. cerevisiae*.

CONCLUSION AND PERSPECTIVES

According to the reports gathered for this review, it appears that every group of insects can bear *S. cerevisiae*, but only further and more detailed studies investigating a higher number of a broader range of insect species, as well as the standardization of isolation and identification methodologies, will consolidate this observation. Also, further studies on the geographical extent of this phenomenon would allow evaluating the existence of different physiological characteristics among insect species that favor or prevent the instauration of the association with the budding yeast. Aiming at this, it will be fundamental to also include groups neglected so far. For instance, planthoppers, mosquitoes, and spiders have not or have only poorly been investigated, albeit they could represent an unprecedented source of information as they visit and forage on a broad range of environmental sources. In addition, *S. cerevisiae* has been shown to have an impact on spiders' behavior and health (Tietjen et al., 1987; Patt et al., 2012), and, especially considering that spiders are mostly carnivores, exploring this association would provide insightful information on the role of this yeast in prey hunting and interactions among species.

Overall, the reports published so far depict a tangled relationship between insect and yeast, in which various factors define the insect attraction to yeasts and the impact of this yeast on insect health. Understanding the factors responsible for the attraction of insects by yeasts, also by further exploring the differences among insect species has also important applications. A better understanding of the factors regulating this complex field will provide relevant information potentially useful to ideate approaches to use *S. cerevisiae* as a promoter of insect health or as a pest control. For instance, dissecting the capability of enhancing the host immune reaction against pathogenic microorganisms would be very useful in the fight against the worldwide decline of honeybees and pollinators (Wagner, 2020).

Only further studies will allow us to fully unravel the influence of *S. cerevisiae* on insects, and the potential applications of strains isolated from this natural source.

AUTHOR CONTRIBUTIONS

IS ideated the review. NM and IS gathered the data. DC, MD, NM, and IS wrote the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank Miss Marta Mocciaro for drawing the insect's images. This work was supported by the University of Turin (Local Research Funds). NM, MD, and DC were supported by the University of Florence and by the Regione Toscana POR FSE 2014-2020, VESPATER project.

REFERENCES

Adelabu, B., Kareem, S. O., Adeogun, A. I., and Wakil, S. M. (2019). Optimization of cellulase enzyme from sorghum straw by yeasts isolated from plant feeding-termite *Zonocerus variegatus*. *Food Appl. Biosci. J.* 7, 81–101.

Alarco, A. M., Marcil, A., Chen, J., Suter, B., Thomas, D., and Whiteway, M. (2004). Immune-deficient *Drosophila melanogaster*: a model for the innate immune response to human fungal pathogens. *J. Immunol.* 172, 5622–5628. doi: 10.4049/jimmunol.172.9.5622

Babcock, T., Gries, R., Borden, J., Plamero, L., Mattiacci, A., Masciocchi, M., et al. (2017). Brewer's yeast, *Saccharomyces cerevisiae*, enhances attraction of two invasive yellowjackets (Hymenoptera: Vespidae) to dried fruit and fruit powder. *J. Insect Sci.* 17:91.

Basukriadi, A., Sjamsuridzal, W., and Beristama Putra, B. (2010). Molecular identification and diversity of yeasts associated with *Apis cerana* foraging on flowers of *Jatropha integerrima*. *Microbiol. Indones.* 4, 44–48. doi: 10.5454/mi.4.1.9

Batista, M. R., Uno, F., Chaves, R. D., Tidon, R., Rosa, C. A., and Klaczko, L. B. (2017). Differential attraction of drosophilids to banana baits inoculated with *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* within a Neotropical forest remnant. *PeerJ* 5:e3063. doi: 10.7717/peerj.3063

Batra, L. R., Batra, S. W. T., and Bohart, G. E. (1973). The mycoflora of domesticated and wild bees (Apoidea). *Mycopathol. Mycol. Appl.* 49, 13–44. doi: 10.1007/bf02057445

Becher, P. G., Flick, G., Rozpêdowska, E., Schmidt, A., Hagman, A., Lebreton, S., et al. (2012). Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Funct. Ecol.*26, 822–828. doi: 10.1111/j.1365-2435.2012.02006.x

Bergin, D., Murphy, L., Keenan, J., Clynes, M., and Kavanagh, K. (2006). Pre-exposure to yeast protects larvae of *Galleria mellonella* from a subsequent lethal infection by Candida albicans and is mediated by the increased expression of antimicrobial peptides. *Microbes Infect.* 8, 2105–2112. doi: 10.1016/j.micinf.2006.03.005

Blackwell, M. (2017). Made for each other: ascomycete yeasts and insects. *Microbiol. Spectr.* 5, 1–18. doi: 10.1128/microbiolspec.FUNK-0081-2016

Briones-Roblero, C. I., Rodríguez-Díaz, R., Santiago-Cruz, J. A., Zúñiga, G., and Rivera-Orduña, F. N. (2017). Degradation capacities of bacteria and yeasts isolated from the gut of *Dendroctonus rhizophagus* (Curculionidae: Scolytinae). *Folia Microbiol.* 62, 1–9. doi: 10.1007/s12223-016-0469-4

Bueno, E., Martin, K. R., Raguso, R. A., Mcmullen, J. G., Hesler, S. P., Loeb, G. M., et al. (2019). Response of wild spotted wing *Drosophila* (*Drosophila suzukii*) to microbial volatiles. *J. Chem. Ecol.* doi: 10.1007/s10886-019-01139-4 [Epub ahead of print].

Buser, C. C., Newcomb, R. D., Gaskett, A. C., and Goddard, M. R. (2014). Niche construction initiates the evolution of mutualistic interactions. *Ecol. Lett.* 17, 1257–1264. doi: 10.1111/ele.12331

Carreiro, S. C., Pagnocca, F. C., Bueno, O. C., Júnior, M. B., Hebling, M. J. A., and da Silva, O. A. (1997). Yeasts associated with nests of the leaf-cutting ant Atta sexdens rubropilosa Forel, 1908. *Antonie Van Leeuwenhoek* 71, 243–248.

Chai, C. Y., Huang, L. N., Cheng, H., Liu, W. J., and Hui, F. L. (2019). *Metschnikowia baotianmanensis* f.a., sp. nov., a new yeast species isolated from the gut of the rhinoceros beetle *Allomyrina dichotoma*. *Int. J. Syst. Evol. Microbiol.* 69, 3087–3092. doi: 10.1099/ijsem.0.003593

Chandler, J. A., Eisen, J. A., and Kopp, A. (2012). Yeast communities of diverse *Drosophila* species: comparison of two symbiont groups in the same hosts. *Appl. Environ. Microbiol.* 78, 7327–7336. doi: 10.1128/aem.01741-12

Charron, G., Leducq, J. B., Bertin, C., Dubé, A. K., and Landry, C. R. (2014). Exploring the northern limit of the distribution of Saccharomyces cerevisiae and Saccharomyces paradoxus in North America. *FEMS Yeast Res.* 14, 281–288. doi: 10.1111/1567-1364.12100

Christiaens, J. F., Franco, L. M., Cools, T. L., De Meester, L., Michiels, J., Wenseleers, T., et al. (2014). The fungal aroma gene ATF1 promotes dispersal of yeast cells through insect vectors. *Cell Rep.* 9, 425–432. doi: 10.1016/j.celrep.2014.09.009

Cline, A. R., Skelley, P. E., Kinnee, S. A., Rooney-Latham, S., Winterton, S. L., Borkent, C. J., et al. (2014). Interactions between a sap beetle, sabal palm, scale insect, filamentous fungi and yeast, with discovery of potential antifungal compounds. *PLoS One* 9:e89295. doi: 10.1371/journal.pone.0089295

Cubillos, F. A., Vasquez, C., Faugeron, S., Ganga, A., and Martínez, C. (2009). Self-fertilization is the main sexual reproduction mechanism in native wine yeast populations. *FEMS Microbiol. Ecol.* 67, 162–170. doi: 10.1111/j.1574-6941.2008.00600.x

Daniel, H. M., Rosa, C. A., Sao Thiago-Calac, P. S., Antonini, J., Bastos, E. M. A. F., Evrard, P., et al. (2013). *Starmerella neotropicalis* f.a., sp. nov., a yeast species found in bees and pollen. Int. J. Syst. Evol. Microbiol. 63, 3896–3903. doi: 10.1099/ijs.0.055897-0

Dapporto, L., Stefanini, I., Rivero, D., Polsinelli, M., Capretti, P., De Marchi, P., et al. (2016). Social wasp intestines host the local phenotypic variability of *Saccharomyces cerevisiae* strains. *Yeast* 33, 277–287. doi: 10.1002/yea.3173

de Vega, C., Guzman, B., Lachance, M. A., Steenhuisen, S. L., Johnson, S. D., and Herrera, C. M. (2012). *Metschnikowia proteae* sp. nov., a nectarivorous insect-associated yeast species from Africa. Int. J. Syst. Evol. Microbiol. 62, 2538–2545. doi: 10.1099/ijs.0.040790-0

Delalibera, I., Handelsman, J., and Raffa, K. F. (2005). Contrasts in cellulolytic activities of gut microorganisms between the wood borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the bark beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae). *Environ. Entomol.* 34, 541–552.

Deutscher, A. T., Reynolds, O. L., and Chapman, T. A. (2017). Yeast: an overlooked component of *Bactrocera tryoni* (Diptera: Tephritidae) larval gut microbiota. *J. Econ. Entomol.* 110, 298–300. doi: 10.1093/jee/tow262

Díaz-Nieto, L. M., D'Alessio, C., Perotti, M. A., and Berón, C. M. (2016). *Culex pipiens* development is greatly influenced by native bacteria and exogenous yeast. *PLoS One* 11:e0153133. doi: 10.1371/journal.pone.0153133

dos Santos, T. T., de Oliveira, D. P., Ramos Cabette, H. S., and de Morais, P. B. (2019). The digestive tract of *Phylloicus* (Trichoptera: Calamoceratidae) harbours different yeast taxa in Cerrado streams, Brazil. *Symbiosis* 77, 147–160. doi: 10.1007/s13199-018-0577-9

Elya, C., Zhang, V., Ludington, W. B., and Eisen, M. B. (2016). Stable host gene expression in the gut of adult *Drosophila melanogaster* with different bacterial mono-associations. *PLoS One* 11:e0167357. doi: 10.1371/journal.pone.0167357

Engel, P., and Moran, N. A. (2013). The gut microbiota of insects–diversity in structure and function. *FEMS Microbiol. Rev.* 37, 699–735. doi: 10.1111/1574-6976.12025

Freitas, L. F., Barriga, E. J. C., Barahona, P. P., Lachance, M. A., and Rosa, C. A. (2013). *Kodamaea transpacifica* fa, sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galápagos Islands and Malaysia: further evidence for ancient human transpacific contacts. Int. J. Syst. Evol. Microbiol. 63, 4324–4329. doi: 10.1099/ijs.0.052282-0

Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B., et al. (2016). Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell* 166, 1397–1410.

Goddard, M. R., Anfang, N., Tang, R., Gardner, R. C., and Jun, C. (2010). A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-aided global dispersal in oak barrels. *Environ. Microbiol.* 12, 63–73. doi: 10.1111/j.1462-2920.2009.02035.x

Goddard, M. R., and Greig, D. (2015). *Saccharomyces cerevisiae*: a nomadic yeast with no niche? *FEMS Yeast Res.* 15:fov009.

Gottar, M., Gobert, V., Matskevich, A. A., Reichhart, J. M., Wang, C., Butt, T. M., et al. (2006). Dual detection of fungal infections in Drosophila via recognition of glucans and sensing of virulence factors. *Cell* 127, 1425–1437. doi: 10.1016/j.cell.2006.10.046

Grangeteau, C., Yahou, F., Everaerts, C., Dupont, S., Farine, J. P., BEney, L., et al. (2018). Yeast quality in juvenile diet affects *Drosophila melanogaster* adult life traits. *Sci. Rep.* 8:13070. doi: 10.1038/s41598-018-31561-9

Gunther, C. S., Knight, S. J., Jones, R., and Goddard, M. R. (2019). Are Drosophila preferences for yeasts stable or contextual? *Ecol. Evol.* 9, 8075–8086. doi: 10.1002/ece3.5366

Hamby, K. A., Hernández, A., Boundy-Mills, K., and Zalom, F. G. (2012). Associations of yeasts with spotted wing Drosophila (*Drosophila suzukii*; Diptera: Drosophilidae) in cherries and raspberries. *Appl. Environ. Microbiol.* 78, 4869–4873.

Hoang, D., Kopp, A., and Chandler, J. A. (2015). Interactions between Drosophila and its natural yeast symbionts – Is *Saccharomyces cerevisiae* a good model for studying the fly–yeast relationship? *PeerJ* 3:e1116. doi: 10.7717/peerj.1116

Hui, F. L., Niu, Q. H., Ke, T., and Liu, Z. (2012). *Candida ficus* sp. nov., a novel yeast species from the gut of *Apriona germari* larvae. Int. J. Syst. Evol. Microbiol. 62, 2805–2809. doi: 10.1099/ijs.0.044214-0

Jimenez, S. I., Carroll, C., Babock, T., Derstine, N., Hadwin, A., Moore, M., et al. (2017). Yeasts harbored by vespine wasps in the Pacific Northwest. *Environ. Entomol.* 46, 217–225. doi: 10.1093/ee/nvw173

Kaltenpoth, M., and Steiger, S. (2013). Unearthing carrion beetles' microbiome: characterization of bacterial and fungal hindgut communities across the Silphidae. *Mol. Ecol.* 23, 1251–1267. doi: 10.1111/mec.12469

Kurtzman, C. P., and Robnett, C. J. (1998). Three new insect-associated species of the yeast genus Candida. *Can. J. Microbiol.* 44, 965–973. doi: 10.1139/cjm-44-10-965

Lachance, M. A., Anderson, T. M., and Starmer, W. T. (2006). A new subclade of haplontic Metschnikowia species associated with insects of morning glory flowers in Africa and description of *Metschnikowia aberdeeniae* sp. nov. *Int. J. Syst. Evol. Microbiol.* 56, 1141–1145. doi: 10.1099/ijs.0.64195-0

Lachance, M. A., Bowles, J. M., Chavarria Diaz, M. M., and Janzen, D. H. (2001a). *Candida cleridarum*, *Candida tilneyi* and *Candida powellii*, three new yeast species isolated from insects associated with flowers. *Int. J. Syst. Evol. Microbiol.* 51, 1201–1207. doi: 10.1099/00207713-51-3-1201

Lachance, M. A., Bowles, J. M., and Starmer, W. T. (2003). *Metschnikowia santaceciliae*, *Candida hawaiiana*, and *Candida kipukae*, three new yeast species associated with insects of tropical morning glory. *FEMS Yeast Res.* 3, 97–103. doi: 10.1111/j.1567-1364.2003.tb00144.x

Lachance, M. A., Gilbert, D. G., and Starmer, W. T. (1995). Yeast communities associated with *Drosophila* species and related flies in an eastern oak-pine forest: a comparison with western communities. *J. Ind. Microbiol.* 14, 484–494. doi: 10.1007/bf01573963

Lachance, M. A., Starmer, W. T., Rosa, C. A., Bowles, J. M., Barker, J. S., and Janzen, D. H. (2001b). Biogeography of the yeasts of ephemeral flowers and their insects. *FEMS Yeast Res.* 1, 1–8. doi: 10.1016/s1567-1356(00)00003-9

Lam, S. S., and Howell, K. S. (2015). *Drosophila*-associated yeast species in vineyard ecosystems. *FEMS Microbiol. Lett.* 362:fnv170. doi: 10.1093/femsle/fnv170

Larsen, B. B., Miller, E. C., Rhodes, M. K., and Wiens, J. J. (2017). Inordinate fondness multiplied and redistributed: the number of species on earth and the new pie of life. *Q. Rev. Biol.* 92, 229–265. doi: 10.1086/693564

Lemaitre, B., and Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol.* 25, 697–743.

Lionakis, M. S. (2011). *Drosophila* and *Galleria* insect model hosts: new tools for the study of fungal virulence, pharmacology and immunology. *Virulence* 2, 521–527. doi: 10.4161/viru.2.6.18520

Liti, G. (2015). The fascinating and secret wild life of the budding yeast *S. cerevisiae*. *eLife* 4:e05835. doi: 10.7554/eLife.05835

Liu, X. J., Yi, Z. H., Ren, Y. C., Li, Y., and Hui, F. L. (2016). Five novel species in the Lodderomyces clade associated with insects. *Int. J. Syst. Evol. Microbiol.* 66, 4881–4889. doi: 10.1099/ijsem.0.001446

Lu, H. L., and St Leger, R. J. (2016). "Insect immunity to entomopathogenic fungi" in *Genetics and Molecular Biology of Entomopathogenic Fungi*, eds B. Lovett, and R. J. S. Leger? (New York, NY: Elsevier), 251–285. doi: 10.1016/bs.adgen.2015.11.002

Meriggi, N., Di Paola, M., Vitali, F., Rivero, D., Cappa, F., Turillazzi, F., et al. (2019). *Saccharomyces cerevisiae* induces immune enhancing and shapes gut microbiota in social wasps. *Front. Microbiol.* 10:2320. doi: 10.3389/fmicb.2019.02320

Morais, B. P., Hagler, A. N., Rosa, C. A., Mendonca-Hagler, L. C., and Klaczko, L. B. (1993). Yeasts associated with *Drosophila* in tropical forests of Rio de Janeiro, Brazil. *Can. J. Microbiol.* 38, 1150–1155. doi: 10.1139/m92-188

Morais, P. B., Rosa, C. A., Hagler, A. N., and Mendonca-Hagler, L. C. (1994). Yeast communities of the cactus *Pilosocereus arrabidae* as resources for larval and adult stages of *Drosophila serido*. *Antonie Van Leeuwenhoek* 66, 313–317. doi: 10.1007/bf00882766

Murgier, J., Everaerts, C., Farine, J.-P., and Ferveur, J.-F. (2019). Live yeast in juvenile diet induces species-specific effects on *Drosophila* adult behaviour and fitness. *Sci Rep.* 9:8873. doi: 10.1038/s41598-019-45140-z

Nguyen, N. H., Suh, S. O., and Blackwell, M. (2007). Five novel Candida species in insect-associated yeast clades isolated from Neuroptera and other insects. *Mycologia* 99, 842–858. doi: 10.3852/mycologia.99.6.842

Nguyen, N. H., Suh, S. O., Erbil, C. K., and Blackwell, M. (2006). *Metschnikowia noctiluminum* sp. nov., *Metschnikowia corniflorae* sp. nov., and *Candida chrysomelidarum* sp. nov., isolated from green lacewings and beetles. *Mycol. Res.* 110, 346–356. doi: 10.1016/j.mycres.2005.11.010

Ninomiya, S., Mikata, K., Kajimura, H., and Kawasaki, H. (2013). Two novel ascomycetous yeast species, *Wickerhamomyces scolytoplatypi* sp. nov. and *Cyberlindnera xylebori* sp. nov., isolated from ambrosia beetle galleries. *Int. J. Syst. Evol. Microbiol.* 63, 2706–2711. doi: 10.1099/ijs.0.050195-0

Palanca, L., Gaskett, A. C., Günther, C. S., Newcomb, R. D., and Goddard, M. R. (2013). Quantifying variation in the ability of yeasts to attract *Drosophila melanogaster*. *PLoS One* 8:e75332. doi: 10.1371/journal.pone.0075332

Palma, M. L., Zamith-Miranda, D., Martins, F. S., Bozza, F. A., Nimrichter, L., Montero-Lomeli, M., et al. (2015). Probiotic *Saccharomyces cerevisiae* strains as biotherapeutic tools: Is there room for improvement? *Appl. Microbiol. Biotechnol.* 99, 6563–6570. doi: 10.1007/s00253-015-6776-x

Parapouli, M., Vasileiadis, A., Afendra, A.-S., and Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* 6, 1–31. doi: 10.3934/microbiol.2020001

Park, R., Dzialo, M. C., Spaepen, S., Nsabimana, D., Gielens, K., Devriese, H., et al. (2019). Microbial communities of the house fly *Musca domestica* vary with geographical location and habitat. *Microbiome* 7:147. doi: 10.1186/s40168-019-0748-9

Patt, J. M., Pfannenstiel, R. S., Meikle, W. J., and Adamczyk, J. J. (2012). Supplemental diets containing yeast, sucrose, and soy powder enhance the survivorship, growth, and development of prey-limited cursorial spiders. *Biol. Control.* 63, 237–245. doi: 10.1016/j.biocontrol.2011.02.004

Phaff, H. J., and Knapp, E. P. (1956). The taxonomy of yeast found in exudates of certain trees and other natural breeding sites of some species of *Drosophila*. *Antonie van Leeuwenhoek* 22, 117–130. doi: 10.1007/bf02538319

Piper, A. M., Farnier, K., Linder, T., Speight, R., and Cunningham, J. P. (2017). Two gut-associated yeasts in a Tephritid fruit fly have contrasting effects on adult attraction and larval survival. *J. Chem. Ecol.* 43, 891–901. doi: 10.1007/s10886-017-0877-1

Piper, M. D. W. (2017). Using artificial diets to understand the nutritional physiology of *Drosophila melanogaster*. *Curr. Opin. Insect Sci.* 23, 104–111. doi: 10.1016/j.cois.2017.07.014

Quan, A. S., and Eisen, M. B. (2018). The ecology of the *Drosophila*-yeast mutualism in wineries. *PLoS One* 13:e0196440. doi: 10.1371/journal.pone.0196440

Ramazzotti, M., Stefanini, I., Di Paola, M., De Filippo, C., Rizzetto, L., Berná, L., et al. (2019). Population genomics reveals evolution and variation of *Saccharomyces cerevisiae* in the human and insects gut. *Environ. Microbiol.* 22, 50–71. doi: 10.1111/1462-2920.14422

Ravenscraft, A., Berry, B., Hammer, T., Peay, K., and Boggs, C. (2018). Structure and function of the bacterial and fungal gut microbiota of neotropical butterflies. *Ecol. Monogr.* 89:e01346. doi: 10.1002/ecm.1346

Ren, Y. C., Liu, S. T., Li, Y., and Hui, F. L. (2015). *Pichia dushanensis* sp. nov. and *Hyphopichia paragotoi* sp. nov., two sexual yeast species associated with insects and rotten wood. Int. J. Syst. Evol. Microbiol. 65, 2875–2881. doi: 10.1099/ijs.0.000349

Ren, Y. C., Wang, Y., Chen, L., Ke, T., and Hui, F. L. (2014). *Wickerhamiella allomyrinae* fa, sp. nov., a yeast species isolated from the gut of the rhinoceros beetle *Allomyrina dichotoma*. *Int. J. Syst. Evol. Microbiol.* 64, 3856–3861. doi: 10.1099/ijs.0.068403-0

Resnick, M. A., and Cox, B. S. (2000). Yeast as an honorary mammal. *Mutat. Res.* 451, 1–11. doi: 10.1016/s0027-5107(00)00036-1

Reuter, M., Bell, G., and Greig, D. (2007). Increased outbreeding in yeast in response to dispersal by an insect vector. *Curr. Biol.* 17, R81–R83.

Rivera, F. N., Gonzalez, E., Gomez, Z., Lopez, N., Hernandez-Rodriguez, C., Berkov, A., et al. (2009). Gut-associated yeast in bark beetles of the genus *Dendroctonus Erichson* (Coleoptera: Curculionidae: Scolytinae). *Biol. J. Linn. Soc.* 98, 325–391.

Roh, K. B., Kim, C. H., Lee, H., Kwon, H. M., Park, J. W., Ryu, J. H., et al. (2009). Proteolytic cascade for the activation of the insect toll pathway induced by the fungal cell wall component. *J. Biol. Chem.* 284, 19474–19481. doi: 10.1074/jbc.m109.007419

Rosa, C. A., Lachance, M. A., Silva, J. O. C., Teixeira, A. C. P., Marini, M. M., Antonini, Y., et al. (2003). Yeast communities associated with stingless bees. *FEMS Yeast Res.* 4, 271–275. doi: 10.1016/S1567-1356(03)00173-9

Rosa, C. A., Lachance, M. A., Teixeira, L. C., Pimenta, R. S., and Morais, P. B. (2007). *Metschnikowia cerradonensis* sp. nov., a yeast species isolated from ephemeral flowers and their nitidulid beetles in Brazil. Int. J. Syst. Evol. Microbiol. 57, 161–165. doi: 10.1099/ijs.0.64624-0

Rosa, C. A., Morais, P. B., Hagler, A. N., Mendonsa-Hagler, L. C., and Monteiro, R. F. (1994). Yeast communities of the cactus *Pilosocereus urruhidae* and

associated insects in the sandy coastal plains of southeastern Brazil. *Antonie Van Leeuwenhoek* 65, 55–62. doi: 10.1007/bf00878279

Saksinchai, S., Suzuki, M., Chantawannakul, P., Ohkuma, M., and Lumyong, S. (2015). A novel ascosporogenous yeast species, *Zygosaccharomyces siamensis*, and the sugar tolerant yeasts associated with raw honey collected in Thailand. *Fungal Divers.* 52, 123–139. doi: 10.1007/s13225-011-0115-z

Sandhu, D. K., and Waraich, M. K. (1985). Yeasts associated with pollinating bees and flower nectar. *Microb. Ecol.* 11, 51–58. doi: 10.1007/bf02015108

Schiabor, K. M., Quan, A. S., and Eisen, M. B. (2014). *Saccharomyces cerevisiae* mitochondria are required for optimal attractiveness to *Drosophila melanogaster*. *PLoS One* 9:e113899. doi: 10.1371/journal.pone.0113899

Siavoshi, F., Sahraee, M., Ebrahimi, H., Sarrafnejad, A., and Saniee, P. (2018). Natural fruits, flowers, honey, and honeybees harbor *Helicobacter pylori*-positive yeasts. *Helicobacter* 23:e12471. doi: 10.1111/hel.12471

Six, D. L. (2003). A comparison of mycangial and phoretic fungi of individual mountain pine beetles. *Can. J. For. Res.* 33, 1331–1334. doi: 10.1139/x03-047

Stefanini, I. (2018). Yeast-insect associations: it takes guts. *Yeast* 35, 315–330. doi: 10.1002/yea.3309

Stefanini, I., Dapporto, L., Berná, L., Polsinelli, M., Turillazzi, S., and Cavalieri, D. (2016). Social wasps are a Saccharomyces mating nest. *Proc. Natl. Acad. Sci. U.S.A.* 113, 2247–2251. doi: 10.1073/pnas.1516453113

Stefanini, I., Dapporto, L., Legras, J. L., Calabretta, A., Di Paola, M., De Filippo, C., et al. (2012). Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13398–13403. doi: 10.1073/pnas.1208362109

Steyn, A., Roets, F., and Botha, A. (2016). Yeasts associated with *Culex pipiens* and *Culex theileri* mosquito larvae and the effect of selected yeast strains on the ontogeny of *Culex pipiens*. *Microb. Ecol.* 71, 747–760. doi: 10.1007/s00248-015-0709-1

Suh, S. O., and Blackwell, M. (2004). Three new beetle-associated yeast species in the *Pichia Guilliermondii* clade. *FEMS Yeast Res.* 5, 87–95. doi: 10.1016/j.femsyr.2004.06.001

Suh, S. O., and Blackwell, M. (2006). Three new asexual arthroconidial yeasts, Geotrichum carabidarum sp. nov., *Geotrichum histeridarum* sp. nov., and *Geotrichum cucujoidarum* sp. nov., isolated from the gut of insects. *Mycol. Res.* 110, 220–228. doi: 10.1016/j.mycres.2005.08.008

Suh, S. O., Houseknecht, J. L., Gujjari, P., and Zhou, J. J. (2013). *Scheffersomyces parashehatae* fa, sp. nov., *Scheffersomyces xylosifermentans* fa, sp. nov., *Candida broadrunensis* sp. nov. and *Candida manassasensis* sp. nov., novel yeasts associated with wood-ingesting insects, and their ecological and biofuel implications. *Int. J. Syst. Evol. Microbiol.* 63, 4330–4339. doi: 10.1099/ijs.0.053009-0

Suh, S. O., Nguyen, N. H., and Blackwell, M. (2005). Nine new Candida species near *C. membranifaciens* isolated from insects. *Mycol. Res.* 109, 1045–1056. doi: 10.1017/S0953756205003254

Suh, S. O., Nguyen, N. H., and Blackwell, M. (2006). A yeast clade near *Candida kruisii* uncovered: nine novel Candida species associated with basidioma-feeding beetles. *Mycol. Res.* 110, 1379–1394. doi: 10.1016/j.mycres.2006.09.009

Suh, S. O., Nguyen, N. H., and Blackwell, M. (2007). Yeasts isolated from plant-associated beetles and other insects: seven novel Candida species near Candida albicans. *FEMS Yeast Res.* 8, 88–102. doi: 10.1111/j.1567-1364.2007.00320.x

Tanahashi, M., and Hawes, C. J. (2016). The presence of a mycangium in European *Sinodendron cylindricum* (Coleoptera: Lucanidae) and the associated yeast symbionts. *J. Insect. Sci.* 16:76. doi: 10.1093/jisesa/iew054

Tatum, E. L. (1941). Vitamin B requirements of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* 27, 193–197. doi: 10.1073/pnas.27.4.193

Tietjen, E. J., Ayyagari, L. R., and Uetz, G. W. (1987). Symbiosis between social spiders and yeast: the role in prey attraction. *Psyche A J. Entomol.* 94, 151–158. doi: 10.1155/1987/67258

Toki, W., Tanahashi, M., Togashi, K., and Fukatsu, T. (2012). Fungal farming in a non-social beetle. *PLoS One* 7:e41893. doi: 10.1371/journal.pone.0041893

Urbina, H., Schuster, J., and Blackwell, M. (2013). The gut of Guatemalan passalid beetles: a habitat colonized by cellobiose-and xylose-fermenting yeasts. *Fungal Ecol.* 6, 339–355. doi: 10.1016/j.funeco.2013.06.005

Wagner, D. L. (2020). Insect declines in the Anthropocene. *Annu. Rev. Entomol.* 65, 457–480. doi: 10.1146/annurev-ento-011019-025151

Wang, Y., Ren, Y. C., Zhang, Z. T., Ke, T., and Hui, F. L. (2016). *Spathaspora allomyrinae* sp. nov., a D-xylose-fermenting yeast species isolated from a scarabeid beetle *Allomyrina dichotoma*. *Int. J. Syst. Evol. Microbiol.* 66, 2008–2012. doi: 10.1099/ijsem.0.000979

Wilson, E. O. (1987). The little things that run the World (The importance and conservation of invertebrates). *Conserv. Biol.* 1, 344–346. doi: 10.1111/j.1523-1739.1987.tb00055.x

Witzgall, P., Proffit, M., Rozpedowska, E., Becher, P. G., Andreadis, S., Coracini, M., et al. (2012). "This is not an apple"–yeast mutualism in codling moth. *J. Chem. Ecol.* 38, 949–957. doi: 10.1007/s10886-012-0158-y

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

MINI REVIEW
published: 07 October 2020
doi: 10.3389/fmicb.2020.575372

To the Land and Beyond: Crab Microbiomes as a Paradigm for the Evolution of Terrestrialization

Stefano Cannicci[1,2], Sara Fratini[2], Niccolò Meriggi[2], Giovanni Bacci[2], Alessio Iannucci[2], Alessio Mengoni[2] and Duccio Cavalieri[2]**

[1]Swire Institute of Marine Science and Division of Ecology and Biodiversity, The University of Hong Kong, Pokfulam, Hong Kong, [2]Department of Biology, University of Florence, Florence, Italy

The transition to terrestrial environments by formerly aquatic species has occurred repeatedly in many animal phyla and lead to the vast diversity of extant terrestrial species. The differences between aquatic and terrestrial habitats are enormous and involved remarkable morphological and physiological changes. Convergent evolution of various traits is evident among phylogenetically distant taxa, but almost no information is available about the role of symbiotic microbiota in such transition. Here, we suggest that intertidal and terrestrial brachyuran crabs are a perfect model to study the evolutionary pathways and the ecological role of animal-microbiome symbioses, since their transition to land is happening right now, through a number of independent lineages. The microorganisms colonizing the gut of intertidal and terrestrial crabs are expected to play a major role to conquer the land, by reducing water losses and permitting the utilization of novel food sources. Indeed, it has been shown that the microbiomes hosted in the digestive system of terrestrial isopods has been critical to digest plant items, but nothing is known about the microbiomes present in the gut of truly terrestrial crabs. Other important physiological regulations that could be facilitated by microbiomes are nitrogen excretion and osmoregulation in the new environment. We also advocate for advances in comparative and functional genomics to uncover physiological aspects of these ongoing evolutionary processes. We think that the multidisciplinary study of microorganisms associated with terrestrial crabs will shed a completely new light on the biological and physiological processes involved in the sea-land transition.

Keywords: brachyuran crabs, holobiont theory, symbiotic microbiota, comparative genomics, functional genomics

OPEN ACCESS

Edited by:
Enrica Pessione, University of Turin, Italy

Reviewed by:
Serafino Teseo, Nanyang Technological University, Singapore
Omry Koren, Bar-Ilan University, Israel

****Correspondence:***
Stefano Cannicci
cannicci@hku.hk
Duccio Cavalieri
duccio.cavalieri@unifi.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *23 June 2020*
Accepted: *15 September 2020*
Published: *07 October 2020*

Citation:
Cannicci S, Fratini S, Meriggi N, Bacci G, Iannucci A, Mengoni A and Cavalieri D (2020) To the Land and Beyond: Crab Microbiomes as a Paradigm for the Evolution of Terrestrialization. Front. Microbiol. 11:575372. doi: 10.3389/fmicb.2020.575372

TO THE LAND AND BEYOND: A TRUE CRAB ENDEAVOR

The present day high diversity of terrestrial species is the result of a repeated series of independent attempts to conquer terrestrial environments accomplished by several formerly aquatic animal phyla (Little, 1990, 2009; Randall et al., 2009; Lozano-Fernandez et al., 2016). Among all phyla, Arthropoda contributes the largest portion to terrestrial biodiversity. In this group, the conquest of land happened multiple times and the first successful attempts date back to a period in between Cambrian and Silurian (Lozano-Fernandez et al., 2016). Despite its long history, terrestrialization by some groups of arthropods is still an on-going process

(Lozano-Fernandez et al., 2016) and in brachyuran crabs is happening right now (Burggren and McMahon, 1988) through a number of independent lineages (Giomi et al., 2014; **Figure 1**).

The differences between aquatic and terrestrial habitats are enormous and nearly every aspect of a crab life needs to cope with such a transition (Little, 2009). During this process, remarkable morphological and physiological changes were required to tackle challenges relevant to locomotion (Burggren and McMahon, 1988), gaseous exchange (Farrelly and Greenaway, 1993, 1994; Paoli et al., 2015), excretion (Wood and Boutilier, 1985; Greenaway, 1988), reproduction (Cannicci et al., 2011; Simoni et al., 2013), foraging (Lindquist et al., 2009), and salt availability (Anger, 1995; Faria et al., 2017), given the huge difference in physical properties of air and water (Little, 1990; **Figure 2**).

One of the most remarkable changes that occurred was related to the gills (**Figure 2**). In marine species, gills perform several functions, such as respiratory activities, ionic and osmotic regulation, pH regulation and, in part, nitrogenous waste excretion. Gills of many intertidal and terrestrial brachyurans lost their respiratory function (Burggren and McMahon, 1988; Little, 1990; Farrelly and Greenaway, 1994) and new respiratory organs were evolved, such as branchiostegal lungs (Farrelly and Greenaway, 2005; Paoli et al., 2015) or tympana on the legs (Maitland, 1986; **Figure 2**). Once the respiratory role was lost, or greatly reduced, the main physiological functions exerted by the gills are related to ion exchanges, CO_2 and nitrogen excretion (Morris, 2001; Weihrauch et al., 2004).

Most of terrestrial and semi-terrestrial crabs are mainly relying on vascular plant tissues for food (Linton and Greenaway, 2007) and this new diet is yet another challenge, since their marine ancestors were mainly feeding on micro- and macroalgae and on animal preys (**Figure 2**). Vascular plants have evolved adaptations to prevent herbivory, from deterrence of ingestion, to low digestibility and unpalatability (Wolcott and O'Connor, 1992; Linton and Greenaway, 2007) and are characterized by a low nitrogen/carbon ratio (Linton and Greenaway, 2007). As a consequence, herbivorous terrestrial crabs developed several morphological and physiological adaptations to cope with low nitrogen intake (Linton and Greenaway, 2007; Kawaida et al., 2019; **Figure 2**). The role of microbes was suggested in support for a low-quality diet, but scarce experimental evidences was gathered (Linton and Greenaway, 2007; **Figure 2**).

Intertidal brachyuran crabs belonging to unrelated taxa share very similar sensory, respiratory, excretory, and osmoregulatory adaptations to the terrestrial environment (Burggren and McMahon, 1988), indicating convergent evolutionary trends (Little, 2009; Cannicci et al., 2011; Giomi et al., 2014). Microbes are known to be strongly involved in various physiological processes of animals, such as herbivory (see for instance Hansen and Moran, 2014) and ammonia detoxification (van Kessel et al., 2016).

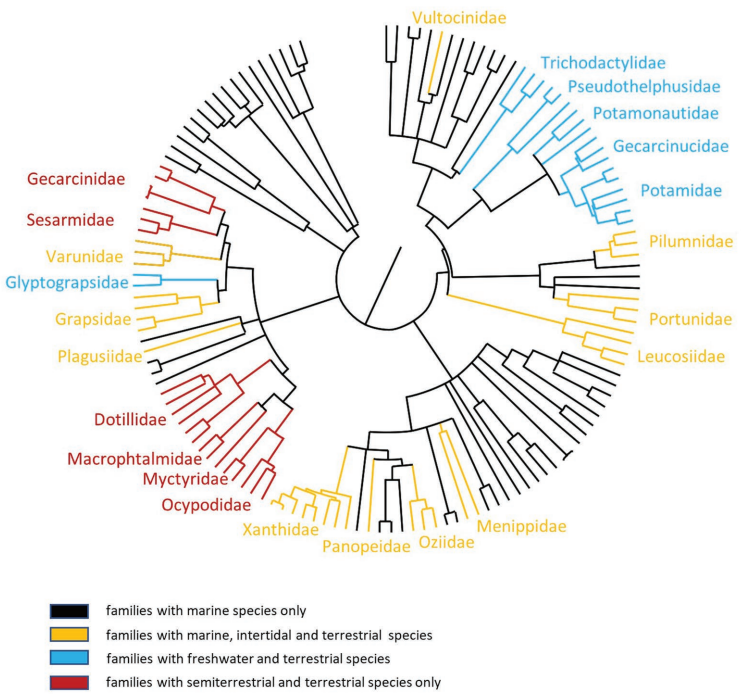


FIGURE 1 | Phylogenetic relationships among semi-terrestrial and terrestrial brachyuran crabs. Unrooted phylogenetic tree of 134 true crab species representative of 57 out of 98 Brachyuran families (see Ng et al., 2008). The order of tree branches is derived from the TimeTree database (http://timetree.org/; Kumar et al., 2017) on the basis of data of Tsang et al. (2014). The color code of the different brachyuran families represents the different habitat they colonized (black lines = families including only marine species; orange lines = families including marine, intertidal and terrestrial species; turquoise lines = families including freshwater and terrestrial species; red lines = families including exclusively semiterrestrial and terrestrial species). Only the taxonomic names of families with terrestrial species are shown on the tree.

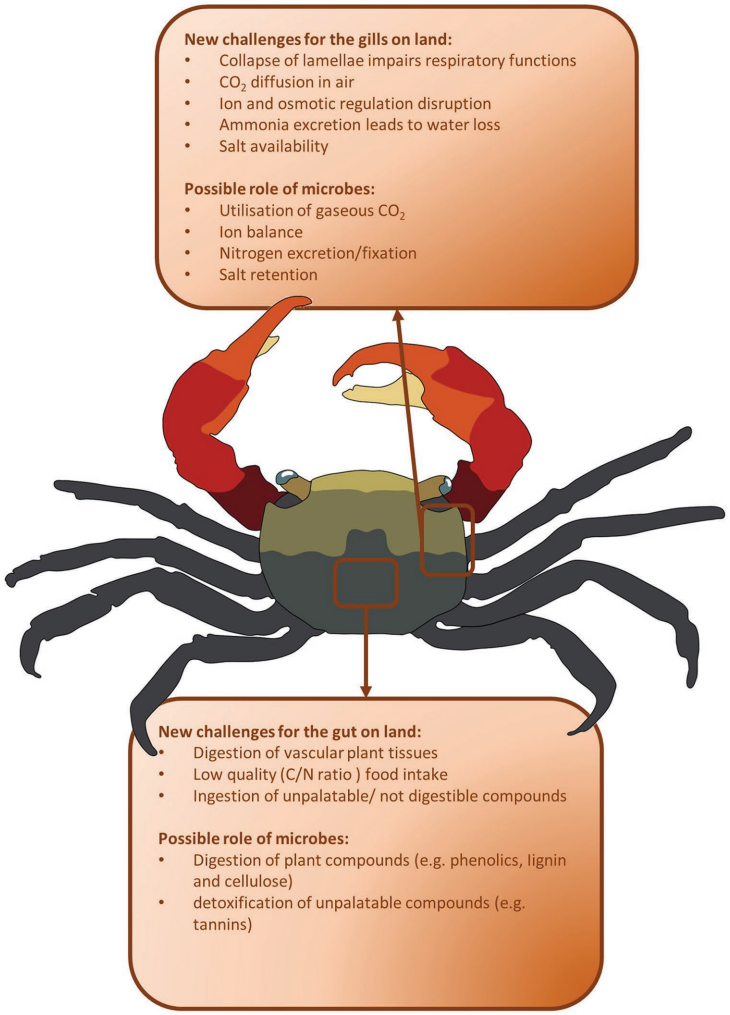


FIGURE 2 | Main challenges met during terrestrialization and possible microbial contribution. Respiratory/urinary and digestive systems are predicted to be the mostly impacted by water-to-land transition. Microbes may help to improve gut functionality toward new nutrient sources (e.g., vascular plant material) and gills functionality in relation to excretion of nitrogen toxic compounds.

However, the role of microbes in the water-to-land evolutionary transition of crabs must still be investigated.

THE MICROBIAL PERSPECTIVE: THE HOLOBIONT THEORY AND THE EVOLUTION OF ARTHROPODA

No multicellular organism is defined by its genes only, but it relies on the genetic functions provided by the microorganisms that are associated to it. This idea has been conceptualized in the holobiont theory of evolution that addresses the host-microbe interaction in an evolutionary perspective. According to this theory, Darwinian evolution acts on the genes of both the host and the commensal microbes (Zilber-Rosenberg and Rosenberg, 2008). Thus, multicellular organisms should be considered as a unique genetic system called hologenome (Zilber-Rosenberg and Rosenberg, 2008; Bordenstein and Theis, 2015) composed by the genetic and functional asset of microorganisms and their host.

Arthropoda represents one of the most relevant animal phyla where host-microbe association has played, and is still playing, a crucial role in environmental adaptation and evolution. In *Drosophila* spp., for instance, variations in the composition of the gut microbiome led to changes in population dynamics that produced allelic divergences, ultimately influencing their ability to adapt to different environments (Rudman et al., 2019). In this phylum, symbiosis can even create paradoxical ecological balances where the benefits of both sides are acquired after a "loss," such as the emblematic case of *Buchnera aphidicola* that lost entire gene clusters to be better tolerated by the aphid (Houk and Griffiths, 1980), which provides it with essential amino acids. On its side, the aphid gave up some of the genes that help to fight bacterial infections, in order to host this

beneficial symbiont. In return, the genome of *B. aphidicola* is tiny, 640.681 bp-640 genes (Shigenobu et al., 2000).

Diet is one of the main factors shaping gut microbiome and is central in the development of stable or transient interactions, critical for the adaptation to the environment. Termites are one of the major examples of organisms depending on resident and cultivated microbiomes (Brune and Dietrich, 2015). They assimilate nutrients from wood substrates (Abe et al., 2000) and play an essential role in ecosystems carbon turnover. This process is made possible by intestinal symbionts which degrade cellulose, hydrolyze xylans and provide CO_2-reductive acetogenesis and N_2 fixation (Warnecke et al., 2007).

Ants, more than any other arthropod, are associated to a broad environmental spectrum. Among them, the genera *Acromyrmex* and *Atta* can cultivate symbiotic fungi of the genus *Attamyces* that grow on leaf litter. In this symbiosis, the fungi produce the enzyme laccase (LgLcc1) that allows leaf cutting ants to detoxify phenolic compounds produced by plants (De Fine Licht et al., 2013). In these ant genera, the ant-fungal association is so intimate that it induced a remodeling of the ant genome, which lost arginine biosynthesis genes. On the other hand, fungi positively selected the pathways of chitinase and lost the key domain of ligninase (Nygaard et al., 2016). Furthermore, *Candidatus Westeberhardia cardiocondylae*, symbiont of the ant *Cardiocondyla obscurior*, offers an excellent example of metabolic complementation. The queens retain this microorganism in the ovarian nurse cells and transmit it to the oocytes. As for *B. aphidicola*, the genome of this microorganism also appears drastically reduced (533 kb), and it is responsable for the development of important metabolic complementations. In fact, the symbiont's ability of producing 4-hydroxyphenylpyruvate, convertible by the host into tyrosine, could contribute to the formation of cuticles during the ant pupal phase (Klein et al., 2016).

Within crustaceans, Isopoda are the most successful group in terms of land colonization and bacteria have been shown to be critical to develop a diet based on vascular plant matrices (Zimmer and Topp, 1998a,b; Zimmer, 2002; Zimmer et al., 2002). Indeed, the ability of several microbial species found in their hepatopancreas to produce Carbohydrate-Active enZymes (CAZymes), involved in lignocellulose degradation is essential for isopods' adaptation to terrestrial life (Bredon et al., 2019). Specialized microbiomes are also critical for the peculiar diet, based solely on lignocellulose, of the woodboring intertidal isopods *Limnoria* spp. (Besser et al., 2018). The substantial absence of intestinal bacteria in true marine isopods (Zimmer et al., 2001) reinforced the hypothesis that hepatopancreatic symbionts were acquired from the environment during terrestrialization and played a central role in this evolutionary process. Also intertidal amphipods showed to depend on environmentally originated microbiomes for cellulose utilization and species of sand hoppers with different food preferences showed contrasting patterns of cellulose degradation gene abundance in their gut microbiome under controlled feeding conditions (Abdelrhman et al., 2017).

Finally, Cuellar-Gempeler and Leibold (2018, 2019) studied the contribution of surface and burrow sediment bacteria to microbial communities associated with fiddler crabs and found that bacterial communities from burrow sediment colonized the crab carapace, while gut bacterial communities mirrored burrow and surface sediment bacteria. Thus, it has been shown that these intertidal crab species can regulate the bacterial assembly of their gut, but nothing is known about the microbiomes present in the gut of truly terrestrial crabs.

THE MICROBIAL PERSPECTIVE: EXPERIMENTAL CHALLENGES

Is there a microbial contribution to crab terrestrialization? Did the same microbial taxonomic groups take part in the evolutionary-independent terrestrialization of crabs, or was the provided microbiome functionality (i.e., the ecosystem service due to the microbial part of the holobiont) the main driver (Doolittle and Booth, 2016), irrespective of the taxonomic group? Trying to answer these questions is not trivial and requires defining experimental models and testing the holobiont theory (Moran and Sloan, 2015).

Technically speaking, in the last two decades metagenomics has emerged as a standalone discipline in studying and understanding the functions of microbiomes, showcasing different methods to survey microbial communities in different environment and in association with different hosts. Despite slight differences across sequencing techniques, metagenomic sequencing approaches are divided into two broad categories: marker gene analysis (also called targeted metagenomics) and whole metagenomics sequencing (also called untargeted metagenomics; Knight et al., 2018). The marker gene analysis is quick and cheap and can rely on very large datasets for comparative studies. Typically, it is based on 16S rRNA gene for prokaryotes and Internal Transcribed Spacer (ITS) for fungi. However, taxonomic information is generally provided only at genus level and biases due to PCR amplification can be present. On the contrary, potentially deep (down to the strain level) taxonomic assignment and functional gene profiles (even entire genome sequences) can be disclosed by the untargeted metagenomics approach, but, at present, at higher operational and computational costs than the targeted metagenomics. To gain a real-time view of the functions provided by the microbiome, metatranscriptomic analyses (as a metagenomic representation of genes expressed at each time by the community) have also been exploited (Shakya et al., 2019).

However, despite the recent advances in metagenomics, issues in statistical and experimental design still represent one of the major obstacles when we need to address questions regarding the co-evolution between the host and its microbiome. The two main types of studies commonly used in microbiome researches are cross-sectional studies and longitudinal studies. The first type consists in grouping individuals together based on one or more factors of interest and sampling only at a single specific time-point, making a snapshot of the bacterial community in different individuals. Conversely in the longitudinal studies one or more single individuals are repeatedly sampled over a specific interval of time, giving a dynamic picture of the changes in their microbiome composition during time. Regardless of the type of experiment, a well-defined design and sound biological models are crucial to extract relevant

biological information. The development status of the crab, diet, sex, environment, and other crab-specific factors must be accounted to control for variation between groups and to minimize biases due to unbalanced groups (Ramette, 2007). Controlling for natural source of variability as well as technical variability in host-microbiome studies involving animal models is not trivial but a well-defined design can help researchers to minimize biases and disentangle the interaction between the microbiome and its host.

THE CRAB PERSPECTIVE: COMPARATIVE AND FUNCTIONAL GENOMICS

Reference genomes proved to be a powerful tool to investigate many biological aspects of target species. In all metazoan taxa, the availability of high-quality reference genomes has led to great advancements in comparative and functional genomics and uncovered several aspects of evolutionary processes of physiological adaptations (Mardis, 2011). The investigation of the terrestrialization process of various groups of organisms also gathered support from genomic data.

Most of the accurate investigation of genomic signature of terrestrialization has been conducted on vertebrates. The analysis of gene expansion and positive selection in the genome of four mudskippers, teleosts uniquely adapted to live on intertidal mudflats, revealed an expansion of innate immune system genes involved in the defense against terrestrial pathogens (You et al., 2014). A positive selection for genes belonging to the ammonia excretion pathway was also detected in the gills, suggesting an important role in mudskippers' tolerance to both environmental and self-produced ammonia, especially when exposed to air (You et al., 2014). Finally, the loss or mutation of vision-related genes showed genomic changes associated with aerial vision, a pivotal characteristic for terrestrial species (You et al., 2014).

Another extensive comparative genomic analysis conducted on five species of coelacanth fish was able to elucidate the time and mode of the evolutionary trajectories, occurring at molecular level, that lead the transition from fish to tetrapods (Nikaido et al., 2013). This study revealed the presence, in the coelacanth genome, of genes related to the olfactory reception of airborne ligands, unknown in ray-finned fish, and of noncoding elements that act as enhancers of key genes for limb development typical of tetrapods but not of ray-finned fishes (Nikaido et al., 2013).

Investigation of genomic traits involved in the terrestrialization process are scarce in invertebrates. Genomic data produced for four species of Ampullaridae, a family of gastropods that includes both aquatic and amphibious snails, revealed the presence of expanded gene families related to environmental sensing and cellulose digestion, which may have played a key role in the water to land transition in this lineage (Sun et al., 2019).

The above examples show how selective pressures exerted by water-land transition can guide the expansion and/or contraction of specific adaptive genes in the genome of metazoans. Horizontal gene transfer (i.e., the acquisition of new genes from foreign sources) could also lead to genome diversification in the context of terrestrialization. Exchange of genetic materials is commonly reported in bacteria and archaea (Gogarten and Townsend, 2005). The frequency and importance of horizontal gene transfers between bacteria and eukaryotes, however, remained controversial and unclear until recently (Husnik and McCutcheon, 2018). Nonetheless, in the last decade some examples of horizontal gene transfers between bacteria and animals have been proved, showing how this process can be involved in the fixation of functional genes especially linked to the evolution of nutritional requests (see Husnik and McCutcheon, 2018 for a review). For example, horizontal gene transfers of bacterial genes involved in carbohydrate metabolism has been found in herbivorous insects (Wybouw et al., 2016) and plant-parasitic nematodes (Danchin et al., 2010; Paganini et al., 2012). Recently, it has been suggested that the marine wood-boring isopod *Limnoria quadripunctata* and the amphipod *Chelura terebrans* have sterile microbe-free digestive systems and they are able to produce all required enzymes for lignocellulose digestion, showing to possess enzymes previously thought to be absent from animal genomes (King et al., 2010; Kern et al., 2013). These enzymes were likely acquired by these species *via* horizontal gene transfer from a protist symbiont.

CONCLUSIONS AND PERSPECTIVES

In this review paper, we are promoting intertidal and terrestrial crabs as novel model systems for the understanding of evolutionary mechanisms at the holobiont level. In the context of terrestrialization of crabs, the presence of multiple taxa which independently underwent, and still undergo, the evolutionary leap from sea to land (**Figure 1**) represents an ideal experimental dataset for cross-sectional studies that aim to compare microbiome composition both among different terrestrial taxa and between them and closely related marine species. Since terrestrialization lead to several, but similar, physiological adaptations (Little, 2009; Cannicci et al., 2011; Giomi et al., 2014), terrestrial crabs represent a suitable model for testing the relationships between microbiome composition and their functions, in order to interpret the ecosystem services provided by the crab-associated microbiome with respect to the new physiological challenges. In terms of organs, the hepatopancreas, where nutrients are stored (Zimmer, 2002), the multifunctional gills, responsible for ion, gas, and nitrogen exchanges (Morris, 2001) and the intestine, which has to cope with non-digestible compounds (Linton and Greenaway, 2007) should be targeted in future research aimed to ascertain the development of host-microbe interactions in these model systems.

The evolution of terrestrialization did not just involve the interaction of crabs with their microbiota, but, necessarily, a selection of specific genomic traits of the crabs themselves. This process is a necessary evolutionary pathway to select for those adaptive traits that play a crucial role in such a dramatic ecological shift. It is also conceivable that genome diversification in crabs that conquered the land could be led by events of horizontal gene transfers. Such transfer events from microbial donors could be likely, for instance, for genes encoding proteins

involved in lignin and cellulose degradation, which represents a big challenge in a diet based on vascular plants, as shown for some herbivorous insects (Wybouw et al., 2016).

Conversely to what happened to insects and isopods (Lozano-Fernandez et al., 2016), many lineages of brachyuran crabs are exploiting the evolutionary opportunity of a transition from sea to the land just now (Giomi et al., 2014; Fusi et al., 2016). This transition is happening so rapidly and involves such a diverse array of taxa and habitats, from tree canopies to deserts, that it is difficult to explain such an adaptive radiation without an intimate relationship, at molecular level, between the true crabs and their microbiome.

The use of a multi-disciplinary approach (combining physiology, microbiology, biochemistry) coupled with both targeted and untargeted metagenomics (including metatranscriptomics) can ultimately clarify the contribution of microbiome on crabs terrestrialization and test part of the holobiont theory of evolution, further delving in the heart of darkness of modern evolutionary theory.

AUTHOR CONTRIBUTIONS

SC and DC ideated the review. All the authors wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

SC was supported by TUYF Charitable Trust funds, Hong Kong, and by the HKU Faculty of Science RAE improvement funds.

ACKNOWLEDGMENTS

The authors would like to thank all the students and colleagues that are currently involved in the study of evolution of terrestrialization in brachyuran crabs.

REFERENCES

Abdelrhman, K. F. A., Bacci, G., Nistri, A., Mengoni, A., and Ugolini, A. (2017). Diet and gut microbiota of two supralittoral amphipods *Orchestia montagui* and *Talitrus saltator* living in different microhabitats. *Estuar. Coast. Shelf Sci.* 197, 119–125. doi: 10.1016/j.ecss.2017.08.016

Abe, T., Bignell, D. E., Higashi, M., Higashi, T., and Abe, Y. (2000). *Termites: Evolution, sociality, symbioses, ecology*. Dordrecht, Boston: Kluwer Academic Publishers.

Anger, K. (1995). The conquest of freshwater and land by marine crabs: adaptations in life-history patterns and larval bioenergetics. *J. Exp. Mar. Biol. Ecol.* 193, 119–145. doi: 10.1016/0022-0981(95)00114-X

Besser, K., Malyon, G. P., Eborall, W. S., Paro da Cunha, G., Filgueiras, J. G., Dowle, A., et al. (2018). Hemocyanin facilitates lignocellulose digestion by wood-boring marine crustaceans. *Nat. Commun.* 9:5125. doi: 10.1038/s41467-018-07575-2

Bordenstein, S. R., and Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol.* 13:e1002226. doi: 10.1371/journal.pbio.1002226

Bredon, M., Herran, B., Lheraud, B., Bertaux, J., Grève, P., Moumen, B., et al. (2019). Lignocellulose degradation in isopods: new insights into the adaptation to terrestrial life. *BMC Genomics* 20:462. doi: 10.1186/s12864-019-5825-8

Brune, A., and Dietrich, C. (2015). The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. *Annu. Rev. Microbiol.* 69, 145–166. doi: 10.1146/annurev-micro-092412-155715

Burggren, W. W., and McMahon, B. R. (1988). *Biology of the land crabs*. Cambridge: Cambridge University Press.

Cannicci, S., Simoni, R., and Giomi, F. (2011). Role of the embryo in crab terrestrialisation: an ontogenetic approach. *Mar. Ecol. Prog. Ser.* 430, 121–131. doi: 10.3354/meps08954

Cuellar-Gempeler, C., and Leibold, M. A. (2018). Multiple colonist pools shape fiddler crab-associated bacterial communities. *ISME J.* 12, 825–837. doi: 10.1038/s41396-017-0014-8

Cuellar-Gempeler, C., and Leibold, M. A. (2019). Key colonist pools and habitat filters mediate the composition of fiddler crab–associated bacterial communities. *Ecology* 100, 1–14. doi: 10.1002/ecy.2628

Danchin, E. G. J., Rosso, M. N., Vieira, P., De Almeida-Engler, J., Coutinho, P. M., Henrissat, B., et al. (2010). Multiple lateral gene transfers and duplications have promoted plant parasitism ability in nematodes. *Proc. Natl. Acad. Sci. U. S. A.* 107, 17651–17656. doi: 10.1073/pnas.1008486107

De Fine Licht, H. H., Schitøt, M., Rogowska-Wrzesinska, A., Nygaard, S., Roepstorff, P., and Boomsma, J. J. (2013). Laccase detoxification mediates the nutritional alliance between leaf-cutting ants and fungus-garden symbionts. *Proc. Natl. Acad. Sci. U. S. A.* 110, 583–587. doi: 10.1073/pnas.1212709110

Doolittle, W. F., and Booth, A. (2016). It's the song, not the singer: an exploration of holobiosis and evolutionary theory. *Biol. Philos.* 32, 5–24. doi: 10.1007/s10539-016-9542-2

Faria, S. C., McNamara, J. C., Faria, S. C., Provete, D. B., and Thurman, C. L. (2017). Phylogenetic patterns and the adaptive evolution of osmoregulation in fiddler crabs (Brachyura, Uca). *PLoS One* 12:e0171870. doi: 10.1371/journal.pone.0171870

Farrelly, C. A., and Greenaway, P. (1993). Land crabs with smooth lungs: grapsidae, gecarcinidae, and sundathelphusidae ultrastructure and vasculature. *J. Morphol.* 215, 245–260. doi: 10.1002/jmor.1052150306

Farrelly, C. A., and Greenaway, P. (1994). Gas exchange through the lungs and gills in air-breathing crabs. *J. Exp. Biol.* 187, 113–130.

Farrelly, C. A., and Greenaway, P. (2005). The morphology and vasculature of the respiratory organs of terrestrial hermit crabs (*Coenobita* and *Birgus*): gills, branchiostegal lungs and abdominal lungs. *Arthropod Struct. Dev.* 34, 63–87. doi: 10.1016/j.asd.2004.11.002

Fusi, M., Cannicci, S., Daffonchio, D., Mostert, B., Pörtner, H. -O., and Giomi, F. (2016). The trade-off between heat tolerance and metabolic cost drives the bimodal life strategy at the air-water interface. *Sci. Rep.* 6:19158. doi: 10.1038/srep19158

Giomi, F., Fusi, M., Barausse, A., Mostert, B., Pörtner, H. -O., and Cannicci, S. (2014). Improved heat tolerance in air drives the recurrent evolution of air-breathing. *Proc. Biol. Sci.* 281:20132927. doi: 10.1098/rspb.2013.2927

Gogarten, J. P., and Townsend, J. P. (2005). Horizontal gene transfer, genome innovation and evolution. *Nat. Rev. Microbiol.* 3, 679–687. doi: 10.1038/nrmicro1204

Greenaway, P. (1988). "Ion and water balance" in *Biology of land crabs*. eds. W. W. Burggren and B. R. McMahon (New York: Cambridge University Press), 211–248.

Hansen, A. K., and Moran, N. A. (2014). The impact of microbial symbionts on host plant utilization by herbivorous insects. *Mol. Ecol.* 23, 1473–1496. doi: 10.1111/mec.12421

Houk, E. J., and Griffiths, G. W. (1980). Intracellular symbiotes of the Homoptera. *Annu. Rev. Entomol.* 25, 161–187. doi: 10.1146/annurev.en.25.010180.001113

Husnik, F., and McCutcheon, J. P. (2018). Functional horizontal gene transfer from bacteria to eukaryotes. *Nat. Rev. Microbiol.* 16, 67–79. doi: 10.1038/nrmicro.2017.137

Kawaida, S., Nanjo, K., Ohtsuchi, N., Kohno, H., and Sano, M. (2019). Cellulose digestion abilities determine the food utilization of mangrove estuarine crabs. *Estuar. Coast. Shelf Sci.* 222, 43–52. doi: 10.1016/j.ecss.2019.04.004

Kern, M., McGeehan, J. E., Streeter, S. D., Martin, R. N. A., Besser, K., Elias, L., et al. (2013). Structural characterization of a unique marine animal family 7 cellobiohydrolase suggests a mechanism of cellulase salt tolerance. *Proc. Natl. Acad. Sci. U. S. A.* 110, 10189–10194. doi: 10.1073/pnas.1301502110

King, A. J., Cragg, S. M., Li, Y., Dymond, J., Guille, M. J., Bowles, D. J., et al. (2010). Molecular insight into lignocellulose digestion by a marine isopod in the absence of gut microbes. *Proc. Natl. Acad. Sci. U. S. A.* 107, 5345–5350. doi: 10.1073/pnas.0914228107

Klein, A., Schrader, L., Gil, R., Manzano-Marín, A., Flórez, L., Wheeler, D., et al. (2016). A novel intracellular mutualistic bacterium in the invasive ant *Cardiocondyla obscurior*. *ISME J.* 10, 376–388. doi: 10.1038/ismej.2015.119

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., et al. (2018). Best practices for analysing microbiomes. *Nat. Rev. Microbiol.* 16, 1–13. doi: 10.1038/s41579-018-0029-9

Kumar, S., Stecher, G., Suleski, M., and Hedges, S. B. (2017). TimeTree: a resource for timelines, timetrees, and divergence times. *Mol. Biol. Evol.* 34, 1812–1819. doi: 10.1093/molbev/msx116

Lindquist, E. S., Krauss, K. W., Green, P. T., O'Dowd, D. J., Sherman, P. M., and Smith, T. J. III (2009). Land crabs as key drivers in tropical coastal forest recruitment. *Biol. Rev. Camb. Philos. Soc.* 84, 203–223. doi: 10.1111/j.1469-185X.2008.00070.x

Linton, S. M., and Greenaway, P. (2007). A review of feeding and nutrition of herbivorous land crabs: adaptations to low quality plant diets. *J. Comp. Physiol. B.* 177, 269–286. doi: 10.1007/s00360-006-0138-z

Little, C. (1990). *The terrestrial invasion. An ecophysiological approach to the origins of land animals*. Cambridge: Cambridge University Press.

Little, C. (2009). *The colonisation of land. Origins and adaptations of terrestrial animals*. Cambridge: Cambridge University Press.

Lozano-Fernandez, J., Carton, R., Tanner, A. R., Puttick, M. N., Blaxter, M., Vinther, J., et al. (2016). A molecular palaeobiological exploration of arthropod terrestrialization. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 371:20150133. doi: 10.1098/rstb.2015.0133

Maitland, D. P. (1986). Crabs that breathe air with their legs-scopimera and dotilla. *Nature* 319, 493–495. doi: 10.1038/319493a0

Mardis, E. R. (2011). A decade's perspective on DNA sequencing technology. *Nature* 470, 198–203. doi: 10.1038/nature09796

Moran, N. A., and Sloan, D. B. (2015). The hologenome concept: helpful or hollow? *PLoS Biol.* 13:e1002311. doi: 10.1371/journal.pbio.1002311

Morris, S. (2001). Neuroendocrine regulation of osmoregulation and the evolution of air-breathing in decapod crustaceans. *J. Exp. Biol.* 204, 979–989.

Nikaido, M., Noguchi, H., Nishihara, H., Toyoda, A., Suzuki, Y., Kajitani, R., et al. (2013). Coelacanth genomes reveal signatures for evolutionary transition from water to land. *Genome Res.* 23, 1740–1748. doi: 10.1101/gr.158105.113

Ng, P. K. L., Guinot, D., and Davie, P. J. F. (2008). Systema Brachyuorum: Part I. An annotated checklist of extant Brachyuran crabs of the world. *Raffles Bull. Zool. Suppl.* 17, 1–286.

Nygaard, S., Hu, H., Li, C., Schiøtt, M., Chen, Z., Yang, Z., et al. (2016). Reciprocal genomic evolution in the ant-fungus agricultural symbiosis. *Nat. Commun.* 7, 1–9. doi: 10.1038/ncomms12233

Paganini, J., Campan-Fournier, A., Da Rocha, M., Gouret, P., Pontarotti, P., Wajnberg, E., et al. (2012). Contribution of lateral gene transfers to the genome composition and parasitic ability of root-knot nematodes. *PLoS One* 7:e50875. doi: 10.1371/journal.pone.0050875

Paoli, F., Wirkner, C. S., and Cannicci, S. (2015). The branchiostegal lung of Uca vocans (*Decapoda*: *Ocypodidae*): unreported complexity revealed by corrosion casting and MicroCT techniques. *Arthropod Struct. Dev.* 44, 622–629. doi: 10.1016/j.asd.2015.09.006

Ramette, A. (2007). Multivariate analyses in microbial ecology. *FEMS Microbiol. Ecol.* 62, 142–160. doi: 10.1111/j.1574-6941.2007.00375.x

Randall, D. J., Burggren, W. W., Farrell, A. P., and Haswell, M. S. (2009). *The evolution of air breathing in vertebrates*. Cambridge: Cambridge University Press.

Rudman, S. M., Greenblum, S., Hughes, R. C., Rajpurohit, S., Kiratli, O., Lowder, D. B., et al. (2019). Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 116, 20025–20032. doi: 10.1073/pnas.1907787116

Shakya, M., Lo, C. -C., and Chain, P. S. G. (2019). Advances and challenges in metatranscriptomic analysis. *Front. Genet.* 10:904. doi: 10.3389/fgene.2019.00904

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y., and Ishikawa, H. (2000). Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* 407, 81–86. doi: 10.1038/35024074

Simoni, R., Giomi, F., Spigoli, D., Pörtner, H. -O., and Cannicci, S. (2013). Adaptations to semi-terrestrial life in embryos of East African mangrove crabs: a comparative approach. *Mar. Biol.* 160, 2483–2492. doi: 10.1007/s00227-013-2243-3

Sun, J., Mu, H., Ip, J. C. H., Li, R., Xu, T., Accorsi, A., et al. (2019). Signatures of divergence, invasiveness, and terrestrialization revealed by four apple snail genomes. *Mol. Biol. Evol.* 36, 1507–1520. doi: 10.1093/molbev/msz084

Tsang, L. M., Schubart, C. D., Ahyong, S. T., Lai, J. C. Y., Au, E. Y. C., Chan, T. Y., et al. (2014). Evolutionary history of true crabs (Crustacea: Decapoda: Brachyura) and the origins of freshwater crabs. *Mol. Biol. Evol.* 31, 1173–1187. doi: 10.1093/molbev/msu068

van Kessel, M. A. H. J., Mesman, R. J., Arshad, A., Metz, J. R., Spanings, F. A. T., van Dalen, S. C. M., et al. (2016). Branchial nitrogen cycle symbionts can remove ammonia in fish gills. *Environ. Microbiol. Rep.* 8, 590–594. doi: 10.1111/1758-2229.12407

Warnecke, F., Luginbühl, P., Ivanova, N., Ghassemian, M., Richardson, T. H., Stege, J. T., et al. (2007). Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* 450, 560–565. doi: 10.1038/nature06269

Weihrauch, D., Morris, S., and Towle, D. W. (2004). Ammonia excretion in aquatic and terrestrial crabs. *J. Exp. Biol.* 207, 4491–4504. doi: 10.1242/jeb.01308

Wolcott, D. L., and O'Connor, N. J. (1992). Herbivory in crabs: adaptations and ecological considerations. *Am. Zool.* 32, 370–381. doi: 10.1093/icb/32.3.370

Wood, C. M., and Boutilier, R. G. (1985). Osmoregulation, ionic exchange, blood chemistry, and nitrogenous waste excration in the land crab Cardisoma carnifex: a field and laboratory study. *Biol. Bull.* 169, 267–290. doi: 10.2307/1541403

Wybouw, N., Pauchet, Y., Heckel, D. G., and Van Leeuwen, T. (2016). Horizontal gene transfer contributes to the evolution of arthropod herbivory. *Genome Biol. Evol.* 8, 1785–1801. doi: 10.1093/gbe/evw119

You, X., Bian, C., Zan, Q., Xu, X., Liu, X., Chen, J., et al. (2014). Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nat. Commun.* 5:5594. doi: 10.1038/ncomms6594

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32, 723–735. doi: 10.1111/j.1574-6976.2008.00123.x

Zimmer, M. (2002). Nutrition in terrestrial isopods (*Isopoda*: *Oniscidea*): an evolutionary-ecological approach. *Biol. Rev. Camb. Philos. Soc.* 77, 455–493. doi: 10.1017/S1464793102005912

Zimmer, M., Danko, J. P., Pennings, S. C., Danford, A. R., Carefoot, T. H., Ziegler, A., et al. (2002). Cellulose digestion and phenol oxidation in coastal isopods (*Crustacea*: *Isopoda*). *Mar. Biol.* 140, 1207–1213. doi: 10.1007/s00227-002-0800-2

Zimmer, M., and Topp, W. (1998a). Microorganisms and cellulose digestion in the gut of the woodlouse *Porcellio scaber*. *J. Chem. Ecol.* 24, 1397–1408. doi: 10.1023/A:1021235001949

Zimmer, M., and Topp, W. (1998b). Nutritional biology of terrestrial isopods (*Isopoda*: *Oniscidea*): copper revisited. *Isr. J. Zool.* 44, 453–462. doi: 10.1080/00212210.1998.10688968

Zimmer, M., Danko, J. P., Pennings, S. C., Danford, A. R., Ziegler, A., Uglow, R. F., et al. (2001). Hepatopancreatic endosymbionts in coastal isopods (*Crustacea*: *Isopoda*), and their contribution to digestion. *Mar. Biol.* 138, 955–963. doi: 10.1007/s002270000519

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 15 October 2020
doi: 10.3389/fmicb.2020.565267

Relationship Between the Fatty Acid Profiles and Gut Bacterial Communities of the Chinese Mitten Crab (*Eriocheir sinensis*) From Ecologically Different Habitats

Shengyan Su[1,2†], Brian Pelekelo Munganga[2†], Fukuan Du[1,2], Juhua Yu[1,2], Jianlin Li[1,2], Fan Yu[1,2], Meiyao Wang[1,2], Xinjin He[2], Xinyuan Li[2], Raouf Bouzoualegh[2], Pao Xu[1,2] and Yongkai Tang[1,2*]*

[1] *Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, China,* [2] *Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, China*

The gut microbiota plays an important role in a variety of physiological functions such as intestinal digestion, metabolic homeostasis, immune response, and responses to disease treatment. Whether there is a relationship between gut microbial communities and fatty acid (FA) profiles of Chinese mitten crab is unclear. Hence, we analyzed the relationship between FA profiles and the gut bacterial communities of six Chinese mitten crab (*Eriocheir sinensis*) populations from different lakes. The crabs were sampled from six different lakes in Jiangsu Province, China. The FA profiles of these crab populations were compared and clustered, and then used to determine the relationship between geographic location and FA composition. We also characterized the gut microbial communities of these crabs using 16S rRNA high-throughput gene sequencing. The FA profiles varied significantly ($P < 0.05$) between crabs from different geographical locations. A similar trend was also observed in the gut microbial communities, which also varied significantly based on their geographical origin ($P < 0.05$). Furthermore, alpha diversity, cluster analysis, and matching bacterial community structures with specific locations revealed patterns that significantly linked FA profiles to the gut microbiota. Further analysis of FA profiles and gut microbial community generated patterns that linked the two parameters. Hence, it was observed that the gut microbial community seems to contribute significantly to the FA composition of the Chinese mitten crab. However, further studies need to be conducted to investigate the interactions between gut microbial communities and the biochemical composition of the Chinese mitten crab, which will ultimately unravel the complexity of microbial ecosystems for potential applications in aquaculture and species conservation.

Keywords: Chinese mitten crab, fatty acid profiles, gut bacterial community, geographic location, fatty acids and gut microbial interactions

OPEN ACCESS

Edited by:
Anna Luganini,
University of Turin, Italy

Reviewed by:
Evgeniya V. Nazarova,
Genentech, United States
Jiteng Wang,
Zhejiang Ocean University, China
Xingkun Jin,
Hohai University, China

****Correspondence:***
Pao Xu
xup13806190669@163.com
Yongkai Tang
tangyk@ffrc.cn

† These authors have contributed equally to this work

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *24 May 2020*
Accepted: *31 August 2020*
Published: *15 October 2020*

Citation:
Su S, Munganga BP, Du F, Yu J, Li J, Yu F, Wang M, He X, Li X, Bouzoualegh R, Xu P and Tang Y (2020) Relationship Between the Fatty Acid Profiles and Gut Bacterial Communities of the Chinese Mitten Crab (Eriocheir sinensis) From Ecologically Different Habitats. Front. Microbiol. 11:565267. doi: 10.3389/fmicb.2020.565267

INTRODUCTION

The Chinese mitten crab (*Eriocheir sinensis*) is one of the most economically important and nutritious crustaceans in China (Chen et al., 2007). It is a medium-sized, freshwater burrowing crab native to eastern coastal rivers and estuaries that feeds into the Yellow Sea and spans both China and Korea (Zhang et al., 2001; Rudnick et al., 2003). It spends most of its life in freshwater, and then migrates to the sea or ocean to reproduce (a catadromous species) (Zhang et al., 2001; Rudnick et al., 2003; Cheng et al., 2008; Sui et al., 2009). The Chinese mitten crab production and value greatly expanded between 1990 and 2012, which reflects the increasing importance of the species to China's aquaculture sector (Chen et al., 2007; Chen et al., 2015; Wang et al., 2015, 2016). In 2015, approximately 820,000 tons of these crabs were harvested, which accounted for more than ¥50 billion in value (People's Republic of China Ministry of Agriculture, 2016; Dong et al., 2018). However, there are some challenges and limitations associated with its culture (e.g., potential disease outbreak), conservation, and management; and the need to improve its nutritional value (Ding et al., 2016; Shen et al., 2017). Consequently, more attention has been given to understanding its health, nutrition, and conservation.

A recent research approach to improving the health, nutrition, and economic values and the conservation of aquatic species is to understand the role of gut microbial communities (Roeselers et al., 2011; Egerton et al., 2018; Zeng et al., 2018; Trevelline et al., 2019). Recent studies have shown that microbial communities play significant roles in animal intestinal digestion, immune response, physiology, and response to disease treatment (Heijtz et al., 2011; Stanley et al., 2014; Waite and Taylor, 2015; Zheng et al., 2017; Ramos and Hemann, 2017; Byndloss et al., 2017). Along this line of thought, some studies have been conducted to understand the principles governing microbial community assembly and maintenance within the gut of the Chinese mitten crab by developing robust model systems to study host-microbial interactions (Chen et al., 2015; Zhang et al., 2016; Dong et al., 2018). A recent study examined the bacterial communities found in the gut of crabs cultured in Lake Tai, China (Chen et al., 2015). In another study, Zhang et al. (2016) assessed the differences between microbiota found in the gut and those found in the surrounding environment. Furthermore, Dong et al. (2018) conducted a comparative gene expression analysis of the intestinal bacterial community and the expression of gut immunity genes in these crabs. However, enormous knowledge gaps still exist regarding the interaction between the gut microbial community and the biochemical composition of these crabs.

In general, no links have been established between gut microbial communities and the proximate composition of the Chinese mitten crab. However, previous studies assessing either the relationship between the proximate composition of animals and geographic location or microbial communities in animals and their locality suggest possible links between the gut microbial community and proximate composition (Zenebe et al., 1998; Riveiro et al., 2011). The proximate composition of the same species living in different environments has been reported to be significantly different (Zenebe et al., 1998; Riveiro et al., 2011; Roeselers et al., 2011; Mohan, 2013, p. 960–963; Antony, 2016). Likewise, the quantity and composition of the gut microbiota of host species have been shown to vary significantly based on their geographic location (Finkel et al., 2011; Franchini et al., 2014). Furthermore, some studies have shown direct links between gut microbiota and proximate composition. For example, in quails and humans, modulation of the gut microbiota induced changes in fatty acid (FA) profiles. In laying Japanese quails (*Coturnix coturnix japonica*), the FA composition of liver lipids can be modified by modulating the gut microbiota (Furuse et al., 1992). Moreover, some polyunsaturated fatty acids (PUFA) derived bacterial metabolites were identified, which were correlated with specific fecal bacteria (*Bifidobacterium* species, *Eubacterium ventriosum,* and *Lactobacillus* species) (Druart et al., 2014).

Understanding gut microbiota composition, abundance, and related environmental factors can provide insights to support the effective management, conservation, and improvement of the economic performance of the crab industry. For example, Zhang et al. (2019) identified an effective mechanism present in the environment that can be utilized to improve a species' growth, which in turn can improve its economic performance. By combining genetics, microbiota, and growth performance, NRT1.1B was found to be a link between root microbiota composition and nitrogen use in rice agriculture (Zhang et al., 2019).

The aim of this study was to establish the relationship between FA profiles and the gut bacterial community of the Chinese mitten crab from ecologically different habitats. For this purpose, we analyzed the FA profiles and gut microbiota of six crab populations sampled from six different lakes in China's Jiangsu province. The FA profiles of these crab populations were compared and clustered, and then used to determine the relationship between geographic location and FA profiles. Furthermore, we characterized the microbial communities from the gut content of the crabs using 16S rRNA high-throughput gene sequencing. Alpha diversity, cluster analysis, and the bacterial community structures in specific locations were observed to determine their contribution to the FA profiles.

MATERIALS AND METHODS

Sample Collection

In the present study, six populations of the Chinese mitten crab (*Eriocheir sinensis*) weighing – 120–150 g were obtained from six different lakes in China's Jiangsu province in December 2018. The lakes are Changdang Lake (C), Gucheng Lake (G), Gaoyou Lake (Gy), Hung-tse Lake (H), Taihu Lake (T), and Yangcheng Lake (Y) (**Figure 1A** and **Table 1**). These lakes are part of the Yangtze River drainage basin system, they form an indirect but continuous water system with Yangtze River. The Chinese mitten crab populations migrate from the six lakes into the Yangtze River estuary to spawn. A total of 180 crabs were collected, 30 from each lake (15 males and 15 females) and brought to the Freshwater Fisheries Research Center of the Chinese Academy of Fishery

Sciences. Five sampling sites were purposely selected in each lake. The biochemical parameters for different lakes were not analyzed, nonetheless, the standard water parameters for the lakes where the crab populations were sampled can be found at (GB3838-2002). Upon arrival, a total of 48 crabs were randomly selected from all the populations (eight crabs from each lake, four males and four females).

Sample Preparation

Gut Microbial Sample

The body surfaces of the randomly selected crabs (48) were washed with sterile water and disinfected with 75% ethanol for 2 min. They were subsequently dissected to remove the digestive tract to collect the gut contents (distal section). The gut contents were collected into sterile tubes and stored in a −80°C freezer and were subsequently used for bacterial DNA extraction. The rest of the crab body parts that remained after collecting gut contents were also stored at −80°C.

FA Sample

The 48 crabs were collected from the freezer 2 days from the day of arrival and allowed to thaw, and then 6–8g of muscle was removed from the appendage carapaces of each crab. These muscle tissues were dried using a vacuum freeze dryer (Labconco Corp., Kansas City, MO, United States) and grounded into powder form with a mortar and pestle. The dried samples were kept in ziplock plastic bags and stored at −20. Thereafter, they were sent to the lab for FA profile analysis.

Fatty Acid Extraction and Analysis

Approximately 2–3g of the ground muscle tissues from each crab were added into tubes and a mixed solution (chloroform/methanol, 2:1 v/v) was added and oscillatory extraction was conducted. Oscillatory extraction was done three times, followed by filtering, and evaporation drying of the filtrates. Thereafter, 2 mL of 0.5 mol/L sodium hydroxide in methanol was added, and the solution was put in a water bath at 60°C for 30 min. The solution was allowed to cool, then 2 mL of 25% boron trifluoride in methanol was added and put in a water bath again at 60°C for 20 min. After cooling, 2 mL of n-hexane and 2 mL of saturated sodium chloride solution were added to obtain fatty acid methyl esters. The fatty acid methyl esters obtained were analyzed by gas chromatography (Chromatographic column: DB-WAX 30 M I. D. 0.32 mm; Shimadzu GC-2030, Europe). The temperature of the column was initially held at 100°C for 3 min; then increased to 180°C at a rate of 10°C/min and held for 1 min; and finally increased to 240°C at a rate of 3°C/min and held for 15 min. The whole analysis process took 95 min. The carrier gas was Nitrogen (N_2) with a flow rate of 3 mL/min, fuel gas was hydrogen (H_2) with a flow rate 40 mL/min and oxidant gas was air with a flow rate 400 mL/min. The injection port and the temperature detector were kept at 250°C. Identification of the FA was based on the known standard time of retention (Sigma-Aldrich Co., St. Louis, MO, United States). FA composition are presented as the percentage of each FA compared to the total fatty acids.

DNA Extraction

Prior to DNA extraction, the intestinal contents and mucosa from each individual crab were collected and mixed completely using a hand-held tissue homogenizer. Each sample of intestinal contents and mucosa from 48 crabs were divided into 14 tubes. The genomic DNA of the sample was extracted using commercially available kit (E.Z.N.A® Genomic DNA Isolation Kits, Omega Bio-Tek), following the kit manufacturer protocols. The appropriate amount (50 μL) of sample DNA was collected into the centrifuge tube. Then the purity and concentration of DNA tested by electrophoresis (1% agrose gel, 5μL Nared (dye), 4 μl of DNA sample, 2 μL of loading buffer and 4 μL of DL-1000 maker). The samples were stored at −20°C and were later sent for further analysis.

Polymerase Chain Reaction (PCR) Amplification, Purification, and Pyrosequencing

Based on the genomic region of DNA, specific primers with barcodes were selected according to the selection of the sequencing region. Universal polymerase chain reaction (PCR) primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3) and 806R (5′-GGACTACHVGGGTWTCTAAT-3) (Magoc and Salzberg, 2011; Caporaso et al., 2012), targeting the V4 hypervariable region of the 16S rRNA gene (400–450 bp) were used to identify bacterial diversity. PCR reactions were performed using the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, United States) according to the manufacturer's instructions. Samples were amplified in triplicate to a total reaction volume of 50 μL. Each 50 μL reaction mixture contained 25 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, United States), 2.5 μL of each forward and reverse primer (10 pmol each), 1 μL DNA template (∼5 ng), and 19 μL nuclease-free water. The thermocycling conditions were as follows: initial denaturing, 98°C for 30 s, followed by 35 cycles of 10 s at 98°C (denaturing), 30 s at 65°C (annealing), 30 s at 72°C (extension), and a final extension for 10 min at 72°C. The amplicons were evaluated on 2% agarose gels. The PCR products were mixed with equal volumes of 1X loading buffer containing SYBR Green prior to loading onto the gels. PCR products were then purified with a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing libraries were created using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, United States) following the manufacturer's guidelines, and index codes were added. DNA was quantified using a Qubit® 2.0 fluorometer with the aid of a Qubit® dsDNA HS Assay (Thermo Fisher Scientific, Waltham, United States) and the Agilent Bioanalyzer 2100 system. Sequencing was carried out on the Illumina HiSeq 2500 platform, and 250 bp paired-end reads were generated.

Statistical Analysis

Sequence analysis [including operational taxonomic unit (OTU) identification], taxonomic allocations, and evaluation of community composition were primarily conducted with

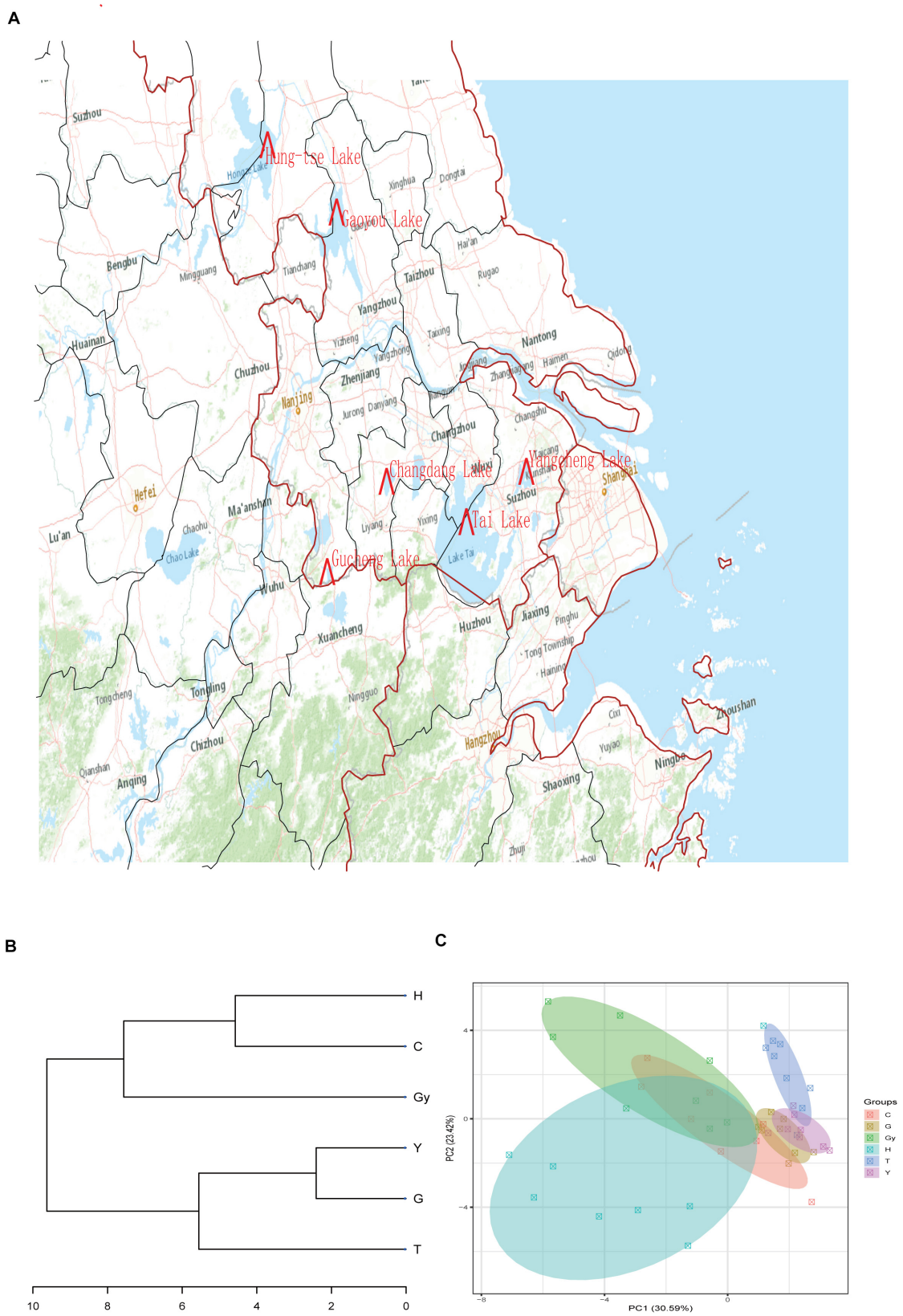


FIGURE 1 | Sample collection sites, dendrogram cluster, and PCA. **(A)** Sample collection sites. **(B)** Dendrogram cluster analysis of the crab populations based on their fatty acid profiles. **(C)** PCA analysis of the crab populations based on their fatty acid profiles.

the MOTHOR software (Kozich et al., 2013). Paired-end reads were allocated to samples based on their specific barcodes and shortened by trimming off the barcode and primer sequence. Paired-end reads were merged using FLASH (V1.2.7)[1] (Magoc and Salzberg, 2011). In order to obtain high-quality clean tags, the raw tags were subjected to quality control processes using the QIIME program (V1.7.0)[2]. Chimeric sequences were identified and removed using the UCHIME algorithm (UCHIME Algorithm)[3] to obtain effective tags. The sequences were analyzed using the Uparse software (Uparse v7.0.1001)[4], and sequences with $\geq$ 97% similarity were allocated to the same OTUs. For annotation, representative sequences for each OTU were screened. For each representative sequence, the GreenGene Database[5] based on the Ribosomal Database Project (RDP) classifier (Version 2.2)[6] algorithm was used to annotate taxonomic information. To investigate the phylogenetic relationship of OTUs, and the differences between dominant bacterial species in the crab population (groups), multiple sequence alignments were performed using the MUSCLE software (Version 3.8.31)[7]. Principal coordinate analysis (PCoA) was conducted to obtain principal coordinates and visualize the complex, multidimensional data. The weighted correlation network analysis (WGCNA), stat, and ggplot2 packages in the R software (Version 2.15.3) were used to display the PCoA analysis. Identified OTUs, abundance-based coverage estimator (ACE), Shannon, Simpson, Goods-coverage, and Chao1 were calculated with QIIME (Version 1.7.0) and visualized using the R software (Version 2.15.3). Rarefaction analysis was performed for all five libraries, and the heatmaps, Venn diagrams, and species rank abundance distribution curves were generated using the R Project for statistical computing[8]. The distances between gut microbial communities in the six crab populations were calculated using the weighted UniFrac beta-diversity metric through QIIME. Non-metric multidimensional scaling (NMDS) was used to visualize the pairwise UniFrac distances among samples. Statistical analysis was also carried out using the Kruskal-Wallis test.

The FA profile data of crab populations obtained was subjected to multivariate ANOVA using the R statistical software (version 3.1.14), to explore the multivariate structure and the potential differences. The results were expressed as mean $\pm$ standard error, and the differences of all means were determined at $P < 0.05$. Furthermore, PCA and other cluster (dendrogram) analyses were carried out to understand the pattern of FA profiles variation among the crab populations.

[1]http://ccb.jhu.edu/software/FLASH/

[2]http://qiime.org/index.html

[3]http://www.drive5.com/usearch/manual/uchime_algo.html

[4]http://drive5.com/uparse/

[5]http://greengenes.lbl.gov/cgi-bin/nph-index.cgi

[6]http://sourceforge.net/projects/rdp-classifier/

[7]http://www.drive5.com/muscle/

[8]http://www.r-project.org/

TABLE 1 | Sampling site information.

Site	Name	Habit	Area (km^2)	Coordinate		N
C	CHANGDANG HU	Lake	89	N31°59′–31°62′	E119°52′–118°60′	8
G	GUCHENG HU	Lake	65	N31°14′–31°18′	E118°53′–118°57′	8
Gy	GAOYOU HU	Lake	674.7	N32°42′–33°41′	E119°06′–119°25′	8
H	HONGZE HU	Lake	1576.9	N33°06′–33°40′	E118°10′–118°52′	8
T	TAI HU	Lake	2425	N30°55′40″–31°	E119°52′32″–120°	8
Y	YANGCHENG HU	Lake	119.04	N30°55′40″–30°	E119°51′32″–120°	8

RESULTS

FA Profiles as Biomarkers for Crab Populations From Different Geographic Locations

Variation in FA Profiles Based on the Geographic Origin of Crab Populations

The FAs [saturated FAs (SFAs), monounsaturated FAs (MUFAs), PUFAs, and unsaturated FAs (UFA)] identified from the six Chinese mitten crab populations, and their total aggregated percentages are shown in **Table 2**. Generally, SFA abundance (C14:0, C16:0, C20:0, and C22:0) did not vary significantly between populations. However, crabs from H had significantly higher levels of margaric acid (C17:0), but lower levels of stearic acid (C18:0) than crabs from C, G, Gy, T, and Y. Pentadecylic acid (C15:0) is another SFA that showed significant differences ($P < 0.05$) between crab populations, and the highest level was found in crabs from H. Furthermore, pentadecylic acid levels were also higher in crabs from Y than in those from C. The percentage of aggregated SFA were significant ($P < 0.05$) and non-significant among the populations. While it was significantly higher in crabs from Gy than in those from G, T, and Y, there was no significant difference when comparing the Gy population with those from C and H. Similarly, there were no significant differences in SFA percentage when comparing crabs from G, T, and Y, with C and H.

The percentage of aggregated MUFAs was significantly higher ($P < 0.05$) in crabs from T than in those from G. However, the MUFA percentage in crabs from T was not significantly different from those sampled from C, Gy, and Y. Furthermore, no differences were observed when comparing crabs from G with those from C, H, and Y. The percentage of PUFAs was significantly higher in crabs from C, G, H, and Y than in those from Gy, however, no significant differences were observed when crabs from T were compared to those from Gy, and when compared to those from C, G, H, and Y. The aggregated percentage of UFAs (MUFAs and PUFAs) was found to be higher in crabs from G, Y, and T than in those from Gy ($P < 0.05$), but there was no difference between Gy, C, and H populations. The

TABLE 2 | Fatty acid profiles of 6 Chinese mitten crab populations from different lakes.

	C	G	Gy	H	T	Y
C12:0	0.018 ± 0.002^{a}	0.017 ± 0.003^{a}	0.016 ± 0.003^{ab}	0.017 ± 0.005^{a}	0.012 ± 0.002^{ab}	0.010 ± 0.003^{b}
C14:0	0.418 ± 0.035	0.483 ± 0.017	0.411 ± 0.023	0.514 ± 0.029	0.460 ± 0.019	0.515 ± 0.036
C15:0	0.207 ± 0.011^{c}	0.275 ± 0.009^{bc}	0.235 ± 0.014^{bc}	0.399 ± 0.033^{a}	0.248 ± 0.009^{bc}	0.285 ± 0.013^{b}
C16:0	12.157 ± 0.237	12.434 ± 0.146	12.870 ± 0.199	12.076 ± 0.144	12.677 ± 0.236	12.222 ± 0.206
C16:1	4.381 ± 0.465^{ab}	3.348 ± 0.114^{b}	5.596 ± 0.692^{a}	5.144 ± 0.676^{ab}	3.509 ± 0.171^{b}	3.250 ± 0.120^{b}
C17:0	0.617 ± 0.032^{b}	0.644 ± 0.028^{b}	0.515 ± 0.042^{b}	0.878 ± 0.092^{a}	0.542 ± 0.025^{b}	0.612 ± 0.022^{b}
C18:0	7.707 ± 0.205^{b}	7.368 ± 0.207^{b}	6.988 ± 0.186^{b}	6.080 ± 0.255^{a}	7.328 ± 0.154^{b}	7.328 ± 0.170^{b}
C18:1	22.979 ± 0.747^{b}	22.610 ± 0.295^{b}	25.708 ± 0.765^{a}	22.702 ± 0.962^{b}	25.215 ± 0.375^{ab}	23.026 ± 0.291^{b}
C18:2	8.090 ± 0.771^{a}	5.674 ± 0.343^{ab}	4.590 ± 0.297^{b}	5.183 ± 1.190^{b}	5.736 ± 0.426^{ab}	6.669 ± 0.433^{ab}
C18:3	1.379 ± 0.085^{ab}	1.225 ± 0.117^{b}	2.245 ± 0.269^{c}	2.463 ± 0.221^{a}	0.847 ± 0.052^{b}	0.956 ± 0.046^{b}
C20:0	0.142 ± 0.014	0.174 ± 0.014	0.128 ± 0.010	0.118 ± 0.015	0.160 ± 0.042	0.197 ± 0.018
C20:1	0.768 ± 0.081^{b}	0.871 ± 0.048^{b}	0.790 ± 0.061^{b}	1.089 ± 0.107^{ab}	1.065 ± 0.046^{ab}	1.367 ± 0.103^{a}
C20:2	1.273 ± 0.129^{bcd}	1.735 ± 0.053^{ab}	1.040 ± 0.090^{cd}	1.558 ± 0.105^{abd}	1.215 ± 0.091^{d}	1.866 ± 0.082^{a}
C20:3	0.582 ± 0.058^{ac}	0.407 ± 0.028^{bc}	0.587 ± 0.026^{ac}	0.486 ± 0.051^{c}	0.291 ± 0.022^{b}	0.353 ± 0.020^{bc}
C20:4	6.337 ± 0.252^{bc}	5.991 ± 0.221^{bc}	6.882 ± 0.338^{ac}	8.721 ± 0.894^{a}	4.741 ± 0.366^{b}	5.735 ± 0.218^{bc}
C20:5	18.933 ± 0.650^{ab}	20.281 ± 0.242^{a}	17.495 ± 0.860^{b}	17.933 ± 0.822^{ab}	18.308 ± 0.261^{ab}	18.731 ± 0.386^{ab}
C22:0	0.109 ± 0.004	0.124 ± 0.003	0.112 ± 0.015	0.090 ± 0.006	0.116 ± 0.006	0.113 ± 0.010
SFA	25.754 ± 0.458^{ab}	24.867 ± 0.306^{b}	26.871 ± 1.258^{a}	25.314 ± 0.462^{ab}	25.051 ± 0.299^{b}	24.532 ± 0.259^{b}
MUFA	23.956 ± 0.764^{bcd}	23.680 ± 0.264^{b}	26.710 ± 0.725^{ad}	24.025 ± 0.994^{bc}	26.485 ± 0.422^{d}	24.659 ± 0.272^{abcd}
PUFA	50.289 ± 1.175^{a}	51.453 ± 0.387^{a}	46.419 ± 1.168^{b}	50.660 ± 1.010^{a}	48.463 ± 0.610^{ab}	50.809 ± 0.444^{a}
UFA	74.245 ± 0.458^{ab}	75.132 ± 0.306^{a}	73.129 ± 0.532^{b}	74.686 ± 0.463^{ab}	74.949 ± 0.299^{a}	75.468 ± 0.259^{a}

Values are means ± SE of 8 crabs from each lake. Means with at least one same superscript in the same row are not significantly different ($p < 0.05$).

percentage of aggregated UFAs was also comparable between C, G, Y, and H populations.

Dendrogram Showing the Relationship Between Crab FA Composition and Geographic Location

The FA profiles of the six crab populations varied significantly according to their geographic origins. This suggests that these FA profiles may be dependent on the geographical distribution of these crabs (**Figure 1A**). To verify this hypothesis, we created a dendrogram using the FA profile dataset (**Figure 1B**), which divided the six populations into two groups: Y, G, and T clustered into the first clad, and H, Gy, and C clustered into another clad. The resulting cluster style conformed to the geographical distribution of the crab populations. Similar patterns were shown by principal component analysis (PCA) (**Figure 1C**). On the dendrogram, the lakes Y and T belonged to geographical branch A, and H and Gy belonged to geographical branch B. G and C belonged to geographic branches that connect A and B branches (**Figure 1B**).

Read Sequencing, Assembly, Mapping, and Characterization

The total numbers of raw sequence reads for the intestinal contents and mucosa samples were 4,218,454 raw reads (with an average of 95,874 reads per sample) and 3,947,857 total clean reads (with an average of 89,724 reads per sample) respectively (**Supplementary Table S1**). The clean reads were deposited into the Sequence Read Archive database at NCBI (SRR12277956-SRR12277999).

Gut Bacterial Community Structure Varied According to the Geographic Origin of the Crab Population

Alpha and Beta Diversity

The alpha diversity parameters (Shannon, Simpson, ACE, and Chao1) were normalized and calculated for all crab populations at a 97% identity threshold (**Supplementary Table S2**). The alpha diversity parameters plotted (**Supplementary Figures S1A–D**, **S3B**). The results showed that crabs from T had the lowest bacterial species richness and alpha diversity. Those from H had the highest bacterial species richness, while those from Y had the highest diversity. Furthermore, crabs from H had the highest weighted and unweighted species diversity (**Supplementary Figures S1C,D**). Crabs from G had the lowest weighted species diversity, and those from T had the lowest unweighted beta diversity. Results from the rarefaction and rank abundance curves indicate that the number of samples taken are reasonable (**Supplementary Figure S2**).

Rarefaction and rank abundance curves are common curves that describe the diversity of samples in a group. The Rarefaction curve clearly shows how reasonable the amount of sequencing data is and indirectly reflects the richness of the sample species. When the curve is flat, it indicates that the sequencing data volume is reasonable, and more data volume will only produce a small number of new species (OTUs). To plot the rarefaction, and rank abundance curves, a certain amount of sequencing data were randomly selected from the sample, and the number of species represented by this data (i.e., the number of OTUs) was counted.

The rank abundance curve shows the relative species abundances (OTU numbers) starting from the most abundant to the least. To obtain the corresponding order number, find the OTU sort code on the abscissa and then the corresponding relative abundance of OTUs (or the species abundance for each sequence) on the ordinate, and connect these points with broken lines. Normally, a rank abundance curve reflects the richness and evenness of species in the sample. The species richness is reflected by the width of the curve across the abscissa. The greater the species richness, the wider the area of the curve across the horizontal axis. In the vertical direction, the smoothness of the curve reflects the evenness of species in the sample. The flatter the curve, the more homogeneous the species distribution (Lundberg et al., 2013).

Our rarefaction curves tended to be flat, indicating that the number of samples sequenced were reasonable and could reflect the real situation of the samples tested (**Supplementary Figure S2A**). The abundance indices for the 6 populations were compared, which first decreased and then increased in crabs from H and Y, with the highest in crabs from H and the lowest in crabs from T (**Supplementary Figure S2B**). Looking along the abscissa, at the width of the rank abundance curve, the crabs from H had the widest curve and the highest species richness, whereas crabs from T had the narrowest curve and the lowest species richness, which was consistent with the results from the dilution curve. The vertical axis shows that the crabs from H had the smoothest curve, whereas, those from Y had the steepest curve. Therefore, in terms of species uniformity, crabs from H showed the greatest uniformity, and those from Y were the least uniformed.

OTU Structure at Different Classification Levels

As described in section "FA Profiles as Biomarkers for Crab Populations From Different Geographic Locations," the FA profiles of the six crab populations can be used to distinguish geographic origin. The differences observed between the FA profiles of different crab populations have been attributed to variations in gut bacteria, which are influenced by different environmental conditions prevalent at each geographical location. Hence, in this study, we analyzed the type and abundance of intestinal microbial communities found in the six crab populations. The analysis revealed that a total of 1066 different bacterial species were shared among the six crab populations, and crabs from T had the lowest number of bacterial species (87) (**Supplementary Figure S3A**).

A species classification tree was constructed (from the top 10 relative abundance at the genus level, by default) (Pond et al., 2006), and the kingdom, phylum, class, order, family, genus, and species were arranged from left to right (**Supplementary Figure S4**). It showed the OTU structure of all six crab populations at different taxonomic levels. At the phylum level, the most dominant bacteria in all crab populations were Tenericutes. However, there were no significant differences in the proportions of Tenericutes among these populations. Crabs from Gy had the highest proportions of Bacteroidetes at all levels below and inclusive of the phylum (class, family, and genus). Crabs from C had the highest number of Firmicutes, followed by crabs from Y and then the other populations. Crabs from G had the highest number of Proteobacteria. At the genus level, the distribution for specific bacterial populations was similar to that of the phylum. Among the identified bacteria, *Shewanella* was only found in crabs from G, Y, and Gy based on the 0.01 level (**Supplementary Figure S4** and **Supplementary Table S3**).

To determine the dominant genus structure in each group, the top 10 and 30 genera based on relative abundance were selected to generate a cumulative histogram (**Figure 2**). *Aeromonas*, *Bacteroides*, *Dysgonomonas*, *Candidatus Hepatoplasma,* and *Candidatus Bacilloplasma* were the dominant genera (**Figure 2A**). Among these, *Candidatus Bacilloplasma* was the most prevalent, present in more than 0.5% of crabs from T. The top 10 genera accounted for 75% of nearly every population. This distribution did not significantly change when a further 20 genera were added to the dataset (**Figure 2B**). To display the phylogenetic relationship of the bacterial species found in the crab populations, the top 100 genera obtained from multiple sequence alignment and analysis of relative abundance were used to construct a LEfSe Cladogram (**Supplementary Figure S5A**).

The relative abundance of each bacterial genus in each crab population is reflected by its proportional representation on the outer ring (**Supplementary Figure S5A**). Tenericutes were the most abundant, Bacteroidetes, the third-most abundant, and Proteobacteria were the least abundant among the crab populations. To identify the genera that were clustered in specific samples, a heatmap (a heat map chart) was created from the top 35 genera, based on their abundance and taxonomic annotation (**Supplementary Figure S5B**).

The Evolutionary Distance and Relationship Between the Bacterial Communities Found in the Different Crab Populations

Genetic Differences Among the Bacterial Communities

Both weighted and unweighted Unifrac distances were used to calculate the dissimilarity coefficient between the two populations. The smaller the value, the smaller the difference in diversity between these two populations. A heatmap based on weighted and unweighted Unifrac distances was created (**Figure 3A**). For weighted Unifrac distance, the dissimilarity coefficient between groups Y and C was 0.484, which was the smallest difference among the six populations, indicating that this pair had the greatest similarity in species diversity. The largest dissimilarity coefficient (2.045) was observed between crabs from G and Gy, indicating that these two populations differed the most in terms of species diversity. For the unweighted Unifrac distance, the dissimilarity coefficient for crabs from C and T was 0.277, which was the smallest value, whereas the highest dissimilarity coefficient (0.680) was between crabs from Y and H.

Biomakers for Different Populations as Explored by LefSe

Having established the dissimilarity coefficients among the six crab populations, specific bacteria and their

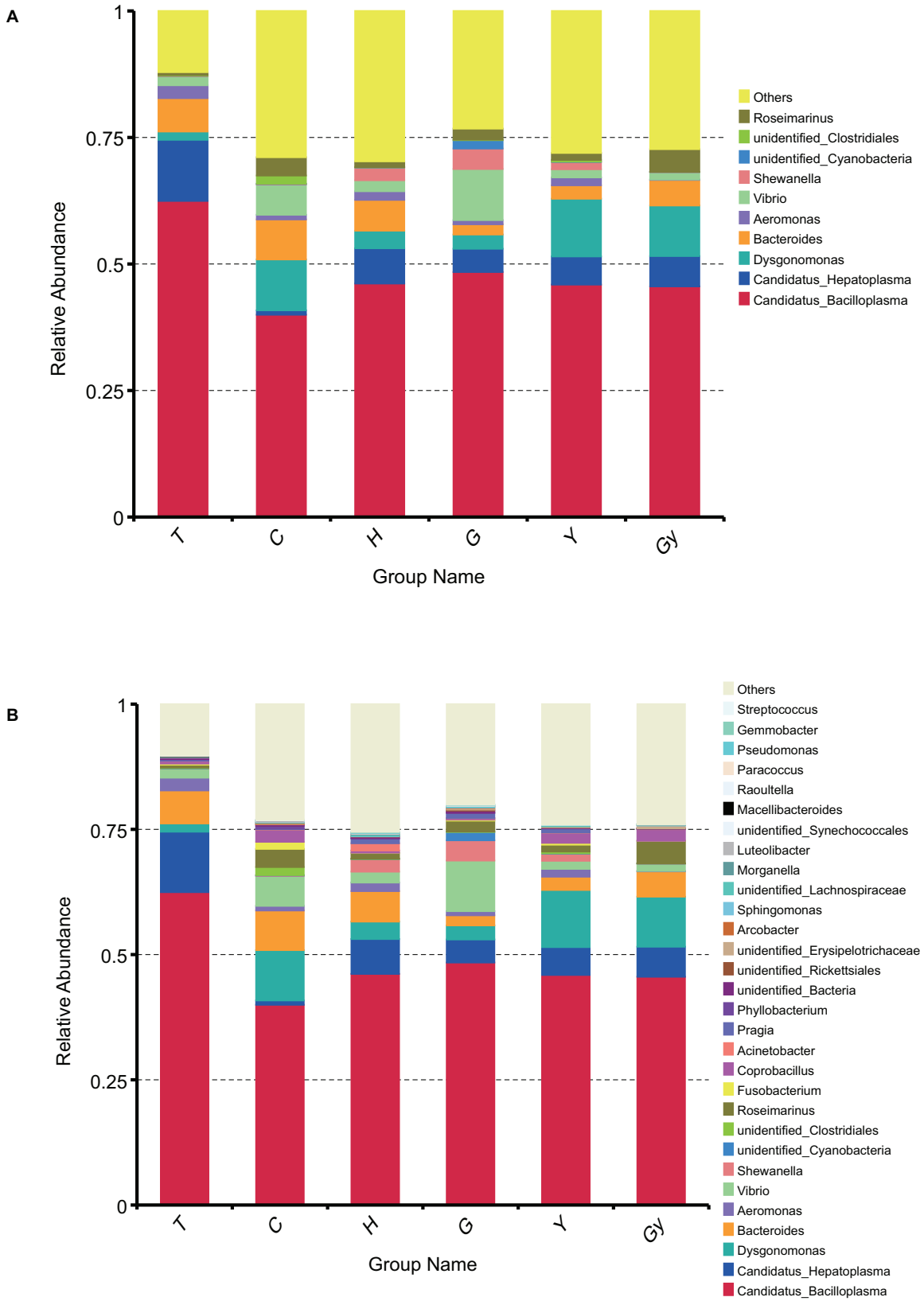


FIGURE 2 | Bar chart of gut bacteria relative abundance on genus level. **(A)** Relative abundance of top 10 genera. **(B)** Relative abundance of top 30 genera.

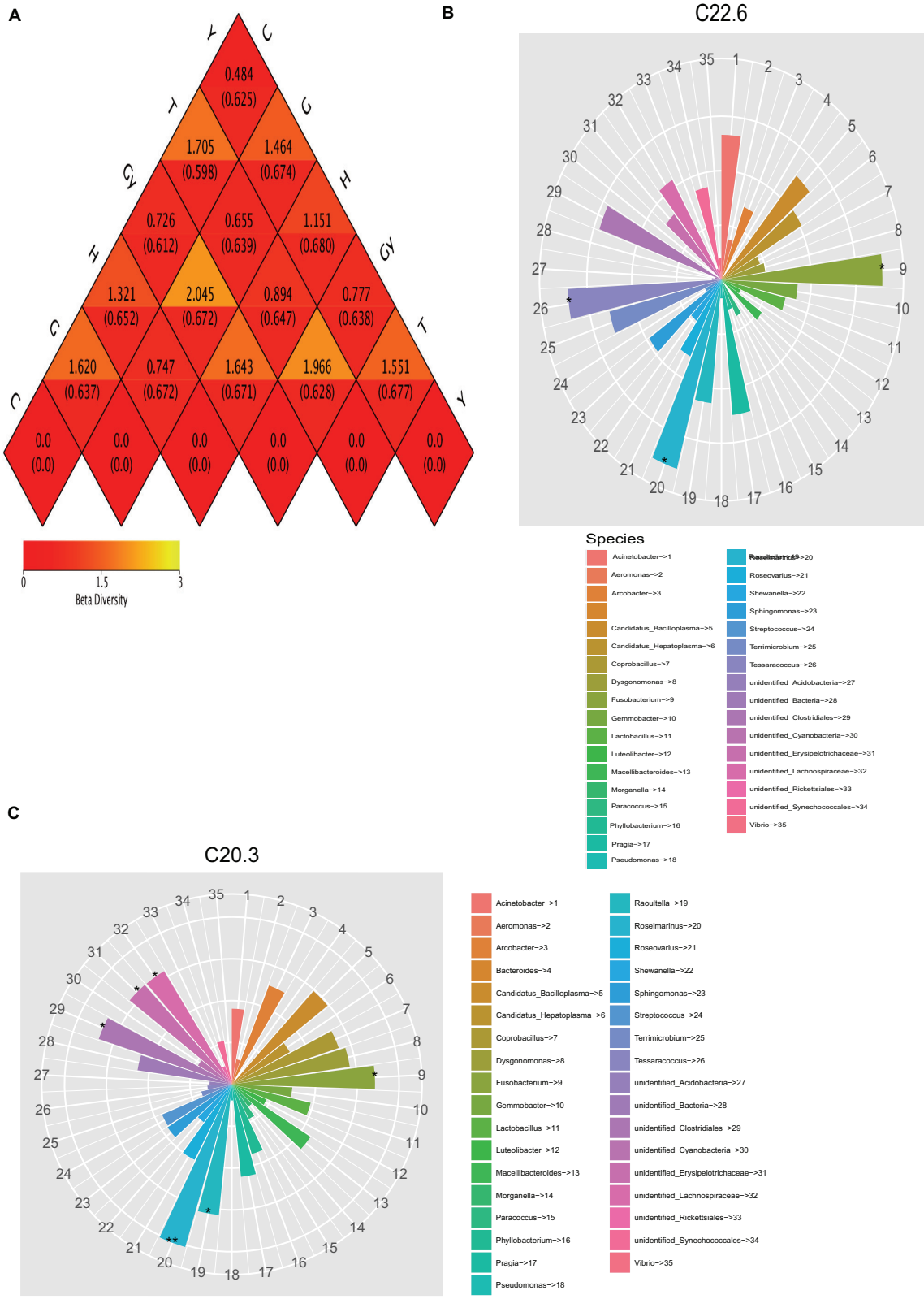


FIGURE 3 | Heatmap of beta diversity and relationship between fatty acid profile and bacterial community. **(A)** Weighted and unweighted Unifrac distances. **(B)** Relationship between C22:6 fatty acids and gut bacterial community. **(C)** Relationship 20:3 fatty acids and gut bacterial community.

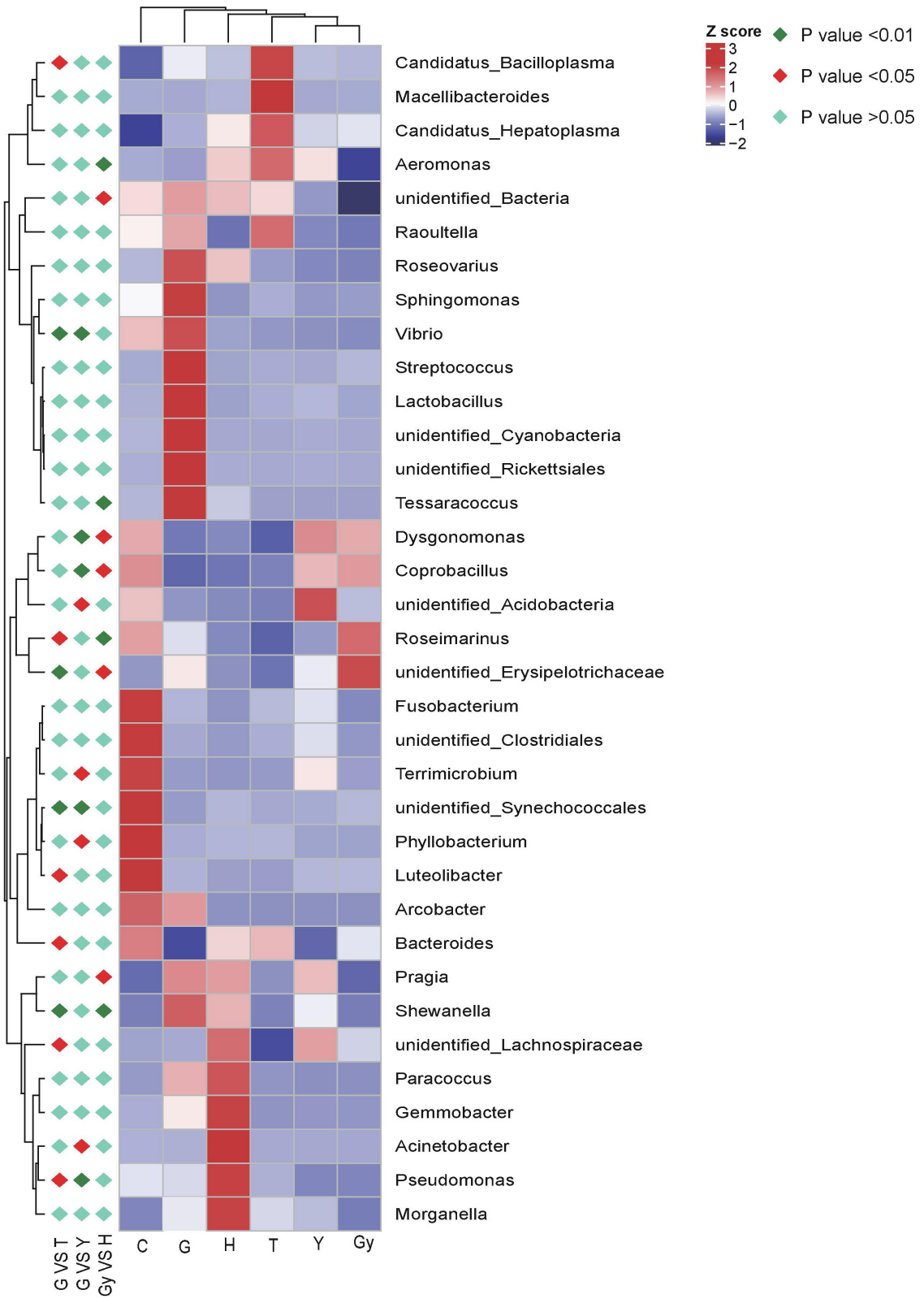


FIGURE 4 | Heat map of the correlation matrix between crab population and the bacteria communities. Red orange tones indicate a positive correlation between crab population and the bacteria communities; blue and yellow white indicate absence of correlation as for example, pragia has no correlation with crabs from Gy, meaning pragia was not found in crabs from Gy.

evolutionary relationships were subsequently analyzed (**Supplementary Figure S6A**). *Prolixibacteraceae*, *Lachnospiraceae*, and *Erysipelotrichaceae* were found in crabs from Gy only, whereas unidentified Bacteroidales were only found in crabs from Y. At the order level, Bacteroidiales and Clostridiales were only found in populations from C. At the phylum level, Tenericutes and Proteobacteria were observed in crabs from T and G; *Alphaproteobacteria* were only found in crabs from H. The relative abundance of each bacterial genus in the six populations is shown in **Supplementary Figure S6B**.

The Bacterial Community Structure in Different Crab Populations and Their Specific Taxa

The metaStat method was used to identify bacteria that were significantly different among the six crab populations. The differences in the abundance of bacterial species distributed among the populations were presented, including the 12 bacterial phyla that showed the most significant differences and those that did not show a difference. All bacterial genera structures that showed significant abundance by pairwise comparison ($n = 5$ per crab population) were plotted (**Figure 4**). Using the metaStat test, 35 genera were identified from 3 crab population pairs (Gy-H, G-Y, and G-T).

Evolutionary Analyses of the Bacterial Community Structures Found in Crab Populations Using PCA

In the present study, we carried out a PCA of the bacterial genera found in microbial communities from the six crab populations (**Figure 5A**). PC1 divided the samples into two groups: one group was composed of crabs from Y, C, and Gy, and the other group consisted of crabs from T, H, and G. PC2 also divided the crab populations into two groups. One group consisted of crabs from T and Y, and a small number of those from Gy and C, while crabs from H and G were segregated to the other group. PC1 and PC2 mainly divided the six crab populations into two groups, which indicate that there were significant differences in bacterial composition among the crab populations.

To study the similarity between the crab populations, the unweighted pair group method with arithmetic mean (UPGMA) method was used. This clustering analysis is based on the unweighted Unifrac distance matrix (**Figure 5B**). The left side is the UPGMA clustering tree, and the right side is the relative abundance of the bacterial communities at the phylum level. Furthermore, it can be s the distance between the C and T groups was the smallest, and they were then grouped with Gy, Y, G, and H. Among these populations, H and C were the furthest apart.

Relationship Between FA Profiles and Bacterial Community Structures

To determine the relationship between crab FA profiles and their gut microbial community compositions, we compared two dendrograms that depict FA profiles and bacterial community structures. The results showed that the positions of lakes of crab origin were the same on the two dendrograms (FA profiles and bacterial community structures dendrograms), except for H and Y that swapped positions on these dendrograms (**Figures 1B**, **2B**). To determine the cause of this difference, the relationship between FA and bacterial community structure was investigated. A mantel test was carried out comparing each FA that showed a significant difference among the crab populations with the 16S sequence OTU dataset (**Supplementary Table S4**). To determine the relationship between FA profiles and bacterial community structure, the analysis was performed in two phases. In the first phase, all FA profiles for each crab population were correlated with the bacterial OTU dataset, which showed that FAs were significantly related to the bacterial community. In the second phase, all FA profiles, which were significantly different among the six crab populations, were correlated with the bacterial OTU dataset. This showed that significant variation in the relative abundance of specific FA could be caused by different bacterial communities. Canonical correspondence analysis (CCA) and distance-based redundancy analysis (dbRDA) were also used to further understand how FA composition is related to the bacterial communities (**Supplementary Tables S5, S6**). All FAs that were significantly different were identified, and the FAs podocarpic acid (C20:3) and cervonic acid (C22:6) that varied in concordance with changes in the bacterial genera structures were analyzed by Spearman's correlation ($P < 0.01$) and they were plotted on the cladograms (**Figures 3B,C**). The genera that correlated with podocarpic acid (C20:3) and cervonic acid (C22:6) changes were *Fusobacterium* and *Roseimarinus*. The results from Spearman's correlation analysis of FA composition and bacterial community structure at the genus level are shown in **Supplementary Table S7**.

DISCUSSION

FA Profiles as Biomarkers for the Geographic Location of Crab Populations

Our study showed that the FA profiles of the six crab populations varied significantly and were dependent on the geographic origin of these crabs (**Table 2** and **Figures 1A,B**, **2**). Similarly, Sillero-Ríos et al. (2018) reported significant variations in the FA composition of *Octopus vulgaris* inhabiting three coastal areas in the West Mediterranean Sea. Significant differences in 22:6 n-3 (DHA, docosahexaenoic acid) and 22:5 n-3 (EPA, eicosapentaenoic acid) FA composition was found in the mantles of the *O. vulgaris* sampled from these areas. Similarly, Rude et al. (2016) demonstrated that the FA profiles of the bluegill differed significantly on the basis of habitat, suggesting that FA profiles can be used as biomarkers for fish habitat. Other studies conducted on terrestrial animals have also shown that the FA composition of milk, muscle, and other tissues can be linked to the geographic origin of these animals (Rutkowska et al., 2015; Kumar et al., 2016; Barłowska et al., 2018). Furthermore, in a study that focused on microbiota and lipids, it was found that the composition of lipids (in particular that of PUFAs) and microbiota in milk differed in animals raised in different geographic locations (Kumar et al., 2016). One of the reasons for this variation is the environment.

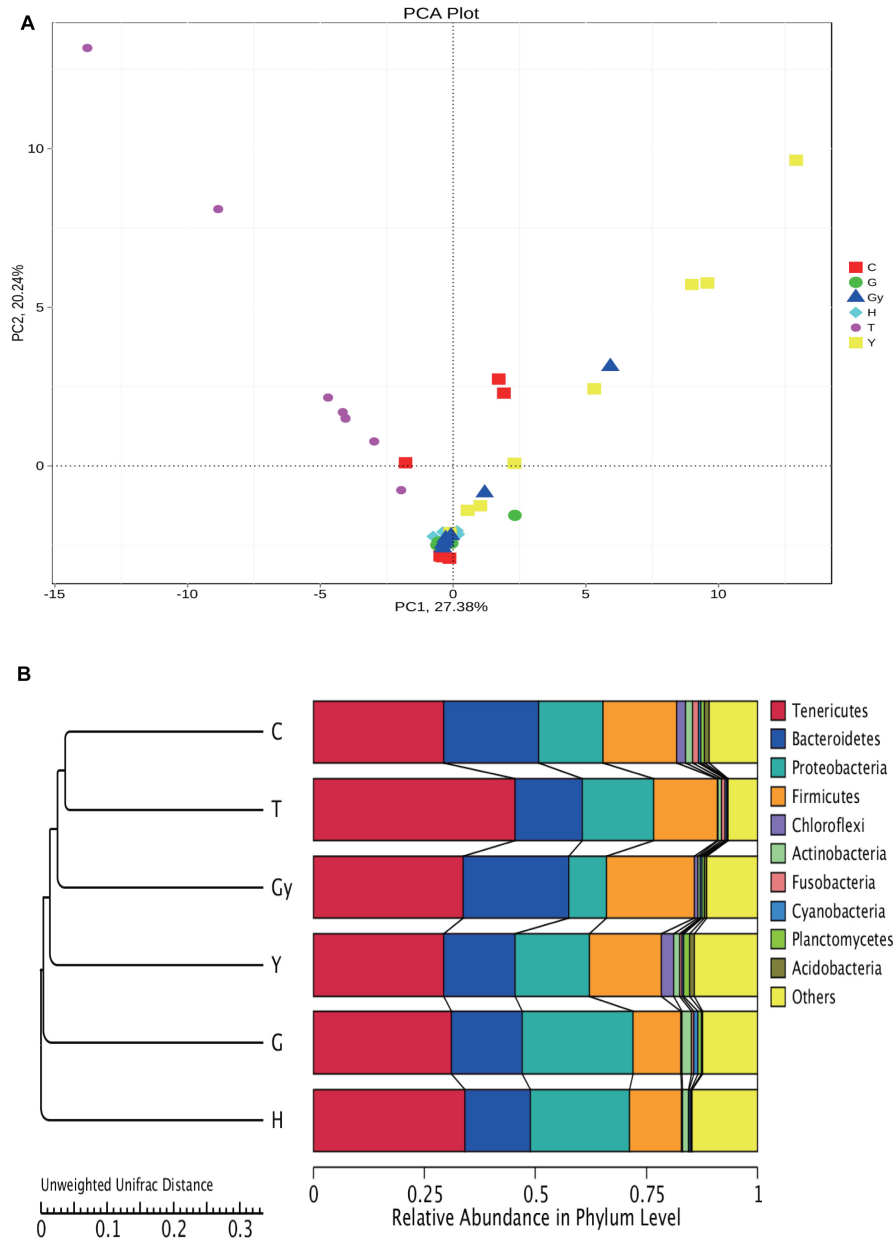


FIGURE 5 | Cluster analysis of all groups based on bacterial community. **(A)** PCA plot is illustrating differences in gut bacterial communities of different crab population. **(B)** UPGMA clustering tree based on weighted Unifrac distance.

In a study directed to see how goat milk is influenced by the environment, it was found that raising goats in certain geographic regions increased the proportion of beneficial FAs in milk and enhanced the qualities valued by consumers (Barłowska et al., 2018). In addition, in cow milk it was discovered that the high levels of linoleic acid (CLA), vaccenic acid (VA), total C18:1trans, and gamma-linolenic acid (GLA, C18:39c12c15c n-6), alpha-linolenic acid (ALA, C18:39c12c15c), were indicative of the Polish mountainous regions while the short-chain saturated FAs (SCFA, C4:0–C11:0), of the lowland (Rutkowska et al., 2015). Therefore, our results suggest that the FA profiles can be used as biomarkers for the geographic origin of crabs.

FA Composition Is Influenced by the Bacterial Community Structure in Crab Guts

Numerous studies have reported that intestinal microbial community structure influences FA composition. For example, using a comprehensive multi-omics approach, gut microbiota has been shown to induce MUFA generation by stearoyl-CoA

desaturase 1 and PUFA elongation by FA elongase 5, leading to significant changes in the glycerophospholipid acyl-chain profiles (Kindt et al., 2018). Similarly, in the present study, our analyses generated patterns that linked FA profiles with gut bacterial communities (**Figures 1B**, **2B** and **Supplementary Table S4**). Similar variations were observed for both FA compositions and gut bacterial communities based on the geographic origin of the crab populations. However, consistent with other associative studies of the gut microbiota, the major challenge is to explain the underlying biological mechanisms and to establish the cause of the observed relationships. Furthermore, information on the relationship between gut bacterial communities and FA profiles in crustaceans is generally lacking. Nevertheless, studies conducted on humans, rodents (mice), zebrafish, and other animals provide possible biological mechanisms that could explain the relationship between the bacterial community and FA composition observed in the present study (Ley et al., 2006; Semova et al., 2012; Chakraborti, 2015). Ley et al. (2005) reported significant differences in the proportions of Firmicutes and Bacteroidetes in mice. In obese mice, the relative proportions of Firmicutes and Bacteroidetes significantly increased and decreased, respectively, in comparison to normal mice. Similarly, obese children had a higher Firmicutes to Bacteroidetes ratio than lean children (Bervoets et al., 2013). The increase in the abundance of Firmicutes has often been linked with an increase in lipid storage due to obesity (Ley et al., 2005, 2006). Other gut bacteria linked to FA metabolism, energy intake, and storage include saccharolytic bacteria, lactobacilli, and staphylococci (Ley et al., 2006; Pachikian et al., 2011; Chakraborti, 2015). One study proposed that under certain conditions in humans and mice, some saccharolytic bacteria species are involved in fat storage and metabolism (Ley et al., 2008). Some *Staphylococcus* species have been positively linked with energy intake in children (Bervoets et al., 2013). Moreover, Million et al. (2012) reported that *Lactobacillus* species, via its role in FA metabolism, are linked to body weight gain and obesity in adults. Additionally, many studies have shown that Firmicutes, in comparison to other bacterial phyla, have a strong association with FA absorption, metabolism, and storage (Ley et al., 2006; Semova et al., 2012; Chakraborti, 2015). In zebrafish, an increase in the abundance of Firmicutes was correlated with an increase in the number of lipid droplets and the promotion of FA absorption (Semova et al., 2012). In the present study, significant differences in the proportions of Firmicutes and Bacteroidetes were observed (**Supplementary Figure S4** and **Supplementary Table S3**). Thus, the variations in gut bacterial communities, especially in the relative abundance of Firmicutes and Bacteroidetes, may be responsible for the variations in the FA composition of the different crab populations.

Furthermore, other studies attempted to verify and explain the relationship between gut bacterial communities and FA profiles. For example, Kindt et al. (2018) used short-term antibiotic treatment to manipulate the gut microbial ecosystem of specific pathogen-free (SPF) animals (mice) and found that the relative FA 16:0, FA 20:3, FA 20:4, and FA 22:6 levels were affected by this treatment. Similarly, in laying Japanese quails (*Coturnix coturnix japonica*), the FA composition of liver lipids can be modified by manipulating gut microflora (Furuse et al., 1992). In the present study, the presence of podocarpic (C20:3) and cervonic (C22:6) acids were explored by CCA and dbRDA overlapping analysis (**Figures 2**, **3A** and **Supplementary Tables S5**, **S6**). We found that changes to the abundance of some *Fusobacterium* and *Roseimarinus* bacteria were correlated with alterations in the composition of FA 20:3 and FA 22:6 in the crabs. Similarly, in Gy and C crab populations, FA 20:3 content was higher than that of other populations, and this correlated with higher Bacteroidetes and Firmicutes abundance (**Figures 4B,C**). The bacteria were identified by Spearman's correlation analysis to determine the influence of FA composition (**Supplementary Table S6**).

Gut Bacterial Community Structure Is Associated With Geographic Location

Generally, the bacterial community structure in the intestinal tracts of fishes from different lakes is different, which may be dependent on many factors (such as food source, water quality, and lifestyle) in the lake. For example, in a study of cichlids living in two crater lakes, the gut microbiota composition, in particular Oceanospirillales (52.28%; halotolerant or halophilic bacteria), differed between the two populations (Franchini et al., 2014). However, both the developmental stage and the geographical location are important determinants of gut bacterial composition in mosquitoes (Bascuñán et al., 2018). Thus, both the developmental stage of an animal and its geographical location determines its gut bacterial composition (Finkel et al., 2011). Variations in microorganisms associated with a host organism were also verified in trees of the same species that were grown in different climate regions. The trees hosted distinct microbial rhizosphere and phyllosphere communities (Finkel et al., 2011). The abovementioned findings are consistent with the findings of our study. In the present study, we found significant variations in gut bacteria at the phylum and lower taxonomical levels according to the geographical origin of the crab population (**Figures 2**, **3A**, **5** and **Supplementary Table S6**). However, geographic location did not have a significant effect on gut microbial communities of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Bass et al., 2018).

Environmental characteristics are among the factors that determine bacterial community composition. A mouse study showed that the type of water consumed significantly influenced the composition of gut microbiota (Dias et al., 2018). In fish, the gut microbial community structure can be affected by water and other substances found in the water. The addition of microalgae to rearing tanks affected the composition and dynamics of the microbial communities in the rearing water (Giménez Papiol et al., 2018). Total organic carbon, n-alkanes, petroleum hydrocarbons, and other compounds were found to be highly related to the bacterial community composition at different depths of water and sediments, originating from various regions of the East Mediterranean Sea (Polymenakou et al., 2005). Food choices also play an important role in shaping the composition and activity of gut microbiota (Zhao et al., 2018). Another factor that played a role in the geographic determination of bacterial composition is ecology. In a study by Sullam et al. (2012), the

composition of fish gut bacteria was found to be shaped by host phylogeny, the water salinity, and the trophic level. In lakes, the most important source of bacteria is the resident community, however, dispersal of these sources have limited immediate effects on the gut bacterial community (Comte et al., 2017).

Fish gut is in a continuous contact with the surrounding water, however, it has been shown that many microbial taxa present in surrounding waters are not found in the gut of fish and vice versa. This suggests that host associated factors strongly influence the gut bacteria community composition compared to environmental factors (Schmidt et al., 2015). In the present only gut bacteria communities were evaluated, the environment parameters and the microbial communities in the surrounding water were not evaluated. This is a major limitation of our study, hence, it was difficult to quantify and identify the exact factors responsible for the observed variations in gut bacteria communities. Nonetheless, the standard water parameters for the lakes where the crab populations were sampled can be found at (GB3838-2002).

While some recent studies suggest both the host associated and environmental factors to be significantly contributing to the fish gut microbial community structure (Dehler et al., 2017), others suggest a stronger influence of the host selective pressures (host genetics) (Li et al., 2017). Therefore, gut microbiota assembly in fish may be primarily controlled by deterministic processes due to host-dependent restrictions (Yan et al., 2016). Individuals of the same fish species occupying different habitats often host variable amount and type of gut microbial community that tend to correspond with variation in their genetic constitution (Dolan, 2005; Allan and Max, 2010; Neuman et al., 2016). The genetic background of the crab populations included in the present was evaluated in our other work (Munganga et al., unpublished), the analysis showed no significant genetic differentiation among the six populations, no geographical background clustering of the populations was observed.

The exact degree with which each of these factors influence the gut microbiome is not known. Clearly, it is difficult to distinguish the host specific and environmental effects on fish gut microbiota. The difference in feeding behavior of different species adds to the problem of investigating the role of each of these factors. It will be essential in future studies to include additional factors such as trophic levels, potential influences of environmental parameters (e.g., diet composition, food chain dynamics, water depth and temperature, geographic location), and compare variations according to season.

CONCLUSION

Similar to mammals, fish gut microbial communities play an integral part in host intestinal digestion, metabolic homeostasis, physiology, immune response, and response to disease treatment. Therefore, understanding the principles governing gut microbial assemblage and maintenance within the intestine of animals and how they interact with the host and the environment has become a primary focus. In concordance with results from previous studies, the current study presents interesting results on the interaction between the gut bacterial community, host organism, and the environment. The FA profiles of six Chinese mitten crab populations varied significantly according to geographical origin. Similar variations were also observed in the composition of the gut, which varied according to geographical origin. Further analysis of FA profiles and gut microbial community generated patterns that linked the two parameters. The FA profiles were found to be significantly related to the composition of the bacterial community in crabs from each geographic area. Hence, it was observed that the gut microbial community seems to contribute significantly to the FA composition of the Chinese mitten crab. This provides new insights that could contribute to future research on the nutrition of Chinese mitten crabs and other related areas. Furthermore, variations in FA composition were also shown to be a potential biomarker for crab geographical background. Our study is only an early analysis of the relationships between FA composition and gut bacteria community in the Chinese mitten crab, and further studies are needed to provide a deeper understanding of these interactions. These studies will help us unravel the complexity of host-gut microbial interactions for possible applications in aquaculture and species conservation.

DATA AVAILABILITY STATEMENT

The sequencing data has been deposited into a publicly accessible NCBI repository (accession: PRJNA646327).

ETHICS STATEMENT

The animal study was reviewed and approved by the Wuxi Municipal Bureau on Science and Technology.

AUTHOR CONTRIBUTIONS

SS, YT, and PX conceived the study and contributed to the design of the experiments. BM, FD, JY, JL, FY, MW, XH, XL, and RB performed all the experiments. All authors contributed to the drafting of the manuscript.

FUNDING

Central Public-interest Scientific Institution Basal Research Fund. This work was supported by grants from Freshwater Fisheries Research Center, CAFS (No. 2019JBFM01), Project for Jiangsu Agricultural New Variety Innovation (PZCZ201749), Jiangsu Fishery Technology (D2018-4), and Jiangsu Modern Agriculture Industry Technology System (JRFS-01-01).

ACKNOWLEDGMENTS

We thank the students and staff of the Aquatic Genetic Laboratory, FFRC for their assistance in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.565267/full#supplementary-material

Supplementary Figure 1 | Boxplot of Alpha-diversity indices. Alpha diversity indexes are multiple indexes reflecting consistency and abundance. **(A)** Ace and **(B)** Shannon, **(C)** Simpson, and **(D)** Chao1 indices reflect the OTU abundance in samples. Boxes represent the interquartile range between the first and third quartiles (25th and 75th percentiles, respectively) and inside the box the horizontal line shows the median.

Supplementary Figure 2 | Dilution and rank abundance curves for all gut bacteria community. **(A)** Rarefaction curves and **(B)** Ranks abundance curves; the x-axis shows the bacterial species and the y-axis shows the relative abundance of the bacteria. Each curve in the graph represents a different crabs and different colors are used to show their lake of origin or group they belong to. As the sequencing depth increased, the number of gut bacteria increased the relative abundance increased in rarefaction curves and decrease in rank abundance curves.

Supplementary Figure 3 | Core bacterial number among all groups and Shannon index box of six crab populations. **(A)** Venn diagram showing gut bacteria in each crab population. **(B)** Box plot of Shannon index showing the differences in the gut bacterial community of the six crab populations.

Supplementary Figure 4 | Gut bacteria species-specific dendrogram of six crab population based on their geographical origin. The pie chart with different colors in the circle consist of crabs from different lakes. The size of each color in the pie is the proportion of the relative abundance of bacteria community from the corresponding lake. The numbers below the taxonomy name represents the average relative abundance percentage of all groups in this level (there are two numbers).

Supplementary Figure 5 | Cladogram of LEfSe and Heat map of the correlation between gut bacteria and crab's geographical origin. **(A)** Cladogram of LEfSe analysis showing abundance of microbial taxa in the six crab populations. The inner circle is the phylogenetic tree constructed by the representative sequences of genera. The color of branches represents their corresponding phylum, each color represents a phylum, and the outer circle represents the relative abundance of each genus in each group. **(B)** Heat map of the correlation matrix between crab population and the bacteria communities at phylum level. The horizontal axis are the different bacteria phyla represented by different color and on the horizontal axis are the lakes of crab origin. Red orange tones indicate a positive correlation between crab population and the bacteria communities; blue and yellow white indicate absence of correlation as for example.

Supplementary Figure 6 | LefSe analysis. **(A)** The cladogram diagram shows the gut bacteria community species with significant differences in the six crab populations at phylum to genus level. The different colors represent different lakes and their corresponding bacteria. **(B)** Species with significant difference among the different crab populations have LDA score greater than the estimated value; the default score is 4.0. The length of the histogram is equivalent to the LDA score.

Supplementary Table 1 | Read sequencing, assembly, mapping, and characterization.

Supplementary Table 2 | Alpha and beta diversity parameters.

Supplementary Table 3 | Out table and relative abundance.

Supplementary Table 4 | Mantel test between significant fatty acid profile difference among all populations and 16S sequence OTU data set.

Supplementary Table 5 | CCA for effective environment factor capturing.

Supplementary Table 6 | dbRDA for effective environment factor capturing.

Supplementary Table 7 | Spearman correlation analysis between fatty acid profile and each bacterial on genus level.

REFERENCES

Allan, G. J., and Max, T. L. (2010). Molecular genetic techniques and markers for ecological research. *Nat. Educ. Knowledge* 3:2.

Antony, M. M. (2016). Comparative evaluation of proximate composition of *Etroplus suratensis* from ecologically different areas of ashtamudi lake, Kerala. *Int. J. Sci. Res.* 5, 959–965.

Barłowska, J., Pastuszka, R., Rysiak, A., Król, J., Brodziak, A., Kêdzierska-Matysek, M., et al. (2018). Physicochemical and sensory properties of goat cheeses and their fatty acid profile in relation to the geographic region of production. *Int. J. Dairy Technol.* 71, 699–708. doi: 10.1111/1471-0307.12506

Bascuñán, P., Niño-Garcia, J. P., Galeano-Castañeda, Y., Serre, D., and Correa, M. M. (2018). Factors shaping the gut bacterial community assembly in two main Colombian malaria vectors. *Microbiome* 6:148. doi: 10.1186/s40168-018-0528-y

Bass, S., Id, E. N., Meziti, A., Antonopoulou, E., Mente, E., and Kormas, K. A. (2018). Gut bacterial communities in geographically distant populations of farmed sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Microorganism* 6:92. doi: 10.3390/microorganisms6030092

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., et al. (2013). Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathog.* 5:10. doi: 10.1186/1757-4749-5-10

Byndloss, M. X., Olsan, E. E., Rivera-Chávez, F., Tiffany, C. R., Cevallos, S. A., Lokken, K. L., et al. (2017). Microbiota-activated PPAR-γ signaling inhibits dysbiotic *Enterobacteriaceae* expansion. *Science* 357, 570–575. doi: 10.1126/science.aam9949

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-lyons, D., Huntley, J., Fierer, N., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6, 1621–1624. doi: 10.1038/ismej.2012.8

Chakraborti, C. K. (2015). New-found link between microbiota and obesity. *World J. Gastrointestinal. Pathophysiol.* 6:110. doi: 10.4291/wjgp.v6.i4.110

Chen, D.-W., Zhang, M., and Shrestha, S. (2007). Compositional characteristics and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*). *Food Chemistry* 103, 1343–1349. doi: 10.1016/J.FOODCHEM.2006.10.047

Chen, X., Di, P., Wang, H., Li, B., and Pan, Y. (2015). Bacterial community associated with the intestinal tract of chinese mitten crab (*Eriocheir sinensis*) farmed in Lake Tai, China. *PLoS One* 10:e0123990. doi: 10.1371/journal.pone.0123990

Cheng, Y., Wu, X., Yang, X., and Hines, A. H. (2008). Current trends in hatchery techniques and stock enhancement for chinese mitten crab, *Eriocheir japonica sinensis*. *Rev. Fish. Sci.* 16, 377–384. doi: 10.1080/10641260701681698

Comte, J., Berga, M., Severin, I., Logue, J. B., and Lindström, E. S. (2017). Contribution of different bacterial dispersal sources to lakes: population and community effects in different seasons. *Environ. Microb.* 19, 2391–2404. doi: 10.1111/1462-2920.13749

Dehler, C. E., Secombes, C. J., and Martin, S. A. (2017). Environmental and physiological factors shape the gut microbiota of Atlantic salmon Parr (Salmo salar L.). *Aquaculture* 467, 149–157. doi: 10.1016/j.aquaculture.2016.07.017

Dias, M. F., Reis, M. P., Acurcio, L. B., Carmo, A. O., Diamantino, C. F., Motta, A. M., et al. (2018). Changes in mouse gut bacterial community in response to different types of drinking water. *Water Res.* 132, 79–89. doi: 10.1016/j.watres.2017.12.052

Ding, Z., Meng, Q., Liu, H., Yuan, S., Zhang, F., Sun, M., et al. (2016). First case of hepatopancreatic necrosis disease in pond-reared Chinese mitten crab, *Eriocheir sinensis*, associated with microsporidian. *J. Fish Dis.* 39, 1043–1051. doi: 10.1111/jfd.12437

Dolan, J. (2005). An introduction to the biogeography of aquatic microbes. *Aquat. Microb. Ecol.* 41, 39–48. doi: 10.3354/ame041039

Dong, J., Li, X., Zhang, R., Zhao, Y., Wu, G., Liu, J., et al. (2018). Comparative analysis of the intestinal bacterial community and expression of gut immunity genes in the Chinese Mitten Crab (*Eriocheir sinensis*). *AMB Exp.* 8:192. doi: 10.1186/s13568-018-0722-0

Druart, C., Dewulf, E. M., Cani, P. D., Neyrinck, A. M., Thissen, J.-P., and Delzenne, N. M. (2014). Gut microbial metabolites of polyunsaturated fatty acids correlate with specific fecal bacteria and serum markers of metabolic syndrome in obese women. *Lipids* 49, 397–402. doi: 10.1007/s11745-014-3881-z

Egerton, S., Culloty, S., Whooley, J., Stanton, C., Ross, R. P., and Ross, R. P. (2018). The gut microbiota of marine fish. *Front. Microbiol.* 9:873. doi: 10.3389/fmicb.2018.00873

Finkel, O. M., Burch, A. Y., Lindow, S. E., Post, A. F., and Belkin, S. (2011). Geographical location determines the population structure in phyllosphere microbial communities of a salt-excreting desert tree. *Appl. Environ. Microbiol.* 77, 7647–7655. doi: 10.1128/AEM.05565-11

Franchini, P., Fruciano, C., Frickey, T., Jones, J. C., and Meyer, A. (2014). The gut microbial community of midas cichlid fish in repeatedly evolved Limnetic-Benthic species pairs. *PLoS One* 9:e95027. doi: 10.1371/journal.pone.0095027

Furuse, M., Murai, A., and Okumura, J. (1992). Gut microflora can modify fatty acid composition in liver and egg yolk lipids of laying Japanese quail (Coturnix coturnix japonica). *Comp. Biochem. Physiol. Comp. Physiol.* 103, 569–571. doi: 10.1016/0300-9629(92)90291-w

Giménez Papiol, G., Roque, A., and Estévez, A. (2018). Variations in bacterial community of rearing water and gut of common Dentex, *Dentex dentex* (Linnaeus 1758), larvae using three microalgae management approaches. *J. World Aquacult. Soc.* 49, 953–963. doi: 10.1111/jwas.12437

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., et al. (2011). Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3047–3052. doi: 10.1073/pnas.1010529108

Kindt, A., Liebisch, G., Clavel, T., Haller, D., Hörmannsperger, G., Yoon, H., et al. (2018). The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. *Nat. Commun.* 9:3760. doi: 10.1038/s41467-018-05767-4

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq illumina sequencing platform. *Appl. Environ. Microbiol.* 79, 5112–5120. doi: 10.1128/AEM.01043-13

Kumar, H., du Toit, E., Kulkarni, A., Aakko, J., Linderborg, K. M., Zhang, Y., et al. (2016). Distinct patterns in human milk microbiota and fatty acid profiles across specific geographic locations. *Front. Microbiol.* 7:1619. doi: 10.3389/fmicb.2016.01619

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., and Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proc. Natl. Acad. Sci. U.S.A.* 102, 11070–11075. doi: 10.1073/pnas.0504978102

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., et al. (2008). Evolution of mammals and their gut microbes. *Science* 320, 1647–1651. doi: 10.1126/science.1155725

Ley, R. E., Turnbaugh, P. J., Klein, S., and Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature* 444, 1022–1023. doi: 10.1038/4441022a

Li, X., Zhou, L., Yu, Y., Ni, J., Xu, W., and Yan, Q. (2017). Composition of gut microbiota in the gibel carp (Carassius auratus gibelio) varies with host development. *Microb. Ecol.* 74, 239–249. doi: 10.1007/s00248-016-0924-4

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., and Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nat. Methods* 10, 999–1002. doi: 10.1038/nmeth.2634

Magoc, T., and Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27, 2957–2963. doi: 10.1093/bioinformatics/btr507

Million, M., Maraninchi, M., Henry, M., Armougom, F., Richet, H., Carrieri, P., et al. (2012). Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int. J. Obes.* 36, 817–825. doi: 10.1038/ijo.2011.153

Mohan, M. (2013). Comparative evaluation of proximate composition of *Etroplus suratensis* from ecologically different areas of Ashtamudi Lake, Kerala. *Int. J. Sci. Res.* 5, 959–965.

Neuman, C., Hatje, E., Zarkasi, K. Z., Smullen, R., Bowman, J. P., and Katouli, M. (2016). The effect of diet and environmental temperature on the faecal microbiota of farmed Tasmanian Atlantic Salmon (*Salmo salar* L.). *Aquac. Res.* 47, 660–672. doi: 10.1111/are.12522

Pachikian, B. D., Neyrinck, A. M., Portois, L., De Backer, F. C., Sohet, F. M., Hacquebard, M., et al. (2011). Involvement of gut microbial fermentation in the metabolic alterations occurring in n-3 polyunsaturated fatty acids-depleted mice. *Nutr. Metabol.* 8:11.

People's Republic of China Ministry of Agriculture (2016). *China Fishery Statistical Yearbook 2016*. Beijing: China Agriculture Press.

Polymenakou, P. N., Bertilsson, S., Tselepides, A., and Stephanou, E. G. (2005). Links between geographic location, environmental factors, and microbial community composition in sediments of the eastern mediterranean sea. *Microb. Ecol.* 49, 367–378. doi: 10.1007/s00248-004-0274-5

Pond, M. J., Stone, D. M., and Alderman, D. J. (2006). Comparison of conventional and molecular techniques to investigate the intestinal microflora of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 261, 194–203. doi: 10.1016/J.AQUACULTURE.2006.06.037

Ramos, A., and Hemann, M. T. (2017). Drugs, bugs, and cancer: *Fusobacterium nucleatum* promotes chemoresistance in colorectal cancer. *Cell* 170, 411–413. doi: 10.1016/j.cell.2017.07.018

Riveiro, I., Guisande, C., Iglesias, P., Basilone, G., Cuttitta, A., Giráldez, A., et al. (2011). Identification of subpopulations in pelagic marine fish species using amino acid composition. *Hydrobiologia* 670, 189–199. doi: 10.1007/s10750-011-0663-y

Roeselers, G., Mittge, E. K., Stephens, W. Z., Parichy, D. M., Cavanaugh, C. M., Guillemin, K., et al. (2011). Evidence for a core gut microbiota in the zebrafish. *ISME J.* 5, 1595–1608. doi: 10.1038/ismej.2011.38

Rude, N. P., Trushenski, J. T., and Whitledge, G. W. (2016). Fatty acid profiles are biomarkers of fish habitat use in a river-floodplain ecosystem. *Hydrobiologia* 773, 63–75. doi: 10.1007/s10750-016-2679-9

Rudnick, D. A., Hieb, K., Grimmer, K. F., and Resh, V. H. (2003). Patterns and processes of biological invasion: the chinese mitten crab in San Francisco Bay. *Basic Appl. Ecol.* 4, 249–262. doi: 10.1078/1439-1791-00152

Rutkowska, J., Bialek, M., Adamska, A., and Zbikowska, A. (2015). Differentiation of geographical origin of cream products in Poland according to their fatty acid profile. *Food Chem.* 178, 26–31. doi: 10.1016/j.foodchem.2015.01.036

Schmidt, V. T., Smith, K. F., Melvin, D. W., and Amaral-Zettler, L. A. (2015). Community assembly of a euryhaline fish microbiome during salinity acclimation. *Mol. Ecol.* 24, 2537–2550. doi: 10.1111/mec.13177

Semova, I., Carten, J. D., Stombaugh, J., MacKey, L. C., Knight, R., Farber, S. A., et al. (2012). Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host Microbe* 12, 277–288. doi: 10.1016/j.chom.2012.08.003

Shen, H., Zang, Y., Song, K., Ma, Y., Dai, T., and Serwadda, A. (2017). A Meta-Transcriptomics survey reveals changes in the microbiota of the chinese mitten crab *Eriocheir sinensis* infected with Hepatopancreatic necrosis disease. *Front. Microbiol.* 8:732. doi: 10.3389/fmicb.2017.00732

Sillero-Ríos, J., Sureda, A., Capó, X., Oliver-Codorniú, M., and Arechavala-Lopez, P. (2018). Biomarkers of physiological responses of Octopus vulgaris to different coastal environments in the western mediterranean sea. *Mar. Pollut. Bull.* 128, 240–247. doi: 10.1016/j.marpolbul.2018.01.032

Stanley, D., Hughes, R. J., and Moore, R. J. (2014). Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease. *Appl. Microbiol.. Biotechnol.* 98, 4301–4310. doi: 10.1007/s00253-014-5646-2

Sui, L., Zhang, F., Wang, X., Bossier, P., Sorgeloos, P., and Hänfling, B. (2009). Genetic diversity and population structure of the Chinese mitten crab Eriocheir sinensis in its native range. *Mar. Biol.* 156, 1573–1583. doi: 10.1007/s00227-009-1193-2

Sullam, K. E., Essinger, S. D., Lozupone, C. A., O'connor, M. P., Rosen, G. L., Knight, R., et al. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Mol. Ecol.* 21, 3363–3378. doi: 10.1111/j.1365-294X.2012.05552.x

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., and Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proc. Biol. Sci.* 286:20182448. doi: 10.1098/rspb.2018.2448

Waite, D. W., and Taylor, M. W. (2015). Exploring the avian gut microbiota: current trends and future directions. *Front. Microbiol.* 6:673. doi: 10.3389/fmicb.2015.00673

Wang, Q., Cheng, L., Liu, J., Li, Z., Xie, S., and De Silva, S. S. (2015). Freshwater aquaculture in PR China: trends and prospects. *Rev. Aquacult.* 7, 283–302. doi: 10.1111/raq.12086

Wang, Q., Liu, J., Zhang, S., Lian, Y., Ding, H., Du, X., et al. (2016). Sustainable farming practices of the chinese mitten crab (*Eriocheir sinensis*) around Hongze Lake, lower Yangtze River Basin, China. *Ambio* 45, 361–373. doi: 10.1007/s13280-015-0722-0

Yan, Q., Li, J., Yu, Y., Wang, J., He, Z., Van Nostrand, J. D., et al. (2016). Environmental filtering decreases with fish development for the assembly of gut microbiota. *Environ. Microbiol.* 18, 4739–4754. doi: 10.1111/1462-2920.13365

Zenebe, T., Ahlgren, G., Gustafsson, I.-B., and Boberg, M. (1998). Fatty acid and lipid content of Oreochromis niloticus L. in Ethiopian lakes - dietary effects of phytoplankton. *Ecol. Freshw. Fish.* 7, 146–158. doi: 10.1111/j.1600-0633.1998.tb00181.x

Zeng, Y., Zeng, D., Zhou, Y., Niu, L., Deng, J., Li, Y., et al. (2018). Microbial biogeography along the gastrointestinal tract of a red panda. *Front. Microbiol.* 9:1411. doi: 10.3389/fmicb.2018.01411

Zhang, J., Liu, Y.-X., Zhang, N., Hu, B., Jin, T., Xu, H., et al. (2019). NRT1.1*B* is associated with root microbiota composition and nitrogen use in field-grown rice. *Nat. Biotechnol.* 37, 676–684. doi: 10.1038/s41587-019-0104-4

Zhang, M., Sun, Y., Chen, L., Cai, C., Qiao, F., and Du, Z. (2016). Symbiotic bacteria in gills and guts of chinese mitten crab (*Eriocheir sinensis*) differ from the free-living bacteria in water. *PLoS One.* 11:e0148135. doi: 10.1371/journal.pone.0148135

Zhang, T., Li, Z., and Cui, Y. (2001). Survival, growth, sex ratio, and maturity of the chinese mitten crab (*Eriocheir sinensis*) reared in a chinese pond. *J. Freshw. Ecol.* 16, 633–640. doi: 10.1080/02705060.2001.9663855

Zhao, Y., Chen, Y., Li, Z., and Peng, W. (2018). Environmental factors have a strong impact on the composition and diversity of the gut bacterial community of Chinese black honeybees. *J. Asia Pacific Entomol.* 21, 261–267. doi: 10.1016/j.aspen.2018.01.002

Zheng, J. H., Nguyen, V. H., Jiang, S.-N., Park, S.-H., Tan, W., Hong, S. H., et al. (2017). Two-step enhanced cancer immunotherapy with engineered *Salmonella typhimurium* secreting heterologous flagellin. *Sci. Transl. Med.* 9:eaak9537. doi: 10.1126/scitranslmed.aak9537

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

REVIEW
published: 20 October 2020
doi: 10.3389/fmicb.2020.573759

The Russian Doll Model: How Bacteria Shape Successful and Sustainable Inter-Kingdom Relationships

*Enrica Pessione**

Department of Life Sciences and Systems Biology, School of Nature Sciences, Università degli Studi di Torino, Turin, Italy

Successful inter-kingdom relationships are based upon a dynamic balance between defense and cooperation. A certain degree of competition is necessary to guarantee life spread and development. On the other hand, cooperation is a powerful tool to ensure a long lasting adaptation to changing environmental conditions and to support evolution to a higher level of complexity. Bacteria can interact with their (true or potential) parasites (i.e., phages) and with their multicellular hosts. In these model interactions, bacteria learnt how to cope with their inner and outer host, transforming dangerous signals into opportunities and modulating responses in order to achieve an agreement that is beneficial for the overall participants, thus giving rise to a more complex "organism" or ecosystem. In this review, particular attention will be addressed to underline the minimal energy expenditure required for these successful interactions [e.g., moonlighting proteins, post-translational modifications (PTMs), and multitasking signals] and the systemic vision of these processes and ways of life in which the system proves to be more than the sum of the single components. Using an inside-out perspective, I will examine the possibility of multilevel interactions, in which viruses help bacteria to cope with the animal host and bacteria support the human immune system to counteract viral infection in a circular vision. In this sophisticated network, bacteria represent the precious link that insures system stability with relative low energy expenditure.

Keywords: phage–bacteria–human host, multitasking signals, evolution, partnership agreement, viral-like particles, moonlight proteins, PTMs (post-translational modifications), MVs (membrane vesicles)

OPEN ACCESS

Edited by:
Shyam Sundar Paul, Central Institute for Research on Buffaloes (ICAR), India

Reviewed by:
Lorena Ruiz, Institute of Dairy Products of Asturias (IPLA), Spain
Changyi Zhang, University of Illinois at Urbana–Champaign, United States

***Correspondence:**
Enrica Pessione
enrica.pessione@unito.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *17 June 2020*
Accepted: *31 August 2020*
Published: *20 October 2020*

Citation:
Pessione E (2020) The Russian Doll Model: How Bacteria Shape Successful and Sustainable Inter-Kingdom Relationships. Front. Microbiol. 11:573759. doi: 10.3389/fmicb.2020.573759

INTRODUCTION

The existence of symbiotic relationships between bacteria and organisms belonging to other kingdoms has long been recognized, although only recently, thanks to the advancement of molecular and *omic* techniques, in-depth insights into their reciprocal interaction at a molecular level have been achieved (Qin et al., 2010; Mendes and Raaijmakers, 2015). The fundamental role that bacteria have played during higher organisms' evolution, and the huge impact that they continue to have in host development, self-recognition, physiology and adaptation to changing environments have been extensively discussed (Zilber-Rosenberg and Rosenberg, 2008; Schnorr, 2015). Paradigmatic for describing these interactions are the examples concerning root (Ramírez-Puebla et al., 2013) and the animal gut (Ley et al., 2008) microbiota where both specificity and

biodiversity have supported the establishment and evolution of what is now called holobiont, the unit on which evolutionary selection acts (Rosenberg and Zilber-Rosenberg, 2016).

In parallel to this multicellular organism-targeted relationship, one of the oldest and most important challenges for bacteria is the interaction with bacteriophages, viruses infecting prokaryotic cells. At a first sight, phages are parasites/predators that, after exploiting host biosynthetic machinery, kill the cell. From this standpoint, they represent a danger to be absolutely avoided by bacteria that have set-up complex mechanisms of phage resistance (Mirzaei and Maurice, 2017) that have in turn generated mutations in bacteriophages both to overcome resistance and to adapt to new bacterial species (Mirzaei and Maurice, 2017). The latter phage strategy promotes "host jumps" that provide a host-range expansion and ensures virus survival (De Sordi et al., 2019a). However, not only bacteria provide bacteriophage diversification, but also phages support bacterial diversification as shown by the high variability of phage receptors in the same bacterial species in a certain ecological niche (De Sordi et al., 2019b). These reciprocal dynamics, by creating a high selective pressure, have deeply contributed to the evolution of both partners resulting in the overall expansion of their genetic diversity, as suggested by Allen and Abedon (2013). This co-evolution has been recently confirmed to be very fast (just 8 days) by Wandro (2019) who observed that the interaction between Efv-phi1 lytic phage and its host *Enterococcus faecium* brought to mutations in the surface bacterial structures and RNA polymerase as well as in the tail proteins of the virus. Beside these surface modifications, bacteria can employ sophisticated strategies aimed to escape phage infection after nucleic acid injection. Among these, are worth mentioning the R-M system and the CRISPR (cluster regularly interspaced short palindromic repeats) sequences. The former involves restriction modification systems providing degradation of the phage genome (De Sordi et al., 2019b). The latter is consistent with abundant phage spacers that are identical to a sequence named the proto-spacer found in the genome of the infecting virulent phage. Therefore, this system counteracts nucleic acid invaders (both DNA and RNA) via a sequence specific strategy (Deveau et al., 2010). However, even in this case resistance mechanisms set up by viruses render both these mechanisms uneffective (Samson et al., 2013). In the case of CRISPR-based bacterial phage resistance, phage mutants exist that carry a single point mutation or a deletion in their proto-spacer and therefore they can successfully complete their lytic cycles (Labrie et al., 2010). The CRISPR system and the phage adaptive response represent one the best examples of co-evolution between bacteria and phages that shape the structure of microbial communities (Andersson and Banfield, 2008). On the other hand, bacteria have learnt how to cope with phage infection renegotiating this stressing event to arrange benefits for both contenders, and how to adapt to mammalian host immune system. This ability to convert a negative affair into an opportunity is part of the strategies referred in 2001 by Hoagland et al. (2001) that described the 16th rules of the living, among which three are worth mentioning: a) different assembling of simple modules to create diversity at low-cost, b) high information exchange to optimize instead that maximize, c) interconnection and integration of new aspects to change a bad event into an opportunity (i.e., changing predation/parasitism into symbiosis) These aspects are particularly relevant in the fast-growing and fast-evolving bacterial populations, however, they constitute a model also for guiding human behavior in a period (climate change and scarcity of resources) that reveals our overall fragility.

When considering bacterial interactions with both host and phage our traditional learning approach seems sometimes inadequate to fully understand the reality. The huge number of interactions and feed-backs, the difficulty to establish a clear cause/effect model, and the overall complexity of the network suggest that a circular and systemic point of view is best fitting for elucidating the whole dynamic. In the present review, I will try to explore, although partly in a reductionist way, the molecular mechanisms underlying this Russian doll model of multilevel interactions, considering on one side the molecular effectors at the host-bacteria interface and on the other side the bacterial advantages linked to phage infection. Finally, some aspects of the complex, systemic and holistic ecosystem made up by host, bacteria and phages will be discussed.

THE INTERACTIVE BIOCHEMISTRY AT THE BACTERIA ANIMAL HOST INTERFACE

Bacteria–host interaction is an unpredictable relationship. What is clear analyzing the literature is that in a symbiosis the whole is more than the sum of the parts and therefore we cannot advance without a systems biology perspective (Relman, 2008). Often, the relationship becomes so intimate that both partners lose some genes and become strongly dependent on the presence of the other partner like in the inter-kingdom syntrophy [for example, lactic acid bacteria (LAB) that cannot synthesize amino acids and need milk proteins or animal inability to synthesize vitamins that are provided by the gut bacteria] (Ellers et al., 2012). This co-evolution has been in some cases so drastic that bacteria lost their identity to become organelles (Margulis' theory) giving origin to a system of higher complexity (Dyall et al., 2004). Therefore, the apparent involution of each partner is sometimes the door to have access to a new life experience.

In humans, the presence of a commensal microbiota increases the host fitness providing new genes, thus conferring new metabolic capabilities, higher flexibility and adaptation to new ecosystems, like a pen-drive that enhances the capacity of a computer without dramatically altering the hard disk (Webster, 2014). However, besides genetics, several host factors (age, circadian rhythms, hormones, diet, and drugs) can affect symbiont bacteria diversity and single cell level phenotypic pattern (Zdziarski et al., 2010), therefore, the relationship should be considered dynamic and often unpredictable. This is the major challenge when exogenous microbiota supply is planned to treat gut dysbiosis (De Vrieze, 2013) or enhance plant fitness using eco-friendly agriculture (Berg et al., 2016). In this scenario, molecular communication and co-metabolism are crucial factors in host-microbe interactions.

Cross-signaling between bacteria and their host is a well-established concern (Mazzoli and Pessione, 2016). From one side, the host can modulate microbiota releasing compounds (top-down control) and, on the other side, bacteria can use their signaling molecules to modify host biology (bottom-up control) also responding to host stimuli (Cryan and Dinan, 2012). Actually, it has been experimentally proven that this reciprocal interaction modifies the gene expression profiles of both partners (Zdziarski et al., 2010). Host-derived factors besides modulating microbiota composition (Hevia et al., 2015) can impact bacterial genomics, transcriptomic and proteomics at a single cell level, altering the virulence profiles (Denou et al., 2008; Sandrini et al., 2014) and thus conditioning microbial evolution and diversification. As an example the expression of virulence genes is often elicited by host factors such as stress-induced catecholamines (Cogan et al., 2007) toward whom bacteria have evolved specific receptors (Clarke et al., 2006). Compounds of exogenous origin such as antibiotics should be included among modulating host-supplied factors. Actually, low-levels of antibiotics (like those acquired from the diet) can induce expression of virulence genes and favor DNA exchanges between bacteria (Goh et al., 2002). In parallel, microbial factors, both surface-bound and secreted, influence host physiology by regulating several functions as better described in the next section (Liu et al., 2020). Although secondary metabolites are specifically produced by microbes to interact with their environment and therefore with organisms sharing the same ecological niche (social adaptation) (O'Brien and Wright, 2011), frequently simple molecules derived from central carbon catabolism also play a role in cross-communication.

Bacterial-Derived Multitasking Signaling Compounds

Several bacterial-produced metabolites deeply affect the overall host physiological status, including metabolism (Bäckhed et al., 2004), immunity (Segain et al., 2000), and mental health (Cryan and Dinan, 2012). The three main families of biochemical compounds (lipids, sugars, and proteins) all contribute to bacteria signaling toward the host. Their multitasking roles, targeting bacteria and host, are reported in **Table 1**.

Short-chain fatty acids (SCFA), namely acetate, lactate, propionate and butyrate, are generally end-products of bacterial carbohydrate catabolism, mainly produced following a fiber-rich diet. By acting as histone deacetylase inhibitors they can induce epigenetic modifications that modulate host functions such as energy storage and immune homeostasis (Davie, 2003). Among SCFA, acetate displays an interesting role in controlling articular diseases by inducing faster resolution of the neutrophil-mediate inflammatory response (Vieira et al., 2017). Butyrate (in concentrations lower than 2 mM) can improve the gut epithelial barrier by enhancing the production and secretion of mucin by goblet cells (Burger-van Paassen et al., 2009) and the expression of tight-junction proteins (Wang et al., 2018) besides stimulating the production of anti-inflammatory cytokines, such as interleukin (IL)-10 (Vipperla and O'keefe, 2012). Butyrate also helps to regulate the balance between proliferation, differentiation, and apoptosis of colonocytes and it can be found in higher quantities in the feces of healthy individuals compared with individuals with colorectal cancer (Serban, 2014).

Exopolysaccharides (EPS), besides being important constituents of the biofilm extracellular polymeric substance and acting as protective shield for preventing cell desiccation and osmotic stress (Pessione, 2012) also have a role as signaling molecules, behaving as growth control agents and immune modulators for the host. *Lactobacillus acidophilus* EPS can inhibit the growth of Caco-2 colon cancer cells (Deepak et al., 2016) while EPS from *L. gasseri* can induce apoptosis in cervical tumor cells, also decreasing the production of TNF-α and increasing the IL-10 production thus controlling inflammation (Sungur et al., 2017). Also *Bifidobacteria*-derived EPS support important functions such as modulation of the composition of the gut microbiota and of communication processes with the host. In this genus the G + C content of the genes encoding EPS synthetic enzymes is different from the one of the whole genome suggesting horizontal gene transfer (HGT) events that underline the importance of acquiring such capability (Hidalgo-Cantabrana et al., 2014).

However, the central role is played by nitrogen compounds such as amino acid (and their metabolic products) peptides and proteins. Glutamate, glutamine, and their derivative gamma aminobutyric acid (GABA), especially produced by LAB, are involved in neuromodulation and in controlling neuro-muscular junctions as well as vascular tension and blood pressure (Mazzoli et al., 2010). The tryptophan-derivative indole can control tight junctions (Bansal et al., 2010). Other amino acids derivatives such as histamine, serotonin, nor-epinephrine as well as inorganic gaseous molecules such as NO and H_2S (generated by bacterial conversion of diet compounds) are responsible for controlling enteric neurotransmission (for exhaustive reading, see Mazzoli and Pessione, 2016).

It is interesting to underline that bacteria produce SCFA and EPS for their own benefit and also amines have a specific function in both pH buffering and energy gain increase for the prokaryote partner (Pessione, 2012). Therefore, this host-targeted effect seems to be an additional and cheap function of an otherwise useful molecule.

A further example concerns quorum sensing (QS) autoinducers. In *Pseudomonas* 3-oxo-C12 homoserine lactone can control different host metabolic pathways including tight-junction proteins modifications, resulting in an altered gut barrier (Vikström et al., 2010), pro- and anti-inflammatory cytokine ratio (Glucksam-Galnoy et al., 2013) and apoptosis (Tateda et al., 2003). Similarly, *Pseudomonas* quinolone signal can downregulate innate immune responses (Kim et al., 2010) and control in a concentration-dependent manner host neutrophil chemotaxis (Hänsch et al., 2014). A review article by Dobson et al. (2012) elegantly illustrates the multiple roles that Gram-positive secreted peptides can have: first, they behave as QS signals at low concentrations and as bacteriocins at higher concentrations by inducing their own biosynthesis when a cell density threshold is reached (Kleerebezem, 2004). Secondarily, at least in *L. plantarum*, they can behave as bacteriocins and immune modulating agents altering cytokine profiles

TABLE 1 | Multitasking bacterial-derived compounds and their different roles in bacteria and host.

Compounds	Function for bacteria	Function on host	References
SCFA	Catabolism end-products	Histone deacetylase inhibitors Control of tight-junction proteins Anti-inflammatory action (increased IL-10, TGF-β, and annexin A1)	Davie (2003), Wang et al. (2018), Vieira et al. (2017)
EPS	Osmoprotectants Biofilm polymeric matrix	Inhibit the growth of Caco-2 colon cancer cells Decrease the production of TNF-α and increase the IL-10 production Innate and adaptive immune responses	Deepak et al. (2016), Sungur et al. (2017), Hidalgo-Cantabrana et al. (2014)
Amino acid derivatives Histamine, serotonin, and nor-epinephrine 3-oxo-C12 homoserine lactone GABA	Alkalinization and energy gain QS autoinducers	Control enteric neurotransmission Modulate pro- and anti-inflammatory cytokine ratio Modulation of neuro-muscular junctions and blood pressure	Mazzoli and Pessione (2016), Glucksam-Galnoy et al. (2013), Mazzoli et al. (2010)
Peptides	QS, bacteriocins	Alter cytokine profiles of both monocytes and dendritic cells	Meijerink et al. (2010), Van Hemert et al. (2010)
Moonlight proteins	Glycolisis, TCA, and stress chaperones (GroEL and DnaK)	Tissue adhesion Dendritic cell maturation Enhance secretion of IL-8 in human macrophages Induce apoptosis in gastric cells	Granato et al. (2004), Floto et al. (2006), Bergonzelli et al. (2006), Basak et al. (2005)

EPS, exopolysaccharides; GABA, γ-amino butyric acid; IL, interleukin; QS, quorum sensing; SCFA, short chain fatty acids; TCA, tricarboxylic acid cycle; TGF-β, tumor growth factor β; TNF-α, tumor necrosis factor α.

of both monocytes (Van Hemert et al., 2010) and dendritic cells (Meijerink et al., 2010). All these examples underline the sustainability of these multitasking molecules that support energy saving for bacteria that use the same compound to achieve many different functions. It is evident that host cells must have evolved receptors for sensing such "non-self" compounds, but it is even smarter the fact that bacteria can use a single molecule to orchestrate relationships with both their siblings and their host, with low energy expenditure, and using the same "language."

Alongside these small molecules, important mediators for bacteria–host interaction are surface-bound and secreted proteins. Surface-layer proteins and glycoproteins can control bacterial adhesion (Beganoviæ et al., 2011) and some secreted proteins like p40 and p75 can cooperate to the gut epithelium physiology by protecting tight junctions and enhancing IgA production (Wang et al., 2017). However, the emblematic example of a cheap bacterial strategy to fulfill a successful interaction with the animal host is the existence of moonlighting proteins (MPs).

Moonlighting Proteins

Moonlighting proteins are proteins that perform different functions in different cellular compartments (Jeffery, 1999). Some exceptions concern MP having a double task in the same cellular compartment such as surface-linked proteases which also behave as adhesins (Kainulainen and Korhonen, 2014). They are ubiquitous in all living kingdoms and their existence has finally overcome the concept one gene-one function, thus providing a clear explanation for the low number of genes present in most living organisms' genomes (Henderson and Martin, 2014). In bacteria, MPs represent a very interesting example of economy that exploits the same macromolecule to achieve housekeeping functions inside the cell and to interact with the host when secreted (**Table 1**). Most of them are central metabolism enzymes (especially glycolytic or TCA cycle enzymes) elongation factors (EF-Tu and Ef-G) and stress-induced proteins (chaperones such as GroEL and DNAk) that after secretion can interact with the animal host favoring bacterial adhesion to mucosal surfaces (when surface-bound) (Jeffery, 2018) and regulating the host immune system (when released) (Henderson et al., 2008). Although it has been recognized that these proteins use different domains to fulfill their intracellular and moonlighting roles, in some cases the two functions are performed by the same molecular region as described for the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) whose adhesive function is inhibited by the co-factor NAD^+ that hinders the catalytic site (Kinoshita et al., 2008).

Moonlighting proteins lack any classical export signal sequence related to the most common Sec or TAT secretion machineries and any surface attachment domain (Jeffery, 2018). Although the secretion mechanism for most MPs is not known, some possible non-canonical export ways have been described. Transient post-translational modifications (PTM) have been hypothesized to occur, acting as a signal for partitioning some proteins (among the pool of a certain metabolic enzyme) outside the cell. Enolase, a glycolytic enzyme, can be spontaneously modified by its substrate (2-phosphoglicerate) at the level of $lysine_{341}$, thus becoming suitable for extracellular release. Catalytically active mutants of enolase bearing glutamine, glutamate, arginine, or alanine in the $residue_{341}$, cannot be extruded (Boël et al., 2004). A second hypothesis suggests that secondary and tertiary structures could be important for recognition and secretion: in *Bacillus subtilis*, a hydrophobic alpha-helical domain within enolase that contributes to its secretion has been identified (Yang et al., 2011). Since secreted and intracellular MPs share the same physical structure, the

existence of chaperones inducing transient rare conformations that are competent for secretion has been hypothesized but not yet proven (Amblee and Jeffery, 2015). Finally, bacterial membrane vesicles (MVs), better described in the next section, can be exploited to release MP (Henderson and Martin, 2014) as previously demonstrated for similar structures present in human cells (Lancaster and Febbraio, 2005).

Regarding surface binding, classical anchoring motifs are lacking in MPs. Some authors ascribe the capability to be retained on the cell surface to specific conformational states of each protein. It has been demonstrated that GroEL1 is prevalently released into the external environment (Cehovin et al., 2010) whereas GroEL2 is a capsule associated protein (Hickey et al., 2010) in Mycobacteria. While the latter favors adhesion to macrophages, the former is the best fitting in inducing cytokine release by monocytes (Lewthwaite et al., 2001). According to others, environmental factors such as pH, also play a role in the cell-anchoring or release of a specific proteins (Antikainen et al., 2007). Because of their different biological tasks, cell-anchored and released MPs both cooperate to achieve a coordinated interaction with the host. Curiously, the MPs released by a bacterial species can re-associate on the surface of a different one, suggesting that the communication occurs among more than two partners (Oliveira et al., 2012).

As far as adhesion is concerned, there is evidence that bacteria displaying surface-bound MPs can auto-aggregate (a pre-requisite for mucosal adhesion) (Waśko et al., 2014) and also co-aggregate with other bacterial species (a feature that potentially quenches pathogens) (Bergonzelli et al., 2006). Adhesion targets in the host are mucus components (Kinoshita et al., 2008), gut epithelial cells (Granato et al., 2004) or extracellular matrix proteins (collagen, laminin, and fibronectin) (Castaldo et al., 2009; Genovese et al., 2013). A peculiar type of interaction has been described between two MPs, the mammalian MP Hsp60 and the bacterial (*Listeria monocytogenes*) MP alcohol-acetaldheyde dehydrogenase, both acting inside the cell as cell stress proteins (Wampler et al., 2004). Actually, in multicellular organisms, membrane exposed MPs act as receptors for bacteria (Jin et al., 2003) and viruses (Reyes-del Valle et al., 2005).

Adhesion ability represents an advantage when microbes live in symbiosis with higher organisms, favoring tissues colonization. It is interesting to underline that, since pathogens and commensal bacteria share most of the moonlighting adhesins, beneficial bacteria can act as competitors for gut epithelium adhesion thus supporting surface exclusion of the pathogens (Servin, 2004). In LAB that are paradigmatic examples of probiotic organisms, MPs localize on lipoteichoic acids and in cell division sites with ionic interactions (Kainulainen and Korhonen, 2014). Their extracellular location (surface-attached or released into the environment) depends upon pH: under acidic conditions (pH lower than their isoelectric point, i.e., 5) some positively charged MPs (like enolase and GAPDH) can interact with the negatively charged lipoteichoic acids, whereas at higher pH the molecules, negatively charged, are released from the cell surface (Antikainen et al., 2007). These considerations, however, are not true for some pathogenic bacteria that retain the MPs cell-anchored even in neutral and alkaline conditions (Jagadeesan et al., 2010). Hence, since cell-wall anchored MPs act as adhesins, competitive displacement of pathogens on host mucosal surfaces by beneficial microbes like LAB can only occur in acidic conditions, underlining the huge importance of the environment in balancing commensal–pathogens–host interactions. In this respect extracellular and envelope proteomic data suggested that a selenium supply in the growth medium can enhance the abundance of some adhesive MPs (alpha-enolase, EF-Tu) in *L. reuteri* Lb2BMDSM16143 (Mangiapane et al., 2014).

Released MPs prevalently act as immune modulating factors. Immune modulation is especially elicited by moonlighting bacterial chaperones. i) GroEL/Hsp60 acts activating human monocytes (Maguire et al., 2002) and stimulates the secretion of IL-8 in human macrophages (Bergonzelli et al., 2006). ii) Bacterial intracellular survival into macrophages is provided by Hsp20 (Schnappinger et al., 2003). iii) A cell-stress protein peptidyl-prolyl isomerase (PPI) also promotes survival inside macrophages (Henderson and Martin, 2011). iv) DNAk stimulates dendritic cells maturation (Floto et al., 2006). Although the last three examples refer to pathogenic bacteria, nevertheless released MPs seem to play an important role in controlling prokaryote–host interaction at multiple levels.

Additional activities of secreted bacterial MPs include i) induction of apoptosis in gastric epithelial cells by PPI (Basak et al., 2005) and ii) the role of GroEL/Hsp60 from *Enterobacter aerogenes* as toxin available for the insect host to paralyze its prey (Yoshida et al., 2001). Finally, according to Henderson et al. (2008) we can conclude that among MPs, stress chaperones, having evolved specifically to interact with other proteins, may have cell signaling functions similar to cytokines, sometimes creating a network of interaction with host-released MPs.

The number of MPs identified by mass spectrometry (MS) and reported to be displayed on the bacterial cell surface is increasing every day (Wang and Jeffery, 2016). Interestingly, bacterial MPs besides interfacing with the mammalian host can also favor symbiotic relationships between bacteria and yeasts (Katakura et al., 2010) suggesting that they double role has evolved to promote whatever inter-kingdom interactions.

PTMs and Cross-Talk

A further fascinating aspect of the bacteria–host cross-talk is the role of PTMs on proteins. At the end of the last century, once achieved exhaustive information about the majority of plant, animal and microbial genomes an unexpected low number of genes was found. If in Eukarya the presence of introns can partially account for this finding, in prokaryotes it cannot. An important step to elucidate this point is the huge presence of PTMs on proteins. Although for long time bacteria were considered unable to post-translationally modify proteins, it is now evident that the paradigm one gene-one protein is wrong even for prokaryotes (Cain et al., 2014).

The concept of protein species (Jungblut et al., 2008), firstly introduced to avoid confusion with the term "isozyme" (that mean the product of a different gene encoding a protein with analogous function, e.g., catalyzing the same reaction with different efficacy and sometimes different direction), refers to different PT modified proteins and is now well accepted. There

is an increasing evidence that PTMs have a huge biological significance because they determine peculiar protein properties like half-life, interaction with other proteins, cellular localization (Kobir et al., 2011) as well as diversified protein functions such as redox signaling, enzyme activity, chemotaxis, and metabolic flux tuning (Dykstra et al., 2013). Actually, each protein species (for instance a protein bearing glycosylation) can solve a specific physiological function not performed by the same gene product that has been processed differently (for instance the same protein when phosphorylated). As an example, methylations or acetylations of cellulosome scaffold proteins have critical functional significance, by impacting differently cellulose utilization efficiency (Dykstra et al., 2013). In this way, only one gene can support several tasks thus guarantee economy of genes and of energy required for transcription and translation (**Figure 1**).

An interesting concern of PTMs is their regulatory/interactive role. Cellular sensing, adjusting and responding to changes in the external environment, is often mediated by reversible PTMs and post-translational regulatory networks in which one PTM can compete with or, on the contrary, facilitate further PTMs by inducing conformational changes on the targeted protein (Soufi et al., 2012) (for comprehensive information on the evolution and functional significance of PTMs see the review by Beltrao et al., 2013). In the specific case of bacteria, the majority of PTMs are transient and occur to ensure rapid adaptation to changing conditions (Grangeasse et al., 2015). Furthermore, in the case of commensal/pathogenic bacteria PTMs on key extracellular proteins can support interaction with the host: serine-glycosylated proteins are important in bacterial adhesion and colonization of host mucosal tissues (Zhou and Wu, 2009), acetylated proteins play a role in virulence (Ren et al., 2017) and multiple PTMs are used by bacteria to alter their surface antigens thus escaping host immune response (Cain et al., 2014).

However, the most intriguing feature is the ability of bacteria to post-translationally modify host proteins (Ribet and Cossart, 2010). This can be achieved by either direct PTM of surface exposed host proteins or by interfering with the host PTM machinery by means of secreted molecules able to penetrate inside the host cell. The preferential targets of these bacterial PTMs are host regulatory proteins like Rho GTPases, since with a single modification on them, bacteria can activate or deactivate the control of a complex network of signals inside the host cell. For instance, RhoGTPases can be either activated by deamidation and/or polyamination or rather inactivated by glycosylation and/or AMPylation thus controlling crucial events such as innate and adaptive immune responses (Ribet and Cossart, 2010). Bacteria can also impact host gene transcription, as occurs by histone-targeted PTMs (Hamon and Cossart, 2008). It is evident that discriminating between "simple" PTMs and epigenetic effects when considering histones is artificial, so the conclusion that bacteria can deeply influence, by epigenetic effects, host gene expression is not so nonsense. Considering that other bacterial modifications on non-proteinaceous molecules have been reported (Pizarro-Cerdá and Cossart, 2004), also nucleic acid modification cannot be totally excluded. As an example, commensal gut bacteria can methylate the DNA of the TLR4 gene thus regulating intestinal inflammation and contributing

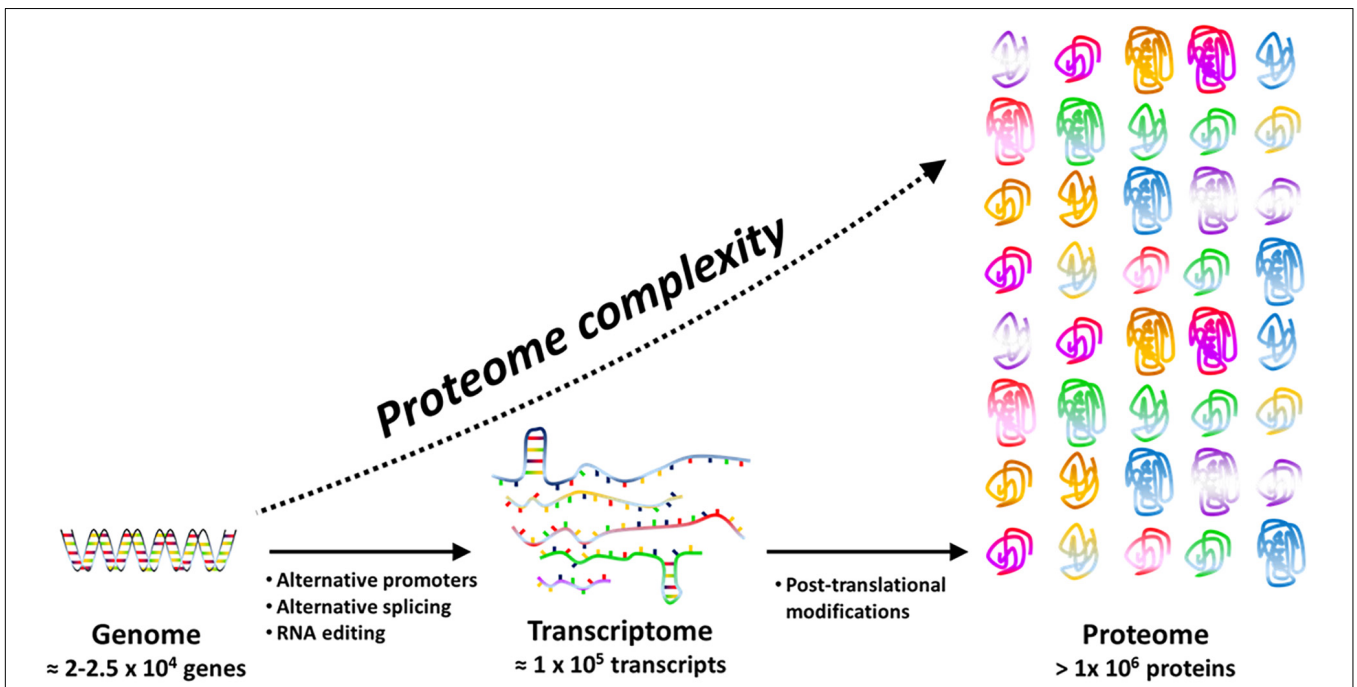


FIGURE 1 | Proteome complexity is the result of transcriptional (e.g. alternative promoters), post-transcriptional (e.g. RNA editing and splicing) and post-translational events. Post-translational events include a large panel of reversible or irreversible chemical modifications that may affect structure, function and cellular location of proteins.

to the maintenance of intestinal symbiosis (Takahashi et al., 2011) and several pathogens can induce histone acetylation and DNA methylation in the host during infection (Schmeck et al., 2005; Bierne et al., 2012). This opens a new area of investigation on the complex interaction network between bacteria and their hosts. Further information in this field, especially concerning the epigenetic modulation of host gene expression through PTM of histones exerted by commensal/symbiont bacteria, will provide added value to the knowledge of the mechanisms involved in bacteria–host reciprocal control and in supporting the theory of a superorganism constituting the holobiome selection unit (Rosenberg and Zilber-Rosenberg, 2016).

THE INNER HOST: BACTERIA-PHAGE CONTRACT, A HIDDEN OPPORTUNITY

Bacteria–phage interaction can result in the well known lytic (phage replication and cell lysis) and lysogenic (phage integration into bacterial host DNA) cycles. Occasionally, in particular conditions also pseudolysogeny (phage enters and remains in the cytoplasm without integrating into the bacterial DNA and without replicating) (Cenens et al., 2013) and the so-called chronic cycle (phages particles are continuously released without causing cell lysis) (Mirzaei and Maurice, 2017), can occur. The former evolves toward lytic or lysogenic cycles whereas the latter can cause a lower growth rate in the host bacterial cell because part of the cell energy is directed to phage functions (Munson-McGee et al., 2018).

The phage lytic cycle involving bacterial killing is frequently observed in open environments (soils and waters) where the number of viral particles is about 50 fold higher than the number of living cells (Srinivasiah et al., 2008). Besides promoting antagonistic co-evolution (De Sordi et al., 2019a,b) the lytic cycle is responsible of nutrient cycling among bacterial populations, mineralization of organic bacterial matter and ultimately enrichment of soils (Allen and Abedon, 2013). However, lytic cycle is not the rule in well-established ecosystems such as the microbial biofilm (Jayaraman, 2008) and the human gut (Mirzaei and Maurice, 2017) where lysogenic events mediated by temperate bacteriophages are prevalent because of several mechanisms of resistance set up by bacteria against virulent viruses (Knowles et al., 2017). Independently from the final outcome (lytic, chronic, pseudolysogenic, or lysogenic cycle) and from the stress that phage attack causes to the bacterial cell, phage infection seems to have ensured during evolution some advantages to the bacterial populations.

Possible Advantages Related to Phage Infection

Bacterial proteins that are involved in nutrient uptake (such as porins present on the outer membrane of Gram-negative bacteria), are often used by phages as receptors. In *Escherichia coli*, as an example, the receptor for the tails of the phages T5, TI, and phi-80 (the ton A/ton B proteins) is used for ferric iron (ferrichrome) uptake (Braun et al., 1976) and in *Salmonella* the T5-like phage receptor is the uptake protein for vitamin B_{12} (Kim and Ryu, 2011). In *E. coli*, it has long been established that the lambda-phage receptor (LamB protein) is a trimeric maltoporin which facilitates maltose uptake (Szmelcman and Hofnung, 1975), although the specific binding sites for phage and for the disaccharide are located on different amino acid residues on this protein (Charbit et al., 1988) (**Figure 2**). It seems therefore that the risk of phage entry is the price to be paid by the bacterial cell to gain food in nutrient-poor environments. Nevertheless, a question arises: why bacteria (whose ability to resist to phage attack with an arsenal of resistance mechanisms is well-known, see for example Mirzaei and Maurice, 2017) continue to harbor a protein that facilitates phage infection, without modifying it to render it suitable only for nutrient uptake? Evidently, the benefits of obtaining essential nutrients overwhelm the negative effects of phage attack, otherwise this system would be lost or modified. It is tempting to speculate that phage attack to the receptor can induce conformational changes on the protein so as to render it most effective in nutrient capturing. However, at present there are not experimental data supporting this hypothesis. Alternatively, these proteins could have been conserved since phage attack, because of the numerous benefits described in the next sections, cannot be seen only/always as a negative event.

A peculiar phenomenon whose origin is still controversial is the presence of viral-like structures inside prokaryotes (**Figure 3**). These protein-based particles, similar to phage capsid (but lacking viral nucleic acid), represent a sort of a primitive compartimentalization strategy allowing the formation of micro-compartments or organelles in bacteria. These protein shells confer evolutionary advantage to the bacterial host because they isolate from the rest of the cytosol peculiar metabolic pathways generating toxic compounds such as aldehydes. It is well known that 3-hydroxypropionaldehyde (produced during the conversion of glycerol to 1,3-propanediol) is toxic for several Gram-negative bacteria such as *Klebsiella*, *Enterobacter*, and *Citrobacter* (Barbirato et al., 1998) and it is even used for food preservation being active also toward *Listeria monocytogenes* and *E. coli* O157:H7 (Vollenweider and Lacroix, 2004). In *L. reuteri* the 3-hydroxypropionaldehyde is named reuterin and, when suitably converted into acrolein, is used as bacteriocin-like weapon conferring ecological advantage to this bacterial species toward surrounding bacterial populations (Engels et al., 2016). In *Salmonella* the enzymes for the metabolization of 1,2 propanediol, generating toxic propionaldehyde are encapsulated in microcompartments (Sampson and Bobik, 2008) that in their aspect and architectural organization (polyhedral protein shells) are very similar to phage particles, especially phage-head. The possible viral origin of these particles is very likely, however, at present, only in Archea the structure (named encapsulin) reveals a common ancestry with the capsid protein of a virus (Sutter et al., 2008). On the other hand, the Gene Transfer Agents are viral-like particles from defective phages that harbor in the capsid bacterial DNA fragments that can be transferred to suitable recipient bacteria sustaining recombination (Lang et al., 2012). These particles do not contain phage nucleic acids, but the bacterial host harbors a prophage in its genome.

Finally, a possible viral remnant present in a certain number of Gram-negative bacteria is the type VI secretion system

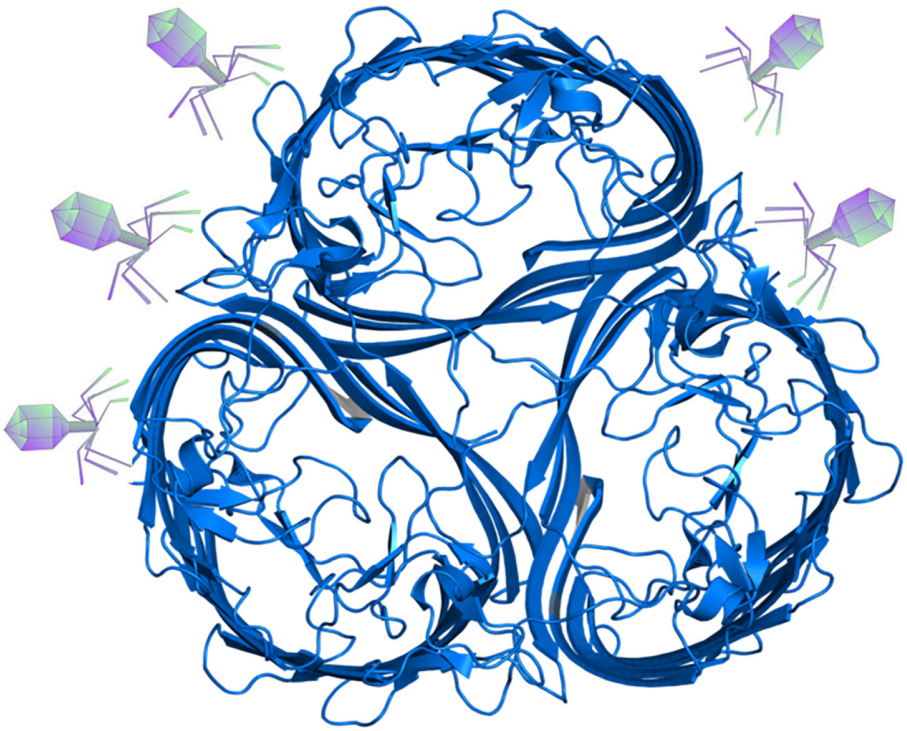


FIGURE 2 | The maltoporin facilitating maltose uptake is a phage receptor.

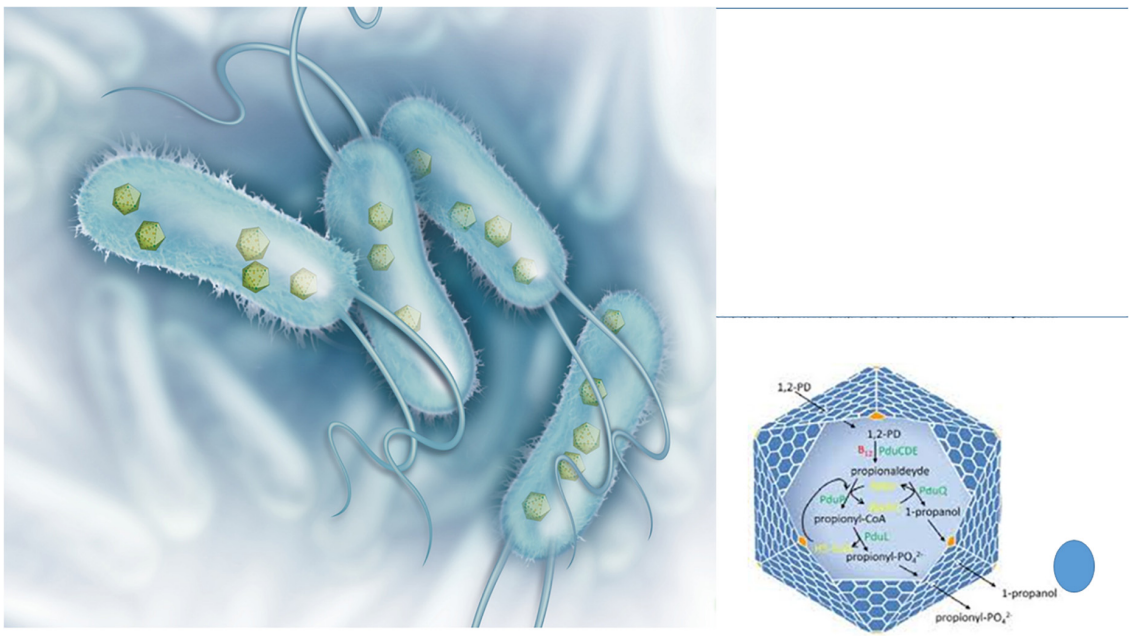


FIGURE 3 | Bacterial structures showing similarities with phages: protein shell microcompartments and the phage head.Microcompartments are used for isolating metabolic pathways generating toxic compounds such as 3-hydroxypropionaldehyde (*on the left*: illustration originally printed in The Scientist by Thom Graves; *on the right*: from Lundin et al., 2020).

(T6SS). This is a phage-like structure very similar to phage T4 contractile nanomachine (Basler et al., 2012). Actually, T6SS can be illustrated as an inverted phage tail on the surface of the bacterial cell, used for secreting proteins in the extracellular environment or for injecting them directly into target (bacterial or host) cells (Records, 2011) (**Figure 4**). Structural homologies

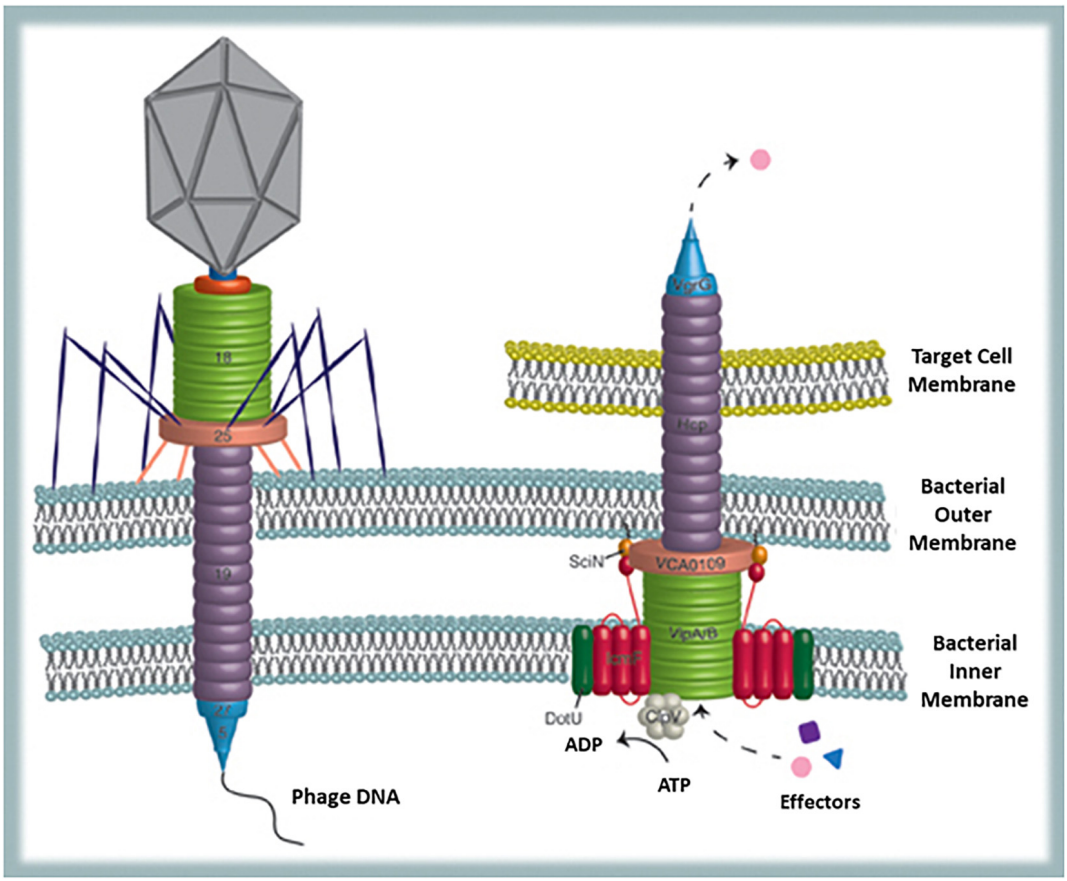


FIGURE 4 | Bacterial structures showing similarities with phages: T6SS and the phage tail.T6SS is used to translocate completely folded proteins across inner and outer membrane of the secreting bacterium and across other bacteria's or eukaryotic host membranes (from Records, 2011).

are common in biology and also the type II secretion system proved to be similar to filamentous phages (Leiman et al., 2009). Nevertheless, besides structural homologies, in the T6SS sequence similarities to phage T4 tail tube are also present (Leiman et al., 2009). Since diversification of functions from one original structural model can occur during evolution, establishing whether the phage tail preceeds the secretion system or *vice-versa* is hard matter. However, according to Records (2011) it is likely that some Gram-negative bacteria have utilized for their own benefit proteins encoded by phage genes integrated into their genomes. This secretion system offers to bacteria numerous advantages. Actually, among the seven known ways that bacteria evolved to export proteins (type I–VII secretion systems) T6SS is one of the most interesting: it provides a very efficient mean (several proteins can be delivered simultaneously into the target cell) to translocate completely folded proteins across inner and outer membrane of the secreting bacterium and also across other bacteria's or eukaryotic host membranes (Basler and Mekalanos, 2012). Sometimes protein transfer is finalized to adjacent bacterial cell killing and acquisition of DNA (that can be used as nitrogen/phosphorus source but also for gene recombination) from the prey (Ringel et al., 2017). This strategy, although initially costly in ATP consumption, supports competition between bacteria as well as competence and HGT (Basler, 2015). Furthermore, proteins secreted by T6SS are exchanged among cells and reused for T6SS assembly thus providing recycling (sustainable and shared use of resources) and promoting cooperation between strains to kill competitors (successful interaction) (Vettiger and Basler, 2016). Furthermore, regarding bacteria–host interaction, it has been shown that mutant strains lacking a T6SS orthologous gene trigger stronger inflammatory responses than usual, suggesting that T6SS may function as modulator of acute inflammation thus promoting long-term interaction between bacteria and host (Chow and Mazmanian, 2010). Finally, since genes for T6SS are also conserved in free-living bacteria, it reasonable to assume that besides being involved in interbacteria and host interaction, T6SS also improves the environmental fitness of the bacteria possessing it.

Lysogeny and Its Numerous Benefits

Lysogeny cannot be considered just as a simple harboring of "selfish DNA" since it offers some basic advantages to the infected bacterium in whatever habitat, such as the immunity toward further viral infections (Richter et al., 2012) and new functions related to stress resistance (Bondy-Denomy and Davidson, 2014).

In specific conditions, other advantages (best fitness) are related to the ecological niche. In open aquatic environments, although the lytic cycle seems to be the general pattern (affecting periodical fluctuations in the bacterial population biodiversity) phage islands controlling genes involved in photosynthesis and light detection have been found in Cyanobacteria genome (Lindell et al., 2007). Ultimately, this results in a better photosynthetic fitness of the bacterial host.

However, it is in complex ecosystems (biofilms, animal-, and vegetal-associated microbiota) that lysogeny reveals its huge potential in driving evolution, suggesting multilevel effects on different partner cells. In the best-studied ecological niche, the human gut, most phages bear integrase genes allowing the prophage lifestyle (Minot et al., 2011). On the other hand, the transition lysogenic-lytic is sometimes dependent also upon QS autoinducers produced by the bacterial host (Silpe and Bassler, 2019). Temperate phages act as vectors for HGT (resulting from encapsidation of bacterial genes by a prophage when the environmental conditions favor a lytic cycles) frequently occurring when bacterial cells are in close proximity. The gene recombination that occurs in a certain ecosystem, favors the exchange of characters crucial for enhancing the metabolic and colonization capabilities of bacteria, meanwhile supporting population diversity and ultimately survival (Harrison and Brockhurst, 2017). However, a recent evaluation, based on a bioinformatics approach, excludes a real role of phages in the propagation of genes involved in antibiotic resistance: actually, the phage genome rarely encodes antibiotic resistance genes and transduction events, being based on erroneous encapsidation of non-viral DNA, seldom target these genes (Enault et al., 2017).

In parallel to the advantages of HGT, phage themselves act as a reservoir of genes important for the host fitness and especially for their social life and interkingdom relationships. Actually, integrated prophages, whose existence depends upon bacterial host survival, can alter virulence gene expression and generally account for a huge number of opportunities offered to their bacterial host (Wandro, 2019). The toxin-antitoxin modules (TA) present in the prokaryote genome have been described as originated from defective viral sources (Villarreal, 2012). They can encode several items. Among these, bacteriocins and immunity factors toward the self-produced bacteriocin (Tikhonenko et al., 1975) can offer advantage to a specific bacterial subpopulation in the fight against other bacteria. The bacteriocin producers therefore, soon become "permanent holder" of the viral genetic element conferring this benefit. Some TA genes such as mazEF induce individual cell death in *E. coli* during early phases of phage P1 infection (before new viral particles are produced and released) to prevent destruction of the overall bacterial population (Hazan and Engelberg-Kulka, 2004). When we consider a biofilm, a well-structured and "intelligent" microbial community, this behavior of programmed and altruistic cell death is related to the so-called sacrificial cells: the presence of this phage-derived module ensures that these cells set up an autolytic strategy to supply nutrients to the rest of the population. A second type of behavior frequent in a biofilm is persistence or dormancy: the bacterial cells are slow growing or in steady-state to prevent virus entry (Thingstad et al., 2015) or to counteract nutrient starvation. Even in this case the TA modules play a central role (Lewis, 2012). Therefore, it seems evident that from one side biofilms ensure perfect ecological niche for prophage maintenance and evolution and from the other side phages seem to have contributed to bacterial evolution toward a community lifestyle.

An interesting and peculiar mechanism recently discovered, i.e., explosive cell lysis (Turnbull et al., 2016) underlines the role played by prophages in the formation and release of bacterial MVs, previously described both in Gram-negative (Schwechheimer and Kuehn, 2015) and in Gram-positive (Lee et al., 2009) bacteria. These MVs (50–800 nm, average size 250 nm) can contain different cargo-molecules such as viral genomes (Gaudin et al., 2014), cytoplasmic MPs (Henderson and Martin, 2014), DNA, RNA (Zavan et al., 2020), and are involved in important functions, e.g., bacterial communication through hydrophobic QS signals, HGT, sharing of nutrients (especially evident in the biofilm) (Toyofuku et al., 2017b, 2020), also protecting the cells from some antibiotics and phages by acting as decoys (Manning and Kuehn, 2011).

In *Pseudomonas aeruginosa* a bacteriophage-associated endolysin, encoded into the pyocin gene cluster and coding for a peptidoglycan hydrolase, triggers explosive cell lysis resulting in MVs biogenesis by curling and self-annealing of shattered membrane fragments. Although the pyocin structural genes are not involved, peptidoglycan hydrolase seems not to be the only enzyme responsible for lysis since, at least in *E. coli*, also holins (facilitating translocation of cell wall hydrolases across the inner membrane) participate to the phenomenon. The inductors of the lytic cycle and vesicle formation can be multiple: light, mutagens, antibiotics and other exogenous stressors, however, endogenous events cannot be excluded (Turnbull et al., 2016).

The described model is not the only one acting on the basis of a phage-mediated stimulus. Toyofuku et al. (2017b) reported that prophages can also induce MVs formation on Gram-positive bacteria, although with a different strategy not implying explosive cell lysis. In *B. subtilis* a holin-endolysin mediated mechanism occurs. First, holin causes the formation of pores in the cytoplasmic membrane thus facilitating endolysin hydrolysis of peptidoglycan. Once achieved the disruption of peptidoglycan, the cytoplasmic membrane is extruded and the vesicles released, while the bacterial cell dies because membrane integrity is lost. Phage-related proteins have been demonstrated within the vesicles by proteomic studies in both *B. subtilis* (Kim et al., 2016) and *Streptococcus pneumoniae* (Resch et al., 2016).

When considering both mechanisms, it is interesting to highlight that only a part of the overall population dies: in *P. aeruginosa* not all cells harbor the pyocin-prophage insert and in the Gram-positive model the damage caused to the cytoplasmic membrane cannot always be lethal (Toyofuku et al., 2017a). In general, it can be stated that the generation of vesicles is beneficial not exactly for the individual cell but for the microbial community (e.g., the biofilm) in which nutrient exchange and HGT are favored. Moreover, MVs being similar to intact cells and bearing phage receptors can act as decoys attracting antibiotics and phages, thus ensuring an escape lane to living cells (Manning and Kuehn, 2011). Furthermore, also phages can beneficiate

of some advantages: since MVs harbor phage receptors, when they fuse with membranes of phage resistant bacterial cells these not-target bacteria become sensitive to phage attack thus allowing the phage population extending the number of hosts and increasing their diffusion among bacterial species. Therefore, in a circular way, also bacterial communities can beneficiate of further HGT by phage transduction. This ultimately results in enhanced fitness of microbial populations as suggested by Nanda et al. (2015). Finally, it is tempting to speculate that MVs can be one of the possible structures giving origin to the lipid envelope present in some phage families. Curiously, the dsRNA phage phi6 (belonging to the *Cystoviridae* family of rare enveloped phages) displaying a lytic behavior on the target Gram-negative *P. aeruginosa* acquires the envelope from the bacterial cytoplasmic membrane inside the host cytoplasm (Laurinavičius et al., 2004). Experiments, recently reported by Lyytinen et al. (2019), highlight that the phage-encoded protein P9 can induce in *E. coli* the formation of intracellular MVs where the protein (possessing a transmembrane domain) is partially incorporated. The presence of internal organelles in prokaryotes are a very rare event observed in Cyanobacteria (thylakoids) and magnetotactic bacteria only. Here again, although the advantages for the host of such a system are still to be fully elucidated, the interaction between bacteria and viruses can give rise to novel opportunities of evolution.

BACTERIA–PHAGE–HOST: A RUSSIAN-DOLL MODEL FOR AN INTER-KINGDOM COORDINATED PROJECT

The complex multilevel communication occurring among bacteria, phages and their animal host have driven evolutionary strategies that are worth to be examined. A huge number of inter-kingdom interactions exists in the complex ecosystem of the human gut where many actors are playing on the stage, including viruses, bacteria, archaea, protozoa, and fungi. However, a significant degree of complexity can be observed even when limiting our attention to the central role of bacteria that cross-talk with their inner (phage) and outer (human) host(s), in a Russian doll model represented in **Figure 5**. First there are some analogies in the behavior that phages and bacteria entertain with their respective host. Similar features characterize phages and bacteria in their ability to set up a form of dormancy (pseudolysogeny in phages and persister cells in bacteria) that allows them waiting for better conditions to activate their reproductive cycle (Łoś and Węgrzyn, 2012). A second analogy is that phage infection is dependent upon the metabolic state of the bacterial-host (De Sordi et al., 2019b) as well as bacterial colonization is dependent upon host metabolism, nutrient availability and circadian rhythms (Rosselot et al., 2016). For instance, planktonic actively duplicating bacterial cells allow to phages high infection efficiency, whereas biofilm-embedded cells are protected from phage (Vidakovic et al., 2018) and viral particle assembly is slow-down during stationary phase (Bryan et al., 2016). In addition, the environment in which bacteria live favors or hinders phage establishment (van Belleghem et al., 2019) like the environment in which the human host lives (hormones, diet, drugs, pollutants, etc. . .) can favor or exclude certain bacterial populations. Although the mucus layer can harbor a reservoir of bacteria that is maintained thanks to the mucin-derived glycans, regardless of food intake, the lumen microbiota is deeply affected by diet (Donaldson et al., 2016). Actually, the crucial role of diet in selecting saccharolytic or proteolytic bacterial populations has long been established (Gibson and Roberfroid, 1995). Similarly, certain host-environmental conditions can induce production of capsules that mask phage receptors on the bacterial surface of certain species compelling phages to find another target (Roach et al., 2013). Even in this case, however, the arms race between bacteria and viruses favors the appearance of mutant phages that possess enzymes hydrolyzing capsules and ensuring phage access to the receptor (Samson et al., 2013).

On the other hand, it is worth underlining that this multilevel relationship deeply affects the fitness of the three partners finally resulting in a dynamic fluctuation generally driven toward homeostasis (Chaturongakul and Ounjai, 2014), although some possible drifts can occur. It has been experimentally detected that healthy humans display a higher bacterial biodiversity, whereas the phage diversity is increased in inflammatory bowel disease (Norman et al., 2014). Nevertheless, filtered fecal transplantation, although lacking bacterial cells, provides benefits in overcoming *Clostridium difficile* colitis, suggesting that bacteriophages play a very important role in the human gut homeostasis (Ott et al., 2017).

Partnership Agreement Between Bacteria and the Human Host

The partnership agreement between bacteria and human host to control several aspects of reciprocal well-being is a well-established concern that also includes bacteriophage control. Actually, humans and other animals have evolved a sophisticated immune system not only toward pathogenic bacteria but against viruses as well. Since host immune system does not discriminate between human-targeting viruses and phages, it is likely that immune defenses can control phage populations thus protecting commensal bacteria. At this purpose, it has been demonstrated that viral nucleic acids are recognized by specific toll-like receptors (TLR) namely DNA by TLR9, dsRNA by TLR3 and ssRNA by TLR7 and TLR8, that after sensing the nucleic acid, trigger production of IFNs and other pro-inflammatory cytokines (De Paepe et al., 2014). More specifically, phages have been demonstrated to interact with the animal immune system, by means of head proteins, inducing immunological responses, even if sometimes this interaction can result in a negative modulation of both humoral and cell-mediated immunity probably inducing tolerance toward phages and their bacterial hosts (Dąbrowska et al., 2006).

Partnership Agreement Between Bacteria and Phages

In a second model, bacteria and phages can set up a partnership agreement that ensures phage survival and better bacterial

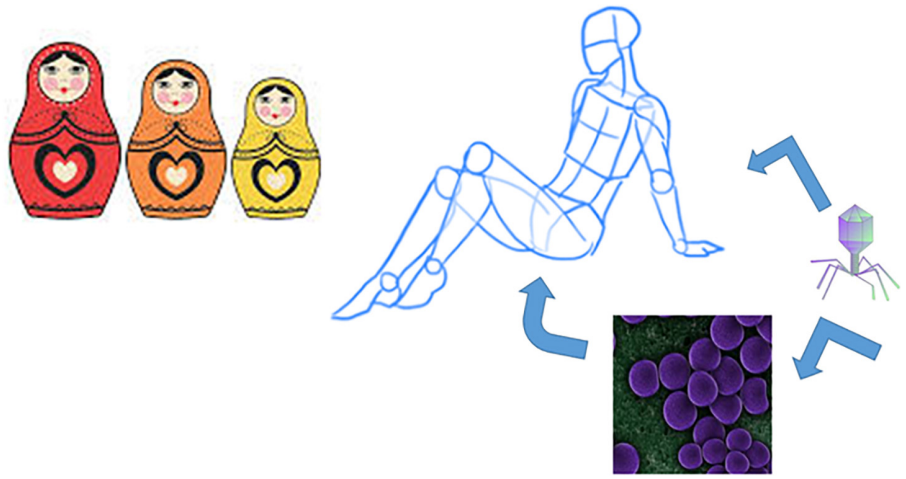


FIGURE 5 | Russian doll model for the multilevel relationships phage–bacteria–human host.

fitness toward the mammalian host. The well-known lysogenic conversion support bacteria in producing toxic molecules (such as cholera, diphtheria, and Shiga toxins) that protect them from the host immune reaction and this also ensures temperate phage stabilization into the bacterial genome (Fortier and Sekulovic, 2013). The severe epidemic outbreak of Shiga-toxin producing *E. coli* O104:H4 occurred in 2011 in Germany was linked to a toxin-encoding phage (Muniesa et al., 2012). An additional example is *P. aeruginosa* PAO1 bearing a prophage, which although less fitting in swimming, swarming and twitching motility, enhances production of the virulence factor pyocianin and increases resistance to macrophages internalization (Hosseinidoust et al., 2013). In the same bacterial model, the phage-like type VI secretion system allows bacterial translocation of virulence factors (Basler et al., 2012). However, virulence factors like toxins can sometimes have also a beneficial action on the animal host such as carcinogenesis suppression as reported by Doulberis et al. (2015).

Besides helping to produce virulence factors, prophages can also modify surface antigenicity of bacteria thus favoring their host in evading the mammalian immune system (De Paepe et al., 2014). In Gram-negative bacteria, modifications in the O-antigen domain of the outer membrane have been described (Davies et al., 2013). A curious example of surface modification involving a three-partner interaction, has been reported by Mitchell et al. (2007): in this case prophage induction occurs and the production of holins provides enhanced cell permeability and secretion of some parts of the phage "body," namely, tail fiber proteins that are exposed on the bacterial (*Streptococcus mitis*) cell surface of not fully lysed cells where they act as adhesins for platelet cells. Furthermore, there is growing evidence that harboring a prophage, besides enhancing aggressiveness toward the host, is a winning strategy for bacteria to improve their resilience to harsh conditions such as presence of bile salts and oxidants, not so rare in the human gut (Wang et al., 2010).

In all the reported examples, phage-bacterial host relationship can be viewed as mutualistic as suggested by Beckett and Williams (2013). Actually, phages provide competitiveness to their bacterial-host in a way that probably proved useless in an abiotic ecological niche (where lysogeny is less observed, see above). On its side, the bacterium continues to cultivate this successful interaction by harboring the virus as a prophage to defend itself from the mammalian host. Therefore, the human/animal host represents a crucial factor for maintaining phage lysogeny. On the other hand, this has been demonstrated also by the abundance of integrase genes detected by metagenomics in the genome of phages living in the gut community (Minot et al., 2011).

Partnership Agreement Between Phages and the Human Host

A third scenario is possible in which the partnership agreement can be signed also between phages and human host. Phages are the most represented entities in the biosphere. It has been established that the phage population in the human gut (where the number of phages reaches 10^9 per gram versus 10^{11} bacteria) (Kim et al., 2011) is higher than that of human-targeting viruses (De Paepe et al., 2014). However, it is difficult to establish which families of viruses are present because of the lack of molecular genomic markers similar to the bacterial 16S ribosomal RNA genes, but also because extracting the genetic content from viral particles is still challenging (Roux et al., 2013). According to Manrique et al. (2017) the most represented families are the dsDNA Myoviridae, Siphoviridae and Podoviride and also the ss DNA Microviridae that represent the healthy gut phageome. In some cases, phages, due to their killing activity (lytic cycle) on bacteria can behave as antimicrobial agents controlling bacterial populations and preventing bacterial infection: this has been related to the high number of bacteriophages present at the gut level on mucosal surfaces and interacting with the mucus layer by means of an immunoglobulin-like capsid domain (Barr et al., 2013). According to these authors, host mucosal surfaces and phage coevolve to maintain phage adherence. From one side,

this benefits the host since phage adherence to mucus provides a non–host-derived antimicrobial defense, limiting the number of mucosal bacteria and contributing to the maintenance of a selected commensal microbiota. Actually, based on the model kill-the-winner (De Paepe et al., 2014), phage can especially control excessive growth of a specific bacterial population, as occurs during a sudden invasion by an exogenous pathogen, but also when dysbiosis favors a particular endogenous lineage. On the other side, it offers to the phage the opportunity to encounter bacteria thus potentially increasing their replicative success (Barr et al., 2013). Alongside this model that represents a true metazoan-phage symbiosis, indirect effects positively affecting host fitness are reported: phage head and tail proteins can stimulate the immune system sometimes inducing antibodies and pro-inflammatory cytokines (Łusiak-Szelachowska et al., 2014) sometimes reducing inflammation caused by bacterial LPS (Miernikiewicz et al., 2016). A recent and very interesting article suggests that dietary fructose and bacterial-derived SCFA can trigger phage transition from lysogenic to lytic (Oh et al., 2019). This is not the only example in which human host-derived molecules such as component of the western diet, food additives ad nicotine can affect phage populations by controlling their bacterial hosts (Mirzaei and Maurice, 2017). Furthermore, sub-inhibitory doses of antibiotics can trigger prophage excision from bacterial DNA and lytic cycle establishment, thus enhancing bacterial killing, both in *E. coli* (Zhang et al., 2000) and in pathogenic Gram-positive models resulting in phage expansion and bacterial infection control (Matos et al., 2013). However, it has been reported that most stressors (oxidants, smoke, and antibiotics) mainly induce prophage lytic cycle on beneficial microbes rather than on opportunistic pathogens thus triggering gut dysbiosis (Mills et al., 2013). Therefore, it seems reasonable to assume that the partnership agreement to be beneficial should occur in specific physiological conditions.

Three-Partner Agreement

Finally, it is of interest considering a three-partner agreement: the one mediated by the relatively recently discovered MVs. As discussed in a previous section, MVs exist because of an integrated prophage into a bacterial genome. Besides being important for phage propagation and bacteria–bacteria interaction, MVs can also play a role in the mammalian host physiology, in pathological states and in the reciprocal interactions occurring between host and bacteria. Actually, it has been demonstrated that MVs release can be induced by animal host signals such as changes in temperature, pH, presence of antimicrobial peptides (Deatherage and Cookson, 2012).

As far as the control of host physiology is concerned, MVs can stimulate protective immunity controlling both innate and adaptive immune responses (Deatherage and Cookson, 2012) and also regulate inflammatory pathways (Alaniz et al., 2007). Moreover, MVs from *Listeria monocytogenes* can inhibit autophagy (Vdovikova et al., 2017) and it has been reported that MVs from *Akkermansia muciniphyla* (but not those from *E. coli*) can regulate tight junctions thus controlling intestinal barrier integrity and gut permeability with consequences on the overall inflammatory state of the host organism (Chelakkot et al., 2018).

Modulation of pathological states of the host, such as cancer, by MVs has also been described. Vdovikova et al. (2018) recently demonstrated that MVs from pathogenic (but not from commensal) bacteria such as *Vibrio cholerae*, can impact gene expression on colon carcinoma cells, by enhancing the transcription of genes involved in cell differentiation. This effect, probably involving epigenetic changes and chromatin accessibility, do not require direct contact between bacteria and cancer cells (Vdovikova et al., 2018). If other cellular structures are involved in the interaction with the bacterial MVs remains to be elucidated. Furthermore, MVs from *V. cholerae* display the ability to induce higher expression of the nuclear receptor for the Vitamin D that promotes differentiation of colon carcinoma cells (Pálmer et al., 2001).

It is evident from all these examples that the phenotypic effect, finally resulting after MVs signaling to the host, strongly depends upon the cargo transported by the MV (toxin, immunomodulating molecule, and non-human-targeted compound). The contact between MV and the host cell can occur either by endocytosis or by membrane fusion (O'Donoghue and Krachler, 2016) however, in both cases this strategy potently enhances the efficiency by which the active compound (transported and protected) is sensed by the target cell.

Membrane vesicles can also shape the pathogen–host interaction, sometimes favoring sometimes attenuating pathogenesis. Two examples are worth mentioning. In the *Salmonella enterica* serovar Thypy model, the Cly A cytotoxin is eight fold more active in causing host cell lysis when embedded in MVs than in the free form. The fusion of MVs with the cell membrane of the eukaryotic host can facilitate the toxic action (Wai et al., 2003). On the contrary, in the *Helicobacter pylori* model the MVs-associated toxin VacA is less active than the soluble form, suggesting that the use of MVs is a strategy used by bacteria to modulate the virulence impact during infection (Ricci et al., 2005). This ambiguity still remains considering the fact that MVs from one side can act as decoys attracting the antibody response, thus allowing the producing bacterium to escape the host immune system, from the other side, they can represent a first signal that allows early detection of the bacterial population by the host immunity that can thus organize and react accordingly (Deatherage and Cookson, 2012). All these data underline that the complex interplay between phages, bacteria and the host is far to be fully understood.

CONCLUSION

From the analysis of the intricate relationships established between bacteria and their inner and outer hosts, two essential aspects emerge: i) bacteria carry out strategies finalized to communication but also to earn energy and make the cheapest choice, ii) bacteria integrate the "enemy" in view of a broader advantage on the long period and in a larger context. Both behaviors are sustainable. From one hand, they take into account the possibility to use simple building blocks to perform multiple duties (i.e., multitasking signaling compounds and MPs), sometimes using module differentiation (PTMs). From

the other hand, the logic of sharing and reuse, together with the conflict mediation has brought during evolution to a shift from a negative event (phage attack) into big opportunities of survival in systemic communities, which ultimately promote a more complex level of organization such as social life and the establishment of a successful integrated lifestyle.

The overall reported data of the last section highlight that a holistic viewpoint can better gave us a framework of what really happens in the phage–bacteria–animal host multilevel community. Phages are the software of bacteria like symbiont bacteria are the software of humans, in a Russian doll-like model. Bacteria are not the same without their prophage(s) and humans are not the same without their tailor-made microbiota. Both can be removed from their respective hosts, however, the hosts pay a cost in reducing its fitness (less resistant/performant bacteria and dysbiosis). Despite their bad reputation due to the fact that both phage and bacteria can kill their host, phages can provide bacteria with new genes, supplying them new opportunities and commensal bacteria can offer to the human hosts new metabolic and signaling pathways not supported by host genome. An agreement often occurs to ensure that the war can be changed into a reciprocally profitable event. Every level sustains intricate and complex relationships with the other levels (and other gut populating organisms as well) and, although more than expected interactions also occur between phages and human host, bacteria are in the center. In this sometimes intricate network of possibilities they prove to be efficient, intelligent in decision-making, and able to respond to both sides. A good lesson also for humans.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

This work has been supported by Ricerca locale (ex 60%) of Turin University. I am grateful to Anna Luganini for helpful discussion and to Roberto Mazzoli for figures improvement.

REFERENCES

Alaniz, R. C., Deatherage, B. L., Lara, J. C., and Cookson, B. T. (2007). Membrane vesicles are immunogenic facsimiles of *Salmonella typhimurium* that potently activate dendritic cells, prime B and T cell responses, and stimulate protective immunity *in vivo*. *J. Immunol.* 179, 7692–7701. doi: 10.4049/jimmunol.179.11.7692

Allen, H. K., and Abedon, S. T. (2013). That's disturbing! An exploration of the bacteriophage biology of change. *Front. Microbiol.* 4:295. doi: 10.3389/fmicb.2013.00295

Amblee, V., and Jeffery, C. J. (2015). Physical features of intracellular proteins that moonlight on the cell surface. *PLoS One* 10:e0130575. doi: 10.1371/journal.pone.0130575

Andersson, A. F., and Banfield, J. F. (2008). Virus population dynamics and acquired virus resistance in natural microbial communities. *Science* 320, 1047–1050. doi: 10.1126/science.1157358

Antikainen, J., Kupannen, V., Lähteenmäki, K., and Korhonen, T. K. (2007). pH-dependent association of enolase and glyceraldehyde-3-phosphate dehydrogenase of Lactobacillus crispatus with the cell wall and lipoteichoic acids. *J. Bacteriol.* 189, 4539–4543. doi: 10.1128/jb.00378-07

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15718–15723. doi: 10.1073/pnas.0407076101

Bansal, T., Alaniz, R. C., Wood, T. K., and Jayaraman, A. (2010). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 107, 228–233. doi: 10.1073/pnas.0906112107

Barbirato, F., Soucaille, P., Camarasa, C., and Bories, A. (1998). Uncoupled glycerol distribution as the origin of the accumulation of 3-hydroxypropionaldehyde during the fermentation of glycerol by *Enterobacter* agglomerans CNCM 1210. *Biotechnol. Bioeng.* 58, 303–305. doi: 10.1002/(sici)1097-0290(19980420)58:2/3<303::aid-bit28>3.0.co;2-b

Barr, J. J., Auro, R., Furlan, M., Whiteson, K. L., Erb, M. L., Pogliano, J., et al. (2013). Bacteriophage adhering to mucus provide a non–host-derived immunity. *Proc. Natl. Acad. Sci. U.S.A.* 110, 10771–10776. doi: 10.1073/pnas.1305923110

Basak, C., Pathak, S. K., Bhattacharyya, A., Pathak, S., Basu, J., and Kundu, M. (2005). The secreted peptidyl prolyl cis, trans-isomerase HP0175 of *Helicobacter pylori* induces apoptosis of gastric epithelial cells in a TLR4-and apoptosis signal-regulating kinase 1-dependent manner. *J. Immunol.* 174, 5672–5680. doi: 10.4049/jimmunol.174.9.5672

Basler, Á., and Mekalanos, J. J. (2012). Type 6 secretion dynamics within and between bacterial cells. *Science* 337, 815–815. doi: 10.1126/science.1222901

Basler, Á., Pilhofer, Á., Henderson, G. P., Jensen, G. J., and Mekalanos, J. J. (2012). Type VI secretion requires a dynamic contractile phage tail-like structure. *Nature* 483, 182–186. doi: 10.1038/nature10846

Basler, M. (2015). Type VI secretion system: secretion by a contractile nanomachine. *Philos. Trans. R. Soc. B Biol. Sci.* 370:20150021. doi: 10.1098/rstb.2015.0021

Beckett, S. J., and Williams, H. T. (2013). Coevolutionary diversification creates nested-modular structure in phage–bacteria interaction networks. *Interface Focus* 3:20130033. doi: 10.1098/rsfs.2013.0033

Beganović, J., Frece, J., Kos, B., Pavunc, A. L., Habjaniè, K., and Šušković, J. (2011). Functionality of the S-layer protein from the probiotic strain *Lactobacillus helveticus* M92. *Antonie Van Leeuwenhoek* 100, 43–53. doi: 10.1007/s10482-011-9563-4

Beltrao, P., Bork, P., Krogan, N. J., and van Noort, V. (2013). Evolution and functional cross-talk of protein post-translational modifications. *Mol. Syst. Biol.* 9:714. doi: 10.1002/msb.201304521

Berg, G., Rybakova, D., Grube, M., and Köberl, M. (2016). The plant microbiome explored: implications for experimental botany. *J. Exp. Bot.* 67, 995–1002. doi: 10.1093/jxb/erv466

Bergonzelli, G. E., Granato, D., Pridmore, R. D., Marvin-Guy, L. F., Donnicola, D., and Corthésy-Theulaz, I. E. (2006). GroEL of *Lactobacillus johnsonii* La1 (NCC 533) is cell surface associated: potential role in interactions with the host and the gastric pathogen *Helicobacter pylori*. *Infect. Immun.* 74, 425–434. doi: 10.1128/iai.74.1.425-434.2006

Bierne, H., Hamon, M., and Cossart, P. (2012). Epigenetics and bacterial infections. *Cold Spring Harb. Perspect. Med.* 2:a010272. doi: 10.1101/cshperspect.a010272

Boël, G., Pichereau, V., Mijakovic, I., Mazé, A., Poncet, S., Gillet, S., et al. (2004). Is 2-phosphoglycerate-dependent automodification of bacterial enolases implicated in their export? *J. Mol. Biol.* 337, 485–496. doi: 10.1016/j.jmb.2003.12.082

Bondy-Denomy, J., and Davidson, A. R. (2014). When a virus is not a parasite: the beneficial effects of prophages on bacterial fitness. *J. Microbiol.* 52, 235–242. doi: 10.1007/s12275-014-4083-3

Braun, V., Hancock, R. E., Hantke, K., and Hartmann, A. (1976). Fuctional organization of the outer membrane of *Escherichia coli*: phage and colicin receptors as components of iron uptake systems. *J. Supramol. Struct.* 5, 37–58. doi: 10.1002/jss.400050105

Bryan, D., El-Shibiny, A., Hobbs, Z., Porter, J., and Kutter, E. M. (2016). Bacteriophage T4 infection of stationary phase *E. coli*: life after log from a phage perspective. *Front. Microbiol.* 7:1391. doi: 10.3389/fmicb.2016.01391

Burger-van Paassen, N., Vincent, A., Puiman, P. J., Van Der Sluis, M., Bouma, J., Boehm, G., et al. (2009). The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem. J.* 420, 211–219. doi: 10.1042/bj20082222

Cain, J. A., Solis, N., and Cordwell, S. J. (2014). Beyond gene expression: the impact of protein post-translational modifications in bacteria. *J. Proteomics* 97, 265–286. doi: 10.1016/j.jprot.2013.08.012

Castaldo, C., Vastano, V., Siciliano, R. A., Candela, M., Vici, M., Muscariello, L., et al. (2009). Surface displaced alfa-enolase of *Lactobacillus plantarum* is a fibronectin binding protein. *Microb. Cell Fact.* 8:14. doi: 10.1186/1475-2859-8-14

Cehovin, A., Coates, A. R., Hu, Y., Riffo-Vasquez, Y., Tormay, P., Botanch, C., et al. (2010). Comparison of the moonlighting actions of the two highly homologous chaperonin 60 proteins of Mycobacterium tuberculosis. *Infect. Immun.* 78, 3196–3206. doi: 10.1128/iai.01379-09

Cenens, W., Makumi, A., Mebrhatu, M. T., Lavigne, R., and Aertsen, A. (2013). Phage–host interactions during pseudolysogeny: lessons from the Pid/dgo interaction. *Bacteriophage* 3:e1003269.

Charbit, A., Gehring, K., Nikaido, H., Ferenci, T., and Hofnung, M. (1988). Maltose transport and starch binding in phage-resistant point mutants of maltoporin: functional and topological implications. *J. Mol. Biol.* 201, 487–493. doi: 10.1016/0022-2836(88)90630-4

Chaturongakul, S., and Ounjai, P. (2014). Phage–host interplay: examples from tailed phages and Gram-negative bacterial pathogens. *Front. Microbiol.* 5:442. doi: 10.3389/fmicb.2014.00442

Chelakkot, C., Choi, Y., Kim, D. K., Park, H. T., Ghim, J., Kwon, Y., et al. (2018). Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Exp. Mol. Med.* 50:e450. doi: 10.1038/emm.2017.282

Chow, J., and Mazmanian, S. K. (2010). A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe* 7, 265–276. doi: 10.1016/j.chom.2010.03.004

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., and Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10420–10425. doi: 10.1073/pnas.0604343103

Cogan, T. A., Thomas, A. O., Rees, L. E., Taylor, A. H., Jepson, M. A., Williams, P. H., et al. (2007). Norepinephrine increases the pathogenic potential of *Campylobacter jejuni*. *Gut* 56, 1060–1065. doi: 10.1136/gut.2006.114926

Cryan, J. F., and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712. doi: 10.1038/nrn3346

Dąbrowska, K., świtała-Jeleń, K., Opolski, A., and Górski, A. (2006). Possible association between phages, Hoc protein, and the immune system. *Arch. Virol.* 151, 209–215. doi: 10.1007/s00705-005-0641-7

Davie, J. R. (2003). Inhibition of histone deacetylase activity by butyrate. *J. Nutr.* 133, 2485S–2493S.

Davies, M. R., Broadbent, S. E., Harris, S. R., Thomson, N. R., and van der Woude, M. W. (2013). Horizontally acquired glycosyltransferase operons drive *Salmonellae* lipopolysaccharide diversity. *PLoS Genet.* 9:e1003568. doi: 10.1371/journal.pgen.1003568

De Paepe, M., Leclerc, M., Tinsley, C. R., and Petit, M. A. (2014). Bacteriophages: an underestimated role in human and animal health? *Front. Cell. Infect. Microbiol.* 4:39. doi: 10.3389/fcimb.2014.00039

De Sordi, L., Lourenço, M., and Debarbieux, L. (2019a). "I will survive": a tale of bacteriophage-bacteria coevolution in the gut. *Gut Microbes* 10, 92–99. doi: 10.1080/19490976.2018.1474322

De Sordi, L., Lourenço, M., and Debarbieux, L. (2019b). The battle within: interactions of bacteriophages and bacteria in the gastrointestinal tract. *Cell Host Microbe* 25, 210–218. doi: 10.1016/j.chom.2019.01.018

De Vrieze, J. (2013). The promise of poop. *Science* 341, 954–957. doi: 10.1126/science.341.6149.954

Deatherage, B. L., and Cookson, B. T. (2012). Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. *Infect. Immun.* 80, 1948–1957. doi: 10.1128/iai.06014-11

Deepak, V., Ramachandran, S., Balahmar, R. M., Pandian, S. R. K., Sivasubramaniam, S. D., Nellaiah, H., et al. (2016). In vitro evaluation of anticancer properties of exopolysaccharides from Lactobacillus acidophilus in colon cancer cell lines. *In Vitro Cell. Dev. Biol. Anim.* 52, 163–173. doi: 10.1007/s11626-015-9970-3

Denou, E., Pridmore, R. D., Berger, B., Panoff, J. M., Arigoni, F., and Brüssow, H. (2008). Identification of genes associated with the long-gut-persistence phenotype of the probiotic *Lactobacillus johnsonii* strain NCC533 using a combination of genomics and transcriptome analysis. *J. Bacteriol.* 190, 3161–3168. doi: 10.1128/jb.01637-07

Deveau, H., Garneau, J. E., and Moineau, S. (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annu. Rev. Microbiol.* 64, 475–493. doi: 10.1146/annurev.micro.112408.134123

Dobson, A., Cotter, P. D., Ross, R. P., and Hill, C. (2012). Bacteriocin production: a probiotic trait? *Appl. Environ. Microbiol.* 78, 1–6. doi: 10.1128/aem.05576-11

Donaldson, G. P., Lee, S. M., and Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 14, 20–32. doi: 10.1038/nrmicro3552

Doulberis, M., Angelopoulou, K., Kaldrymidou, E., Tsingotjidou, A., Abas, Z., Erdman, S. E., et al. (2015). Cholera-toxin suppresses carcinogenesis in a mouse model of inflammation-driven sporadic colon cancer. *Carcinogenesis* 36, 280–290. doi: 10.1093/carcin/bgu325

Dyall, S. D., Brown, M. T., and Johnson, P. J. (2004). Ancient invasions: from endosymbionts to organelles. *Science* 304, 253–257. doi: 10.1126/science.1094884

Dykstra, A. B., Rodriguez, M. Jr., Raman, B., Cook, K. D., and Hettich, R. L. (2013). Characterizing the range of extracellular protein post-translational modifications in a cellulose-degrading bacteria using a multiple proteolyic digestion/peptide fragmentation approach. *Anal. Chem.* 85, 3144–3151. doi: 10.1021/ac3032838

Ellers, J., Toby Kiers, E., Currie, C. R., McDonald, B. R., and Visser, B. (2012). Ecological interactions drive evolutionary loss of traits. *Ecol. Lett.* 15, 1071–1082. doi: 10.1111/j.1461-0248.2012.01830.x

Enault, F., Briet, A., Bouteille, L., Roux, S., Sullivan, M. B., and Petit, M. A. (2017). Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J.* 11, 237–247. doi: 10.1038/ismej.2016.90

Engels, C., Schwab, C., Zhang, J., Stevens, M. J., Bieri, C., Ebert, M. O., et al. (2016). Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. *Sci. Rep.* 6:36246.

Floto, R. A., MacAry, P. A., Boname, J. M., Mien, T. S., Kampmann, B., Hair, J. R., et al. (2006). Dendritic cell stimulation by mycobacterial Hsp70 is mediated through CCR5. *Science* 314, 454–458. doi: 10.1126/science.1133515

Fortier, L. C., and Sekulovic, O. (2013). Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence* 4, 354–365. doi: 10.4161/viru.24498

Gaudin, M., Krupovic, M., Marguet, E., Gauliard, E., Cvirkaite-Krupovic, V., Le Cam, E., et al. (2014). Extracellular membrane vesicles harbouring viral genomes. *Environ. Microbiol.* 16, 1167–1175. doi: 10.1111/1462-2920.12235

Genovese, F., Coïsson, J. D., Majumder, A., Pessione, A., Svensson, B., Jacobsen, S., et al. (2013). An exoproteome approach to monitor safety of a cheese-isolated *Lactococcus lactis*. *Food Res. Int.* 54, 1072–1079. doi: 10.1016/j.foodres.2012.12.017

Gibson, G. R., and Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125, 1401–1412. doi: 10.1093/jn/125.6.1401

Glucksam-Galnoy, Y., Sananes, R., Silberstein, N., Krief, P., Kravchenko, V. V., Meijler, M. M., et al. (2013). The bacterial quorum-sensing signal molecule N-3-oxo-dodecanoyl-L-homoserine lactone reciprocally modulates pro-and anti-inflammatory cytokines in activated macrophages. *J. Immunol.* 191, 337–344. doi: 10.4049/jimmunol.1300368

Goh, E. B., Yim, G., Tsui, W., McClure, J., Surette, M. G., and Davies, J. (2002). Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. *Proc. Natl. Acad. Sci. U.S.A.* 99, 17025–17030. doi: 10.1073/pnas.252607699

Granato, D., Bergonzelli, G. E., Pridmore, R. D., Marvin, L., Rouvet, M., and Corthésy-Theulaz, I. E. (2004). Cell surface-associated elongation factor Tu mediates the attachment of *Lactobacillus johnsonii* NCC533 (La1) to human intestinal cells and mucins. *Infect. Immun.* 72, 2160–2169. doi: 10.1128/iai.72.4.2160-2169.2004

Grangeasse, C., Stülke, J., and Mijakovic, I. (2015). Regulatory potential of post-translational modifications in bacteria. *Front. Microbiol.* 6:500.

Hamon, M. A., and Cossart, P. (2008). Histone modifications and chromatin remodeling during bacterial infections. *Cell Host Microbe* 4, 100–109. doi: 10.1016/j.chom.2008.07.009

Hänsch, G. M., Prior, B., Brenner-Weiss, G., Obst, U., and Overhage, J. (2014). The *Pseudomonas* quinolone signal (PQS) stimulates chemotaxis of polymorphonuclear neutrophils. *J. Appl. Biomater. Funct. Mater.* 12, 21–26. doi: 10.5301/jabfm.5000204

Harrison, E., and Brockhurst, M. A. (2017). Ecological and evolutionary benefits of temperate phage: what does or doesn't kill you makes you stronger. *Bioessays* 39:1700112. doi: 10.1002/bies.201700112

Hazan, R., and Engelberg-Kulka, H. (2004). *Escherichia coli* mazEF-mediated cell death as a defense mechanism that inhibits the spread of phage P1. *Mol. Genet. Genomics* 272, 227–234. doi: 10.1007/s00438-004-1048-y

Henderson, B., Derek, J. G., and Chadwick, J. (2008). "Cell stress proteins as modulators of bacteria-host interactions," in *Novartis Foundation Symposium*, Vol. 291, eds D. Chadwick, and J. A. Goode (Chichester: John Wiley), 141. doi: 10.1002/9780470754030.ch11

Henderson, B., and Martin, A. (2011). Bacterial virulence in the moonlight: multitasking bacterial moonlighting proteins are virulence determinants in infectious disease. *Infect. Immun.* 79, 3476–3491. doi: 10.1128/iai.00179-11

Henderson, B., and Martin, A. C. (2014). Protein moonlighting: a new factor in biology and medicine. *Biochem. Soc. Trans.* 42, 1671–1678. doi: 10.1042/bst20140273

Hevia, A., Delgado, S., Sánchez, B., and Margolles, A. (2015). Molecular players involved in the interaction between beneficial bacteria and the immune system. *Front. Microbiol.* 6:1285.

Hickey, T. B., Ziltener, H. J., Speert, D. P., and Stokes, R. W. (2010). Mycobacterium tuberculosis employs Cpn60. 2 as an adhesin that binds CD43 on the macrophage surface. *Cell. Microbiol.* 12, 1634–1647. doi: 10.1111/j.1462-5822.2010.01496.x

Hidalgo-Cantabrana, C., Sánchez, B., Milani, C., Ventura, M., Margolles, A., and Ruas-Madiedo, P. (2014). Genomic overview, and biological functions of exopolysaccharide biosynthesis in *Bifidobacterium* spp. *Appl. Environ. Microbiol.* 80, 9–18. doi: 10.1128/AEM.02977-13

Hoagland, M. B., Dodson, B., and Hauch, J. (2001). *Exploring the Way Life Works: The Science of Biology*. Burlington, MA: Jones and Bartlett Learning.

Hosseinidoust, Z., Tufenkji, N., and Van De Ven, T. G. (2013). Predation in homogeneous and heterogeneous phage environments affects virulence determinants of *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* 79, 2862–2871. doi: 10.1128/aem.03817-12

Jagadeesan, B., Koo, O. K., Kim, K. P., Burkholder, K. M., Mishra, K. K., Aroonnual, A., et al. (2010). LAP, an alcohol acetaldehyde dehydrogenase enzyme in Listeria, promotes bacterial adhesion to enterocyte-like Caco-2 cells only in pathogenic species. *Microbiology* 156, 2782–2795. doi: 10.1099/mic.0.036509-0

Jayaraman, R. (2008). Bacterial persistence: some new insights into an old phenomenon. *J. Biosci.* 33, 795–805. doi: 10.1007/s12038-008-0099-3

Jeffery, C. (2018). Intracellular proteins moonlighting as bacterial adhesion factors. *AIMS Microbiol.* 4, 362–376. doi: 10.3934/microbiol.2018.2.362

Jeffery, C. J. (1999). Moonlighting proteins. *Trends Biochem. Sci.* 24, 8–11.

Jin, S., Song, Y. C., Emili, A., Sherman, P. M., and Chan, V. L. (2003). JlpA of *Campylobacter jejuni* interacts with surface exposed heat shock protein 90α and triggers signalling pathways leading to the activation of NF-κB and p38 MAP kinase in epithelial cells. *Cell. Microbiol.* 5, 165–174. doi: 10.1046/j.1462-5822.2003.00265.x

Jungblut, P. R., Holzhütter, H. G., Apweiler, R., and Schlüter, H. (2008). The speciation of the proteome. *Chem. Cent. J.* 2, 1–10.

Kainulainen, V., and Korhonen, T. K. (2014). Dancing to another tune—adhesive moonlighting proteins in bacteria. *Biology* 3, 178–204. doi: 10.3390/biology3010178

Katakura, Y., Sano, R., Hashimoto, T., Ninomiya, K., and Shioya, S. (2010). Lactic acid bacteria display on the cell surface cytosolic proteins that recognize yeast mannan. *Appl. Microbiol. Biotechnol.* 86, 319–326. doi: 10.1007/s00253-009-2295-y

Kim, K., Kim, Y. U., Koh, B. H., Hwang, S. S., Kim, S. H., Lépine, F., et al. (2010). HHQ and PQS, two *Pseudomonas aeruginosa* quorum-sensing molecules, down-regulate the innate immune responses through the nuclear factor-κB pathway. *Immunology* 129, 578–588. doi: 10.1111/j.1365-2567.2009.03160.x

Kim, M., and Ryu, S. (2011). Characterization of a T5-like coliphage, SPC35, and differential development of resistance to SPC35 in *Salmonella enterica* serovar *Typhimurium* and *Escherichia coli*. *Appl. Environ. Microbiol.* 77, 2042–2050. doi: 10.1128/aem.02504-10

Kim, M. S., Park, E. J., Roh, S. W., and Bae, J. W. (2011). Diversity and abundance of single-stranded DNA viruses in human feces. *Appl. Environ. Microbiol.* 77, 8062–8070. doi: 10.1128/aem.06331-11

Kim, Y., Edwards, N., and Fenselau, C. (2016). Extracellular vesicle proteomes reflect developmental phases of *Bacillus subtilis*. *Clin. Proteomics* 13:6.

Kinoshita, H., Uchida, H., Kawai, Y., Kawasaki, T., Wakahara, N., Matsuo, H., et al. (2008). Cell surface *Lactobacillus plantarum* LA 318 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) adheres to human colonic mucin. *J. Appl. Microbiol.* 104, 1667–1674. doi: 10.1111/j.1365-2672.2007.03679.x

Kleerebezem, M. (2004). Quorum sensing control of lantibiotic production: nisin and subtilin autoregulate their own biosynthesis. *Peptides* 25, 1405–1414. doi: 10.1016/j.peptides.2003.10.021

Knowles, B., Bailey, B., Boling, L., Breitbart, M., Cobián-Güemes, A., Del Campo, J., et al. (2017). Variability and host density independence in inductions-based estimates of environmental lysogeny. *Nat. Microbiol.* 2:17064.

Kobir, A., Shi, L., Boskovic, A., Grangeasse, C., Franjevic, D., and Mijakovic, I. (2011). Protein phosphorylation in bacterial signal transduction. *Biochim. Biophys. Acta* 1810, 989–994.

Labrie, S. J., Samson, J. E., and Moineau, S. (2010). Bacteriophage resistance mechanisms. *Nat. Rev. Microbiol.* 8, 317–327. doi: 10.1038/nrmicro2315

Lancaster, G. I., and Febbraio, M. A. (2005). Exosome-dependent trafficking of HSP70 A novel secretory pathway for cellular stress proteins. *J. Biol. Chem.* 280, 23349–23355. doi: 10.1074/jbc.m502017200

Lang, A. S., Zhaxybayeva, O., and Beatty, J. T. (2012). Gene transfer agents: phage-like elements of genetic exchange. *Nat. Rev. Microbiol.* 10, 472–482. doi: 10.1038/nrmicro2802

Laurinavičius, S., Käkelä, R., Bamford, D. H., and Somerharju, P. (2004). The origin of phospholipids of the enveloped bacteriophage phi6. *Virology* 326, 182–190. doi: 10.1016/j.virol.2004.05.021

Lee, E. Y., Choi, D. Y., Kim, D. K., Kim, J. W., Park, J. O., Kim, S., et al. (2009). Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. *Proteomics* 9, 5425–5436. doi: 10.1002/pmic.200900338

Leiman, P. G., Basler, M., Ramagopal, U. A., Bonanno, J. B., Sauder, J. M., Pukatzki, S., et al. (2009). Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4154–4159. doi: 10.1073/pnas.0813360106

Lewis, K. (2012). "Persister cells: molecular mechanisms related to antibiotic tolerance," in *Antibiotic Resistance*, ed. A. R. M. Coates (Heidelberg: Springer), 121–133. doi: 10.1007/978-3-642-28951-4_8

Lewthwaite, J. C., Coates, A. R., Tormay, P., Singh, M., Mascagni, P., Poole, S., et al. (2001). Mycobacterium tuberculosis Chaperonin 60.1 Is a More Potent Cytokine Stimulator than Chaperonin 60.2 (Hsp 65) and Contains a CD14-Binding Domain. *Infect. Immun.* 69, 7349–7355. doi: 10.1128/iai.69.12.7349-7355.2001

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., and Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6, 776–788. doi: 10.1038/nrmicro1978

Lindell, D., Jaffe, J. D., Coleman, M. L., Futschik, M. E., Axmann, I. M., Rector, T., et al. (2007). Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature* 449, 83–86. doi: 10.1038/nature06130

Liu, Q., Yu, Z., Tian, F., Zhao, J., Zhang, H., Zhai, Q., et al. (2020). Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. *Microb. Cell Fact.* 19:23.

Łoś, M., and Węgrzyn, G. (2012). Pseudolysogeny. *Adv. Virus Res.* 82, 339–349. doi: 10.1016/b978-0-12-394621-8.00019-4

Lundin, A. P., Stewart, K. L., Stewart, A. M., Herring, T. I., Chowdhury, C., and Bobik, T. A. (2020). Genetic characterization of a glycyl radical microcompartment used for 1, 2-propanediol fermentation by uropathogenic *Escherichia coli* CFT073. *J. Bacteriol.* 202. doi: 10.1128/JB.00017-20

Łusiak-Szelachowska, M., Żaczek, M., Weber-Dąbrowska, B., Miêdzybrodzki, R., Kłak, M., Fortuna, W., et al. (2014). Phage neutralization by sera of patients receiving phage therapy. *Viral Immunol.* 27, 295–304. doi: 10.1089/vim.2013.0128

Lyytinen, O. L., Starkova, D., and Poranen, M. M. (2019). Microbial production of lipid-protein vesicles using enveloped bacteriophage phi6. *Microb. Cell Fact.* 18:29. doi: 10.1186/s12934-019-1079-z

Maguire, M., Coates, A. R., and Henderson, B. (2002). Chaperonin 60 unfolds its secrets of cellular communication. *Cell Stress Chaperones* 7, 317–329. doi: 10.1379/1466-1268(2002)007<0317:cuisoc>2.0.co;2

Mangiapane, E., Lamberti, C., Pessione, A., Galano, E., Amoresano, A., and Pessione, E. (2014). Selenium effects on the metabolism of a Se-metabolizing *Lactobacillus reuteri*: analysis of envelope-enriched and extracellular proteomes. *Mol. Biosyst.* 10, 1272–1280. doi: 10.1039/c3mb70557a

Manning, A. J., and Kuehn, M. J. (2011). Contribution of bacterial outer membrane vesicles to innate bacterial defense. *BMC Microbiol.* 11:258. doi: 10.1186/1471-2180-11-258

Manrique, P., Dills, M., and Young, M. J. (2017). The human gut phage community and its implications for health and disease. *Viruses* 9:141. doi: 10.3390/v9060141

Matos, R. C., Lapaque, N., Rigottier-Gois, L., Debarbieux, L., Meylheuc, T., Gonzalez-Zorn, B., et al. (2013). Enterococcus faecalis prophage dynamics and contributions to pathogenic traits. *PLoS Genet.* 9:e1003539. doi: 10.1371/journal.pgen.1003539

Mazzoli, R., and Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. *Front. Microbiol.* 7:1934. doi: 10.3389/fmicb.2016.01934

Mazzoli, R., Pessione, E., Dufour, M., Laroute, V., Giuffrida, M. G., Giunta, C., et al. (2010). Glutamate-induced metabolic changes in *Lactococcus lactis* NCDO 2118 during GABA production: combined transcriptomic and proteomic analysis. *Amino Acids* 39, 727–737. doi: 10.1007/s00726-010-0507-5

Meijerink, M., Van Hemert, S., Taverne, N., Wels, M., De Vos, P., Bron, P. A., et al. (2010). Identification of genetic loci in *Lactobacillus plantarum* that modulate the immune response of dendritic cells using comparative genome hybridization. *PLoS One* 5:e10632. doi: 10.1371/journal.pone.0010632

Mendes, R., and Raaijmakers, J. M. (2015). Cross-kingdom similarities in microbiome functions. *ISME J.* 9, 1905–1907. doi: 10.1038/ismej.2015.7

Miernikiewicz, P., Kłopot, A., Soluch, R., Szkuta, P., Kêska, W., Hodyra-Stefaniak, K., et al. (2016). T4 phage tail adhesin gp12 counteracts LPS-induced inflammation *in vivo*. *Front. Microbiol.* 7:1112. doi: 10.3389/fmicb.2016.01112

Mills, S., Shanahan, F., Stanton, C., Hill, C., Coffey, A., and Ross, R. P. (2013). Movers and shakers: influence of bacteriophages in shaping the mammalian gut microbiota. *Gut Microbes* 4, 4–16. doi: 10.4161/gmic.22371

Minot, S., Sinha, R., Chen, J., Li, H., Keilbaugh, S. A., Wu, G. D., et al. (2011). The human gut virome: inter-individual variation and dynamic response to diet. *Genome Res.* 21, 1616–1625. doi: 10.1101/gr.122705.111

Mirzaei, M. K., and Maurice, C. F. (2017). Ménage à trois in the human gut: interactions between host, bacteria and phages. *Nat. Rev. Microbiol.* 15, 397–408. doi: 10.1038/nrmicro.2017.30

Mitchell, J., Siboo, I. R., Takamatsu, D., Chambers, H. F., and Sullam, P. M. (2007). Mechanism of cell surface expression of the Streptococcus mitis platelet binding proteins PblA and PblB. *Mol. Microbiol.* 64, 844–857. doi: 10.1111/j.1365-2958.2007.05703.x

Muniesa, M., Hammerl, J. A., Hertwig, S., Appel, B., and Brüssow, H. (2012). Shiga toxin-producing *Escherichia coli* O104: H4: a new challenge for microbiology. *Appl. Environ. Microbiol.* 78, 4065–4073. doi: 10.1128/aem.00217-12

Munson-McGee, J. H., Snyder, J. C., and Young, M. J. (2018). Archaeal viruses from high-temperature environments. *Genes* 9:128. doi: 10.3390/genes9030128

Nanda, A. M., Thormann, K., and Frunzke, J. (2015). Impact of spontaneous prophage induction on the fitness of bacterial populations and host-microbe interactions. *J. Bacteriol.* 197, 410–419. doi: 10.1128/jb.02230-14

Norman, J. M., Handley, S. A., and Virgin, H. W. (2014). Kingdom-agnostic metagenomics and the importance of complete characterization of enteric microbial communities. *Gastroenterology* 146, 1459–1469. doi: 10.1053/j.gastro.2014.02.001

O'Brien, J., and Wright, G. D. (2011). An ecological perspective of microbial secondary metabolism. *Curr. Opin. Biotechnol.* 22, 552–558. doi: 10.1016/j.copbio.2011.03.010

O'Donoghue, E. J., and Krachler, A. M. (2016). Mechanisms of outer membrane vesicle entry into host cells. *Cell. Microbiol.* 18, 1508–1517. doi: 10.1111/cmi.12655

Oh, J. H., Alexander, L. M., Pan, M., Schueler, K. L., Keller, M. P., Attie, A. D., et al. (2019). Dietary fructose and microbiota-derived short-chain fatty acids promote bacteriophage production in the gut symbiont *Lactobacillus reuteri*. *Cell Host Microbe* 25, 273–284. doi: 10.1016/j.chom.2018.11.016

Oliveira, L., Madureira, P., Andrade, E. B., Bouaboud, A., Morello, E., Ferreira, P., et al. (2012). Group B streptococcus GAPDH is released upon cell lysis, associates with bacterial surface, and induces apoptosis in murine macrophages. *PLoS One* 7:e29963. doi: 10.1371/journal.pone.0029963

Ott, S. J., Waetzig, G. H., Rehman, A., Moltzau-Anderson, J., Bharti, R., Grasis, J. A., et al. (2017). Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. *Gastroenterology* 152, 799–811. doi: 10.1053/j.gastro.2016.11.010

Pálmer, H. G., González-Sancho, J. M., Espada, J., Berciano, M. T., Puig, I., Baulida, J., et al. (2001). Vitamin D3 promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of β-catenin signaling. *J. Cell Biol.* 154, 369–388. doi: 10.1083/jcb.200102028

Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Front. Cell. Infect. Microbiol.* 2:86. doi: 10.3389/fcimb.2012.00086

Pizarro-Cerdá, J., and Cossart, P. (2004). Subversion of phosphoinositide metabolism by intracellular bacterial pathogens. *Nat. Cell Biol.* 6, 1026–1033. doi: 10.1038/ncb1104-1026

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65.

Ramírez-Puebla, S. T., Servín-Garcidueñas, L. E., Jiménez-Marín, B., Bolaños, L. M., Rosenblueth, M., Martínez, J., et al. (2013). Gut and root microbiota commonalities. *Appl. Environ. Microbiol.* 79, 2–9. doi: 10.1128/aem.02553-12

Records, A. R. (2011). The type VI secretion system: a multipurpose delivery system with a phage-like machinery. *Mol. Plant Microbe Interact.* 24, 751–757. doi: 10.1094/mpmi-11-10-0262

Relman, D. A. (2008). 'Til death do us part': coming to terms with symbiotic relationships. *Nat. Rev. Microbiol.* 6, 721–724. doi: 10.1038/nrmicro1990

Ren, J., Sang, Y., Lu, J., and Yao, Y. F. (2017). Protein acetylation and its role in bacterial virulence. *Trends Microbiol.* 25, 768–779. doi: 10.1016/j.tim.2017.04.001

Resch, U., Tsatsaronis, J. A., Le Rhun, A., Stübiger, G., Rohde, M., Kasvandik, S., et al. (2016). A two-component regulatory system impacts extracellular membrane-derived vesicle production in group A Streptococcus. *mBio* 7:e00207-16.

Reyes-del Valle, J., Chávez-Salinas, S., Medina, F., and Del Angel, R. M. (2005). Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J. Virol.* 79, 4557–4567. doi: 10.1128/jvi.79.8.4557-4567.2005

Ribet, D., and Cossart, P. (2010). Pathogen-mediated posttranslational modifications: a re-emerging field. *Cell* 143, 694–702. doi: 10.1016/j.cell.2010.11.019

Ricci, V., Chiozzi, V., Necchi, V., Oldani, A., Romano, M., Solcia, E., et al. (2005). Free-soluble and outer membrane vesicle-associated VacA from *Helicobacter pylori*: two forms of release, a different activity. *Biochem. Biophys. Res. Commun.* 337, 173–178. doi: 10.1016/j.bbrc.2005.09.035

Richter, C., Chang, J. T., and Fineran, P. C. (2012). Function and regulation of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated (Cas) systems. *Viruses* 4, 2291–2311. doi: 10.3390/v4102291

Ringel, P. D., Hu, D., and Basler, M. (2017). The role of type VI secretion system effectors in target cell lysis and subsequent horizontal gene transfer. *Cell Rep.* 21, 3927–3940. doi: 10.1016/j.celrep.2017.12.020

Roach, D. R., Sjaarda, D. R., Castle, A. J., and Svircev, A. M. (2013). Host exopolysaccharide quantity and composition impacts bacteriophage pathogenesis of *Erwinia amylovora*. *Appl. Environ. Microbiol.* 79, 3249–3256. doi: 10.1128/aem.00067-13

Rosenberg, E., and Zilber-Rosenberg, I. (2016). Microbes drive evolution of animals and plants: the hologenome concept. *mBio* 7:e01395.

Rosselot, A. E., Hong, C. I., and Moore, S. R. (2016). Rhythm and bugs: circadian clocks, gut microbiota, and enteric infections. *Curr. Opin. Gastroenterol.* 32, 7–11. doi: 10.1097/mog.0000000000000227

Roux, S., Krupovic, M., Debroas, D., Forterre, P., and Enault, F. (2013). Assessment of viral community functional potential from viral metagenomes may be hampered by contamination with cellular sequences. *Open Biol.* 3:130160. doi: 10.1098/rsob.130160

Sampson, E. M., and Bobik, T. A. (2008). Microcompartments for B12-dependent 1, 2-propanediol degradation provide protection from DNA and cellular damage by a reactive metabolic intermediate. *J. Bacteriol.* 190, 2966–2971. doi: 10.1128/jb.01925-07

Samson, J. E., Magadán, A. H., Sabri, M., and Moineau, S. (2013). Revenge of the phages: defeating bacterial defences. *Nat. Rev. Microbiol.* 11, 675–687. doi: 10.1038/nrmicro3096

Sandrini, S., Alghofaili, F., Freestone, P., and Yesilkaya, H. (2014). Host stress hormone norepinephrine stimulates pneumococcal growth, biofilm formation and virulence gene expression. *BMC Microbiol.* 14:180. doi: 10.1186/1471-2180-14-180

Schmeck, B., Beermann, W., van Laak, V., Zahlten, J., Opitz, B., Witzenrath, M., et al. (2005). Intracellular bacteria differentially regulated endothelial cytokine release by MAPK-dependent histone modification. *J. Immunol.* 175, 2843–2850. doi: 10.4049/jimmunol.175.5.2843

Schnappinger, D., Ehrt, S., Voskuil, M. I., Liu, Y., Mangan, J. A., Monahan, I. M., et al. (2003). Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: insights into the phagosomal environment. *J. Exp. Med.* 198, 693–704. doi: 10.1084/jem.20030846

Schnorr, S. L. (2015). The diverse microbiome of the hunter-gatherer. *Nature* 518, S14–S15.

Schwechheimer, C., and Kuehn, M. J. (2015). Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. *Nat. Rev. Microbiol.* 13, 605–619. doi: 10.1038/nrmicro3525

Segain, J. P., De La Blétiere, D. R., Bourreille, A., Leray, V., Gervois, N., Rosales, C., et al. (2000). Butyrate inhibits inflammatory responses through NFκB inhibition: implications for Crohn's disease. *Gut* 47, 397–403. doi: 10.1136/gut.47.3.397

Serban, D. E. (2014). Gastrointestinal cancers: influence of gut microbiota, probiotics and prebi-otics. *Cancer Lett.* 345, 258–270. doi: 10.1016/j.canlet.2013.08.013

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol. Rev.* 28, 405–440. doi: 10.1016/j.femsre.2004.01.003

Silpe, J. E., and Bassler, B. L. (2019). A host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. *Cell* 176, 268–280. doi: 10.1016/j.cell.2018.10.059

Soufi, B., Soares, N. C., Ravikumar, V., and Macek, B. (2012). Proteomics reveals evidence of cross-talk between protein modifications in bacteria: focus on acetylation and phosphorylation. *Curr. Opin. Microbiol.* 15, 357–363. doi: 10.1016/j.mib.2012.05.003

Srinivasiah, S., Bhavsar, J., Thapar, K., Liles, M., Schoenfeld, T., and Wommack, K. E. (2008). Phages across the biosphere: contrasts of viruses in soil and aquatic environments. *Res. Microbiol.* 159, 349–357. doi: 10.1016/j.resmic.2008.04.010

Sungur, T., Aslim, B., Karaaslan, C., and Aktas, B. (2017). Impact of Exopolysaccharides (EPSs) of Lactobacillus gasseri strains isolated from human vagina on cervical tumor cells (HeLa). *Anaerobe* 47, 137–144. doi: 10.1016/j.anaerobe.2017.05.013

Sutter, M., Boehringer, D., Gutmann, S., Günther, S., Prangishvili, D., Loessner, M. J., et al. (2008). Structural basis of enzyme encapsulation into a bacterial nanocompartment. *Nat. Struct. Mol. Biol.* 15, 939–947. doi: 10.1038/nsmb.1473

Szmelcman, S., and Hofnung, M. (1975). Maltose transport in *Escherichia coli* K-12: involvement of the bacteriophage lambda receptor. *J. Bacteriol.* 124, 112–118. doi: 10.1128/jb.124.1.112-118.1975

Takahashi, K., Sugi, Y., Nakano, K., Tsuda, M., Kurihara, K., Hosono, A., et al. (2011). Epigenetic control of the host gene by commensal bacteria in large intestinal epithelial cells. *J. Biol. Chem.* 286, 35755–35762. doi: 10.1074/jbc.m111.271007

Tateda, K., Ishii, Y., Horikawa, M., Matsumoto, T., Miyairi, S., Pechere, J. C., et al. (2003). The *Pseudomonas aeruginosa* autoinducer N-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. *Infect. Immun.* 71, 5785–5793. doi: 10.1128/iai.71.10.5785-5793.2003

Thingstad, T. F., Pree, B., Giske, J., and Våge, S. (2015). What difference does it make if viruses are strain-, rather than species-specific? *Front. Microbiol.* 6:320. doi: 10.3389/fmicb.2015.00320

Tikhonenko, A. S., Belyaeva, N. N., and Ivanovics, G. (1975). Electron microscopy of phages liberated by megacin A producing lysogenic *Bacillus megaterium* strains. *Acta Microbiol. Acad. Sci. Hung.* 22, 58–59.

Toyofuku, M., Cárcamo-Oyarce, G., Yamamoto, T., Eisenstein, F., Hsiao, C. C., Kurosawa, M., et al. (2017a). Prophage-triggered membrane vesicle formation through peptidoglycan damage in *Bacillus subtilis*. *Nat. Commun.* 8:481.

Toyofuku, M., Morinaga, K., Hashimoto, Y., Uhl, J., Shimamura, H., Inaba, H., et al. (2017b). Membrane vesicle-mediated bacterial communication. *ISME J.* 11, 1504–1509. doi: 10.1038/ismej.2017.13

Toyofuku, M., Tashiro, Y., Nomura, N., and Eberl, L. (2020). "Functions of MVs in Inter-bacterial communication," in *Bacterial Membrane Vesicles*, eds M. Kaparakis-Liaskos, and T. Kufer (Cham: Springer), 101–117. doi: 10.1007/978-3-030-36331-4_5

Turnbull, L., Toyofuku, M., Hynen, A. L., Kurosawa, M., Pessi, G., Petty, N. K., et al. (2016). Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. *Nat. Commun.* 7:11220.

van Belleghem, J. D., Dąbrowska, K., Vaneechoutte, M., Barr, J. J., and Bollyky, P. L. (2019). Interactions between bacteriophage, bacteria, and the mammalian immune system. *Viruses* 11:10. doi: 10.3390/v11010010

Van Hemert, S., Meijerink, M., Molenaar, D., Bron, P. A., de Vos, P., Kleerebezem, M., et al. (2010). Identification of *Lactobacillus plantarum* genes modulating the cytokine response of human peripheral blood mononuclear cells. *BMC Microbiol.* 10:293. doi: 10.1186/1471-2180-10-293

Vdovikova, S., Gilfillan, S., Wang, S., Dongre, M., Wai, S. N., and Hurtado, A. (2018). Modulation of gene transcription and epigenetics of colon carcinoma cells by bacterial membrane vesicles. *Sci. Rep.* 8:7434.

Vdovikova, S., Luhr, M., Szalai, P., Nygård Skalman, L., Francis, M. K., Lundmark, R., et al. (2017). A novel role of *Listeria monocytogenes* membrane vesicles in inhibition of autophagy and cell death. *Front. Cell. Infect. Microbiol.* 7:154. doi: 10.3389/fcimb.2017.00154

Vettiger, A., and Basler, M. (2016). Type VI secretion system substrates are transferred and reused among sister cells. *Cell* 167, 99–110. doi: 10.1016/j.cell.2016.08.023

Vidakovic, L., Singh, P. K., Hartmann, R., Nadell, C. D., and Drescher, K. (2018). Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. *Nat. Microbiol.* 3, 26–31. doi: 10.1038/s41564-017-0050-1

Vieira, A. T., Galvão, I., Macia, L. M., Sernaglia, E. M., Vinolo, M. A. R., Garcia, C. C., et al. (2017). Dietary fiber and the short-chain fatty acid acetate promote resolution of neutrophilic inflammation in a model of gout in mice. *J. Leukoc. Biol.* 101, 275–284. doi: 10.1189/jlb.3a1015-453rrr

Vikström, E., Bui, L., Konradsson, P., and Magnusson, K. E. (2010). Role of calcium signalling and phosphorylations in disruption of the epithelial junctions by *Pseudomonas aeruginosa* quorum sensing molecule. *Eur. J. Cell Biol.* 89, 584–597. doi: 10.1016/j.ejcb.2010.03.002

Villarreal, L. P. (2012). "The addiction module as a social force," in *Viruses: Essential Agents of Life*, ed. G. Witzany (Dordrecht: Springer), 107–145. doi: 10.1007/978-94-007-4899-6_6

Vipperla, K., and O'keefe, S. J. (2012). The microbiota and its metabolites in colonic mucosal health and cancer risk. *Nutr. Clin. Pract.* 27, 624–635. doi: 10.1177/0884533612452012

Vollenweider, S., and Lacroix, C. (2004). 3-Hydroxypropionaldehyde: applications and perspectives of biotechnological production. *Appl. Microbiol. Biotechnol.* 64, 16–27. doi: 10.1007/s00253-003-1497-y

Wai, S. N., Lindmark, B., Söderblom, T., Takade, A., Westermark, M., Oscarsson, J., et al. (2003). Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial ClyA cytotoxin. *Cell* 115, 25–35. doi: 10.1016/s0092-8674(03)00754-2

Wampler, J. L., Kim, K. P., Jaradat, Z., and Bhunia, A. K. (2004). Heat shock protein 60 acts as a receptor for the Listeria adhesion protein in Caco-2 cells. *Infect. Immun.* 72, 931–936. doi: 10.1128/iai.72.2.931-936.2004

Wandro, S. J. (2019). *Evolution of Bacteriophages Infecting Enterococcus from the Human Microbiome*. Ph.D. dissertation, University of California Irvine, Irvine, CA.

Wang, J., Ji, H., Wang, S., Liu, H., Zhang, W., Zhang, D., et al. (2018). Probiotic *Lactobacillus plantarum* promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. *Front. Microbiol.* 9:1953. doi: 10.3389/fmicb.2018.01953

Wang, W., and Jeffery, C. J. (2016). An analysis of surface proteomics results reveals novel candidates for intracellular/surface moonlighting proteins in bacteria. *Mol. Biosyst.* 12, 1420–1431. doi: 10.1039/c5mb00550g

Wang, X., Kim, Y., Ma, Q., Hong, S. H., Pokusaeva, K., Sturino, J. M., et al. (2010). Cryptic prophages help bacteria cope with adverse environments. *Nat. Commun.* 1:147.

Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., et al. (2017). Antioxidant properties of probiotic bacteria. *Nutrients* 9:521. doi: 10.3390/nu9050521

Waśko, A., Polak-Berecka, M., Paduch, R., and Jóźwiak, K. (2014). The effect of moonlighting proteins on the adhesion and aggregation ability of *Lactobacillus helveticus*. *Anaerobe* 30, 161–168. doi: 10.1016/j.anaerobe.2014.10.002

Webster, N. S. (2014). Cooperation, communication, and co-evolution: grand challenges in microbial symbiosis research. *Front. Microbiol.* 5:164. doi: 10.3389/fmicb.2014.00164

Yang, C. K., Ewis, H. E., Zhang, X., Lu, C. D., Hu, H. J., Pan, Y., et al. (2011). Nonclassical protein secretion by *Bacillus subtilis* in the stationary phase is not due to cell lysis. *J. Bacteriol.* 193, 5607–5615. doi: 10.1128/jb.05897-11

Yoshida, N., Oeda, K., Watanabe, E., Mikami, T., Fukita, Y., Nishimura, K., et al. (2001). Chaperonin turned insect toxin. *Nature* 411, 44–44. doi: 10.1038/35075148

Zavan, L., Bitto, N. J., and Kaparakis-Liaskos, M. (2020). "Introduction, History, and Discovery of Bacterial Membrane Vesicles," in *Bacterial Membrane Vesicles: Biogenesis, Functions and Applications*, eds M. Kaparakis-Liaskos and T. A. Kufer (Cham: Springer), 1–21. doi: 10.1007/978-3-030-36331-4_1

Zdziarski, J., Brzuszkiewicz, E., Wullt, B., Liesegang, H., Biran, D., Voigt, B., et al. (2010). Host imprints on bacterial genomes—rapid, divergent evolution in individual patients. *PLoS Pathog.* 6:e1001078. doi: 10.1371/journal.ppat.1001078

Zhang, X., McDaniel, A. D., Wolf, L. E., Keusch, G. T., Waldor, M. K., and Acheson, D. W. (2000). Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. *J. Infect. Dis.* 181, 664–670. doi: 10.1086/315239

Zhou, M., and Wu, H. (2009). Glycosylation and biogenesis of a family of serine-rich bacterial adhesins. *Microbiology* 155, 317–327. doi: 10.1099/mic.0.025221-0

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32, 723–735. doi: 10.1111/j.1574-6976.2008.00123.x

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 10 December 2020
doi: 10.3389/fmicb.2020.594052

Thiamine-Mediated Cooperation Between Auxotrophic *Rhodococcus ruber* ZM07 and *Escherichia coli* K12 Drives Efficient Tetrahydrofuran Degradation

*Hui Huang, Minbo Qi, Yiming Liu, Haixia Wang, Xuejun Wang, Yiyang Qiu and Zhenmei Lu**

MOE Laboratory of Biosystem Homeostasis and Protection, Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, China

Tetrahydrofuran (THF) is a universal solvent widely used in the synthesis of chemicals and pharmaceuticals. As a refractory organic contaminant, it can only be degraded by a small group of microbes. In this study, a thiamine auxotrophic THF-degrading bacterium, *Rhodococcus ruber* ZM07, was isolated from an enrichment culture H-1. It was cocultured with *Escherichia coli* K12 (which cannot degrade THF but can produce thiamine) and/or *Escherichia coli* K12Δ*thiE* (which can neither degrade THF nor produce thiamine) with or without exogenous thiamine. This study aims to understand the interaction mechanisms between ZM07 and K12. We found that K12 accounted for 30% of the total when cocultured and transferred with ZM07 in thiamine-free systems; in addition, in the three-strain (ZM07, K12, and K12Δ*thiE*) cocultured system without thiamine, K12Δ*thiE* disappeared in the 8th transfer, while K12 could still stably exist (the relative abundance remained at approximately 30%). The growth of K12 was significantly inhibited in the thiamine-rich system. Its proportion was almost below 4% after the fourth transfer in both the two-strain (ZM07 and K12) and three-strain (ZM07, K12, and K12Δ*thiE*) systems; K12Δ*thiE*'s percentage was higher than K12's in the three-strain (ZM07, K12, and K12Δ*thiE*) cocultured system with exogenous thiamine, and both represented only a small proportion (less than 1% by the fourth transfer). The results of the coculture of K12 and K12Δ*thiE* in thiamine-free medium indicated that intraspecific competition between them may be one of the main reasons for the extinction of K12Δ*thiE* in the three-strain (ZM07, K12, and K12Δ*thiE*) system without exogenous thiamine. Furthermore, we found that ZM07 could cooperate with K12 through extracellular metabolites exchanges without physical contact. This study provides novel insight into how microbes cooperate and compete with one another during THF degradation.

Keywords: tetrahydrofuran degradation, thiamine auxotroph, cooperation, interaction mechanism, *Rhodococcus ruber* ZM07, *Escherichia coli* K12

OPEN ACCESS

Edited by:
Obulisamy Parthiba Karthikeyan, University of Houston, United States

Reviewed by:
Dhiraj Kumar Chaudhary, Korea University, South Korea
Patrick C. Cirino, University of Houston, United States

****Correspondence:***
Zhenmei Lu
lzhenmei@zju.edu.cn

Specialty section:
This article was submitted to Microbiotechnology, a section of the journal Frontiers in Microbiology

Received: *17 August 2020*
Accepted: *16 November 2020*
Published: *10 December 2020*

Citation:
Huang H, Qi M, Liu Y, Wang H, Wang X, Qiu Y and Lu Z (2020) Thiamine-Mediated Cooperation Between Auxotrophic Rhodococcus ruber ZM07 and Escherichia coli K12 Drives Efficient Tetrahydrofuran Degradation. Front. Microbiol. 11:594052. doi: 10.3389/fmicb.2020.594052

INTRODUCTION

As an important solvent, tetrahydrofuran (THF) is widely applied in the chemical industry. According to toxicity tests, THF induces cell proliferation and causes DNA damage, thereby increasing the risk of cancer in laboratory animals (Gamer et al., 2002; Hermida et al., 2006); moreover, severe central nervous system disorders can be triggered by acute exposure to high concentrations of THF (Katahira et al., 1982; Chhabra et al., 1990). In recent years, biodegradation has been universally acknowledged as an ecofriendly remediation strategy for contaminant removal with high degradation rates (Oh et al., 2010). However, a few kinds of THF-degrading microorganisms (including sixteen bacteria and three fungi) have been reported (Bernhardt and Diekmann, 1991; Parales et al., 1994; Kohlweyer et al., 2000; Skinner et al., 2009; Yao et al., 2009; Masuda et al., 2012; Tajima et al., 2012; Sei et al., 2013; Ren et al., 2020). Furthermore, the biodegradation pathway of THF has not been investigated thoroughly (Skinner et al., 2009). One of the most widely accepted THF metabolic pathways is the oxidation pathway, whereby THF is initially oxidized into 2-hydroxytetrahydrofuran (2-OH THF), which then can form γ-butyrolactone or γ-hydroxybutyraldehyde. Both of these compounds can form γ-hydroxybutyrate, which can be further oxidized to succinate and enter the tricarboxylic acid cycle (Bernhardt and Diekmann, 1991; Thiemer et al., 2003; Morenohorn et al., 2005; Tajima et al., 2012). ' According to previous studies, bacteria are ubiquitous and coexist to survive in nature (Dubey and Ben-Yehuda, 2011; Schuster et al., 2013; Amin et al., 2015; Bruger and Waters, 2018). Cooperative behaviors among microorganisms are commonplace (Brown and Johnstone, 2001; Velicer, 2003). For microbes, communication and cooperation are essential in their natural lives. Through cooperation, microorganisms can more efficiently perform biological functions, such as foraging, biofilm formation, pathopoiesis, anabolism and biodegradation (Webb et al., 2003; Du et al., 2012; Rakoff-Nahoum et al., 2014; Filkins et al., 2015; Tao et al., 2017). Many crucial genetic functions of cooperators are leaky, some productions are produced by microbes of community and become the "public good," which represents an important type of cooperation between microorganisms (Morris et al., 2012; Morris, 2015). Not only the producers but also other nearby members can benefit from diffusible substances released into the environment (Van Gestel et al., 2014; Lujan et al., 2015). Cross-feeding (or syntrophy) is another important type of interaction among microorganisms (Sieuwerts et al., 2008; Morris et al., 2013). Metabolites are released into the extracellular environment and then utilized by others as nutrients or energy sources (Mee et al., 2014; Pande et al., 2016). Previous studies found that a number of microorganisms are auxotrophic in nature and rely on external nutrients (i.e., amino acids, vitamins and other cofactors) for growth (D'Souza et al., 2014; Wexler and Goodman, 2017; Liu et al., 2018). Hydrocarbon-degrading bacterial consortia are built by microbial interactions among amino acids and vitamin auxotrophy and their cooperators (Hubalek et al., 2017). However, the question remains as to how auxotrophic microorganisms, especially contaminant-degrading bacteria, perform their functions in microbial communities. In this study, a THF-degrading bacterium, *Rhodococcus ruber* ZM07, was isolated from a THF-degrading bacterial culture H-1 (Huang et al., 2019). This strain cannot be continuously passaged in basal salt medium (BSM) with THF as the sole carbon source, although it can cooperate with many kinds of other bacteria, such as *Escherichia coli* K12, to form a stable symbiotic system. Strain ZM07 has been verified as a thiamine auxotroph. Therefore, it is very likely that some of the microorganisms that degrade refractory pollutants, e.g., THF, but have not yet been isolated might also be auxotrophic and depend on other non-degrading microbes to perform their functions in nature. Strain ZM07 was deemed highly suitable for this study, which investigated this interesting phenomenon and the ecological significance of auxotrophic strains.

Cooperative behavior can potentially be exploited by uncooperative cheaters (Fiegna and Velicer, 2003; West et al., 2006), which gain benefits from other cooperators without contributing; thus, they achieve a competitive advantage and can invade the community (Smith, 2001; Velicer, 2003; West et al., 2006). If cheaters are unrestricted in the bacterial community, they may quickly overgrow, ultimately leading to a population collapse of the community (Hardin, 1968). In a previous study, cheaters could help to counteract competition among microorganisms and foster biodiversity in well-mixed media by invading the community of cooperating siderophore producers (Leinweber et al., 2017). However, another study indicated that cheaters could be resisted by the conditional privatization strategy under stressful conditions (Jin et al., 2018). Therefore, it might be important to investigate the behavior pattern of uncooperative organisms in systems. We could deduce the stability of the cooperative community of auxotrophic degrading bacteria and nondegrading bacteria in actual environments, such as in wastewater treatment, by studying the noncooperator K12Δ*thiE* constructed in this study.

Auxotrophic microorganisms that degrade refractory pollutants depend on other nondegrading microbes to perform their functions in nature. However, the understanding of the interaction mechanisms between degrading and nondegrading microorganisms as well as the ecological significance of auxotrophic strains is limited. In this study, different cooperative and competitive systems were constructed to explore the interspecies and transspecies communications among the thiamine auxotrophic THF-degrading bacterium ZM07 and its cooperator K12 as well as the stability of the cooperative community of these two strains. This research was proposed to investigate the functions of auxotrophic degrading bacteria and nondegrading bacteria in a cooperative system for THF degradation. The proposed model of auxotrophic THF-degrading and non-THF-degrading microorganisms may provide an insight into the interaction mechanisms in multispecies ecosystems.

MATERIALS AND METHODS

Strains and Plasmids

The strains and plasmids used in this study are listed in **Table 1**. The THF-degrading strain *Rhodococcus ruber* ZM07

TABLE 1 | Strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	References or source
Strains		
Rhodococcus ruber ZM07	THF-degrading, non-thiamine-synthesizing strain G^+	This study
Escherichia coli K12	Wild-type, non-THF-degrading, thiamine-synthesizing strain, G^-	Bachmann et al., 1976
Escherichia coli K12Δ*thiE*	*Escherichia coli* K12 mutant with *thiE* gene replaced by chloramphenicol resistance gene from plasmid pGemT7cat, Chl^R	This study
Plasmids		
pDK46	λ Red recombinase expression, Amp^R	Datsenko and Wanner, 2000
pGemT7cat	Source of chloramphenicol resistance gene	Król et al., 2010

(collection number CCTCC AB 2019217) was isolated from an enrichment culture designated H-1 (Huang et al., 2019). The non-THF-degrading strain *Escherichia coli* K12 (Bachmann et al., 1976) and its knockout strain K12Δ*thiE* (which can neither degrade THF nor produce thiamine) were both cocultured with ZM07, and the latter strain was constructed as described in the **Supplementary Methods**. The λ-Red recombination plasmid pDK46 (Datsenko and Wanner, 2000) used for *thiE* disruption was purchased from Youbio Biological Technology Co., Ltd. (Changsha, China). Plasmid pGemT7cat (Król et al., 2010) was used as a template for PCR amplification of the fused chloramphenicol-selectable marker.

Subculture Conditions and Experiments

The strains were initially cultivated in lysogeny broth (LB) culture medium and then transferred into BSM supplemented with THF as the sole carbon source. One liter of LB contained 10 g tryptone, 5 g yeast extract, and 10 g NaCl, and the initial pH of the media was 7.4. One liter of BSM contained 3.240 g K_2HPO_4, 1.000 g $NaH_2PO_4{\cdot}H_2O$, 2.000 g NH_4Cl, 0.123 g $C_6H_8NNa_3O_7$, 0.200 g $MgSO_4{\cdot}7H_2O$, 0.012 g $FeSO_4{\cdot}7H_2O$, 0.003 g $MnSO_4{\cdot}H_2O$, 0.003 g $ZnSO_4{\cdot}7H_2O$, and 0.001 g $CoCl_2{\cdot}6H_2O$ (Parales et al., 1994; Huang et al., 2019). For experiments under thiamine-rich conditions, 0.01 mM external thiamine was added to the BSM. All strains used in this study were cultured in 100 mL of BSM with an initial pH of 7.0 at 30°C, referred to the conditions of THF-degrading bacterial culture H-1 (Huang et al., 2019). Most experiments were performed using 500 mL shaking flasks at 160 rpm, while specifically designed two-phase reactors were used in the experiment to verify the interaction mode between strains ZM07 and K12 with a shaking rate of 100 rpm.

(1) To investigate the interaction mechanism between ZM07 and K12, this study used a two-phase reactor that can separate cells but allow extracellular metabolites to pass through. The reactor is divided into two chambers (each 500 mL by volume and containing 100 mL of BSM) by track-etched polyethylene terephthalate (PET) nucleopore membranes (with a pore size distribution at 0.22 μm), which were purchased from Wuwei Kejin Xinfa Technology Co., Ltd. (Gansu, China). The test experiments of the reactor performance are detailed in **Supplementary Methods**. Strains ZM07 and K12 were inoculated separately into each side of the reactor at a ratio of 1:1 (the initial inoculum size of each strain was OD_{600} = 0.06). Samples were collected every 24 h for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations.

(2) Coculture of ZM07 and K12: Strains were cultivated in LB, harvested by centrifugation (7,000 × g, 10 min), and washed with BSM three times. The resulting cells were resuspended with BSM (OD_{600} = 3); subsequently, 100 mL of BSM with 20 mM THF was inoculated with 1 mL of strain ZM07 and 1 mL of strain K12 to generate a cell ratio of 1:1 for coculture as the first transfer. The corresponding monoculture systems were incubated with the same inoculum of ZM07 and 1 mL of BSM. Subsequently, the coculture and monoculture were transferred (OD_{600} = 6 and 1 mL of cell suspension) in the same way for the second and third transfers. Samples were collected every 24 h for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations.

(3) Coculture of K12 and K12Δ*thiE*: To test the survivability of K12Δ*thiE* in the thiamine-limited and thiamine-rich media used in this study, we cocultured K12 wild type and K12Δ*thiE* in BSM supplemented with 20 mM succinate as the sole carbon source. K12 and K12Δ*thiE* were inoculated with a cell ratio of 1:1 either supplemented with (0.01 mM) thiamine or without thiamine. We used the method described above to passage coculture strains for 8 transfers every 12 h, and the initial cell densities and cell ratios of K12 and K12Δ*thiE* are shown in **Supplementary Table S1**. Samples were collected at the end of cultivation of each transfer for analysis of biomass (OD_{600}) and relative abundance.

(4) Coculture of ZM07, K12, and K12Δ*thiE*: To assess the synergetic and competitive relationships in the three-strain (ZM07, K12, and K12Δ*thiE*) and two-strain (ZM07 and K12, ZM07 and K12Δ*thiE*) communities during THF degradation with or without extra thiamine, we conducted experiments on three different combinations of the three strains (ZM07 and K12, ZM07 and K12Δ*thiE*, ZM07 and K12 and K12Δ*thiE*) with sufficient (0.01 mM) thiamine or without thiamine. The initial inoculum size and strain ratio of the three strains in every experiment are shown in **Supplementary Table S1**. Each combination with thiamine was transferred to fresh medium every 3 days, and 2 mL samples were collected at 48 h for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations and relative strain abundances. Analogously, the cultures without additional thiamine were transferred every 4 days, and samples were collected at 72 h for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations and relative strain abundances.

Determining the Composition of Different Strains in Coculture Systems

The relative abundances of ZM07, K12, and K12Δ*thiE* were determined by quantitative PCR (qPCR). Genomic DNA of the bacterial consortium was isolated with an E.Z.N.A® Bacterial DNA Kit (Omega Bio-Tek) according to the manufacturer's instructions. Primers for the qPCR assay are shown in **Supplementary Table S2**. The qPCR reactions were carried out in a volume of 20 μL containing 10 μL of TB Green™ Premix Ex Taq™ (TaKaRa, Dalian, China), 0.4 μL each of forward and reverse gene-specific primers (10 μM), 0.4 μL of DNA (50–100 ng/μL) and 8.8 μL of ddH_2O. The following conditions were used for PCR: 95°C for 2 min, followed by 40 cycles of 10 s at 95°C, 20 s at 58°C and 20 s at 72°C. Three independent DNA samples were assayed, and the $2^{-\Delta\Delta CT}$ method was used to calculate the gene abundance level of the strains (Livak and Schmittgen, 2001).

KEGG Metabolic Pathways Analysis

The metabolic pathways of strains ZM07 and K12 were analyzed using the method described in previous research (Li et al., 2018). The whole-genome sequencing results of strains ZM07 and K12 were annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG[1]). The draft genome sequence data of strain ZM07 have been included in the National Center for Biotechnology Information (NCBI), accession number JACVXT000000000, and strain K12 genome sequence data can also be found in NCBI (GenBank accession number NC_000913.3). We then compared the results to find differences and annotated them in KEGG Mapper with different colors (pathways that are unique in ZM07 and K12 are colored green and blue, respectively, while pathways harbored by both strains are colored brown). We searched for the anabolic pathways that were completely present only in the K12 strain.

Verification of Thiamine Auxotroph

Experiments were conducted to verify that strain ZM07 is a thiamine auxotroph. Different amounts of thiamine were added to the third transfer of ZM07 (the strain could barely grow in BSM with 40 mM THF as the sole carbon source after continuous passage culture). Furthermore, strain ZM07 was cocultured with K12 and K12Δ*thiE* with 40 mM THF, respectively, for two transfers. Samples were collected 72 h after each transfer for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations.

Invasion Experiments With Trace Amounts of K12 in Microbial Communities

To verify whether trace wild-type K12 can form a stable interaction system with ZM07, we added 0.5% (OD_{600} = 0.0003) K12 wild type to ZM07 culture in the first transfer and passaged the culture for 8 transfers. In addition, we added 0.5% of the K12 wild type to the third transfer of the ZM07 culture, which was cultivated for 4 days alone with almost no growth. In other tests, 0.5% K12Δ*thiE* was added to the ZM07 and K12 coculture in the first and third transfers and passaged for 8 transfers to determine how trace K12Δ*thiE* behaves when invading the ZM07 and K12 system. The initial cell densities and cell ratios of the three strains in every experiment are shown in **Supplementary Table S1**. Each combination was transferred to fresh medium every 4 days, and samples were collected at 72 h for analysis of the biomass (OD_{600}) and determination of the residual THF concentrations and relative strain abundances.

Intermediate Metabolites of THF Used to Culture Strain K12

Strain ZM07 in the first transfer was cultured in 100 mL of BSM supplemented with 20 mM THF for 1, 2, 3, and 4 days. Then, the supernatant of ZM07, which was obtained using a 0.22 μm vacuum bottle filter (BIOFIL, China), was used to cultivate strain K12 (the initial inoculum size of K12 was OD_{600} = 0.06) for 3 days. Furthermore, THF, 2-OH THF, γ-butyrolactone and succinate (three intermediate metabolites of THF) were chosen to test the ability of strain K12 to use these compounds (5 mM each). Strain K12 was cultured in 100 mL of BSM at 30°C and 160 rpm, with samples collected after 12, 24, and 72 h to measure the OD_{600}. Furthermore, the effects of THF, 2-OH THF and γ-butyrolactone on the growth and morphology of K12 and K12Δ*thiE* were also studied as described in **Supplementary Methods**.

Detection and Identification of THF and Related Metabolites and Statistical Analysis

The cultures were centrifuged at 10,000 × g for 10 min, and 500 μL of the supernatant was subjected to THF concentration determination using gas chromatograph (GC-2014C; SHIMADZU, Japan). The temperatures of the injector, oven, and detector were set to 250, 160, and 250°C, respectively. The degradation ratio was calculated as described in our previous study (Huang et al., 2019). The THF intermediate metabolites produced by strain ZM07 were detected as described in **Supplementary Methods**. The biomass was monitored by recording the OD_{600} using a UV spectrometer (UV-3100PC; MAPADA; Shanghai). Succinate detection is shown below: Samples of extracellular supernatants and intracellular extracts were derivatized using pyridine and N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and analyzed by gas chromatography-mass spectrometry (GC-MS). Each experiment was performed in triplicate. *P*-values for all assays were determined using a two-tailed Student's *t*-test.

RESULTS

KEGG Metabolic Pathway Analysis Revealed That the THF-Degrading Bacterium ZM07 Is a Thiamine Auxotroph Strain

The THF-degrading strain *Rhodococcus ruber* ZM07 was isolated from an enrichment culture H-1 (Huang et al., 2019), and cannot

[1]www.genome.jp/kegg

be subcultured serially in BSM with THF as the sole carbon source. Before carrying out experiments, monoculture growths of strains ZM07, K12, and K12Δ*thiE* in BSM (not supplemented with THF or any other carbon sources) with or without thiamine were tested. The results showed that these three strains cannot grow in BSM without THF or any other carbon sources (**Supplementary Figure S1**). Monoculture experiments showed that ZM07 degraded 20 mM THF completely within 3 days in the first transfer, while its THF-degrading ability was inhibited when its growth rate slowed in the second transfer; furthermore, its growth was inhibited when using THF in the third transfer (**Figure 1A**). We speculated that intracellular thiamine, which may still remain during the first transfer, supported the growth of strain ZM07 in the starting phase. In contrast, when cocultured with strain K12, ZM07 was able to proliferate well and continually degrade THF during passaging (**Figure 1B**).

Accordingly, we suspected that the THF-degrading bacterium ZM07 may not be able to produce some growth factors that are critical to its growth. To verify this hypothesis, we plotted a comparative metabolic pathway network for ZM07 and K12 with the pathways harbored by the different strains, which are colored differently (**Supplementary Figure S2**). The map of thiamine metabolism supports our hypothesis since the thiamine synthesis pathway is incomplete in strain ZM07 but intact in strain K12 (**Figure 2**). As an essential growth factor, thiamine pyrophosphate is produced through the condensation reaction of 4-amino-5-hydroxymethyl pyrimidine pyrophosphate and 4-methyl-5-(beta-hydroxyethyl)-thiazole monophosphate (Begley et al., 1999). As shown in **Figure 2**, strain ZM07 lacks the genes *thiF*, *thiH,* and *thiI* (highlighted by the purple boxes), which are required for 4-methyl-5-(beta-hydroxyethyl)-thiazole synthesis (Webb et al., 1997; Dorrestein et al., 2004; Martinez-Gomez et al., 2004).

To further verify our hypothesis, we performed two experiments. First, we cocultured the wild-type strain K12 and the *thiE* (highlighted by the red boxes in **Figure 2**)-deficient strain K12Δ*thiE* separately with strain ZM07. The *thiE* gene encodes thiamine phosphate synthase (ThiE), which is responsible for linking the thiazole and pyrimidine moieties of thiamine monophosphate (TMP) generated in respective metabolic pathways (Chiu et al., 1999; Jurgenson et al., 2009). The results showed that strain ZM07 could grow and degrade THF normally when cocultured and transferred with K12 wild type (**Supplementary Figure S3D**), while it could not restore its growth and THF degradation ability when cocultured with K12Δ*thiE* (which is very obvious after the second transfer) (**Supplementary Figure S3E**). The coculture of ZM07 and K12Δ*thiE* (basically no growth) quickly became cloudy with additional thiamine on the 1st day, with a final OD_{600} of 2.541 ± 0.056 (data not shown), while the growth of coculture without additional thiamine was very slow (**Supplementary Figure S4A**). Further experiments were performed to confirm that THF-degrading bacterium ZM07 is indeed a thiamine auxotroph strain by adding thiamine directly to the culture medium. The results showed that when supplied with more than 10^{-5} mM thiamine, strain ZM07 grew well and degraded THF normally (**Supplementary Figure S5**). Nevertheless, the growth and THF-degrading ability of ZM07 were still inhibited when thiamine concentrations were below 10^{-5} mM (**Supplementary Figure S5**). The results indicated that THF-degrading bacterium ZM07 cannot grow alone with THF as the sole carbon source since it is a thiamine auxotroph strain.

Contact-Independent Interaction Mode Between Strains ZM07 and K12

To determine the interaction mode between strains ZM07 and K12, the metabolic intermediates of THF were detected and identified at different cultivation time of strain ZM07 when utilizing 20 mM THF as substrates firstly. The results showed that no metabolites were detected in extracellular supernatants (**Supplementary Figure S6A**), while one peak that appeared at retention times of 6.816 min were observed in intracellular extracts (**Supplementary Figure S6B**). According to the retention times of standards, the sources of the peak was identified as γ-butyrolactone. Furthermore, succinate was identified in both

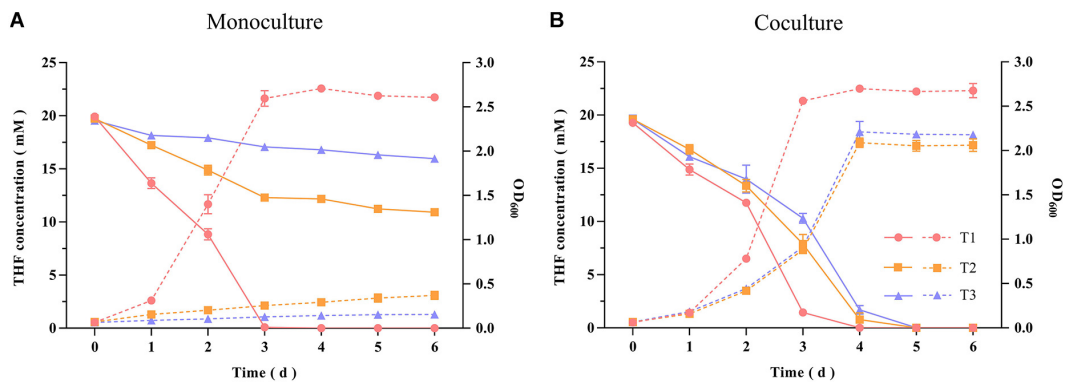


FIGURE 1 | THF degradation and growth curves of strain ZM07 monocultured **(A)** and cocultured with strain K12 **(B)** in the first, second and third transfers. The solid lines represent THF concentration curves; the dotted lines represent growth curves (OD_{600}); the circles represent the first transfer; the squares represent the second transfer; and the triangles represent the third transfer. Error bars: SD from three independent replicates, which may be smaller than the marker.

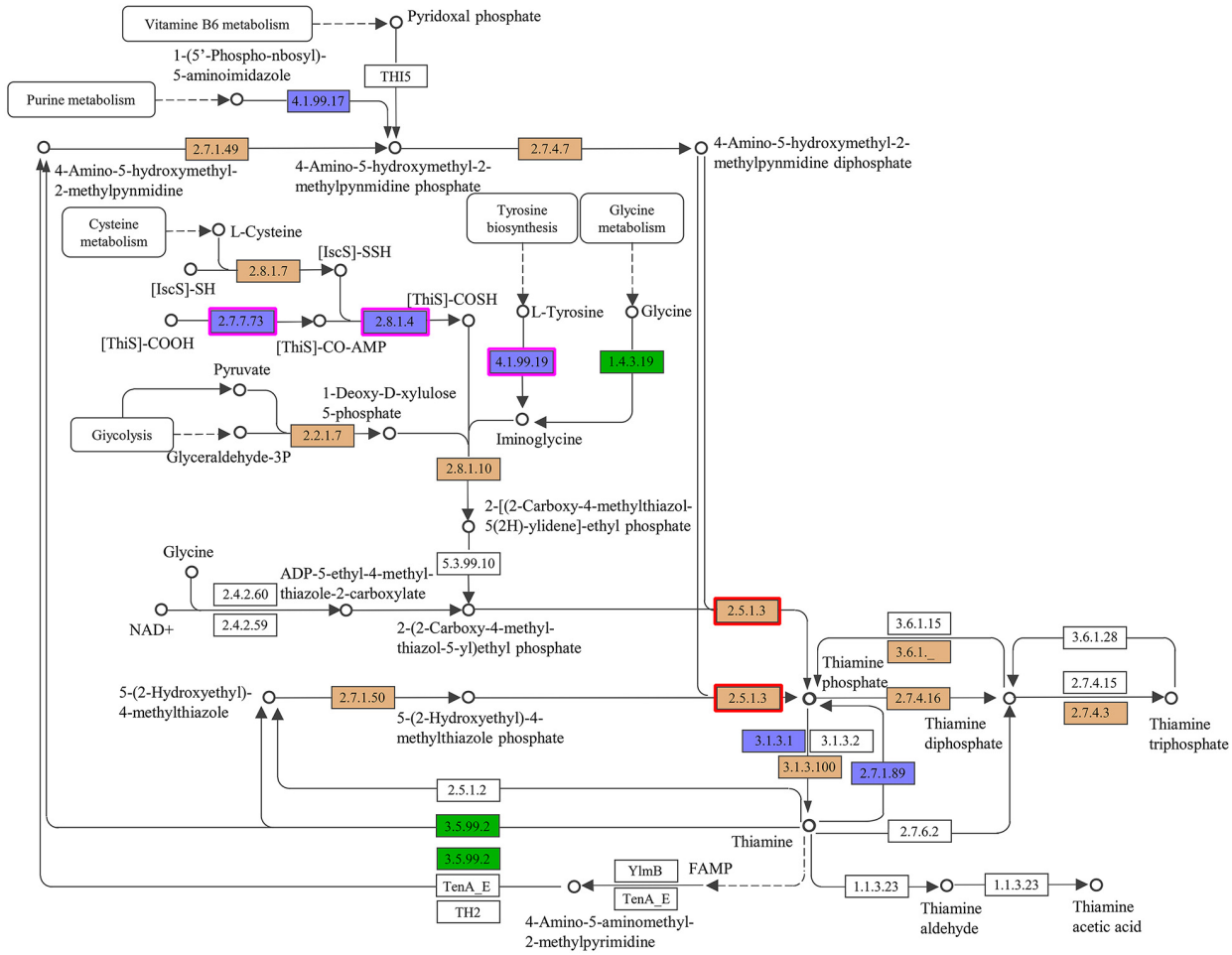


FIGURE 2 | Comparison of the KEGG metabolic pathways reconstructed from the complete genomes of ZM07 and K12. Here, thiamine metabolism (map00730) is shown. The ZM07-specific pathways are colored in green; the K12-specific pathways are colored in blue; shared pathways are colored in brown; and pathways present in neither ZM07 nor K12 are colored in white. The genes for *thiF* (No. 2.7.7.73), *thiH* (No. 4.1.99.19), and *thiI* (No. 2.8.1.4) are highlighted by purple boxes, and the gene for *thiE* (No. 2.5.1.3) is highlighted by red boxes.

of the samples of extracellular supernatants and intracellular extracts (**Supplementary Figures S6D,E**). To investigate whether K12 could utilize the intermediate metabolites of THF degraded by ZM07 in the cocultured system, we used THF and its intermediate metabolites (2-OH THF, γ-butyrolactone and succinate) as carbon sources to cultivate strain K12. The results showed that K12 could utilize only succinate to survive, with the highest OD_{600} value reaching 0.305 ± 0.002, while it could not utilize THF, 2-OH THF, or γ-butyrolactone as sole carbon sources (**Supplementary Table S3**). The effects of several different concentrations (0, 1.25, 2.5, and 5 mM) of THF, 2-OH THF and γ-butyrolactone on growth of K12 and its mutant were tested. The results showed that different THF, 2-OH THF, and γ-butyrolactone concentrations (0, 1.25, 2.5, and 5 mM) did not significantly influence the biomass of K12 or K12Δ*thiE* (**Supplementary Figure S7**). Otherwise, the transmission electron microscopy (TEM) images of two strains morphology after exposuring the cell to these three compounds indicated that there are no obvious changes of both two strains exposing to 5 mM THF, 2-OH THF, and γ-butyrolactone (**Supplementary Figure S8**).

In addition, we used culture supernatants of ZM07 (cultured for 1, 2, 3, and 4 days) to culture strain K12 (**Supplementary Table S4**), and it showed no growth probably because THF intermediate metabolites in the supernatants of strain ZM07, which could be used by strain K12, are easily accessible carbon sources and cannot be accumulated, hence the instantaneous concentration is too low to support the growth of K12. Finally, two-phase reactors were used to explore whether physical contact was needed to mediate the interaction between these two species. The test experiments of the reactor performance indicated that the membrane can separate cells and allow only extracellular

metabolite exchange (**Supplementary Figures S9, S10**). The results showed that the separately cultured strain K12 could help ZM07 grow and degrade THF (**Figures 3A,B**). A comparison with the contacting coculture of ZM07 and K12 showed that THF was degraded even faster in the group of the non-contacting coculture (**Figure 3B**), which indicated that there might be some cooperation and competition mechanisms in these two strains. The highest OD_{600} value of K12 reached 0.313 ± 0.026 (**Figure 3C**), indicating that physical contact is not a prerequisite for the interaction between strains ZM07 and K12. Separately cultured strain K12 could grow well when continuous supplying with THF intermediate metabolites as carbon sources from ZM07 through membrane, while it was not growing with discontinuous supplies cultured in the supernatants of strain ZM07. Thus, the exchange of extracellular metabolites is one way these two strains interact.

Intraspecific Competition Between K12 and K12ΔthiE

Succinate, one of the intermediate metabolites of THF, was used as a carbon source to explore the intraspecific competition between wild-type K12 and its knockout strain. The results showed that under thiamine-limited conditions, strain K12 wild type outcompeted strain K12Δ*thiE*. The proportion of K12Δ*thiE* was drastically reduced during passage and disappeared almost entirely by the fourth transfer (**Figure 4A**), while its proportion remained stable in the coculture system under thiamine-rich conditions. Moreover, the composition of K12Δ*thiE* and K12 remained stable (at a ratio of approximately 6:4) during passage with sufficient thiamine (**Figure 4B**). However, there was no difference in the biomass between K12 and K12Δ*thiE* with or without thiamine in the first transfer (**Supplementary Figures S11A,B**). Furthermore, strain K12Δ*thiE* cannot be continuously passaged in BSM without thiamine, and the final biomass of 1 day of cultivation between K12 and the coculture of K12 and K12Δ*thiE* showed no difference (**Supplementary Figure S11C**). In conclusion, K12Δ*thiE* has obvious disadvantages in thiamine-limited medium. However, additional thiamine can significantly increase the competitiveness of K12Δ*thiE* against its wild-type relative.

Competition and Cooperation Among ZM07, K12, and K12ΔthiE in Thiamine-Limited Medium

In thiamine-limited medium, experiments revealed that the relative abundance of K12 remained at approximately 30% in both the two-strain system (ZM07 and K12) and the three-strain

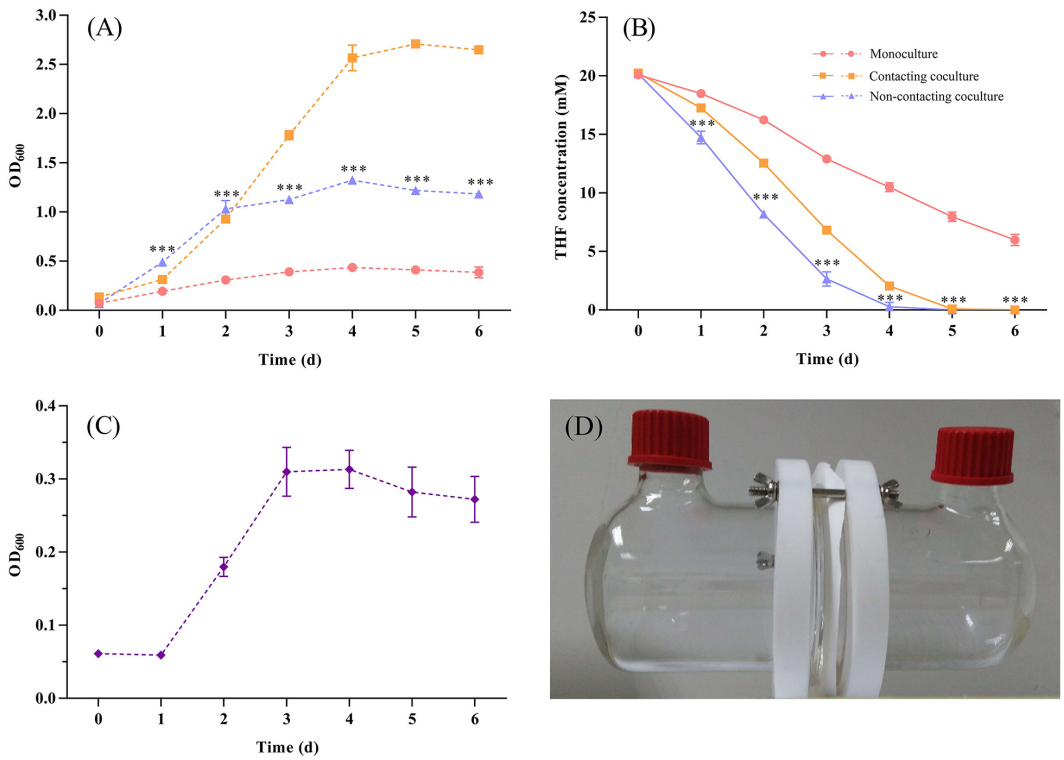


FIGURE 3 | (A) Growth curves of strain ZM07 monocultured (pink line) and cocultured with K12 under the non-contact condition (blue line) in two-phase reactors and the growth curve of the two strains cocultured under the contact condition (yellow line). **(B)** THF degradation curves of strain ZM07 in the three cases described above. **(C)** Growth curve of strain K12 cocultured with ZM07 under the non-contact condition in a two-phase reactor. **(D)** Two-phase reactor used to separate the cultured strains in this study. The *P*-value indicates a significant difference between monocultured strain ZM07 and strain ZM07 cocultured with K12 in two-phase reactors and was determined using Student's *t*-test ($n = 3$, $^{***}p < 0.001$). Error bars: SD from three independent replicates, which may be smaller than the marker.

system (ZM07, K12, and K12Δ*thiE*) during passage without additional thiamine (**Figures 5A,B**). In the invasion experiments, a trace amount (the initial inoculum size of the strain was OD_{600} = 0.0003, and same as below) of K12 was added to the ZM07 culture in the 1st transfer, and the results showed that the relative abundances of ZM07 and K12 finally became similar to those of the coculture of ZM07 and K12 without additional thiamine (**Figure 5A**) during passage (**Figure 5C**). In addition, the addition of trace amounts of K12 to the third transfer of the ZM07 culture that was cultivated for 4 days alone with almost no growth could easily restore the growth and THF-degrading ability of ZM07 (**Supplementary Figure S4B**). These results indicated that strain K12 could help auxotrophic strain ZM07 rapidly regain its growth and THF degradation abilities. Then, the results for the coculture of ZM07, K12, and K12Δ*thiE* showed that K12Δ*thiE* could not continuously exist in the symbiotic system composed of ZM07 and K12 in thiamine-limited medium and instead disappeared after the 8th transfer (**Figure 5B**). In the

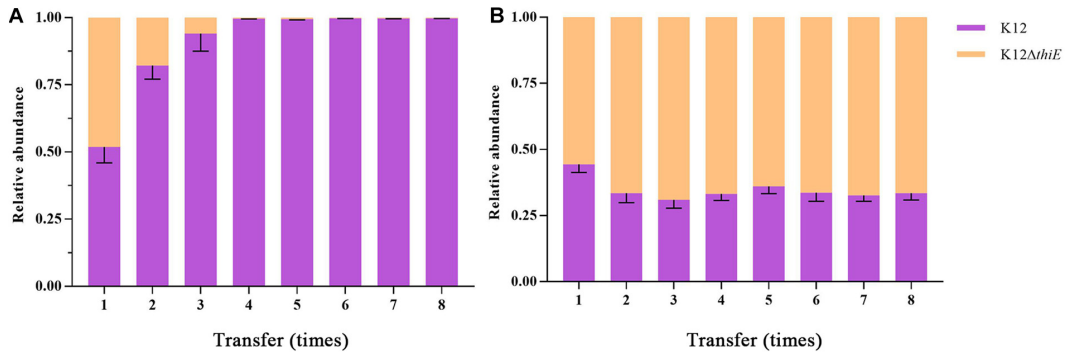


FIGURE 4 | Relative abundances of K12 and K12Δ*thiE* cocultured under 20 mM succinate without **(A)** or with **(B)** 0.01 mM thiamine across different transfers. Error bars: SD from three independent replicates.

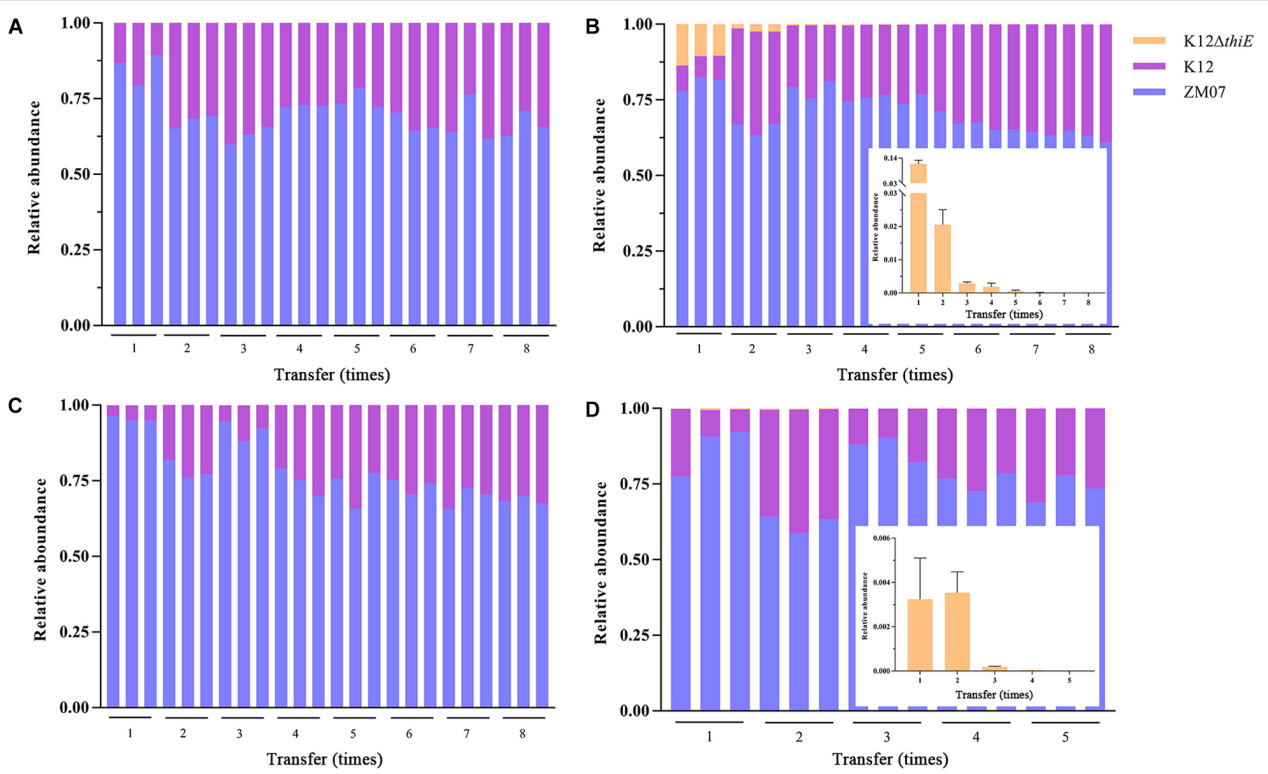


FIGURE 5 | Relative abundances of ZM07, K12, and K12Δ*thiE* in a two-strain system (ZM07 and K12) **(A)** and in a three-strain system **(B)** across different transfers without thiamine. Relative abundances of three strains in a two-strain system (ZM07 and trace amount of K12) **(C)** and in a three-strain system (ZM07, K12 and trace amount of K12Δ*thiE*) **(D)** across different transfers. The plots within **(B,D)** show details of the relative abundance of K12Δ*thiE* in corresponding experiments. Three independent replicates were performed for each group. Error bars: SD from three independent replicates.

invasion experiments, when trace amounts of K12Δ*thiE* were added to the ZM07 and K12 system in the 1st transfer, K12Δ*thiE* disappeared by the 8th transfer during passage (**Figure 5D**). Additionally, to eliminate the influence of intracellular thiamine, which may still remain during the first two transfers, experiments were performed in which trace amounts of K12Δ*thiE* were added to the ZM07 and K12 system in the 3rd transfer; however, K12Δ*thiE* disappeared even faster (**Supplementary Figure S12**). These results indicated that K12Δ*thiE* cannot invade the symbiotic system of strains ZM07 and K12.

Competition and Cooperation Among ZM07, K12, and K12ΔthiE in Thiamine-Rich Medium

In thiamine-rich medium, the results showed that additional thiamine drastically increased the advantage of ZM07 and decreased the proportion of K12 in the coculture of ZM07 and K12 (**Figure 6A**), which indicated that strain K12 was significantly inhibited by additional thiamine. Moreover, despite occupying only a small proportion of the cocultured systems, strain K12Δ*thiE* existed throughout the whole transfer process in the two-strain system of ZM07 and K12Δ*thiE* (**Figure 6B**) or in the three-strain system of ZM07, K12, and K12Δ*thiE* (**Figure 6C**) with additional thiamine, which differed from its fate in thiamine-limited systems (**Figure 5B**). Furthermore, the proportion of K12 in the cocultured system of ZM07 and K12 showed no significant differences from the proportion of K12Δ*thiE* in the cocultured system of ZM07 and K12Δ*thiE* in thiamine-rich medium (**Figure 6D**); however, K12Δ*thiE* grew better than K12 in the three-strain system with additional thiamine (**Figure 6E**), which was similar to the results of the intraspecific competition experiment of K12 and K12Δ*thiE* (**Figure 4B**). The results indicated that additional thiamine could increase the competitiveness of K12Δ*thiE* against its wild-type relative. Additionally, the THF degradation ratio and the total biomass of the systems showed no significant difference among different groups (monoculture of ZM07; two-strain coculture of ZM07 and K12, or ZM07 and K12Δ*thiE*; and three-strain coculture of ZM07, K12, and K12Δ*thiE*) in thiamine-rich medium (**Supplementary Figure S13**), and all combinations of these three strains mentioned above were able to completely degrade 20 mM THF within 4 days. We can conclude that exogenous thiamine might eliminate the effect of K12 and K12Δ*thiE* on the THF degradation ability of strain ZM07 in the cocultured systems.

DISCUSSION

Auxotrophic strains rely on external nutrients for growth, and they are also very likely to benefit their cooperators, which facilitates the establishment of a stable interaction system between them. Rhodococci have a wide range of degradation spectra (Martínková et al., 2009), compatibility with foreign genes (Larkin et al., 2006), and effective degradability; therefore, they have enormous application potential for the treatment of environmental pollutants. Thiamine auxotrophic microorganisms are ubiquitous in the *Rhodococcus* genus

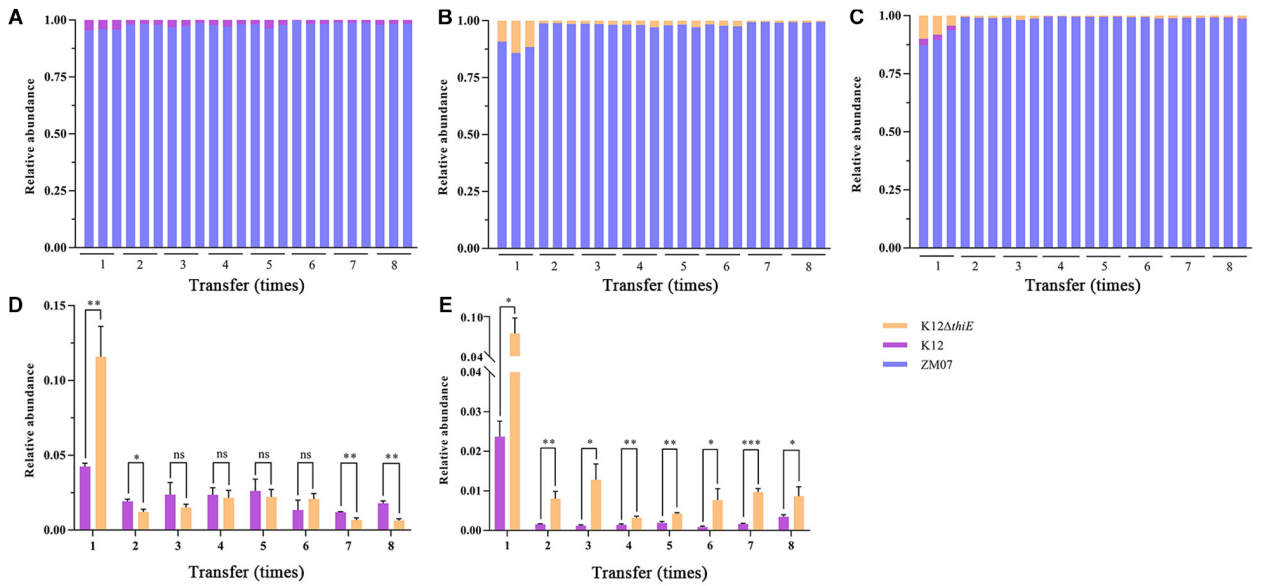


FIGURE 6 | Relative abundances of ZM07, K12, and K12Δ*thiE* in two-strain systems (ZM07 and K12) **(A)**, (ZM07 and K12Δ*thiE*) **(B)** and a three-strain system **(C)** in different transfers with 0.01 mM thiamine. **(D)** Relative abundances of K12 and K12Δ*thiE* in the above experiments in two-strain systems (the relative abundance of strain K12 in a cocultured system of ZM07 and K12 in **(A)** and the relative abundance of strain K12Δ*thiE* in a cocultured system of ZM07 and K12Δ*thiE* in **panel (B)** in different transfers with 0.01 mM thiamine. **(E)** Relative abundances of K12 and K12Δ*thiE* in the above experiments in the three-strain system (ZM07, K12, and K12Δ*thiE* in **(C)** in different transfers with 0.01 mM thiamine. Three independent replicates were performed for each group. Error bars: SD from three independent replicates. The *P* value indicates statistical significance between K12 and K12Δ*thiE* determined using Student's *t*-test ($n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

(Hummel et al., 1987; Denome et al., 1994). The *Rhodococcus ruber* strain ZM07 used in this study is a natural thiamine auxotrophic THF-degrading strain. Compared with other strains in the enrichment culture H-1, strain ZM07 seems to have greater survival advantages in nutrient-poor environments with high THF concentrations (Huang et al., 2019). Therefore, during the long-term evolution process, why have many species of *Rhodococcus* genus lost thiamine synthesis ability, even though it is extremely important for their survival and they cannot live independently without a supply of thiamine provided by other microbes? The "Black Queen Hypothesis" and streamlining theory might provide a reference for this topic; according to these theories, genome reduction might result in higher fitness and optimized resource allocation (Morris et al., 2012; Wolf and Koonin, 2013; Batut et al., 2014; Giovannoni et al., 2014; Otero-Bravo et al., 2018; Zengler and Zaramela, 2018). *Prochlorococcus* mutants gradually lost the ability to reduce HOOH in water; however, they can still enjoy the benefits of decomposing H_2O_2 because other microorganisms in the community still have this function (Morris et al., 2012). Accordingly, we conjecture that "landlords," such as the strain ZM07 and *Prochlorococcus* mutants, may play vital roles in the microbial community. Their lost functions can be compensated for by "leakage" from synthesis-capable organisms since thiamine, a public good, is a readily available substance in the environment. In this manner, the auxotrophic functional bacterium ZM07 may be able to degrade THF more effectively and easily by forming a stable symbiotic system with non-THF-degrading bacteria in nature.

According to previous studies, cooperation through public good is ubiquitous among microbial members in nature (Crespi, 2001; West et al., 2006). In this study, we proposed a contact-independent interaction mode between ZM07 and K12. Based on the results of two-phase reactors, these two strains' cooperation relies on extracellular metabolic interactions, which do not require physical contact between strains (**Figure 3**). During THF degradation, cooperator K12 provides thiamine as a public good to ZM07, which is critical to growth and could pass through the membrane without difficulty (**Supplementary Figure S10**). Through the results of THF intermediate metabolites detection and using abilities of K12, THF-degrading bacterium ZM07 most likely provided available metabolites of THF that are easily used, such as succinate for K12 (**Supplementary Table S3** and **Supplementary Figure S6**). In addition, the highest OD_{600} value of ZM07 cultured separately with K12 in two-phase reactors reached only 1.323 ± 0.022 (**Figure 3A**), while it could reach up to 2.708 ± 0.012 with normal growth in the first transfer (**Figure 1A**). The low public goods exchange rate between the two strains might represent an important reason for its poor growth. Alternatively, there might be some other contact-dependent interaction mechanisms between these two strains that need to be further studied. However, cooperators (who produce public goods) would generate a metabolic cost, while all the microorganisms in the community benefit from it, regardless of whether they are producers (Brockhurst et al., 2010; Popat et al., 2012). Hence, such cooperative systems can easily be invaded by cheaters. The invasion of cheaters usually causes low proportions of cooperators who contribute public goods to maintain growth stability, consequently damaging the interests of the cooperative system (Dunny et al., 2008). In the cocultured system of ZM07, K12, and K12Δ*thiE* in thiamine-limited medium, the non-cooperator K12Δ*thiE* did not cause the entire community to crash; instead, it gradually disappeared during the passages (**Figure 5B**), indicating that the symbiotic system of strains ZM07 and K12 was stable and K12Δ*thiE* could not invade it. The reasons for the success of the cooperative system that resists the non-cooperator might be as follows. First, intraspecific competition experiments between the K12 wild type and defective strain showed that under thiamine-limited conditions, K12Δ*thiE* faced fierce intraspecific competition and was inhibited during coculture with K12 (**Figure 4**). Intraspecific competition experiments indicate that one of the reasons for the failure of K12Δ*thiE* is the insufficient thiamine secreted by K12 to the culture medium. A previous study showed that thiamine tends to be stored intracellularly rather than secreted extracellularly (Schyns et al., 2005), which also supports our conjectures. Furthermore, the results showed that the growth of strain ZM07 was inhibited by the shortage of thiamine in the community (**Supplementary Figure S5**) and that the degrading bacterial strain ZM07 cannot keep the entire community functioning with such low activity in the thiamine-limited medium. At the beginning of inoculation, strain ZM07 lacked thiamine, strain K12 lacked a carbon source, and both strains depended on each other to grow; hence, it was impossible to eliminate either of the two strains through rapid proliferation, which eventually might result in a stable symbiotic system. Experiments using trace amounts of K12 to rapidly restore the growth and THF-degrading ability of ZM07 (**Figure 5C**) also demonstrated the stability of the community of ZM07 and K12. In the cocultured system, strain ZM07 might have stronger thiamine utilization ability than K12Δ*thiE*, and since it can degrade THF, strain ZM07 could gain some preferential access to the broken-down products. These two reasons might explain why strain ZM07 shows a faster growth rate than K12Δ*thiE*, which might be an important factor in resisting non-cooperator invasion. In the fierce competition for dual nutrients (carbon source and thiamine), the non-cooperator could not find a sufficient niche in the coculture system without additional thiamine. Moreover, the dual pressures of carbon deficiency and thiamine deficiency might have accelerated K12Δ*thiE* extinction.

In this study, the addition of thiamine weakened the advantage of K12, whether it was cocultured with ZM07 alone or cocultured with both ZM07 and K12Δ*thiE*, and the relative abundance of K12 was decreased to a very low level in the cocultured systems (**Figures 6A,C**). Otherwise, no difference occurred in the proportions of K12 and K12Δ*thiE* when they were cocultured separately with ZM07 in thiamine-rich medium (**Figure 6D**), indicating that adding thiamine eliminates the advantage of the K12 wild type, which can only attain its position when needed by ZM07 in a cocultured system. Why could the cooperative system recognize the role of K12 and inhibit it under thiamine-rich conditions? We speculate that the THF-degrading bacterium ZM07 might reduce the secretion of intermediate metabolites of THF when thiamine is easily available. Conversely, strain

ZM07 has to maintain its THF-degrading ability through a metabolite cross-feeding interaction with K12 under thiamine-limited conditions. Furthermore, K12Δ*thiE* grew better than K12 in the three-strain system with additional thiamine (**Figure 6E**), indicating that there is a metabolic cost for thiamine synthesis as discussed above. In the three-strain communities in this study, the non-cooperator K12Δ*thiE* disappeared by the 8th transfer in thiamine-limited medium (**Figure 5B**) but remained in the medium supplemented with thiamine (**Figure 6C**), indicating that the additional thiamine might slow down the elimination of K12Δ*thiE* in cooperative communities. Accordingly, in the kingdom of the THF degradation system, cooperators may utilize thiamine as "rent" that they exchange for "food" (carbon sources) from the "landlord," the THF-degrading bacterium. Non-cooperators who are unable to provide "rent" are likely to be gradually eliminated.

Based on the above results, we propose a model depicting the succession process of these three strains during passaging in the communities. In a cocultured system, K12 provides thiamine, an essential growth factor, as a public good to thiamine auxotrophic bacteria ZM07 and K12Δ*thiE*; THF-degrading bacterium ZM07 degrades THF (the sole carbon source in the cocultured system) and provides easily accessible intermediates (succinate and other downstream products) to the cooperator strain K12 and the non-cooperator K12Δ*thiE* as carbon sources. Eventually, the non-cooperator K12Δ*thiE* (which can neither degrade THF nor produce thiamine) disappears during passaging, indicating that non-cooperator strain K12Δ*thiE* cannot invade the symbiotic ZM07 and K12 system (**Figure 7**). In thiamine-rich medium, both K12 and K12Δ*thiE* represented only a small proportion of the coculture system, while ZM07 became dominant (**Figure 7**). Based on the observed interactions between strains ZM07 and K12, "rent" must be paid to maintain a place in the symbiotic system of THF degradation; non-cooperators who cannot pay the "rent" might be excluded by the cooperative system. However, thiamine auxotrophic THF-degrading bacterium ZM07 might not need cooperators when surrounded by sufficient thiamine. Taken together, we show that cooperation and competition mechanisms exist in THF multispecies ecosystems to maintain the stability of communities. Auxotrophic degrading bacteria

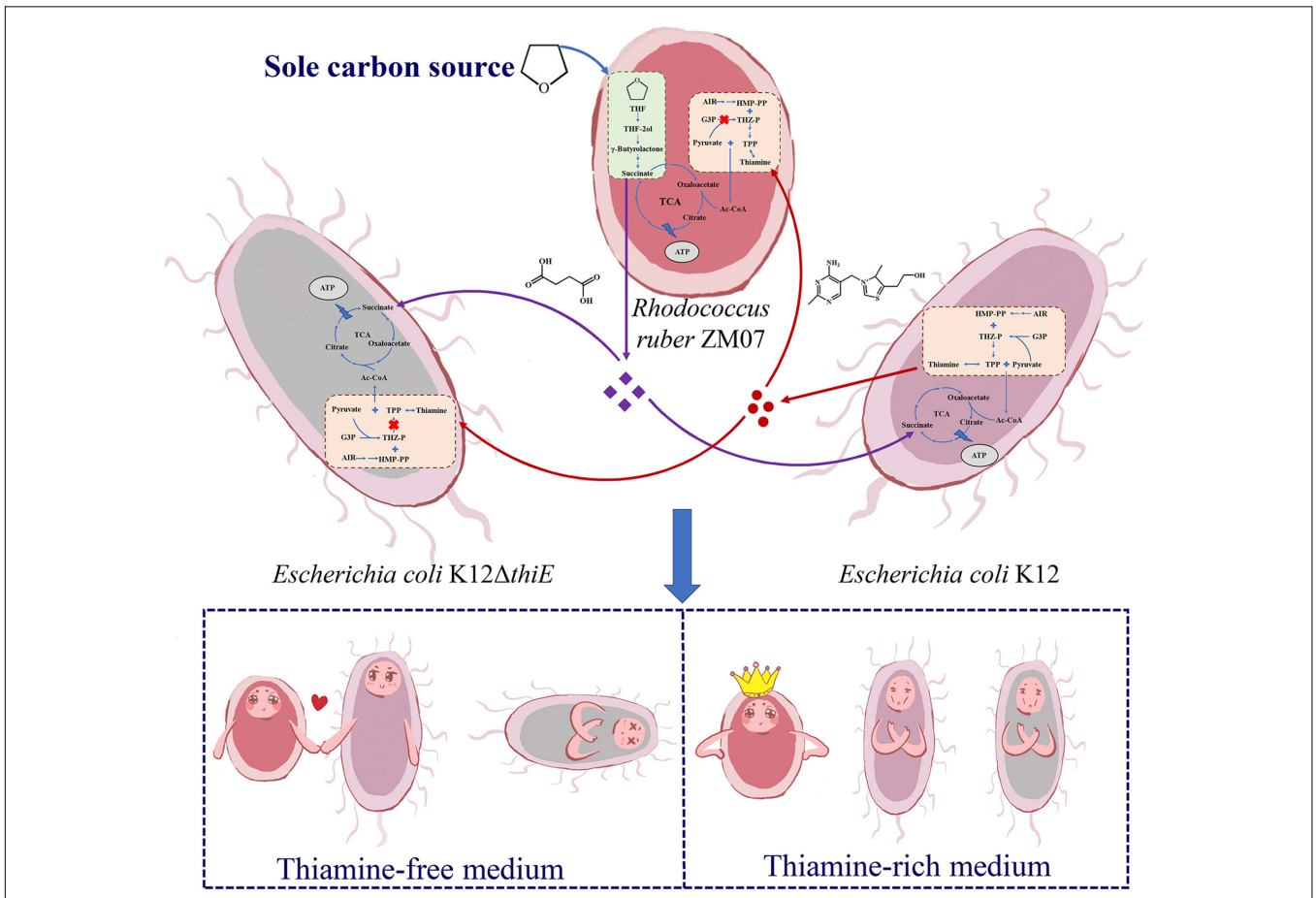


FIGURE 7 | Proposed schematic depiction of the interaction mechanism among strains ZM07, K12, and K12Δ*thiE* during THF degradation. Strain K12 provides thiamine (red circles) to ZM07 and K12Δ*thiE*, and strain ZM07 might provide succinate or other downstream products (purple diamonds) to K12 and K12Δ*thiE* as a carbon source by degrading THF. In thiamine-free medium, ZM07 and K12 formed a stable symbiotic system, and K12Δ*thiE* disappeared during passaging; in thiamine-rich media, both K12 and K12Δ*thiE* represented only a small proportion of the coculture system.

and their cooperators can play an important role in species coexistence in microbial communities.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

ZL and HH conceived and designed the experiments. HH and YL performed the experiments. HH, MQ, and YL analyzed the data. HH, HW, XW, and YQ wrote the manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This work was financially supported by the National Natural Science Foundation of China (Nos. 41630637 and 31370151).

ACKNOWLEDGMENTS

This is a short text to acknowledge the contributions of specific colleagues, institutions, or agencies that aided the efforts of the authors.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.594052/full#supplementary-material

REFERENCES

Amin, S., Hmelo, L., Van Tol, H., Durham, B., Carlson, L., Heal, K., et al. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522, 98–101. doi: 10.1038/nature14488

Bachmann, B. J., Low, K. B., and Taylor, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriol. Rev.* 40, 116–167. doi: 10.1128/mmbr.40.1.116-167.1976

Batut, B., Knibbe, C., Marais, G., and Daubin, V. (2014). Reductive genome evolution at both ends of the bacterial population size spectrum. *Nat. Rev. Microbiol.* 12, 841–850. doi: 10.1038/nrmicro3331

Begley, T. P., Downs, D. M., Ealick, S. E., Mclafferty, F. W., Van Loon, A. P., Taylor, S., et al. (1999). Thiamin biosynthesis in prokaryotes. *Arch. Microbiol.* 171, 293–300. doi: 10.1007/s002030050713

Bernhardt, D., and Diekmann, H. (1991). Degradation of dioxane, tetrahydrofuran and other cyclic ethers by an environmental *Rhodococcus* strain. *Appl. Microbiol. Biotechnol.* 36, 120–123. doi: 10.1007/BF00164711

Brockhurst, M. A., Habets, M. G., Libberton, B., Buckling, A., and Gardner, A. (2010). Ecological drivers of the evolution of public-goods cooperation in bacteria. *Ecology* 91, 334–340. doi: 10.1890/09-0293.1

Brown, S. P., and Johnstone, R. A. (2001). Cooperation in the dark: signalling and collective action in quorum-sensing bacteria. *Proc. R. Soc. Lond. B* 268, 961–965. doi: 10.1098/rspb.2001.1609

Bruger, E. L., and Waters, C. M. (2018). Maximizing growth yield and dispersal via quorum sensing promotes cooperation in Vibrio bacteria. *Appl. Environ. Microbiol.* 84:e00402-18. doi: 10.1128/AEM.00402-18

Chhabra, R. S., Elwell, M. R., Chou, B. J., Miller, R. A., and Renne, R. A. (1990). Subchronic toxicity of tetrahydrofuran vapors in rats and mice. *Toxicol. Sci.* 14, 338–345. doi: 10.1016/0272-0590(90)90213-4

Chiu, H.-J., Reddick, J. J., Begley, T. P., and Ealick, S. E. (1999). Crystal structure of thiamin phosphate synthase from Bacillus subtilis at 1.*25* Å resolution. *Biochemistry* 38, 6460–6470. doi: 10.1021/bi982903z

Crespi, B. J. (2001). The evolution of social behavior in microorganisms. *Trends Ecol. & Evol.* 16, 178–183. doi: 10.1016/S0169-5347(01)02115-2

Datsenko, K. A., and Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A* 97, 6640–6645. doi: 10.1073/pnas.120163297

Denome, S. A., Oldfield, C., Nash, L. J., and Young, K. D. (1994). Characterization of the desulfurization genes from *Rhodococcus* sp. strain IGTS8. *J. bacteriol.* 176, 6707–6716. doi: 10.1128/jb.176.21.6707-6716.1994

Dorrestein, P. C., Zhai, H., Mclafferty, F. W., and Begley, T. P. (2004). The biosynthesis of the thiazole phosphate moiety of thiamin: the sulfur transfer mediated by the sulfur carrier protein ThiS. *Chem. Biol.* 11, 1373–1381. doi: 10.1016/j.chembiol.2004.08.009

D'Souza, G., Waschina, S., Pande, S., Bohl, K., Kaleta, C., and Kost, C. (2014). Less is more: selective advantages can explain the prevalent loss of biosynthetic genes in bacteria. *Evolution* 68, 2559–2570. doi: 10.1111/evo.12468

Du, J., Zhou, J., Xue, J., Song, H., and Yuan, Y. (2012). Metabolomic profiling elucidates community dynamics of the Ketogulonicigenium vulgare–Bacillus megaterium consortium. *Metabolomics* 8, 960–973. doi: 10.1007/s11306-011-0392-2

Dubey, G. P., and Ben-Yehuda, S. (2011). Intercellular nanotubes mediate bacterial communication. *Cell* 144, 590–600. doi: 10.1016/j.cell.2011.01.015

Dunny, G. M., Brickman, T. J., and Dworkin, M. (2008). Multicellular behavior in bacteria: communication, cooperation, competition and cheating. *Bioessays* 30, 296–298. doi: 10.1002/bies.20740

Fiegna, F., and Velicer, G. J. (2003). Competitive fates of bacterial social parasites: persistence and self–induced extinction of Myxococcus xanthus cheaters. *Proc. R. Soc. Lond. B* 270, 1527–1534. doi: 10.1098/rspb.2003.2387

Filkins, L. M., Graber, J. A., Olson, D. G., Dolben, E. L., Lynd, L. R., Bhuju, S., et al. (2015). Coculture of *Staphylococcus aureus* with *Pseudomonas aeruginosa* drives *S. aureus* towards fermentative metabolism and reduced viability in a cystic fibrosis model. *J. Bacteriol.* 197, 2252–2264. doi: 10.1128/JB.00059-15

Gamer, A. O., Jaeckh, R., Leibold, E., Kaufmann, W., Gembardt, C., Bahnemann, R., et al. (2002). Investigations on cell proliferation and enzyme induction in male rat kidney and female mouse liver caused by tetrahydrofuran. *Toxicol. Sci.* 70, 140–149. doi: 10.1093/toxsci/70.1.140

Giovannoni, S. J., Thrash, J. C., and Temperton, B. (2014). Implications of streamlining theory for microbial ecology. *ISME J.* 8, 1553–1565. doi: 10.1038/ismej.2014.60

Hardin, G. (1968). The tragedy of the commons. *Science* 162, 1243–1248. doi: 10.1126/science.162.3859.1243

Hermida, S. A. S., Possari, E. P. M., Souza, D. B., Campos, I. P. D. A., Gomes, O. F., Mascio, P. D., et al. (2006). 2'-Deoxyguanosine, 2'-deoxycytidine, and 2'-deoxyadenosine adducts resulting from the reaction of tetrahydrofuran with DNA bases. *Chem. Res. Toxicol.* 19, 927–936. doi: 10.1021/tx060033d

Huang, H., Yu, H., Qi, M., Liu, Z., Wang, H., and Lu, Z. (2019). Enrichment and characterization of a highly efficient tetrahydrofuran-degrading bacterial culture. *Biodegradation* 30, 467–479. doi: 10.1007/s10532-019-09888-5

Hubalek, V., Buck, M., Tan, B., Foght, J., Wendeberg, A., Berry, D., et al. (2017). Vitamin and amino acid auxotrophy in anaerobic consortia operating under methanogenic conditions. *mSystems* 2:e00038-17. doi: 10.1128/mSystems.00038-17

Hummel, W., Schütte, H., Schmidt, E., Wandrey, C., and Kula, M.-R. (1987). Isolation of L-phenylalanine dehydrogenase from *Rhodococcus* sp. *M*4 and its application for the production of L-phenylalanine. *Appl. Microbiol. Biotechnol.* 26, 409–416. doi: 10.1007/BF00253523

Jin, Z., Li, J., Ni, L., Zhang, R., Xia, A., and Jin, F. (2018). Conditional privatization of a public siderophore enables *Pseudomonas aeruginosa* to resist cheater invasion. *Nat. Commun.* 9:1383. doi: 10.1038/s41467-018-03791-y

Jurgenson, C. T., Begley, T. P., and Ealick, S. E. (2009). The structural and biochemical foundations of thiamin biosynthesis. *Annu. Rev. Biochem.* 78, 569–603. doi: 10.1146/annurev.biochem.78.072407.102340

Katahira, T., Teramoto, K., and Horiguchi, S. (1982). Experimental studies on the acute toxicity of tetrahydrofuran in animals. *Sangyo lgaku* 24:373. doi: 10.1539/joh1959.24.373

Kohlweyer, U., Thiemer, B., Schrader, T., and Andreesen, J. R. (2000). Tetrahydrofuran degradation by a newly isolated culture of *Pseudonocardia* sp. strain K1. *FEMS Microbiol. Lett.* 186, 301–306. doi: 10.1111/j.1574-6968.2000.tb09121.x

Król, J. E., Rogers, L. M., Krone, S. M., and Top, E. M. (2010). Dual reporter system for in situ detection of plasmid transfer under aerobic and anaerobic conditions. *Appl. Environ. Microbiol.* 76, 4553–4556. doi: 10.1128/AEM.00226-10

Larkin, M. J., Kulakov, L. A., and Allen, C. C. (2006). Biodegradation by members of the genus *Rhodococcus*: biochemistry, physiology, and genetic adaptation. *Adv. Appl. Microbiol.* 59, 1–29. doi: 10.1016/S0065-2164(06)59001-X

Leinweber, A., Inglis, R. F., and Kümmerli, R. (2017). Cheating fosters species co-existence in well-mixed bacterial communities. *ISME J.* 11, 1179–1188. doi: 10.1038/ismej.2016.195

Li, Q., Lin, F., Yang, C., Wang, J., Lin, Y., Shen, M., et al. (2018). A large-scale comparative metagenomic study reveals the functional interactions in six bloom-forming microcystis-epibiont communities. *Front. Microbiol.* 9:746. doi: 10.3389/fmicb.2018.00746

Liu, Y.-F., Galzerani, D. D., Mbadinga, S. M., Zaramela, L. S., Gu, J.-D., Mu, B.-Z., et al. (2018). Metabolic capability and in situ activity of microorganisms in an oil reservoir. *Microbiome* 6:5. doi: 10.1186/s40168-017-0392-1

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Lujan, A. M., Gomez, P. S., and Buckling, A. (2015). Siderophore cooperation of the bacterium *Pseudomonas* fluorescens in soil. *Biol. Lett.* 11:20140934. doi: 10.1098/rsbl.2014.0934

Martinez-Gomez, N. C., Robers, M., and Downs, D. M. (2004). Mutational analysis of ThiH, a member of the radical S-adenosylmethionine (AdoMet) protein superfamily. *J. Biol. Chem.* 279, 40505–40510. doi: 10.1074/jbc.M403985200

Martínková, L., Uhnáková, B., Pátek, M., Nešvera, J., and Křen, V. (2009). Biodegradation potential of the genus *Rhodococcus*. *Environ. Int.* 35, 162–177. doi: 10.1016/j.envint.2008.07.018

Masuda, H., Mcclay, K., Steffan, R. J., and Zylstra, G. J. (2012). Biodegradation of tetrahydrofuran and 1,4-dioxane by soluble diiron monooxygenase in *Pseudonocardia* sp. strain ENV478. *J. Mol. Microbiol. Biotechnol.* 22, 312–316. doi: 10.1159/000343817

Mee, M. T., Collins, J. J., Church, G. M., and Wang, H. H. (2014). Syntrophic exchange in synthetic microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 111:201405641. doi: 10.1073/pnas.1405641111

Morenohorn, M., Garbe, L. A., Tressl, R., and Gorisch, H. (2005). Transient accumulation of γ-butyrolactone during degradation of bis(4-chloro-n-butyl) ether by diethylether-grown Rhodococcus sp. *strain DTB. Appl. Microbiol. Biotechnol.* 69, 335–340. doi: 10.1007/s00253-005-1984-4

Morris, B. E. L., Henneberger, R., Huber, H., and Moissleichinger, C. (2013). Microbial syntrophy: interaction for the common good. *FEMS Microbiol. Rev.* 37, 384–406. doi: 10.1111/1574-6976.12019

Morris, J. J. (2015). Black Queen evolution: the role of leakiness in structuring microbial communities. *Trends Genet.* 31, 475–482. doi: 10.1016/j.tig.2015.05.004

Morris, J. J., Lenski, R. E., and Zinser, E. R. (2012). The Black Queen hypothesis: evolution of dependencies through adaptive gene loss. *mBio* 3:e00036-12. doi: 10.1128/mBio.00036-12

Oh, S., Kim, J. R., Premier, G. C., Lee, T., Kim, C., and Sloan, W. T. (2010). Sustainable wastewater treatment: how might microbial fuel cells contribute. *Biotechnol. Adv.* 28, 871–881. doi: 10.1016/j.biotechadv.2010.07.008

Otero-Bravo, A., Goffredi, S., and Sabree, Z. L. (2018). Cladogenesis and genomic streamlining in extracellular endosymbionts of tropical stink bugs. *Genome Biol. Evol.* 10, 680–693. doi: 10.1093/gbe/evy033

Pande, S., Kaftan, F., Lang, S. N., Svatos, A., Germerodt, S., and Kost, C. (2016). Privatization of cooperative benefits stabilizes mutualistic cross-feeding interactions in spatially structured environments. *ISME J.* 10, 1413–1423. doi: 10.1038/ismej.2015.212

Parales, R., Adamus, J., White, N., and May, H. (1994). Degradation of 1, 4-dioxane by an actinomycete in pure culture. *Appl. Environ. Microbiol.* 60, 4527–4530. doi: 10.1128/aem.60.12.4527-4530.1994

Popat, R., Crusz, S. A., Messina, M., Williams, P., West, S. A., and Diggle, S. P. (2012). Quorum-sensing and cheating in bacterial biofilms. *Pro. R. Soc. Lond. B* 279, 4765–4771. doi: 10.1098/rspb.2012.1976

Rakoff-Nahoum, S., Coyne, M., and Comstock, L. (2014). An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr. Biol.* 24, 40–49. doi: 10.1016/j.cub.2013.10.077

Ren, H., Li, H., Wang, H., Huang, H., and Lu, Z. (2020). Biodegradation of tetrahydrofuran by the newly isolated filamentous fungus *Pseudallescheria boydii* ZM01. *Microorganisms* 8:1190. doi: 10.3390/microorganisms8081190

Schuster, M., Joseph Sexton, D., Diggle, S. P., and Peter Greenberg, E. (2013). Acyl-homoserine lactone quorum sensing: from evolution to application. *Annu. Rev. Microbiol.* 67, 43–63. doi: 10.1146/annurev-micro-092412-155635

Schyns, G., Potot, S., Geng, Y., Barbosa, T. M., Henriques, A., and Perkins, J. B. (2005). Isolation and characterization of new thiamine-deregulated mutants of *Bacillus subtilis*. *J. Bacteriol.* 187, 8127–8136. doi: 10.1128/JB.187.23.8127-8136.2005

Sei, K., Miyagaki, K., Kakinoki, T., Fukugasako, K., Inoue, D., and Ike, M. (2013). Isolation and characterization of bacterial strains that have high ability to degrade 1,4-dioxane as a sole carbon and energy source. *Biodegradation* 24, 665–674. doi: 10.1007/s10532-012-9614-1

Sieuwerts, S., De Bok, F. A. M., Hugenholtz, J., and Vlieg, J. E. T. V. H. (2008). Unraveling microbial interactions in food fermentations: from classical to genomics approaches. *Appl. Environ. Microbiol.* 74, 4997–5007. doi: 10.1128/AEM.00113-08

Skinner, K., Cuiffetti, L., and Hyman, M. R. (2009). Metabolism and cometabolism of cyclic ethers by a filamentous fungus, a *Graphium* sp. *Appl. Environ. Microbiol.* 75, 5514–5522. doi: 10.1128/AEM.00078-09

Smith, J. (2001). The social evolution of bacterial pathogenesis. *Proc. R. Soc. Lond. B* 268, 61–69. doi: 10.1098/rspb.2000.1330

Tajima, T., Hayashida, N., Matsumura, R., Omura, A., Nakashimada, Y., and Kato, J. (2012). Isolation and characterization of tetrahydrofuran-degrading *Rhodococcus aetherivorans* strain M8. *Process. Biochem.* 47, 1665–1669. doi: 10.1016/j.procbio.2011.08.009

Tao, K., Liu, X., Chen, X., Hu, X., Cao, L., and Yuan, X. (2017). Biodegradation of crude oil by a defined co-culture of indigenous bacterial consortium and exogenous *Bacillus subtilis*. *Bioresour. Technol.* 224, 327–332. doi: 10.1016/j.biortech.2016.10.073

Thiemer, B., Andreesen, J. R., and Schrader, T. (2003). Cloning and characterization of a gene cluster involved in tetrahydrofuran degradation in *Pseudonocardia* sp. strain K1. *Arch. Microbiol.* 179, 266–277. doi: 10.1007/s00203-003-0526-7

Van Gestel, J., Weissing, F. J., Kuipers, O. P., and Kovacs, A. T. (2014). Density of founder cells affects spatial pattern formation and cooperation in *Bacillus subtilis* biofilms. *ISME J.* 8, 2069–2079. doi: 10.1038/ismej.2014.52

Velicer, G. J. (2003). Social strife in the microbial world. *Trends Microbiol.* 11, 330–337. doi: 10.1016/S0966-842X(03)00152-5

Webb, E., Claas, K., and Downs, D. M. (1997). Characterization of thiI, a new gene involved in thiazole biosynthesis in *Salmonella typhimurium*. *J. Bacteriol.* 179, 4399–4402. doi: 10.1128/jb.179.13.4399-4402.1997

Webb, J. S., Givskov, M., and Kjelleberg, S. (2003). Bacterial biofilms: prokaryotic adventures in multicellularity. *Curr. Opin. Microbiol.* 6, 578–585. doi: 10.1016/j.mib.2003.10.014

West, S. A., Griffin, A. S., Gardner, A., and Diggle, S. P. (2006). Social evolution theory for microorganisms. *Nat. Rev. Microbiol.* 4, 597–607. doi: 10.1038/nrmicro1461

Wexler, A. G., and Goodman, A. L. (2017). An insider's perspective: bacteroides as a window into the microbiome. *Nat. Microbiol.* 2:17026. doi: 10.1038/nmicrobiol.2017.26

Wolf, Y. I., and Koonin, E. V. (2013). Genome reduction as the dominant mode of evolution. *Bioessays* 35, 829–837. doi: 10.1002/bies.201300037

Yao, Y., Lv, Z., Min, H., Lv, Z., and Jiao, H. (2009). Isolation, identification and characterization of a novel *Rhodococcus* sp. strain in biodegradation of tetrahydrofuran and its medium optimization using sequential statistics-based experimental designs. *Bioresour. Technol.* 100, 2762–2769. doi: 10.1016/j.biortech.2009.01.006

Zengler, K., and Zaramela, L. S. (2018). The social network of microorganisms—how auxotrophies shape complex communities. *Nat. Rev. Microbiol.* 16:383. doi: 10.1038/s41579-018-0004-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

REVIEW
published: 20 January 2021
doi: 10.3389/fmicb.2020.571417

The Less Expensive Choice: Bacterial Strategies to Achieve Successful and Sustainable Reciprocal Interactions

*Enrica Pessione**

Department of Life Sciences and Systems Biology, Università degli Studi di Torino, Torino, Italy

Bacteria, the first organisms that appeared on Earth, continue to play a central role in ensuring life on the planet, both as biogeochemical agents and as higher organisms' symbionts. In the last decades, they have been employed both as bioremediation agents for cleaning polluted sites and as bioconversion effectors for obtaining a variety of products from wastes (including eco-friendly plastics and green energies). However, some recent reports suggest that bacterial biodiversity can be negatively affected by the present environmental crisis (global warming, soil desertification, and ocean acidification). This review analyzes the behaviors positively selected by evolution that render bacteria good models of sustainable practices (urgent in these times of climate change and scarcity of resources). Actually, bacteria display a tendency to optimize rather than maximize, to economize energy and building blocks (by using the same molecule for performing multiple functions), and to recycle and share metabolites, and these are winning strategies when dealing with sustainability. Furthermore, their ability to establish successful reciprocal relationships by means of anticipation, collective actions, and cooperation can also constitute an example highlighting how evolutionary selection favors behaviors that can be strategic to contain the present environmental crisis.

Keywords: economize, sharing, storing, cooperative behaviors, system communication

OPEN ACCESS

Edited by:
Dimitrios Georgios Karpouzas, University of Thessaly, Greece

Reviewed by:
James Q. Boedicker, University of Southern California, Los Angeles, United States
Valeria Souza, National Autonomous University of Mexico, Mexico
Antonis Chatzinotas, Helmholtz Centre for Environmental Research (UFZ), Germany

***Correspondence:**
Enrica Pessione
enrica.pessione@unito.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *10 June 2020*
Accepted: *11 December 2020*
Published: *20 January 2021*

Citation:
Pessione E (2021) The Less Expensive Choice: Bacterial Strategies to Achieve Successful and Sustainable Reciprocal Interactions. Front. Microbiol. 11:571417. doi: 10.3389/fmicb.2020.571417

INTRODUCTION

The role of microorganisms as actors in environmental sustainability has long been established (Kuhad, 2012). Since the last century, both bacteria and fungi have been employed in bioremediation of polluted sites with particular reference to hydrocarbons and heavy metals (Dash et al., 2013). In the changing environment of the 21st century, deeply marked by environmental challenges such as climate change, resource exhaustion, and demographic pressure, advances in applied research allow exploiting the microbial potential to maintain favorable conditions for life on the Earth. Bacteria can be used in the bioconversion of wastes for (i) generation of renewable energy (microbial fuel cells, methane, biobutanol, bioethanol, and biohydrogen production) (Cho et al., 2020; Mazzoli et al., 2012), (ii) synthesis of bio-based and biodegradable plastic polymers from lignocellulosic wastes (Mazzoli et al., 2014; Abdel-Rahman and Sonomoto, 2016), (iii) production of antibiotic alternatives (Cotter et al., 2013), and (iv) supporting organic agriculture and reducing greenhouse gases (methanotrophic archaea and photosynthetic plankton) (Kuhad, 2012).

However, it has been recently pointed out that microbial communities can suffer from unfavorable life conditions such as those arising from the over-exploitation of soils in conventional intensive agriculture (Kuhad, 2012) and from the growing global warming (Cavicchioli et al., 2019). Actually, the reduction of microbial biodiversity could impact the ability of other species to survive in both terrestrial and aquatic environments, because changes at the microbial community level can affect overall ecosystem biochemical fluxes important in supporting biogeochemical cycles and, ultimately, life on the planet (Cavicchioli et al., 2019). Although bacteria, as extensively reported in the present review, display excellent capabilities to adapt to changing conditions, their potential to face a very high number of environmental challenges can be threatened.

The present review explores a different standpoint on the role of bacteria in sustainability: considering them not as effectors of eco-friendly technological approaches but as models of behaviors that were positively selected by a very long evolutionary history and are still successful. Fast-changing environments, especially for non-symbiotic microorganisms, are the rule in bacterial life, as well as resource exhaustion and demographic pressure. In this scenario, their tendency for optimization rather than maximization, their interconnections, cross-feeding, sharing, and recycling together with their propensity to economize can be seen as valuable examples of successful ways of living, even in scarcity of resources and in rapid environmental changes as those we are currently facing.

Here, I will first try to examine the concept of bacterial intelligence and then I will describe some examples of cheap strategies and cooperative behaviors set up by bacteria at different levels to reach better fitness and more complex degree of organization.

BACTERIAL INTELLIGENCE AND INTERACTIVE CONNECTIONS

Although the term intelligence is generally applied to brain-bearing animals, evidence supports the idea of different forms of intelligence in brainless living organisms such as plants (Trewavas, 2002) and microbes (Hellingwerf, 2005). As reported by Shapiro (2007), bacteria are "small but not stupid": they can explore their environment and then adapt accordingly to optimize survival and fitness. The more complex are the external conditions, the more sophisticated is the network to cope with. Not only the genetic size (especially richness/abundance in genes encoding proteins involved in signal transduction) but also long-term (transcription and translation control) and short-term (post-translational modifications, proteolysis, allosteric effects) regulations can account for this adaptation abilities.

This intelligence is not an immaterial concept but is based on precise molecular structures and devices (for the role of molecular conformational states in bacterial intelligence, see Westerhoff et al., 2014; for exhaustive review on bacterial intelligence and signal transduction systems in prokaryotes, see Lyon, 2015; Pinto and Mascher, 2016). The module "sense, integrate information and coherently respond," also called adaptive behavior, is a well-recognized form of intelligence based on the same scheme as neurons, largely spread in bacteria where a sensory receptor, an information processing unit and a motor activity constitute the simplest system sometimes referred as "nanobrain" (Webre et al., 2003). As an example, in *Escherichia coli*, the flagellar motor turns clockwise generating tumbling movements. When a signal molecule (i.e., a nutrient) is sensed by a surface receptor, a phosphorylation transduction cascade is used to trigger counterclockwise rotation of flagellar motor, thus allowing swimming motility toward the nutrient (chemotaxis) (Bourret and Stock, 2002). A protein-based information processing unit constituting a sort of "sensing–integrating–responding" system is the stressosome found in *Bacillus subtilis*. The latter is a micro-organ with higher degree of complexity than the simple motility device finalized to chemiotaxis, able to set differentiated responses to different stressors (Marles-Wright et al., 2008). Additional functional possibilities can be found in prokaryotes such as memory (Wolf et al., 2008), learning (Hoffer et al., 2001), anticipation (Mitchell et al., 2009; Goo et al., 2012), decision making (Adler and Tso, 1974; Ross-Gillespie and Kümmerli, 2014), coordinated movements (Kaiser and Warrick, 2014), and cooperative interactions (Jacob et al., 2004).

The concept *memory–learning–anticipate* strongly underlines that prokaryote reactions take into account not only space (the environment) but also time (Tagkopoulos et al., 2008; Mitchell et al., 2009; Lyon, 2015; Pinto and Mascher, 2016). As an example, saving resources for the future is a possible mechanism elicited by the "awareness" of possible future scarcity. When environmental conditions (e.g., anaerobiosis) inhibit TCA cycle and make ATP synthesis impossible, *Acinetobacter* strains consume all the cellular ATP to survive and store carbon resources as polyhydroxylkanoates (PHA) (Deinema et al., 1980). Meanwhile, they use this steady-state period to activate a "luxury uptake system" of phosphorus that will allow them an "overplus accumulation" of phosphorus into polyphosphates when oxygen is supplied and normal energetic conditions are restored (Deinema et al., 1980). In this second phase, stocked PHA are consumed and ATP synthesis can proceed. Hence, phosphorus is not only used to restore the ATP stock but also intracellularly accumulated as polyphosphate (Deinema et al., 1980). This strategy protects bacteria from further starvation risks (**Figure 1**). The duration of memory (i.e., ability to retain and store information) varies depending on the situation, from the time necessary for protein modification to long-term (transgenerational) epigenetic modifications (Wolf et al., 2008). Learning (i.e., the ability to assimilate new information) is based on gene/operon autoamplification (Hoffer et al., 2001) and represents the obligate path to be able to respond with anticipatory behavior.

As far as *problem solving–decision making* is concerned, there are several examples highlighting that bacteria can sense the environment and evaluate the costs and benefits, generally making the less expensive choice. In *Pseudomonas aeruginosa*, there are two different siderophores: pyochelin

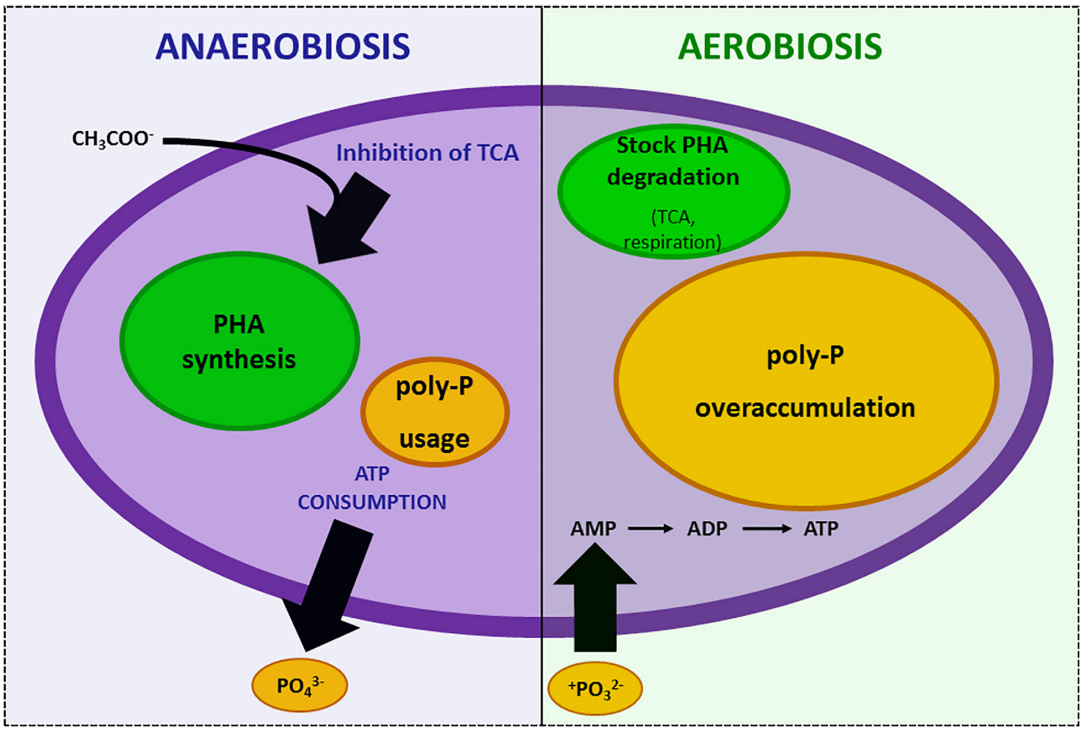


FIGURE 1 | Example of "memory" in strictly aerobic *Acinetobacter* sp. during anaerobiosis–aerobiosis cycles. In anaerobiosis, carbon nutrients cannot be metabolized in the TCA cycle and are accumulated as polyhydroxyalkanoates (PHA) while cells consume all the ATP and phosphorus stocks. This stress condition induces luxury uptake of phosphorus when oxygen availability restores respiration and over-accumulation of phosphate as polyphosphate (poly-P).

is a low-cost (only six genes involved in its biosynthesis) and low-efficiency system and pyoverdine is a high-cost (14 genes involved) and high-efficiency device. It has been demonstrated that this bacterium normally utilizes the low-cost low-efficiency mechanism using the high-cost high-efficiency system only in extreme iron-limiting conditions (Dumas et al., 2013). Similarly, *E. coli* can "choose" among three different systems to uptake/assimilate ammonia that are hierarchically regulated to prevent ATP waste and futile cycles (van Heeswijk et al., 2013). These two examples underline how evolution has shaped bacterial intelligence to limit energy loss and waste production.

A further degree of complexity concerns the so-called *bacterial social intelligence*, i.e., capability of bacteria to cross-talk and to *act collectively* as observed in population decision making (Goo et al., 2012), movement coordination (swarming) (Kearns, 2010), and cooperation (Tarnita, 2017). These collective behaviors, which include both synergies and conflicts of interest, require coordination and flexibility since the external conditions may rapidly change and the choice between a "faster-and-less-accurate" response (if there is need of rapid but transient coordination) and "more-accurate-but-slower" response (in case of necessity to maintain long-term cooperation) should be clearly established (Ross-Gillespie and Kümmerli, 2014).

The discovery of quorum sensing (QS) (Fuqua et al., 1994; Miller and Bassler, 2001) has shed light on a phenomenon known among humans as "unity is strength." Collective decision making has been successfully selected by evolution since individual actions have generally low impact and are seldom winning strategies. If a low number of bacterial cells begins to produce toxins or bacteriocins, the most probable event is the defeat. Actually, host immune system or surrounding bacteria sense this and rapidly react accordingly. Briefly, the result is that the attacking cells become victims. If, conversely, the cells begin to produce and secrete weapons when they are in significant number, it is probable that the battle will be won. Once a threshold concentration of diffusible autoinducers (molecules of different chemical structure, for comprehensive reading see Visick and Fuqua, 2005) is reached, revealing that the population number is high, sensing and amplification phenomena occur, thus triggering transcriptional responses resulting in several phenotypic changes such as light or pigment production, bacteriocin/toxin release, competence, sporulation and biofilm formation (**Figure 2**). Sometimes, the system is so sophisticated that different virulence factors are sequentially produced to obtain a time course effect (Waters and Bassler, 2005). In complex ecosystems, bacteria have to select QS signals from a background noise created by the large number of molecules present in the environment; however, despite these disturbances, feedbacks reveal that this mechanism is able to set up and optimize complex responses as well as to coordinate social behaviors such as collective decision making (Visick and Fuqua, 2005).

A paradigmatic example of collective anticipative behavior is described in *Burkholderia.* When a QS-mediated information

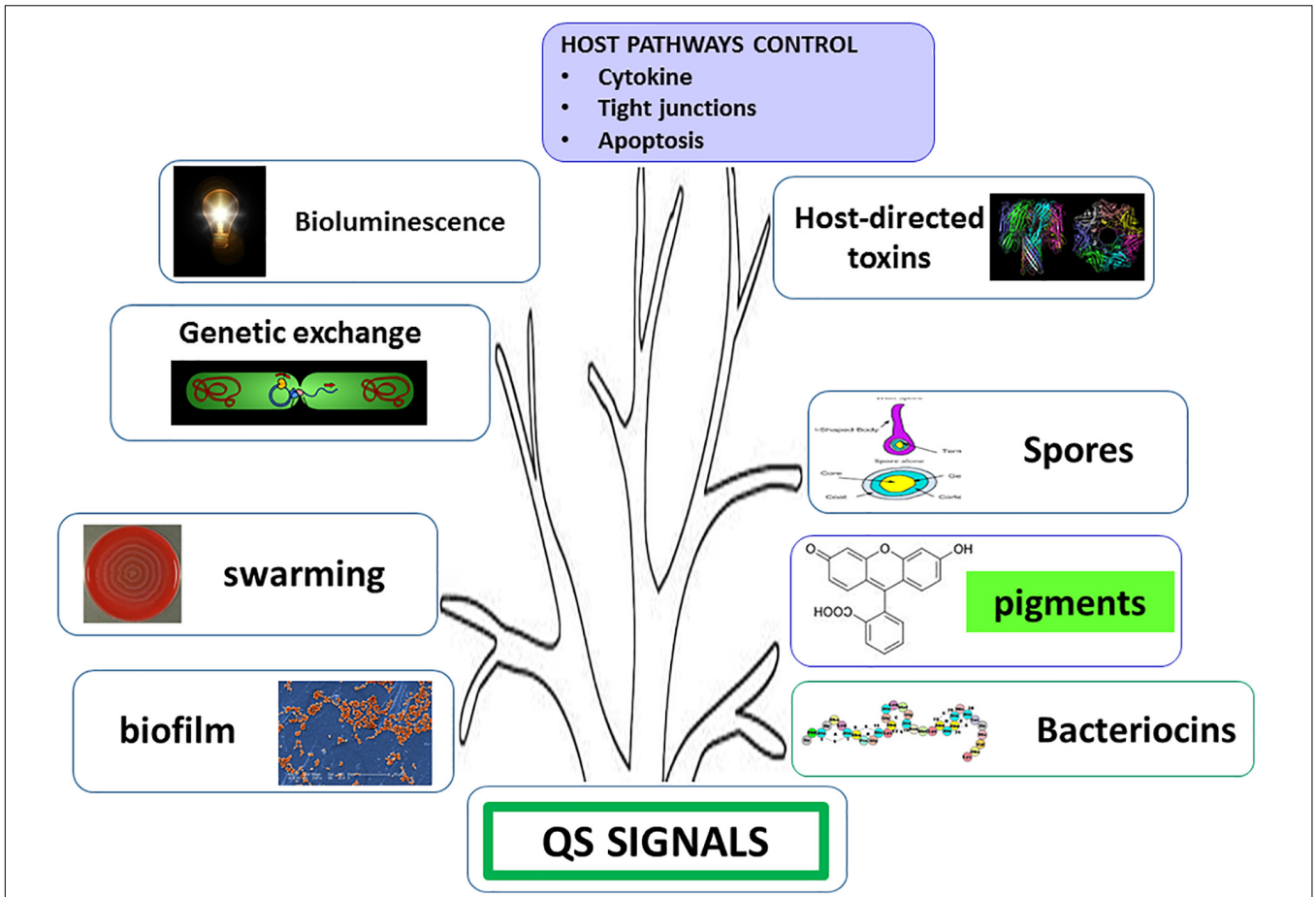


FIGURE 2 | Phenotypic traits under quorum sensing (QS) control. Enhanced interbacterial cooperation (genetic exchange, swarming, and biofilm production) and antagonism (production of pigments and bacteriocins), increased environmental resistance (sporulation), and host cross-talk are induced by QS signals.

is spread in the population, revealing that a threshold biomass is reached and stationary phase is approaching, cells begin to synthesize oxalic acid. This is achieved in conditions of neutral pH (and therefore not as a simple mechanistic response induced by high pH) to preventively buffer alkalization that will occur in stationary phase due to ammonia accumulation following cell lysis and protein/amino acid degradation (Goo et al., 2012). Similarly, a collective decision based on QS and concerning competence occurs in *B. subtilis* where only some cells in the overall population acquire the "competent cell" phenotype (Leisner et al., 2008).

Regarding motility, apart from passive movements especially observed in non-flagellated bacteria (gliding, darting, sliding, floating, and twitching) (Jarrell and McBride, 2008), generally chemotactic behavior is achieved by individual swimming (Bourret and Stock, 2002). Besides this, coordinated collective motility known as swarming is present in several Gram-negative Enterobacteria such as *Proteus* and *Salmonella* (Kim and Surette, 2004; Sturgill and Rather, 2004). Swarming motility is not chemotactic but set up as a means to facilitate access to nutrients and oxygen to all population components, thus preventing intercellular competition (Kaiser and Warrick, 2014). Individual cells align each other (like birds during migration flights) using type IV pili, thus giving origin to a structured expanded movement where the motility apparatus continuously changes position, causing a reverse of direction about every 8 min, probably finalized to avoid collisions. In this mechanism, best studied in *Myxococcus xanthus*, about 40 genes are involved, including a cytoplasmic protein acting as a "pacemaker" (Kaiser and Warrick, 2011). Swarming bacteria are phenotypically different from swimming cells: proteomic analyses revealed that, in *Salmonella*, several proteins are 5- to 20-fold differentially abundant and swarmers displayed higher antibiotic resistance (Kim and Surette, 2004). In *Proteus mirabilis*, the phenotypic changes observed during swarming are controlled by putrescine (the decarboxylation product of ornithine) that act as a signaling system (Sturgill and Rather, 2004).

Discussing cooperative behaviors in bacteria is challenging because cooperation and competition are often intertwined, and the former does not exclude conflicts. In general, cooperation benefits have to outweigh cooperation costs considering that both environmental and time-related factors are crucial in driving eco-evolutionary forces. Therefore, defining bacterial

social behavior without a clear ecological context is erroneous (Werner et al., 2014; Tarnita, 2017). According to some authors, synthesizing antagonistic molecules is expensive and decrease biomass production. Therefore, competition or selfish behavior is confined to periods of nutrient abundance (Mora et al., 2013). These authors describe how, in lactic acid bacteria (LAB), the abundance of the carbon substrate has shaped the phenotype from cooperative to selfish. Based on the assumption that respiration is a cooperative behavior allowing sharing of resources in the ecological niche, whereas fermentation is a selfish behavior consuming resources and producing antagonistic compounds (the pH-lowering lactic and acetic acid), the authors speculate on the evolutionary origin of these alternative pathways. Actually, most food-related LAB such as *Streptococcus thermophilus* have lost the capability (present in other pathogenic streptococci best adapted to the animal ecological niche) to synthesize toxins (hemolysins) able to subtract heme from the animal host. For this reason, they cannot have functional respiratory chains (cytochromes contain heme) and are compelled to perform lactic fermentation or mixed-type fermentation (Pessione, 2012). Their adaptation to the milk environment has occurred because, in parallel with the loss of toxin-encoding genes, they have acquired genes for lactose uptake and utilization together with genes involved in pH balancing (Arioli et al., 2010). The overall consequence is that food-related LAB display an antagonistic behavior consuming nutrient resources and producing pH-lowering compounds, while in different conditions, LAB can cooperate with their partners sharing the same ecological niche. Nevertheless, according to other authors, competition increases during scarcity of nutrients and harsh conditions (Escalante and Travisano, 2017). An example is the emergence of GASP cells in *E. coli* during stationary phase. In general, in nutrient scarcity and waste accumulation conditions, wild-type cells stop growing to control population density, provide a slow decay, and guarantee long-term sustainability. However, GASP mutants with selfish behavior, lacking growth inhibitory mechanisms, appear, thus accelerating abrupt death due to overcrowding (ecological collapse) (Vulić and Kolter, 2001). In spite of these opposite considerations, it is well recognized that conflict resolution and adaptive cooperation to maintain phenotypic diversity is a winning road to increase biological complexity during evolution, including major evolutionary transitions toward multicellular organisms (Escalante and Travisano, 2017). Several examples concerning the trend toward cooperation, biofilm development, and maintenance will be examined in subsequent sections.

COOPERATIVE STRATEGIES: SO ADVANTAGEOUS AND SO DIFFICULT TO ACHIEVE

Mutual benefit and altruism are the two types of cooperative behavior. Mutual cross-feeding (reciprocal exchange of nutrients) and syntrophic chains (one species utilizes the waste end products of another species supplying its wastes to a third one, and so on, in a vertical temporal sequence and receiving fitness benefits at each step) (Smith et al., 2019) are reciprocally advantageous, supporting optimized exploitation of resources and reducing waste accumulation in a certain ecological niche (**Figure 3a**). Sulfur-reducing and sulfur-oxidizing bacteria spatially distribute in consortia facilitating metabolite exchanges (Müller and Overmann, 2011). These evolution-driven behaviors from one side can be exploited in the circular economy approach to sustainability (e.g., converting carbon wastes into methane, through a long synthrophic chain that includes acidogenic and acetogenic bacteria), from another they can be seen as examples of sharing and recycling with minimal waste production. In addition to nutritional cooperation, when

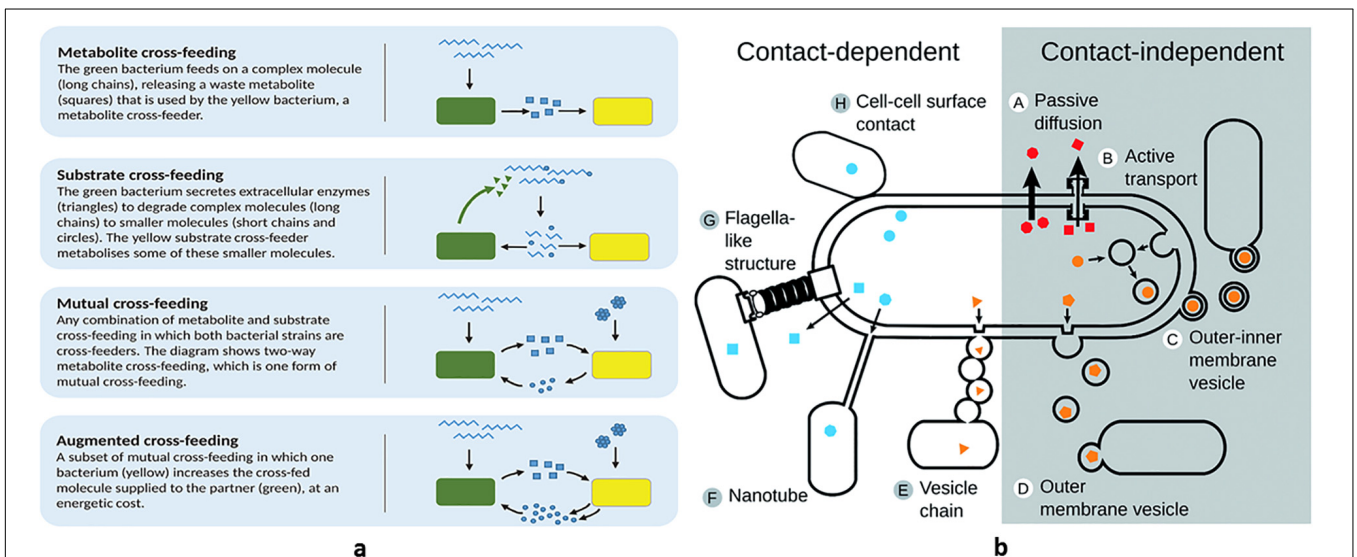


FIGURE 3 | Bacterial syntrophic and interactive behaviors: examples of **(a)** syntrophies and **(b)** metabolite exchange.

preceded by gene duplication, gene exchange and recombination is a reciprocally advantageous event as well, supporting all the population with new possibilities to cope with the external environment (capability to utilize new nutrients and antibiotic resistance).

Conjugation offers an emblematic example of intelligent cross-talk finalized to enhance population fitness. Actually, plasmid-bearing donors are not always ready to transfer their DNA to recipients, demonstrating that conjugation is not a simple mechanistic and random event. To convert donors into transfer competent cells, the two partners have to communicate. In the *E. faecalis* model, the recipient secretes peptide pheromones that, once up-taken by the donor cell, induce transcription of the conjugative apparatus by removing a transcriptional repressor (Clewell, 2011). Conversely, in the *B. subtilis* model is the donor that secretes an inhibitory peptide that, once taken up by the recipient, allows the regulatory network to act inducing expression of the gene transfer apparatus (Singh et al., 2013). In both these ways, plasmid is successfully transferred, avoiding random distribution. Furthermore, since transfer-competent cells bear some disadvantages such as increased risk to be attacked by phages at the pilus level (Frost and Koraimann, 2010), higher sensitivity to bile salts (Bidlack and Silverman, 2004), and general stressors (Zahrl et al., 2006), this condition is triggered only when strictly necessary and this is beneficial for both the donor and the recipient. An additional mechanism concerning gene transfer is that in the overall population of potential donors, only a small fraction (0.1–1%) is converted into transfer-competent cells (Wagner et al., 2013). This allows the majority of cells to maintain the plasmid (even for many generations) without paying the costs of its replication and of the expression of the transfer machinery (Koraimann and Wagner, 2014). Whether this subpopulation of transfer-competent cells arises randomly or is affected by environmental/phenotypic factors (e.g., position of the colony in solid media) has to be fully elucidated yet (Reisner et al., 2012).

A more recently discovered mechanism that supports both nutrient sharing and genetic exchanges is the one linked to membrane vesicles. This system also ensures communication between bacterial cells, since, inside the vesicles, different cargo molecules can be transported (nutrients, DNA, and hydrophobic QS peptides) (Toyofuku et al., 2020). Vesicles are present in Gram-negative and Gram-positive bacteria and can originate both in the outer (Manning and Kuehn, 2011) and in the cytoplasmic membrane (Lee et al., 2009). The role of lysogenic phages in membrane vesicle biogenesis has been ascertained and recently reviewed (Pessione, 2020). The system, used to translocate the transported molecules directly inside the target cell by means of membrane fusion, proved to be more efficient than simple metabolite secretion since inside the vesicles the different compounds are protected both from enzymatic degradation and from host factors (for instance, quorum quenching in case of symbiont bacteria) (**Figure 3b**). Thus, membrane vesicles proved to be useful tools to avoid loss of important and costly molecules.

Sometimes, however, mutual benefits can shift into altruistic behaviors that can damage the effectors and, over long time, the overall population. This is the case of the so-called shared good (or public goods) that especially in liquid habitats can easily diffuse and reach a relative distance from the producer. In *Pseudomonas*, it has been demonstrated that the production of the siderophore pyoverdine can enhance fitness, facilitating iron uptake and utilization, especially in iron-limiting conditions. However, these molecules are not cell-bound but released into the external environment thus becoming accessible for surrounding bacteria (West and Buckling, 2003). This sharing could be advantageous if all the cells contribute to the public good secretion. On the contrary, some mutant cells (called cheaters and consisting in about 9% of the population) emerge that benefit of the secreted siderophores without contributing to their production (Butaité et al., 2017). Among these mutants, some have lost their pyoverdine-synthesis cluster (structural non-producers) whereas others display a complete but inactive locus, producing only residual amounts of the siderophore (silent non-producers). Because of the lower effort in pyoverdine biosynthesis, cheaters have better opportunity to use energy for duplication, thus becoming more and more abundant in the overall population. When this occurs in liquid cultures, the original siderophore producers go extinct and the gene for siderophore biosynthesis is soon lost, rendering the community unable to survive during iron scarcity (Griffin et al., 2004). Despite this so-called "tragedy of the commons," possible escape lanes for saving the altruistic and therefore the overall population have been reported: (i) presence of low-affinity iron receptors in the cheaters not allowing them to outcompete the pyoverdine producers and (ii) appearance of producers secreting pyoverdines that repress rather than promote the growth of cheaters (Butaité et al., 2017). These resistance mechanisms toward cheaters finally support the cooperative behavior favoring maintenance of biodiversity in the bacterial community.

Different strategies to control cheaters and to specifically direct nutrients toward selected partners have been described, all implicating spatial structuring that limits public good diffusion (Lion and Baalen, 2008). Several examples underline the importance of the close proximity of bacterial cells to achieve the best performance: hydrogen donors and autotrophic methanogenic Archea organize in flocks (Boetius et al., 2000) and a peculiar physical connection through pili that provides direct electron transfer between bacteria (microbial nanowires) also exists (Summers et al., 2010) (**Figure 3b**). However, the best solution for limiting loss of shared good and for facilitating time-course cooperation is attachment to (biotic and abiotic) solid surfaces as it occurs in biofilms (Werner et al., 2014). This strategy, discussed in a succeeding section, also prevents wasting of precious and costly molecules.

Actually, a different scenario is observed in solid environments, suggesting that the sessile way of life is a fundamental step of evolution that can partly control the cheater damage, reduce energetic costs, and favor cooperative behaviors. Actually, in solid media, surrounding cells are "relatives" (i.e., offspring of the parent cell); hence, producers

share the public good with genetically related cells, rendering the behavior (resulting in indirect fitness effects = kin selection) not altruistic but mutualistic (Hamilton, 1964). A further more complex mechanism to isolate cheaters is the cell spatial distribution on solid surfaces, where an assembling of cooperative cells minimizes parasitic behaviors (Kovács, 2014). It has been reported that EPS-producing *B. subtilis* (i.e., those able to generate a biofilm) are advantageously selected over EPS non-producers only if high spatial segregation occurs (thus allowing EPS to be available only to cooperative cells), whereas in low-assortment conditions, cheaters can be favored due to public good availability and lower energetic investment in EPS production. Furthermore, in this model, optimization rather than maximization of EPS production is observed, suggesting that the fitness costs required by the biosynthetic effort is high (Van Gestel et al., 2014). An important factor favoring segregation is the high amount of nutrients: in nutritionally poor media, cross-feeding becomes necessary, thus facilitating low assortment. Even in this case, however, exclusion of non-producers can also occur in mixed communities if a preferential cross-feeding between two cooperators is established (Kovács, 2014). All these mechanisms ensure that good-sharing attitude will not be lost, without the necessity to neutralize cheaters by secreting antimicrobial compounds.

BACTERIAL BIOFILMS: A MODEL OF "HIGH FREEDOM DEGREE" COMMUNITY?

Besides being considered for long time as unicellular organisms, bacteria revealed a complex social organization based on partial differentiation of cell types and exchanging a huge number of data through a complex network of both hydrophobic and water-soluble molecular signals (Shapiro, 1998). This community, called biofilm, has the merit to have depicted in an unambiguous way that cooperative behavior is as important as competition in the evolution of microbial living systems (Crespi, 2001). Actually, biofilm is a successful systemic structure anchored to biotic or abiotic surfaces (O'Toole et al., 2000) that allows nutrient sharing (Costerton et al., 1995), favors genetic exchanges among single cells (Merod and Wuertz, 2014), and protects bacteria from exogenous stressors like animal immune systems (Clutterbuck et al., 2007) and antibiotics (Olsen, 2015). Biofilm lifestyle constitutes, after all, the preferred way of living of prokaryotes for most of their life cycle (Costerton et al., 1995).

The main advantage of bacterial biofilms in relation to multicellularity of higher organisms lies in the relative higher freedom degree of the cells living in these communities. Contact and continuity are ensured because cells are embedded into a self-produced extracellular polymeric matrix (for differences in its composition, see Hobley et al., 2015) that acts as a connective tissue; however, differentiation is relatively limited. In higher organisms, cell differentiation is necessary for workflow partitioning. Actually, single cells belonging to multicellular organisms (a part from stem cells) cannot perform all the functions encoded in their genomes since most activities are repressed to address energy toward very specific tasks, according to the tissue/organ they belong, such as thyroid hormone synthesis, keratin production, and so on. They acquire a strong tissue identity and do not move from the site they live except during metastasis. The paradigm of single-species biofilm-living bacteria is different: (i) each cell does not deprive itself of the majority of its physiological functions, (ii) differentiation giving rise to a certain degree of phenotypic heterogeneity is generally a reversible event or only concerns some clones, and (iii) the process of living in community can be stopped, allowing some of the cells to revert back to the planktonic lifestyle (Jefferson, 2004).

It is worth highlighting that biofilm-living cells display a different gene expression profile from planktonic cells: as an example, a higher expression of genes involved in iron metabolism is observed since iron level is critical for expression of adhesion factors important in biofilm formation (Nakamura et al., 2016). A second feature that differs is motility: in *B. subtilis*, the operon involved in biofilm matrix biosynthesis also encoded an inhibitor of motility demonstrating that sessile lifestyle and planktonic lifestyle are oppositely regulated since matrix-embedded lifestyle hinders movements (Blair et al., 2008). However, transcriptomic data also confirm that flagella-related gene expression is dependent on the biofilm growth phase, highlighting the different importance of flagella during the adherence, maturation, and dispersal steps (Nakamura et al., 2016). Curiously, recently dispersed planktonic cells proved to be different both from biofilm parent cells and from truly planktonic ones (Guilhen et al., 2016, 2017), being more similar to cells giving rise to biofilm formation (Sauer et al., 2002).

It is evident from the above reported data that when talking about biofilm, considering the dynamic temporal evolution of phenotypes is of fundamental importance. The three main phases of a biofilm life, namely, adherence, maturation, and dispersal, are consistent with different phenotypes, similarly with what occurs in higher animals during aging (Steenackers et al., 2016). However, also at each developmental stage, bacterial subpopulations may arise, generating cell heterogeneity finalized to cooperation and to obtain best fitness for the whole community. Van Gestel et al. (2015) proposed the term "division of labor" to indicate the functions of cells specialized in different tasks and based on cell types displaying different gene expression profiles.

It is well recognized that differentiation into various cell phenotypes is mainly due to environmental gradients. In heterogeneous biofilms (made up of cells belonging to different species and, sometimes, different kingdoms), cells colonize different biofilm areas in relation to their oxygen demand, acidic tolerance, and metabolic features (Watnick and Kolter, 2000). Nevertheless, also in single-species biofilms, cell adaptation to chemical gradients can trigger differences in gene expression as well as mutation and selection for the fittest variant resulting in different spatial pattern formation among phenotypes (Stewart and Franklin, 2008). However, cells responding differently to the same spatial and temporal environmental conditions indicate that local physiological adaptation is not the only

environmental factor affecting the cell differentiation at a metabolic/biosynthetic level.

Van Gestel et al. (2015) studied two bacterial models: *B. subtilis* and *P. aeruginosa*. In *P. aeruginosa* biofilm, two phenotypes of motile and non-motile cells concur to produce a mushroom-shaped structure. Non-motile cells, a subpopulation that is low metabolically active but has a high cell density, give rise to a sort of "stalk." The stalk cells mainly produce QS signals, siderophores, exopolysaccharides (EPS), and surfactants (rhamnolipids) that become public goods available for the entire population. After a period of about 4 days, a second phenotype of motile cells appears and migrate, probably by twitching motility-mediated chemotaxis, on the top of stalk cells to have access to more nutrients, thus giving rise to the formation of "caps" in a mushroom-shaped three-dimensional biofilm. It has been observed that among non-motile stalk bacteria, sacrificial cells also appear (before the motile ones) and undergo cell lysis, thus liberating their DNA. This extracellular DNA is localized at the edge of the stalk and has the function, together with the surfactants, to facilitate climbing by the motile cells on the top of stalk cells. How this occurs is not fully understood; however, DNase treatment prevents climbing and exogenous DNA supply restores the migration (Van Gestel et al., 2015). The signal triggering cell lysis in sacrificial cells is a QS quinolone molecule produced by the stalk cells (Van Gestel et al., 2015). Besides low metabolically active, sacrificial, and motile cells, persister cells can be part of a biofilm as well. These are steady-state cells that survive without growing, setting their metabolism to minimal levels and that become resistant against environmental stressors (such as antibiotics), thus ensuring survival of the population in case of stress (Balaban et al., 2004). It is evident that these heterogeneous subpopulations contribute inside a biofilm to control regulatory circuits resulting in cooperative interactions finalized to work in concert and to improve fitness. However, about 7 days after the biofilm establishment, when waste catabolites begin to accumulate or when nutrients become scarce, some cells die whereas the most metabolically active ones start to disperse reverting to planktonic cells to look for a new nutrient-rich place to establish (Van Gestel et al., 2015). In this case, cell lysis occurs after induction (by QS signaling) of a filamentous prophage that is generally non-lytic in planktonic-style living cells (Webb et al., 2003). Cell lysis, together with extracellular matrix degradation, enhances the total number of dispersal units, while surfactants, such as rhamnolipids, produced by the stalk cells before dying, can facilitate cell dispersal. The subpopulation of dispersing cells remains viable, increasing the synthesis of flagellin and polysaccharide lyases (hydrolytic enzymes that break down the biofilm matrix), allowing dispersion from the matrix, and improving the possibility to move away and colonize new habitats (Boyd and Chakrabarty, 1994; Petrova and Sauer, 2016). This cooperative interaction should result in an emergent benefit supporting the migrating cells.

In the *B. subtilis* model, three phenotypes have been described: (i) matrix (EPS)-producing cells that also produce communication signals, proteases, and bacteria-directed toxins; (ii) motile cells; and (iii) sporulating cells. There is a sort of spatial distribution (although not so well-defined as in *P. aeruginosa*) where matrix producers stay in the center, motile cells on the edges and sporulating cells on the top of biofilms (Vlamakis et al., 2008). Here, again, when the biofilm grows old, a division of labor can be observed in which altruistic producers, sacrificial cells, and survivors (migrating but especially sporulating cells) are simultaneously present. Indeed, toxins secreted by the producers can kill sibling (unintentional sacrificial cells) that do not express the necessary immunity genes (Ellermeier et al., 2006) resulting in enhanced availability of nutrients that can delay sporulation. Both proteases and toxins can supply nutrients (degraded matrix and intracellular content of siblings) to accomplish sporulation and, in case, migration. Nevertheless, some sacrificial cells within *B. subtilis* biofilms also set up an autolytic behavior that is independent of the toxin and possibly comparable to programmed cell death: actually, caspase-like enzymes inducing apoptosis have also been described in prokaryotes (Rice and Bayles, 2003, 2008). The interaction between various subpopulations that occurs during biofilm growth promotes behaviors that prove to be cooperative, revealing population plasticity that finally results in enhanced survival and fitness of the overall community.

CONCLUSION

By a continuous flow of mass and energy, as biogeochemical agents, as symbionts of plants and animals, as dead organic matter degraders, bacteria support essential functions that provide successful and sustainable ways of living on the Earth. Nevertheless, bacteria have to cope with an extremely changing environment (pH, temperature, and oxygen may rapidly change), facing a high number of challenges (nutrient scarcity, phage and/or host immune system attack, and environmentally released toxic xenobiotic compounds) and paying high costs to successfully overcome difficulties. According to some reports, competition and single-cell interest are prevalent during scarcity, whereas according to others, scarcity can favor bacterial cooperation and the formation of communities to ensure survival. However, it has to be underlined that cooperation, although sometimes helping in counteracting harsh conditions, is not free from conflicts.

Analyzing the strategies that bacteria use to overcome a variety of environmental stresses and to adjust reciprocal relationships can shed light on how evolutionary forces have shaped a winning model of life. Earning energy and resources, anticipating, cooperatively interacting with other bacterial cells to achieve systems of higher degree of complexity, sharing, and recycling seem to be the rule in prokaryote life. The main concept that emerges by observing bacteria is the necessity of a continuous adaptation to face new scenarios in a fast-changing environment. Each new difficulty encountered (phage attack, "cheaters," and host immune system) can be an opportunity for evolution. The present environmental crisis (global warming, demographic pressure, desertification, and ocean acidification) also compels humans to face fast-changing conditions, seldom experimented

before. The behaviors that evolution has selected in bacteria can suggest, among multiple possible strategies that humans can set up, interesting paths on how to overcome the present challenges.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

FUNDING

This work has been supported by Ricerca Locale Unito (ex 60%).

ACKNOWLEDGMENTS

I am indebted to Roberto Mazzoli for improving the figures and for critical reading of the manuscript.

REFERENCES

Abdel-Rahman, M. A., and Sonomoto, K. (2016). Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. *J. Biotechnol.* 236, 176–192. doi: 10.1016/j.jbiotec.2016.08.008

Adler, J., and Tso, W. W. (1974). "Decision"-making in bacteria: chemotactic response of *Escherichia coli* to conflicting stimuli. *Science* 184, 1292–1294. doi: 10.1126/science.184.4143.1292

Arioli, S., Ragg, E., Scaglioni, L., Fessas, D., Signorelli, M., Karp, M., et al. (2010). Alkalizing reactions streamline cellular metabolism in acidogenic microorganisms. *PLoS One* 5:e15520. doi: 10.1371/journal.pone.0015520

Balaban, N. Q., Merrin, J., Chait, R., Kowalik, L., and Leibler, S. (2004). Bacterial persistence as a phenotypic switch. *Science* 305, 1622–1625. doi: 10.1126/science.1099390

Bidlack, J. E., and Silverman, P. M. (2004). An active type IV secretion system encoded by the F plasmid sensitizes *Escherichia coli* to bile salts. *J. Bacteriol.* 186, 5202–5209. doi: 10.1128/jb.186.16.5202-5209.2004

Blair, K. M., Turner, L., Winkelman, J. T., Berg, H. C., and Kearns, D. B. (2008). A molecular clutch disables flagella in the *Bacillus subtilis* biofilm. *Science* 320, 1636–1638. doi: 10.1126/science.1157877

Boetius, A., Ravenschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., et al. (2000). A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407, 623–626. doi: 10.1038/35036572

Bourret, R. B., and Stock, A. M. (2002). Molecular information processing: lessons from bacterial chemotaxis. *J. Biol. Chem.* 277, 9625–9628. doi: 10.1074/jbc.r100066200

Boyd, A., and Chakrabarty, A. Á (1994). Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* 60, 2355–2359. doi: 10.1128/aem.60.7.2355-2359.1994

Butaité, E., Baumgartner, M., Wyder, S., and Kummerli, R. (2017). Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nat. Commun.* 8:414.

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., et al. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nat. Rev. Microbiol.* 17, 569–586.

Cho, E. J., Trinh, L. T. P., Song, Y., Lee, Y. G., and Bae, H. J. (2020). Bioconversion of biomass waste into high value chemicals. *Bioresour. Technol.* 298:122386. doi: 10.1016/j.biortech.2019.122386

Clewell, D. B. (2011). Tales of conjugation and sex pheromones: a plasmid and *Enterococcal odyssey*. *Mobile Genet. Elements* 1, 38–54. doi: 10.4161/mge.1.1.15409

Clutterbuck, A. L., Woods, E. J., Knottenbelt, D. C., Clegg, P. D., Cochrane, C. A., and Percival, S. L. (2007). Biofilms and their relevance to veterinary medicine. *Vet. Microbiol.* 121, 1–17. doi: 10.1016/j.vetmic.2006.12.029

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., and Lappin-Scott, H. M. (1995). Microbial biofilms. *Ann. Rev. Microbiol.* 49, 711–745.

Cotter, P. D., Ross, R. P., and Hill, C. (2013). Bacteriocins—a viable alternative to antibiotics? *Nat. Rev. Microbiol.* 11, 95–105. doi: 10.1038/nrmicro2937

Crespi, B. J. (2001). The evolution of social behavior in microorganisms. *Trends Ecol. Evolut.* 16, 178–183. doi: 10.1016/s0169-5347(01)02115-2

Dash, H. R., Mangwani, N., Chakraborty, J., Kumari, S., and Das, S. (2013). Marine bacteria: potential candidates for enhanced bioremediation. *Appl. Microbiol. Biotechnol.* 97, 561–571. doi: 10.1007/s00253-012-4584-0

Deinema, M. H., Habets, L. H. A., Scholten, J., Turkstra, E., and Webers, H. A. A. M. (1980). The accumulation of polyphosphate in *Acinetobacter* spp. *FEMS Microbiol. Lett.* 9, 275–279.

Dumas, Z., Ross-Gillespie, A., and Kümmerli, R. (2013). Switching between apparently redundant iron-uptake mechanisms benefits bacteria in changeable environments. *Proc. R. Soc. B Biol. Sci.* 280:1055.

Ellermeier, C. D., Hobbs, E. C., Gonzalez-Pastor, J. E., and Losick, R. (2006). A three-protein signaling pathway governing immunity to a bacterial cannibalism toxin. *Cell* 124, 549–559. doi: 10.1016/j.cell.2005.11.041

Escalante, A. E., and Travisano, M. (2017). Conflict and Cooperation in Microbial Societies. *Front. Microbiol.* 8:141. doi: 10.3389/fmicb.2017.00141

Frost, L. S., and Koraimann, G. (2010). Regulation of bacterial conjugation: balancing opportunity with adversity. *Future Microbiol.* 5, 1057–1071. doi: 10.2217/fmb.10.70

Fuqua, W. C., Winans, S. C., and Greenberg, E. P. (1994). Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176:269. doi: 10.1128/jb.176.2.269-275.1994

Goo, E., Majerczyk, C. D., An, J. H., Chandler, J. R., Seo, Y. S., Ham, H., et al. (2012). Bacterial quorum sensing, cooperativity, and anticipation of stationary-phase stress. *Proc. Natl. Acad. Sci. U.S.A.* 109, 19775–19780. doi: 10.1073/pnas.1218092109

Griffin, A. S., West, S. A., and Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature* 430, 1024–1027. doi: 10.1038/nature02744

Guilhen, C., Charbonnel, N., Parisot, N., Gueguen, N., Iltis, A., Forestier, C., et al. (2016). Transcriptional profiling of *Klebsiella pneumoniae* defines signatures for planktonic, sessile and biofilm-dispersed cells. *BMC Genom.* 17:237. doi: 10.1186/s12864-016-2557-x

Guilhen, C., Forestier, C., and Balestrino, D. (2017). Biofilm dispersal: multiple elaborate strategies for dissemination of bacteria with unique properties. *Mol. Microbiol.* 105, 188–210. doi: 10.1111/mmi.13698

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7, 17–52. doi: 10.1016/0022-5193(64)90039-6

Hellingwerf, K. J. (2005). Bacterial observations: a rudimentary form of intelligence? *Trends Microbiol.* 13, 152–158. doi: 10.1016/j.tim.2005.02.001

Hobley, L., Harkins, C., MacPhee, C. E., and Stanley-Wall, N. R. (2015). Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS Microbiol. Rev.* 39, 649–669. doi: 10.1093/femsre/fuv015

Hoffer, S. M., Westerhoff, H. V., Hellingwerf, K. J., Postma, P. W., and Tommassen, J. A. N. (2001). Autoamplification of a two-component regulatory system results in "learning" behavior. *J. Bacteriol.* 183, 4914–4917. doi: 10.1128/jb.183.16.4914-4917.2001

Jacob, E. B., Becker, I., Shapira, Y., and Levine, H. (2004). Bacterial linguistic communication and social intelligence. *Trends Microbiol.* 12, 366–372. doi: 10.1016/j.tim.2004.06.006

Jarrell, K. F., and McBride, M. J. (2008). The surprisingly diverse ways that prokaryotes move. *Nat. Rev. Microbiol.* 6, 466–476. doi: 10.1038/nrmicro1900

Jefferson, K. K. (2004). What drives bacteria to produce a biofilm? *FEMS Microbiol. Lett.* 236, 163–173. doi: 10.1111/j.1574-6968.2004.tb09643.x

Kaiser, D., and Warrick, H. (2011). *Myxococcus xanthus* swarms are driven by growth and regulated by a pacemaker. *J. Bacteriol.* 193, 5898–5904. doi: 10.1128/jb.00168-11

Kaiser, D., and Warrick, H. (2014). Transmission of a signal that synchronizes cell movements in swarms of *Myxococcus xanthus*. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13105–13110. doi: 10.1073/pnas.1411925111

Kearns, D. B. (2010). A field guide to bacterial swarming motility. *Nat. Rev. Microbiol.* 8, 634–644. doi: 10.1038/nrmicro2405

Kim, W., and Surette, M. G. (2004). Metabolic differentiation in actively swarming *Salmonella*. *Mol. Microbiol.* 54, 702–714. doi: 10.1111/j.1365-2958.2004.04295.x

Koraimann, G., and Wagner, M. A. (2014). Social behavior and decision making in bacterial conjugation. *Front. Cell. Inf. Microbiol.* 4:54. doi: 10.3389/fcimb.2014.00054

Kovács, ÁT. (2014). Impact of spatial distribution on the development of mutualism in microbes. *Front. Microbiol.* 5:649. doi: 10.3389/fmicb.2014.00649

Kuhad, R. C. (2012). Microbes and their role in sustainable development. *Indian J. Microbiol.* 52, 309–313. doi: 10.1007/s12088-012-0267-x

Lee, E. Y., Choi, D. Y., Kim, D. K., Kim, J. W., Park, J. O., Kim, S., et al. (2009). Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of *Staphylococcus aureus*-derived membrane vesicles. *Proteomics* 9, 5425–5436. doi: 10.1002/pmic.200900338

Leisner, M., Stingl, K., Frey, E., and Maier, B. (2008). Stochastic switching to competence. *Curr. Opin. Microbiol.* 11, 553–559. doi: 10.1016/j.mib.2008.09.020

Lion, S., and Baalen, M. V. (2008). Self-structuring in spatial evolutionary ecology. *Ecol. Lett.* 11, 277–295. doi: 10.1111/j.1461-0248.2007.01132.x

Lyon, P. (2015). The cognitive cell: bacterial behavior reconsidered. *Front. Microbiol.* 6:264. doi: 10.3389/fmicb.2015.00264

Manning, A. J., and Kuehn, M. J. (2011). Contribution of bacterial outer membrane vesicles to innate bacterial defense. *BMC Microbiol.* 11:258. doi: 10.1186/1471-2180-11-258

Marles-Wright, J., Grant, T., Delumeau, O., Van Duinen, G., Firbank, S. J., Lewis, P. J., et al. (2008). Molecular architecture of the "Stressosome," a signal integration and transduction hub. *Science* 322, 92–96. doi: 10.1126/science.1159572

Mazzoli, R., Bosco, F., Mitzrahi, I., Bayer, E. A., and Pessione, E. (2014). Towards lactic acid bacteria-based biorefineries. *Biotechnol. Adv.* 32, 1216–1236. doi: 10.1016/j.biotechadv.2014.07.005

Mazzoli, R., Lamberti, C., and Pessione, E. (2012). Engineering new metabolic capabilities in bacteria: lessons from recombinant cellulolytic strategies. *Trends Biotechnol.* 30, 111–119. doi: 10.1016/j.tibtech.2011.08.003

Merod, R. T., and Wuertz, S. (2014). Extracellular polymeric substance architecture influences natural genetic transformation of *Acinetobacter baylyi* in biofilms. *Appl. Environ. Microbiol.* 80, 7752–7757. doi: 10.1128/aem.01984-14

Miller, M. B., and Bassler, B. L. (2001). Quorum sensing in bacteria. *Ann. Rev. Microbiol.* 55, 165–199.

Mitchell, A., Romano, G. H., Groisman, B., Yona, A., Dekel, E., Kupiec, M., et al. (2009). Adaptive prediction of environmental changes by microorganisms. *Nature* 460, 220–224. doi: 10.1038/nature08112

Mora, D., Arioli, S., and Compagno, C. (2013). Food environments select microorganisms based on selfish energetic behavior. *Front. Microbiol.* 4:348. doi: 10.3389/fmicb.2013.00348

Müller, J., and Overmann, J. (2011). Close interspecies interactions between prokaryotes from sulfureous environments. *Front. Microbiol.* 2:146. doi: 10.3389/fmicb.2011.00146

Nakamura, Y., Yamamoto, N., Kino, Y., Yamamoto, N., Kamei, S., Mori, H., et al. (2016). Establishment of a multi-species biofilm model and metatranscriptomic analysis of biofilm and planktonic cell communities. *Appl. Microbiol. Biotechnol.* 100, 7263–7279. doi: 10.1007/s00253-016-7532-6

Olsen, I. (2015). Biofilm-specific antibiotic tolerance and resistance. *Eur. J. Clin. Microbiol. Infect. Dis.* 34, 877–886. doi: 10.1007/s10096-015-2323-z

O'Toole, G., Kaplan, H. B., and Kolter, R. (2000). Biofilm formation as microbial development. *Ann. Rev. Microbiol.* 54, 49–79.

Pessione, E. (2012). Lactic acid bacteria contribution to gut complexity: lights and shadows. *Front. Cell. Inf. Microbiol.* 2:86. doi: 10.3389/fmicb.2011.00086

Pessione, E. (2020). The russian doll model: how bacteria shape successful and sustainable inter-kingdom relationships. *Front. Microbiol.* 11:2325. doi: 10.3389/fmicb.2011.2325

Petrova, O. E., and Sauer, K. (2016). Escaping the biofilm in more than one way: desorption, detachment or dispersion. *Curr. Opin. Microbiol.* 30, 67–78. doi: 10.1016/j.mib.2016.01.004

Pinto, D., and Mascher, T. (2016). Actino Bacterial "intelligence": using comparative genomics to unravel the information processing capacities of microbes. *Curr. Genet.* 62, 487–498. doi: 10.1007/s00294-016-0569-3

Reisner, A., Wolinski, H., and Zechner, E. L. (2012). *In situ* monitoring of IncF plasmid transfer on semi-solid agar surfaces reveals a limited invasion of plasmids in recipient colonies. *Plasmid* 67, 155–161. doi: 10.1016/j.plasmid.2012.01.001

Rice, K. C., and Bayles, K. W. (2003). Death's toolbox: examining the molecular components of bacterial programmed cell death. *Mol. Microbiol.* 50, 729–738. doi: 10.1046/j.1365-2958.2003.t01-1-03720.x

Rice, K. C., and Bayles, K. W. (2008). Molecular control of bacterial death and lysis. *Microbiol. Mol. Biol. Rev.* 72, 85–109. doi: 10.1128/mmbr.00030-07

Ross-Gillespie, A., and Kümmerli, R. (2014). Collective decision-making in microbes. *Front. Microbiol.* 5:54. doi: 10.3389/fmicb.2011.00054

Sauer, K., Camper, A. K., Ehrlich, G. D., Costerton, J. W., and Davies, D. G. (2002). *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. *J. Bacteriol.* 184, 1140–1154. doi: 10.1128/jb.184.4.1140-1154.2002

Shapiro, J. A. (1998). Thinking about bacterial populations as multicellular organisms. *Ann. Rev. Microbiol.* 52, 81–104. doi: 10.1146/annurev.micro.52.1.81

Shapiro, J. A. (2007). Bacteria are small but not stupid: cognition, natural genetic engineering and socio-bacteriology. *Stud. Hist. Philos. Sci. Part C* 38, 807–819. doi: 10.1016/j.shpsc.2007.09.010

Singh, P. K., Ramachandran, G., Ramos-Ruiz, R., Peiro-Pastor, R., Abia, D., Wu, L. J., et al. (2013). Mobility of the native *Bacillus subtilis* conjugative plasmid pLS20 is regulated by intercellular signaling. *PLoS Genet.* 9:e1003892. doi: 10.1371/journal.pgen.1003892

Smith, N. W., Shorten, P. R., Altermann, E., Roy, N. C., and McNabb, W. C. (2019). The classification and evolution of bacterial cross-feeding. *Front. Ecol. Evol.* 7:153. doi: 10.3389/fmicb.2011.00153

Steenackers, H. P., Parijs, I., Foster, K. R., and Vanderleyden, J. (2016). Experimental evolution in biofilm populations. *FEMS Microbiol. Rev.* 40, 373–397. doi: 10.1093/femsre/fuw002

Stewart, P. S., and Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* 6, 199–210. doi: 10.1038/nrmicro1838

Sturgill, G., and Rather, P. N. (2004). Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Mol. Microbiol.* 51, 437–446. doi: 10.1046/j.1365-2958.2003.03835.x

Summers, Z. M., Fogarty, H. E., Leang, C., Franks, A. E., Malvankar, N. S., and Lovley, D. R. (2010). Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* 330, 1413–1415. doi: 10.1126/science.1196526

Tagkopoulos, I., Liu, Y. C., and Tavazoie, S. (2008). Predictive behavior within microbial genetic networks. *Science* 320, 1313–1317. doi: 10.1126/science.1154456

Tarnita, C. E. (2017). The ecology and evolution of social behavior in microbes. *J. Exper. Biol.* 220, 18–24. doi: 10.1242/jeb.145631

Toyofuku, M., Tashiro, Y., Nomura, N., and Eberl, L. (2020). "Functions of MVs in inter-bacterial communication," in *Bacterial Membrane Vesicles*, eds M. Kaparakis-Liaskos and T. Kufer (Cham: Springer), 101–117. doi: 10.1007/978-3-030-36331-4_5

Trewavas, A. (2002). Plant intelligence: mindless mastery. *Nature* 415, 841–841. doi: 10.1038/415841a

Van Gestel, J., Weissing, F. J., Kuipers, O. P., and Kovács, A. T. (2014). Density of founder cells affects spatial pattern formation and cooperation in *Bacillus subtilis* biofilms. *ISME J.* 8, 2069–2079. doi: 10.1038/ismej.2014.52

Van Gestel, J. V., Vlamakis, H., and Kolter, R. (2015). Division of labor in biofilms: the ecology of cell differentiation. *Microb. Biofilms* 3:MB-0002-2014.

van Heeswijk, W. C., Westerhoff, H. V., and Boogerd, F. C. (2013). Nitrogen assimilation in *Escherichia coli*: putting molecular data into a systems perspective. *Microbiol. Mol. Biol. Rev.* 77, 628–695. doi: 10.1128/mmbr.00025-13

Visick, K. L., and Fuqua, C. (2005). Decoding microbial chatter: cell-cell communication in bacteria. *J. Bacteriol.* 187, 5507–5519. doi: 10.1128/jb.187.16.5507-5519.2005

Vlamakis, H., Aguilar, C., Losick, R., and Kolter, R. (2008). Control of cell fate by the formation of an architecturally complex bacterial community. *Genes Dev.* 22, 945–953. doi: 10.1101/gad.1645008

Vulić, M., and Kolter, R. (2001). Evolutionary cheating in *Escherichia coli* stationary phase cultures. *Genetics* 158, 519–526.

Wagner, M. A., Bischof, K., Kati, D., and Koraimann, G. (2013). Silencing and activating type IV secretion genes of the F-like conjugative resistance plasmid R1. *Microbiology* 159, 2481–2491. doi: 10.1099/mic.0.071738-0

Waters, C. M., and Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annu. Rev. Cell Dev. Biol.* 21, 319–346. doi: 10.1146/annurev.cellbio.21.012704.131001

Watnick, P., and Kolter, R. (2000). Biofilm, city of microbes. *J. Bacteriol.* 182, 2675–2679. doi: 10.1128/jb.182.10.2675-2679.2000

Webb, J. S., Thompson, L. S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., et al. (2003). Cell death in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* 185, 4585–4592. doi: 10.1128/jb.185.15.4585-4592.2003

Webre, D. J., Wolanin, P. M., and Stock, J. B. (2003). Bacterial chemotaxis. *Curr. Biol.* 13, R47–R49.

Werner, G. D., Strassmann, J. E., Ivens, A. B., Engelmoer, D. J., Verbruggen, E., Queller, D., et al. (2014). Evolution of microbial markets. *Proc. Natl. Acad. Sci. U.S.A.* 111, 1237–1244.

West, S. A., and Buckling, A. (2003). Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 270, 37–44. doi: 10.1098/rspb.2002.2209

Westerhoff, H. V., Brooks, A. N., Simeonidis, E., García-Contreras, R., He, F., Boogerd, F., et al. (2014). Macromolecular networks and intelligence in microorganisms. *Front. Microbiol.* 5:379. doi: 10.3389/fmicb.2011.00379

Wolf, D. M., Fontaine-Bodin, L., Bischofs, I., Price, G., Keasling, J., and Arkin, A. P. (2008). Memory in microbes: quantifying history-dependent behavior in a bacterium. *PLoS One* 3:e1700. doi: 10.1371/journal.pone.0001700

Zahrl, D., Wagner, M., Bischof, K., and Koraimann, G. (2006). Expression and assembly of a functional type IV secretion system elicit extracytoplasmic and cytoplasmic stress responses in *Escherichia coli*. *J. Bacteriol.* 188, 6611–6621. doi: 10.1128/jb.00632-06

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

MINI REVIEW
published: 28 January 2021
doi: 10.3389/fmicb.2021.635432

We Are One: Multispecies Metabolism of a Biofilm Consortium and Their Treatment Strategies

Ruchika Vinod Joshi[1†], *Cindy Gunawan*[1,2] *and Riti Mann*[1*†]

[1] *iThree Institute, University of Technology Sydney, Sydney, NSW, Australia,* [2] *School of Chemical Engineering, University of New South Wales, Sydney, NSW, Australia*

The ecological and medical significance of bacterial biofilms have been well recognized. Biofilms are harder to control than their planktonic free-living counterparts and quite recently, the focus of the study has shifted to the multispecies consortia, which represent the vast majority of real-case infection scenarios. Studies have begun to explore the complex interspecies interactions within these biofilms. However, only little attention is currently given to the role of cellular metabolites in the cell-to-cell communication. The concentration gradients of metabolic substrates and products affect the spatial growth of bacteria in multispecies biofilm. This, if looked into more deeply, can lead to identification of potential therapies targeting the specific metabolites and hence the coordinated protection in the bacterial community. Herein, we review the interspecies communications, including their metabolic cross-talking, in multispecies biofilm, to signify the importance of such interactions on the initial formation and subsequent growth of these biofilms. Multispecies biofilms with their species heterogeneity are more resilient to antimicrobial agents than their single species biofilm counterparts and this characteristic is of particular interest when dealing with pathogenic bacteria. In this Review, we also discuss the treatment options available, to include current and emerging avenues to combat pathogenic multispecies biofilms in the clinical, environmental, as well as industrial settings.

Keywords: biofilms, multispecies, metabolism, treatment, interactions

OPEN ACCESS

Edited by:
Enrica Pessione,
University of Turin, Italy

Reviewed by:
Giuliana Banche,
University of Turin, Italy
Ilana Kolodkin-Gal,
Weizmann Institute of Science, Israel

***Correspondence:**
Riti Mann
Riti.Mann@uts.edu.au

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *30 November 2020*
Accepted: *11 January 2021*
Published: *28 January 2021*

Citation:
Joshi RV, Gunawan C and Mann R (2021) We Are One: Multispecies Metabolism of a Biofilm Consortium and Their Treatment Strategies. Front. Microbiol. 12:635432. doi: 10.3389/fmicb.2021.635432

INTRODUCTION

Bacteria typically live in complex biological communities, known as biofilms; which dominate all habitats on the surface of the Earth, except the oceans, where 20–80% of bacterial cells exist as biofilms (Hall et al., 2014; Flemming and Wuertz, 2019). Biofilms are often comprised of multiple microbial species, each carrying its own unique features, imparting certain evolved and unique functions that are not present in their mono-species counterparts (Flemming et al., 2016). Such biofilms, referred to as the multispecies biofilms, are commonly found on a wide range of medical devices and are associated with a significant amount of human bacterial infections, posing a serious human health concern and economic burden to the health-care systems (Bryers, 2008; Hall et al., 2014; Kvich et al., 2020).

The formation of multispecies biofilms is a complex process, coordinated by the sequential interaction of different species. These interactions in the bacterial community are highly specific and often change the structural and functional dynamics of the whole biofilm community, enhancing protection as well as virulence characteristics (Yang et al., 2011). These spatial interactions, arising from a high level of species heterogeneity in these biofilms, renders these biofilms highly resilient to conventional antimicrobial treatments, urging the need for effective alternative therapies (Flemming et al., 2016). Understanding the interspecies communications in multispecies biofilms will enable the discovery of novel targets for controlling biofilms in the environmental, industrial and clinical settings. Herein, we discuss important recent literatures to showcase our current understanding of the interspecies interactions in a multispecies biofilm. Later in the review, we describe the metabolic heterogeneity in such biofilms, a factor influencing their antibiotic susceptibility; and finally, we highlight the recent advancements in the treatment of biofilm-related infections, centering more on the discovery of non-antibiotic alternative treatment options.

BUILDING THE MULTISPECIES BIOFILMS

Critical to the formation and development of multispecies biofilms is the cell-to-cell interactions, termed as co-adhesion and co-aggregation, which together foster mutualistic communications between adjacent cells in a biofilm. The adherence of bacterial cells to immobilized cells is called as co-adhesion whereas the binding of microbial cells in suspension is known as co-aggregation (Kolenbrander et al., 2010). These two binding interactions provide diverse attachment sites for the planktonic bacteria to adhere to in the process of biofilm development (Foster and Kolenbrander, 2004). The formation of multispecies biofilms is a complex process that in general is categorized into three steps: (1) the attachment of primary colonizers to the surface, their clonal growth and the production of exopolysaccharides, protein adhesins, amyloids and nucleic acids, which together form the EPS (Extracellular Polymeric Substance); resulting in the formation of microcolonies, (2) the attachment of secondary colonizers to these microcolonies, followed by their proliferation, and (3) dispersion of biofilm, mediated by environmental stimulus, which allows the cells to detach and establish a new biofilm at other sites (Hobley et al., 2015; Steinberg and Kolodkin-Gal, 2015; Salinas et al., 2020). The first step, dependent on the bacterial physiochemical interactions, is highly specific; such that the primary colonizers can only co-aggregate with other primary colonizers and not with any secondary colonizing bacteria. The co-adhesion of initial primary colonizers is crucial for the biofilm colonization, whereas an increase in EPS production is essential for the attachment of secondary colonizers to the microcolonies, as EPS works as an intercellular-cement in biofilm proliferation by sticking the cells together and mediating a successive co-aggregation as the biofilm matures (Rickard et al., 2003).

INTERSPECIES INTERACTIONS IN MULTISPECIES BIOFILMS

The interspecies interactions within a biofilm have been a recent focus of many studies. Bacteria in a multispecies biofilm consortium mainly communicate via four highly specific mechanisms, namely, the physical interactions, exchange of genetic material, metabolic networking and by using diffusible signals, which in many cases, only take place when the respective bacterial species form a multispecies biofilm (Blehert et al., 2003; Flemming et al., 2016) (**Figure 1**). These interspecies communications, depending on the intricate molecular mechanisms, can cause social behaviors that can be neutral, cooperative or competitive for the species involved (Burmølle et al., 2014), with the latter two types mainly shaping the organization and functionality of a multispecies biofilm community.

Cooperation within the biofilm community is facilitated through synergistic interactions that modulate the differential gene expression and cellular responses of each species, allowing them to evolve and better adapt to the biofilm conditions. One such example is the association between *Pseudomonas putida* KT2440 and *Acinetobacter* sp. C6, wherein *P. putida* evolves in the presence of *Acinetobacter* by altering its outer core lipopolysaccharide synthesis. This results in the formation of rough variants that show enhanced fitness by acquiring more benzoate – a by-product of *Acinetobacter*, making the overall community more stable and productive (Hansen et al., 2007). Synergistic interactions can also result from transfer of genetic material between different species, either through plasmid conjugation or DNA transformation, providing stability to the biofilm and helps in resisting attacks from phages, antibiotics and toxins (Wang et al., 2002; Molin and Tolker-Nielsen, 2003; Reisner et al., 2006). For instance, the biofilm-stimulating effects were observed due to the conjugative transfer of F-like and IncIα plasmids between genetically diverse strains of *Escherichia coli* (Reisner et al., 2006). Cell-to-cell physical interactions are also an important factor in the synergistic interactions in biofilms, resulting in the formation of cellular aggregates. This, for example, has been seen in multispecies biofilms causing dental plaque in oral cavities (Kolenbrander et al., 2010). The inter-cellular communication, a key process in the formation of oral biofilms, between *Actinomyces naeslundii* and *Streptococcus oralis* [via the universal intergeneric signaling molecule – Autoinducer 2 (AI2)], only occurs when these bacteria co-aggregate (Hardie and Heurlier, 2008). Research inquiries have indicated that synergistic interactions cause a particular bacterium to thrive better in the presence of other bacteria than they would on their own. For instance, *Bacillus cereus* is known to release thiazolyl peptide antibiotics – thiocillins, which increase the population of matrix-producing cells of *Bacillus subtilis*, thereby enhancing its biofilm forming properties (Bleich et al., 2015). Synergistic interactions could also manifest

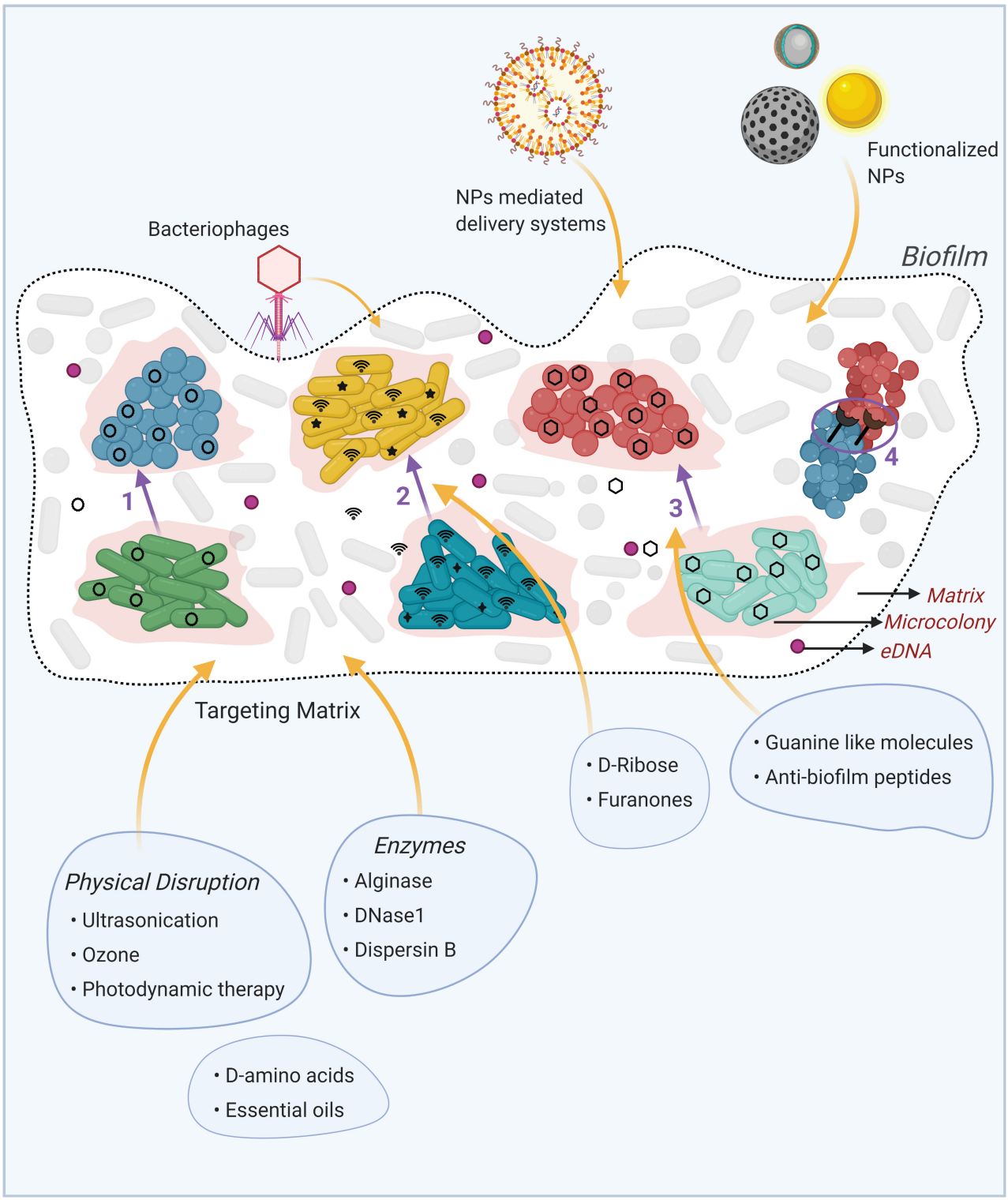


FIGURE 1 | Schematic representation of the mechanisms of interspecies interactions in multispecies biofilms and innovative biofilm-therapeutic strategies. **(1)** Horizontal gene transfer via plasmid conjugation, where plasmid (black circles) is transferred from one species (green cells) to another (blue cells). **(2)** Quorum sensing through intraspecific (black stars) and interspecific (wifi signals) communication by diffusible molecules. **(3)** Metabolic cooperation where the by-product of one species (green cells) serve as nutrient (black hexagons) for another species (red cells). **(4)** Physical interactions, where specific cell-to-cell interactions occurs between cells of different species through specific cell surface receptors. Available treatment options to combat biofilms are depicted in illustrations around the biofilm, with yellow arrows pointing to their target in the biofilm. NPs: Nanoparticles, eDNA: extracellular DNA. Created using Biorender.

in the form of an enhanced growth rate when the species are present together, as demonstrated by a threefold increase in multispecies biofilm of four soil isolates: *Stenotrophomonas rhizophila*, *Xanthomonas retroflexus*, *Microbacterium oxydans*, and *Paenibacillus amylolyticus*, compared to their respective single species biofilms. This synergistic effect is suggested to result from their shared evolutionary history that facilitates nutrient cross-feeding between them (Ren et al., 2014).

Competitive interactions result from antagonistic relationships in a biofilm, whereby one bacterial species produces molecules that inhibit the growth of other species. This has been observed in the dual species biofilm of *Lactobacilli* and *Streptococcus*, in which the biofilm forming ability of *Streptococcus* on glass surfaces was inhibited by *Lactobacilli* in a pH-dependent manner (Söderling et al., 2011). In other case, *Pseudomonas aeruginosa* displays a "blanketing" effect on *Agrobacterium tumefaciens* microcolonies, when grown as dual biofilms, facilitating better growth of the former bacterium. This effect is thought to result from the motile nature of *P. aeruginosa* cells, as the flagellar and type IV pili mutants of the bacterium did not exhibit the "blanketing" effect (An et al., 2006). Antagonistic relationship is also observed in the dual biofilm of *P. aeruginosa* and *Candida albicans*, in which *P. aeruginosa* restricts the maturation of *C. albicans* biofilms by regulating the expression of adhesion molecules, quorum sensing (QS) molecules and the virulence genes (Holcombe et al., 2010). In the meat processing industry, *Salmonella* biofilms are shown to be inhibited by *P. aeruginosa* through the production of acyl-homoserine lactone (AHL), which is hypothesized to modulate the cell division in *Salmonella*, also affecting the chemical composition of EPS, reducing the adhesion ability of *Salmonella* (Wang et al., 2013). Antagonistic activity of several molecules released by the soil-bacterium *B. subtilis* has been demonstrated against a range of different bacterial genera. This includes; surfactin, which is shown to arrest the development of aerial hyphae in *Streptomyces coelicolor* (Straight and Joanne, 2006), chlorotetain, which degrades the colonies of *Staphylococcus epidermidis* when these two bacteria come in proximity on the human skin (Hernandez-Valdes et al., 2020), surfactin and plipastatin, which alters the virulence factors of *Staphylococcus aureus* (Gonzalez et al., 2011), and surfactin and cannibalism toxin, both of which eliminate the colonies of *Bacillus simplex* and *Bacillus toyonensis* (Rosenberg et al., 2016). On the other hand, research enquiries have also identified the antagonistic effect of compounds released by other bacterial species on the growth, physiology and biofilm formation of *B. subtilis*. For example, the active compound 2,4-diacetylphloroglucinol (DAPG), secreted by *Pseudomonas protegens*, is shown to cause phenotypic alterations and inhibit biofilm formation in *B. subtilis* (Powers et al., 2015), and linearmycins, a family of polyketides, produced by the soil bacterium *Streptomyces* sp. has been seen to cause cellular lysis of *B. subtilis* (Stubbendieck and Straight, 2015).

Communication through chemical signaling, referred to as QS, plays an important role in the establishment of multispecies biofilms. QS systems in *P. aeruginosa*, a strong biofilm former, are highly complex and among the most studied systems. The production of amino-4-methoxy-trans-3-butenoic acid, a QS-regulated toxic compound produced by *P. aeruginosa*, has been associated with inhibition of other pathogenic microbes (Rojas Murcia et al., 2015). The dual biofilm of *Streptococcus mitis* and *P. aeruginosa* are commonly found in the endotracheal tubes of infants. Although *S. mitis* is not a pathogen by itself, it releases the QS autoinducer-2 (AI-2) molecule, which aids the growth of *P. aeruginosa*, enhancing its biofilm forming capability and apparently, its pathogenicity (Wang et al., 2016). QS has a decisive role to play in the pathogenicity of *P. aeruginosa*, as indicated by the differential QS profiling of its clinical isolates and lab-cultured strains, primarily arising from the relative abundance of a QS molecule AHL (Singh et al., 2000).

In addition to the specific molecular mechanisms and physical interactions discussed above, metabolic communication also facilitates inter-species cross-talk in a biofilm. These metabolic interactions, dealt in the next section of this review, play important roles in spatial organization of microbes and a proper functioning of a biofilm.

METABOLIC COMMUNICATIONS IN MULTISPECIES BIOFILMS

Matrix production, in addition to establishing the biofilm structure, also results in metabolic diversification by controlling the physical interactions between bacterial cells and their immediate environment. This enables metabolic cross-feeding, promoting the development of metabolically differentiated subpopulations in a biofilm and making the biofilms a metabolically heterogeneous community [refer to the recent reviews by Evans et al. (2020) and Povolotsky et al. (2021) for a comprehensive discussion on metabolic heterogeneity in biofilms]. Interspecies interactions, along with the biofilm structure, influence the signals that promote metabolic differentiation, eventually shaping the nutrient and chemical gradient of a biofilm. Interspecies interactions facilitate metabolic cooperation in a biofilm when the metabolic by-products of one species are used as nutrients by the other species (Christensen et al., 2002). One example is the use of lactic acid from *S. oralis* by *Veillonella* sp. in the oral biofilm formed by these two species (Periasamy and Kolenbrander, 2010). Structurally, EPS helps in the absorption of nutrients, creating a nutrient gradient, whereby, by-products of one species can be used as nutrient by the other species, reducing unwanted, toxic waste in biofilms (Elias and Banin, 2012). The spatial organization and composition of *P. protegens*, *P. aeruginosa*, and *Klebsiella pneumoniae* multispecies biofilm is influenced by nutrient availability, which has an effect on their survival under stressful conditions (Lee et al., 2014). Metabolically distinct subzones, based on oxygen availability, were observed in *P. aeruginosa* PA14 biofilms; whereby cells in anoxic regions produced lactate by expressing lactate dehydrogenase (LdhA). The lactate was then cross-fed to cells in the oxic conditions, activating the expression of *lldE*, the gene that encodes for lactate oxidizing enzyme – lactate dehydrogenase, involved in utilization of lactate (Lin et al., 2018). This metabolic cross-feeding

allows the use of a carbon source – lactate, which would else persist as a toxic metabolic waste product within the biofilm. Similarly, metabolic cross-feeding mediated by high redox potential compounds – phenazines, has been observed in between the oxic and anoxic regions of a *P. aeruginosa* biofilm. Phenazines, produced in oxic regions of the biofilm, were observed to migrate to oxygen-limited regions, where they served as alternate electron acceptors, supporting the metabolic activity in these zones (Williamson et al., 2012; Schiessl et al., 2019).

As the metabolic state of a cell is the determinant of its antibiotic susceptibility (Stokes et al., 2019), numerous studies have looked into the metabolic status of individual cells as well as the metabolic cross-feeding in multicellular systems (Evans et al., 2020); however similar research on biofilms is still scanty, and only a few metabolites involved have been identified. Biofilm formation in various bacterial species is related to an increase in the activity of cyclic diguanylate monophosphate (c-di-GMP) (Ross et al., 1987). In Gram-negative bacteria, this secondary messenger molecule regulates biofilm formation by acting as the main switch between sessile and motile mode of bacterial growth, enabling attachment of cells on surfaces through a signaling cascade (Simm et al., 2004). In *P. aeruginosa*, c-di-GMP regulates the production of exopolysaccharide alginate, a major component of the biofilm matrix and is a factor in the persistence of *P. aeruginosa* biofilms, frequently seen in lung infections (Römling and Balsalobre, 2012). An elevated level of c-di-GMP is also noted in the rough small colony variants (RSCV) of *P. aeruginosa*, a hyper biofilm former, showing an increased tolerance to antimicrobials (Starkey et al., 2009). *S. aureus* produces c-di-AMP (cyclic diadenosine monophosphate) as a secondary messenger, instead of c-di-GMP, which produces components, most likely adhesins, required for biofilm formation (Corrigan et al., 2011).

Cyclic adenosine monophosphate (cAMP) is another important secondary messenger molecule that has been shown to affect biofilm formation process through multiple signal transduction cascades (Jackson et al., 2002; McDonough and Rodriguez, 2012; Kalivoda et al., 2013). In *Vibrio cholerae*, it activates biofilm formation by negatively regulating the biofilm repressor HapR (QS transcriptional regulator) and positively regulating the biofilm activator VpsR (transcriptional regulator of the *Vibrio* polysaccharide synthesis operon) (Liang et al., 2007). It also acts as a biofilm repressor by negatively regulating an activator – diguanylate cyclase CdgA of biofilm formation (Fong and Yildiz, 2008). cAMP was also found to inhibit EPS synthesis and the formation of a multilayer biofilm (Houot and Watnick, 2008). Another metabolite, ppGpp plays crucial role in the formation and maintenance of biofilms, as the ppGpp mutants were observed to form loose biofilms due to their decreased ability to adhere to a surface (De la Fuente-Núñez et al., 2014). Another study found that eliminating ppGpp synthesis in a biofilm, reduced bacterial growth compared to the wild type cells, and the cells that grew were tolerant to the DNA replication targeting antibiotic ofloxacin (Nguyen et al., 2011).

The interspecies interactions in a biofilm, described so far, enhance the survival of bacterial biofilms, which pose a significant issue in industrial and clinical settings (Kolenbrander and London, 1993; Galié et al., 2018). Hence, in the subsequent section, we discuss the recent technological advancements in controlling biofilms and identify potential interspecies interactions that can be targeted to combat a vast array of biofilm-related infections.

INNOVATIVE TREATMENT STRATEGIES FOR CONTROLLING BIOFILMS

The complex biofilm matrix makes the biofilms resilient to almost all antimicrobial treatments. Besides the use of antibiotics, research work on novel biofilm eradication strategies have been primarily focused on the disruption of the protective EPS matrix, leading to biofilm disintegration. These approaches, considered an effective strategy to control biofilms, are schematically summarized in **Figure 1**. Targeting alginate, one of the major component of the EPS in *P. aeruginosa* biofilm, using the alginase enzyme, has been identified as a potential strategy for the treatment of cystic fibrosis patients (Glonti et al., 2010). Combination therapies, comprising of a matrix degrading agent and an antibacterial agent, have also shown efficacy in dealing with biofilm infections. An antibiotic – Dnase1 (degrades extracellular DNA) combination therapy was shown to disrupt the EPS, enhancing the antibiofilm effects of antibiotics in clearing bacterial single-species biofilms (Fanaei Pirlar et al., 2020). A combination of DNase1 and plant-based essential oils also disrupted the biofilm of methicillin-resistant *S. aureus* (Rubini et al., 2018). Using Dispersin B, a biofilm-dispersing enzyme, in combination with peptides, eradicated ∼70% of *S. epidermidis* biofilms compared to only ∼35% by Dispersin B alone (Chen and Lee, 2018). Further, D-amino acids from *B. subtilis*, known to signal for biofilm disassembly, were found to inhibit the development of *S. aureus* biofilms (Chen et al., 2020). Intriguingly, honey – a natural product, has also shown anti-biofilm effects by inhibiting *P. aeruginosa* biofilm formation and reducing its established biofilms (Lu et al., 2019). The cell-free supernatant of the yeast *Saccharomyces cerevisiae* has been shown to exhibit anti-biofilm effects on *Listeria monocytogenes* biofilms, primarily by decreasing the EPS production (Kim et al., 2021). Another innovative approach in treatment of biofilm-related infections is the use of iron chelators, which have shown significant anti-biofilm activity on both Gram-positive and Gram-negative bacteria (Richter et al., 2017).

Other treatment strategy involves the development of nanoparticle (NP)-based systems to target biofilms. Proteinase K-capped gold NPs were shown to degrade the mature biofilms of *P. fluorescens* by disrupting its EPS components (Habimana et al., 2018), while silver (Ag) NPs at concentrations as low as 1μg/mL have shown efficacy in inhibiting the formation of *P. aeruginosa* biofilms (Kora and Arunachalam, 2011). In addition to their use as anti-biofilm agents, nanosystems have also been successfully applied as carriers to enhance antibiotic delivery in biofilm systems by co-mobilizing a matrix-disrupting

agent and an antibacterial agent onto NPs (Baelo et al., 2015; Tan et al., 2018, 2020).

Studies have shown the use of QS inhibitors in the treatment of biofilm-forming pathogenic infections. In the dual biofilm of *S. mitis* and *P. aeruginosa*, whereby the AI-2 molecule released from *S. mitis* promotes the pathogenicity of *P. aeruginosa*; D-ribose, has been shown to inhibit the activity of AI-2 by competing for its receptor site (Wang et al., 2016). Naturally occurring halogenated molecules, the furanones, can inhibit the QS signaling molecule "AHL," resulting in reduced biofilm thickness and swarming motility of *E. coli*, *V. cholerae*, and *P. aeruginosa* (Proctor et al., 2020). Bacteriophages have also shown promising results in treatment of highly antibiotic-resistant biofilm infections, as they prevented *Klebsiella* biofilm formation on urinary catheters and demonstrated significant clinical improvements in chronic otitis patients (caused by *P. aeruginosa* and *S. aureus* biofilms) (Wright et al., 2009; Townsend et al., 2020). The use of physical techniques for biofilm dispersal has also gained attention in the last decade. Combination ultrasonication-ozone treatment, for example, has been shown to eliminate *L. monocytogenes* biofilms from stainless steel surfaces through disruption of proteins in the EPS (Baumann et al., 2009; Yu et al., 2020). Photodynamic therapy, using the photosensitizing molecule 5-aminolevulinic acid was able to inactivate cells in mono-species antibiotic-resistant *S. aureus* and *S. epidermidis* biofilms (Li et al., 2013).

The cellular metabolites involved in interspecies interactions in multispecies biofilms can be a potential target options for the treatment of the biofilms, for example, c-di-GMP, a signaling molecule required for biofilm formation is the prime target candidate. A recent *in silico* study identified "guanine-like" molecules that could limit diguanylate cyclase activity, leading to reduced intracellular c-di-GMP signals, which in turn, inhibited the initial attachment and induced dispersion of *P. aeruginosa* biofilm (Sambanthamoorthy et al., 2014). Anti-biofilm peptides, a subset of host defense peptides, have been shown to interact with and degrade the ppGpp molecule, which plays a role in biofilm establishment. As low as 0.8 μg/mL concentration of the peptide was able to initiate dispersal of *P. aeruginosa* biofilms, while treatment at 10 μg/mL caused complete destruction of the biofilms (De la Fuente-Núñez et al., 2014). However, despite showing efficacy, these peptides are susceptible to degradation by the innate presence of bacterial proteases. To address this, studies have been developing d-enantiomeric protease resistant peptides, which show a 10-fold decreased biofilm inhibition concentration compared to the protease susceptible peptides (de la Fuente-Núñez et al., 2015).

Above suggested treatments are generally applicable for both single-species and multispecies biofilms. However, it is now well-established that during infections, bacteria are mostly found coexisting with other species, showing interspecies interactions, metabolic heterogeneity and cross-feeding; which all can enhance the cellular pathogenicity and antibiotic tolerance. Indeed, studies have identified that metabolites involved in interspecies interactions can interfere with 'drug-cell' interactions and metabolic differentiation does contribute to antibiotic tolerance in multicellular systems, which can critically influence our ability to treat infections (Co et al., 2019; Schiessl et al., 2019). Hence, the treatment outcome from a multispecies biofilm with complex cross-species interactions would be different from a monospecies biofilm, highlighting the need to incorporate these interactions while designing our treatment strategies.

CONCLUSION

The negative effects of bacterial biofilms are well recognized. Many biofilm treatment strategies have been focused on targeting the protective polymer matrix that shields the bacterial community from antimicrobial agents. Apart from such structural targeting, we found from this review that the physiological cell-to-cell interactions in biofilm can indeed serve as another important avenue worth exploring as the potential treatment target. A number of physiological interactions, primarily the receptor-mediated cell aggregation, intercellular signaling, metabolic communication and horizontal gene transfer, are known to maintain a tightly regulated and functional biofilm biomass, for a community-associated protection against stress, including from antimicrobial agents. While inhibitors of intercellular signaling (QS inhibitors) have been identified, only little progress however, has been made on the targeting of other intercellular interaction pathways. As an example, the targeting of metabolic communications such as those via the global transcriptional regulators such as cyclic AMP or c-di-GMP, is anticipated to inhibit the cell-to-cell interactions, even those between different species, presenting a potential implication in the treatment of multispecies biofilms. The Review highlights the need to shift biofilm eradication strategies from the current targeting of biofilm structural entities to targeting metabolic communications that underlie the cell-to-cell interactions, which is anticipated to offer long-term treatment solutions.

AUTHOR CONTRIBUTIONS

RM conceived, revised, and edited the manuscript. RJ conducted the literature study. RJ and RM wrote the manuscript. CG provided feedback and edited the manuscript. All authors gave final approval of the version to be submitted and published.

FUNDING

This work was supported by the Australian Research Council Discovery Project (DP 180100474).

REFERENCES

An, D., Danhorn, T., Fuqua, C., and Parsek, M. R. (2006). Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3828–3833. doi: 10.1073/pnas.0511323103

Baelo, A., Levato, R., Julián, E., Crespo, A., Astola, J., Gavaldà, J., et al. (2015). Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. *J. Control. Release* 209, 150–158. doi: 10.1016/j.jconrel.2015.04.028

Baumann, A. R., Martin, S. E., and Feng, H. A. O. (2009). Removal of *Listeria monocytogenes* biofilms from stainless steel by use of ultrasound and ozone. *J. Food Prot.* 72, 1306–1309. doi: 10.4315/0362-028X-72.6.1306

Blehert, D. S., Palmer, R. J., Xavier, J. B., Almeida, J. S., and Kolenbrander, P. E. (2003). Autoinducer 2 production by *Streptococcusgordonii* DL1 and the biofilm phenotype of a luxS mutant are influenced by nutritional conditions. *J. Bacteriol.* 185, 4851–4860. doi: 10.1128/JB.185.16.4851-4860.2003

Bleich, R., Watrous, J. D., Dorrestein, P. C., Bowers, A. A., and Shank, E. A. (2015). Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* 112, 3086–3091. doi: 10.1073/pnas.1414272112

Bryers, J. D. (2008). Medical biofilms. *Biotechnol. Bioeng.* 100, 1–18. doi: 10.1002/bit.21838

Burmølle, M., Ren, D., Bjarnsholt, T., and Sørensen, S. J. (2014). Interactions in multispecies biofilms: do they actually matter? *Trends Microbiol.* 22, 84–91. doi: 10.1016/j.tim.2013.12.004

Chen, K.-J., and Lee, C.-K. (2018). Twofold enhanced dispersin B activity by N-terminal fusion to silver-binding peptide for biofilm eradication. *Int. J. Biol. Macromol.* 118, 419–426. doi: 10.1016/j.ijbiomac.2018.06.066

Chen, M., Zhang, S., and He, Z. (2020). Controlled block polypeptide composed of d-type amino acids: a therapeutics delivery platform to inhibit biofilm formation of drug-resistant bacteria. *ACS Appl. Bio Mater.* 3, 6343–6350. doi: 10.1021/acsabm.0c00795

Christensen, B. B., Haagensen, J. A. J., Heydorn, A., and Molin, S. (2002). Metabolic commensalism and competition in a two-species microbial consortium. *Appl. Environ. Microbiol.* 68, 2495–2502. doi: 10.1128/AEM.68.5.2495-2502.2002

Co, A. D., Vliet, S. V., and Ackermann, M. (2019). Emergent microscale gradients give rise to metabolic cross-feeding and antibiotic tolerance in clonal bacterial populations. *Philos. Trans. R. Soc. B Biol. Sci.* 374:20190080. doi: 10.1098/rstb.2019.0080

Corrigan, R. M., Abbott, J. C., Burhenne, H., Kaever, V., and Gründling, A. (2011). c-di-AMP is a new second messenger in *Staphylococcus aureus* with a role in controlling cell size and envelope stress. *PLoS Pathog.* 7:e1002217. doi: 10.1371/journal.ppat.1002217

De la Fuente-Núñez, C., Reffuveille, F., Haney, E. F., Straus, S. K., and Hancock, R. E. (2014). Broad-spectrum anti-biofilm peptide that targets a cellular stress response. *PLoS Pathog.* 10:e1004152. doi: 10.1371/journal.ppat.1004152

de la Fuente-Núñez, C., Reffuveille, F., Mansour, S. C., Reckseidler-Zenteno, S. L., Hernández, D., Brackman, G., et al. (2015). D-enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal *Pseudomonas aeruginosa* infections. *Chem. Biol.* 22, 196–205. doi: 10.1016/j.chembiol.2015.01.002

Elias, S., and Banin, E. (2012). Multi-species biofilms: living with friendly neighbors. *FEMS Microbiol. Rev.* 36, 990–1004. doi: 10.1111/j.1574-6976.2012.00325.x

Evans, C. R., Kempes, C. P., Price-Whelan, A., and Dietrich, L. E. P. (2020). Metabolic heterogeneity and cross-feeding in bacterial multicellular systems. *Trends Microbiol.* 28, 732–743. doi: 10.1016/j.tim.2020.03.008

Fanaei Pirlar, R., Emaneini, M., Beigverdi, R., Banar, M., van Leeuwen, W. B., and Jabalameli, F. (2020). Combinatorial effects of antibiotics and enzymes against dual-species *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms in the wound-like medium. *PLoS One* 15:e0235093. doi: 10.1371/journal.pone.0235093

Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. *Nat. Rev. Microbiol.* 14, 563–575. doi: 10.1038/nrmicro.2016.94

Flemming, H.-C., and Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. *Nat. Rev. Microbiol.* 17, 247–260. doi: 10.1038/s41579-019-0158-9

Fong, J. C. N., and Yildiz, F. H. (2008). Interplay between cyclic AMP-cyclic AMP receptor protein and cyclic di-GMP signaling in *Vibrio cholerae* biofilm formation. *J. Bacteriol.* 190, 6646–6659. doi: 10.1128/JB.00466-08

Foster, J. S., and Kolenbrander, P. E. (2004). Development of a multispecies oral bacterial community in a saliva-conditioned flow cell. *Appl. Environ. Microbiol.* 70, 4340–4348. doi: 10.1128/AEM.70.7.4340-4348.2004

Galié, S., García-Gutiérrez, C., Miguélez, E. M., Villar, C. J., and Lombó, F. (2018). Biofilms in the food industry: health aspects and control methods. *Front. Microbiol.* 9:898. doi: 10.3389/fmicb.2018.00898

Glonti, T., Chanishvili, N., and Taylor, P. W. (2010). Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J. Appl. Microbiol.* 108, 695–702. doi: 10.1111/j.1365-2672.2009.04469.x

Gonzalez, D. J., Haste, N. M., Hollands, A., Fleming, T. C., Hamby, M., and Pogliano, K. (2011). Microbial competition between *Bacillus subtilis* and *Staphylococcus aureus* monitored by imaging mass spectrometry. *Microbiology* 157, 2485–2492. doi: 10.1099/mic.0.048736-0

Habimana, O., Zanoni, M., Vitale, S., O'Neill, T., Scholz, D., Xu, B., et al. (2018). One particle, two targets: a combined action of functionalised gold nanoparticles, against *Pseudomonas fluorescens* biofilms. *J. Colloid Interface Sci.* 526, 419–428. doi: 10.1016/j.jcis.2018.05.014

Hall, M. R., McGillicuddy, E., and Kaplan, L. J. (2014). Biofilm: basic principles, pathophysiology, and implications for clinicians. *Surg. Infect.* 15, 1–7. doi: 10.1089/sur.2012.129

Hansen, S. K., Rainey, P. B., Haagensen, J. A. J., and Molin, S. (2007). Evolution of species interactions in a biofilm community. *Nature* 445, 533–536. doi: 10.1038/nature05514

Hardie, K. R., and Heurlier, K. (2008). Establishing bacterial communities by 'word of mouth': LuxS and autoinducer 2 in biofilm development. *Nat. Rev. Microbiol.* 6, 635–643. doi: 10.1038/nrmicro1916

Hernandez-Valdes, J. A., Zhou, L., de Vries, M. P., and Kuipers, O. P. (2020). Impact of spatial proximity on territoriality among human skin bacteria. *NPJ Biofilms Microbiomes* 6:30. doi: 10.1038/s41522-020-00140-0

Hobley, L., Harkins, C., MacPhee, C. E., and Stanley-Wall, N. R. (2015). Giving structure to the biofilm matrix: an overview of individual strategies and emerging common themes. *FEMS Microbiol. Rev.* 39, 649–669. doi: 10.1093/femsre/fuv015

Holcombe, L. J., McAlester, G., Munro, C. A., Enjalbert, B., Brown, A., Gow, N. A., et al. (2010). *Pseudomonas aeruginosa* secreted factors impair biofilm development in *Candida albicans*. *Microbiology* 156, 1476–1486. doi: 10.1099/mic.0.037549-0

Houot, L., and Watnick, P. I. (2008). A novel role for enzyme I of the *Vibrio cholerae* phosphoenolpyruvate phosphotransferase system in regulation of growth in a biofilm. *J. Bacteriol.* 190, 311–320.

Jackson, D. W., Simecka, J. W., and Romeo, T. (2002). Catabolite repression of *Escherichia coli* biofilm formation. *J. Bacteriol.* 184, 3406–3410. doi: 10.1128/JB.184.12.3406-3410.2002

Kalivoda, E. J., Brothers, K. M., Stella, N. A., Schmitt, M. J., and Shanks, R. M. Q. (2013). Bacterial cyclic AMP-phosphodiesterase activity coordinates biofilm formation. *PLoS One* 8:e71267. doi: 10.1371/journal.pone.0071267

Kim, Y. J., Yu, H. H., Song, Y. J., Park, Y. J., Lee, N.-K., and Paik, H.-D. (2021). Anti-biofilm effect of the cell-free supernatant of probiotic *Saccharomyces cerevisiae* against *Listeria monocytogenes*. *Food Control* 121:107667. doi: 10.1016/j.foodcont.2020.107667

Kolenbrander, P. E., and London, J. (1993). Adhere today, here tomorrow: oral bacterial adherence. *J. Bacteriol.* 175, 3247–3252. doi: 10.1128/jb.175.11.3247-3252.1993

Kolenbrander, P. E., Palmer, R. J., Periasamy, S., and Jakubovics, N. S. (2010). Oral multispecies biofilm development and the key role of cell–cell distance. *Nat. Rev. Microbiol.* 8, 471–480. doi: 10.1038/nrmicro2381

Kora, A. J., and Arunachalam, J. (2011). Assessment of antibacterial activity of silver nanoparticles on *Pseudomonas aeruginosa* and its mechanism of action. *World J. Microbiol. Biotechnol.* 27, 1209–1216. doi: 10.1007/s11274-010-0569-2

Kvich, L., Burmølle, M., Bjarnsholt, T., and Lichtenberg, M. (2020). Do mixed-species biofilms dominate in chronic infections?-Need for *in situ* visualization of bacterial organization. *Front. Cell. Infect. Microbiol.* 10:396. doi: 10.3389/fcimb.2020.00396

Lee, K. W. K., Periasamy, S., Mukherjee, M., Xie, C., Kjelleberg, S., and Rice, S. A. (2014). Biofilm development and enhanced stress resistance of a model, mixed-species community biofilm. *ISME J.* 8, 894–907. doi: 10.1038/ismej.2013.194

Li, X., Guo, H., Tian, Q., Zheng, G., Hu, Y., Fu, Y., et al. (2013). Effects of 5-aminolevulinic acid–mediated photodynamic therapy on antibiotic-resistant staphylococcal biofilm: an *in vitro* study. *J. Surg. Res.* 184, 1013–1021. doi: 10.1016/j.jss.2013.03.094

Liang, W., Silva, A. J., and Benitez, J. A. (2007). The cyclic AMP receptor protein modulates colonial morphology in *Vibrio cholerae*. *Appl. Environ. Microbiol.* 73, 7482–7487. doi: 10.1128/AEM.01564-07

Lin, Y.-C., Cornell, W. C., Jo, J., Price-Whelan, A., and Dietrich, L. E. P. (2018). The *Pseudomonas aeruginosa* complement of lactate dehydrogenases enables use of d- and l-Lactate and metabolic cross-feeding. *mBio* 9:e00961-18. doi: 10.1128/mBio.00961-18

Lu, J., Cokcetin, N. N., Burke, C. M., Turnbull, L., Liu, M., and Carter, D. A. (2019). Honey can inhibit and eliminate biofilms produced by *Pseudomonas aeruginosa*. *Sci. Rep.* 9:18160. doi: 10.1038/s41598-019-54576-2

McDonough, K. A., and Rodriguez, A. (2012). The myriad roles of cyclic AMP in microbial pathogens: from signal to sword. *Nat. Rev. Microbiol.* 10, 27–38. doi: 10.1038/nrmicro2688

Molin, S., and Tolker-Nielsen, T. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Curr. Opin. Biotechnol.* 14, 255–261. doi: 10.1016/S0958-1669(03)00036-3

Nguyen, D., Joshi-Datar, A., Lepine, F., Bauerle, E., Olakanmi, O., Beer, K., et al. (2011). Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. *Science* 334, 982–986.

Periasamy, S., and Kolenbrander, P. E. (2010). Central role of the early colonizer *Veillonella* sp. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. *J. Bacteriol.* 192, 2965–2972. doi: 10.1128/JB.01631-09

Povolotsky, T. L., Keren-Paz, A., and Kolodkin-Gal, I. (2021). Metabolic microenvironments drive microbial differentiation and antibiotic resistance. *Trends Genet.* 37, 4–8.

Powers, M. J., Sanabria-Valentín, E., Bowers, A. A., and Shank, E. A. (2015). Inhibition of cell differentiation in *Bacillus subtilis* by *Pseudomonas protegens*. *J. Bacteriol.* 197, 2129–2138. doi: 10.1128/JB.02535-14

Proctor, C. R., McCarron, P. A., and Ternan, N. G. (2020). Furanone quorum-sensing inhibitors with potential as novel therapeutics against *Pseudomonas aeruginosa*. *J. Med. Microbiol.* 69, 195–206. doi: 10.1099/jmm.0.001144

Reisner, A., Höller, B. M., Molin, S., and Zechner, E. L. (2006). Synergistic effects in mixed *Escherichia coli* biofilms: conjugative plasmid transfer drives biofilm expansion. *J. Bacteriol.* 188, 3582–3588. doi: 10.1128/JB.188.10.3582-3588.2006

Ren, D., Madsen, J. S., de la Cruz-Perera, C. I., Bergmark, L., Sørensen, S. J., and Burmølle, M. (2014). High-throughput screening of multispecies biofilm formation and quantitative PCR-based assessment of individual species proportions, useful for exploring interspecific bacterial interactions. *Microb. Ecol.* 68, 146–154. doi: 10.1007/s00248-013-0315-z

Richter, K., Thomas, N., Claeys, J., McGuane, J., Prestidge, C. A., Coenye, T., et al. (2017). A topical hydrogel with deferiprone and gallium-protoporphyrin targets bacterial iron metabolism and has antibiofilm activity. *Antimicrob. Agents Chemother.* 61:e00481-17. doi: 10.1128/AAC.00481-17

Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E., and Handley, P. S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol.* 11, 94–100. doi: 10.1016/S0966-842X(02)00034-3

Rojas Murcia, N., Lee, X., Waridel, P., Maspoli, A., Imker, H. J., Chai, T., et al. (2015). The *Pseudomonas aeruginosa* antimetabolite L -2-amino-4-methoxy-trans-3-butenoic acid (AMB) is made from glutamate and two alanine residues via a thiotemplate-linked tripeptide precursor. *Front. Microbiol.* 6:170. doi: 10.3389/fmicb.2015.00170

Römling, U., and Balsalobre, C. (2012). Biofilm infections, their resilience to therapy and innovative treatment strategies. *J. Intern. Med.* 272, 541–561. doi: 10.1111/joim.12004

Rosenberg, G., Steinberg, N., Oppenheimer-Shaanan, Y., Olender, T., Doron, S., Ben-Ari, J., et al. (2016). Not so simple, not so subtle: the interspecies competition between *Bacillus simplex* and *Bacillus subtilis* and its impact on the evolution of biofilms. *NPJ Biofilms Microbiomes* 2:15027. doi: 10.1038/npjbiofilms.2015.27

Ross, P., Weinhouse, H., Aloni, Y., Michaeli, D., Weinberger-Ohana, P., Mayer, R., et al. (1987). Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature* 325, 279–281. doi: 10.1038/325279a0

Rubini, D., Banu, S. F., Nisha, P., Murugan, R., Thamotharan, S., Percino, M. J., et al. (2018). Essential oils from unexplored aromatic plants quench biofilm formation and virulence of Methicillin resistant *Staphylococcus aureus*. *Microb. Pathog.* 122, 162–173. doi: 10.1016/j.micpath.2018.06.028

Salinas, N., Povolotsky, T. L., Landau, M., and Kolodkin-Gal, I. (2020). Emerging roles of functional bacterial amyloids in gene regulation, toxicity, and immunomodulation. *Microbiol. Mol. Biol. Rev.* 85:e00062-20. doi: 10.1128/MMBR.00062-20

Sambanthamoorthy, K., Luo, C., Pattabiraman, N., Feng, X., Koestler, B., Waters, C. M., et al. (2014). Identification of small molecules inhibiting diguanylate cyclases to control bacterial biofilm development. *Biofouling* 30, 17–28. doi: 10.1080/08927014.2013.832224

Schiessl, K. T., Hu, F., Jo, J., Nazia, S. Z., Wang, B., Price-Whelan, A., et al. (2019). Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in *Pseudomonas aeruginosa* biofilms. *Nat. Commun.* 10:762. doi: 10.1038/s41467-019-08733-w

Simm, R., Morr, M., Kader, A., Nimtz, M., and Römling, U. (2004). GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. *Mol. Microbiol.* 53, 1123–1134. doi: 10.1111/j.1365-2958.2004.04206.x

Singh, P. K., Schaefer, A. L., Parsek, M. R., Moninger, T. O., Welsh, M. J., and Greenberg, E. P. (2000). Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 407, 762–764. doi: 10.1038/35037627

Söderling, E. M., Marttinen, A. M., and Haukioja, A. L. (2011). Probiotic *Lactobacilli* interfere with *Streptococcus mutans* biofilm formation *in vitro*. *Curr. Microbiol.* 62, 618–622. doi: 10.1007/s00284-010-9752-9

Starkey, M., Hickman, J. H., Ma, L., Zhang, N., De Long, S., Hinz, A., et al. (2009). *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *J. Bacteriol.* 191, 3492–3503. doi: 10.1128/jb.00119-09

Steinberg, N., and Kolodkin-Gal, I. (2015). The matrix reloaded: how sensing the extracellular matrix synchronizes bacterial communities. *J. Bacteriol.* 197, 2092–2103. doi: 10.1128/JB.02516-14

Stokes, J. M., Lopatkin, A. J., Lobritz, M. A., and Collins, J. J. (2019). Bacterial metabolism and antibiotic efficacy. *Cell Metab.* 30, 251–259. doi: 10.1016/j.cmet.2019.06.009

Straight, P. D., and Joanne, M. (2006). Interactions between *Streptomyces Coelicolor* and *Bacillus Subtilis*: role of surfactants in raising aerial structures. *J. Bacteriol.* 188, 4918–4925.

Stubbendieck, R. M., and Straight, P. D. (2015). Escape from lethal bacterial competition through coupled activation of antibiotic resistance and a mobilized subpopulation. *PLoS Genet.* 11:e1005722. doi: 10.1371/journal.pgen.1005722

Tan, Y., Ma, S., Leonhard, M., Moser, D., Haselmann, G. M., Wang, J., et al. (2018). Enhancing antibiofilm activity with functional chitosan nanoparticles targeting biofilm cells and biofilm matrix. *Carbohydr. Polym.* 200, 35–42. doi: 10.1016/j.carbpol.2018.07.072

Tan, Y., Ma, S., Leonhard, M., Moser, D., Ludwig, R., and Schneider-Stickler, B. (2020). Co-immobilization of cellobiose dehydrogenase and deoxyribonuclease I on chitosan nanoparticles against fungal/bacterial polymicrobial biofilms targeting both biofilm matrix and microorganisms. *Mater. Sci. Eng. C* 108:110499. doi: 10.1016/j.msec.2019.110499

Townsend, E. M., Moat, J., and Jameson, E. (2020). CAUTI's next top model – model dependent *Klebsiella* biofilm inhibition by bacteriophages and antimicrobials. *Biofilm* 2:100038. doi: 10.1016/j.bioflm.2020.100038

Wang, B. Y., Chi, B., and Kuramitsu, H. K. (2002). Genetic exchange between *Treponema denticola* and *Streptococcus gordonii* in biofilms. *Oral Microbiol. Immunol.* 17, 108–112. doi: 10.1046/j.0902-0055.2001.00001.x

Wang, H.-H., Ye, K.-P., Zhang, Q.-Q., Dong, Y., Xu, X.-L., and Zhou, G.-H. (2013). Biofilm formation of meat-borne *Salmonella enterica* and inhibition by the

cell-free supernatant from *Pseudomonas aeruginosa*. *Food Control* 32, 650–658. doi: 10.1016/j.foodcont.2013.01.047

Wang, Z., Xiang, Q., Yang, T., Li, L., Yang, J., Li, H., et al. (2016). Autoinducer-2 of *Streptococcus mitis* as a target molecule to inhibit pathogenic multi-species biofilm formation *in vitro* and in an endotracheal intubation rat model. *Front. Microbiol.* 7:88. doi: 10.3389/fmicb.2016.00088

Williamson, K. S., Richards, L. A., Perez-Osorio, A. C., Pitts, B., McInnerney, K., Stewart, P. S., et al. (2012). Heterogeneity in *Pseudomonas aeruginosa* biofilms includes expression of ribosome hibernation factors in the antibiotic-tolerant subpopulation and hypoxia-induced stress response in the metabolically active population. *J. Bacteriol.* 194, 2062–2073. doi: 10.1128/JB.00022-12

Wright, A., Hawkins, C. H., Änggård, E. E., and Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clin. Otolaryngol.* 34, 349–357. doi: 10.1111/j.1749-4486.2009.01973.x

Yang, L., Liu, Y., Wu, H., Hóiby, N., Molin, S., and Song, Z. -j (2011). Current understanding of multi-species biofilms. *Int. J. Oral Sci.* 3, 74–81. doi: 10.4248/IJOS11027

Yu, H., Liu, Y., Li, L., Guo, Y., Xie, Y., Cheng, Y., et al. (2020). Ultrasound-involved emerging strategies for controlling foodborne microbial biofilms. *Trends Food Sci. Technol.* 96, 91–101. doi: 10.1016/j.tifs.2019.12.010

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

REVIEW
published: 30 March 2021
doi: 10.3389/fmicb.2021.623839

The Lichens' Microbiota, Still a Mystery?

Maria Grimm[1], Martin Grube[2], Ulf Schiefelbein[3], Daniela Zühlke[1], Jörg Bernhardt[1] and Katharina Riedel[1]*

[1] Institute of Microbiology, University Greifswald, Greifswald, Germany, [2] Institute of Plant Sciences, Karl-Franzens-University Graz, Graz, Austria, [3] Botanical Garden, University of Rostock, Rostock, Germany

Lichens represent self-supporting symbioses, which occur in a wide range of terrestrial habitats and which contribute significantly to mineral cycling and energy flow at a global scale. Lichens usually grow much slower than higher plants. Nevertheless, lichens can contribute substantially to biomass production. This review focuses on the lichen symbiosis in general and especially on the model species *Lobaria pulmonaria* L. Hoffm., which is a large foliose lichen that occurs worldwide on tree trunks in undisturbed forests with long ecological continuity. In comparison to many other lichens, *L. pulmonaria* is less tolerant to desiccation and highly sensitive to air pollution. The name-giving mycobiont (belonging to the Ascomycota), provides a protective layer covering a layer of the green-algal photobiont (*Dictyochloropsis reticulata*) and interspersed cyanobacterial cell clusters (*Nostoc* spec.). Recently performed metaproteome analyses confirm the partition of functions in lichen partnerships. The ample functional diversity of the mycobiont contrasts the predominant function of the photobiont in production (and secretion) of energy-rich carbohydrates, and the cyanobiont's contribution by nitrogen fixation. In addition, high throughput and state-of-the-art metagenomics and community fingerprinting, metatranscriptomics, and MS-based metaproteomics identify the bacterial community present on *L. pulmonaria* as a surprisingly abundant and structurally integrated element of the lichen symbiosis. Comparative metaproteome analyses of lichens from different sampling sites suggest the presence of a relatively stable core microbiome and a sampling site-specific portion of the microbiome. Moreover, these studies indicate how the microbiota may contribute to the symbiotic system, to improve its health, growth and fitness.

Keywords: lichens, symbiosis, microbiome, lichen-associated bacteria, *Lobaria pulmonaria*, omics

OPEN ACCESS

Edited by:
Nathalie Connil, Université de Rouen, France

Reviewed by:
Dirk Benndorf, Otto von Guericke University Magdeburg, Germany
Guilherme Lanzi Sassaki, Federal University of Paraná, Brazil

****Correspondence:***
Maria Grimm
maria.grimm@uni-greifswald.de

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *30 October 2020*
Accepted: *10 March 2021*
Published: *30 March 2021*

Citation:
Grimm M, Grube M, Schiefelbein U, Zühlke D, Bernhardt J and Riedel K (2021) The Lichens' Microbiota, Still a Mystery? Front. Microbiol. 12:623839. doi: 10.3389/fmicb.2021.623839

LICHENS: A FASCINATING SYMBIOSIS

Introduction

Evolution is replenished with examples of symbiotic life forms, which often include great examples of emergent metabolic solutions and joint morphologies. Being terrestrial symbioses and widespread in natural habitats, lichens are eye-catching examples that developed characteristic growth styles of their joined symbionts at least 415 million years ago (Honegger et al., 2013). According to the classic definition, lichens are a mutualistic relationship of one exhabitant heterotrophic mycobiont (i.e., a fungus, in most cases an ascomycete) and an autotrophic

photobiont. Because the fungal partner determines the morphology of lichens, classification is integrated in the system of fungi. Accordingly, lichens may also be understood as fungi forming self-sustained life-forms with algae. The large majority of the approximately 20.000 described lichen species are formed with a green alga as photobiont, whereas only 10% of lichens represent symbiotic associations with cyanobacteria. Two to four percent of lichenized fungi may associate with both types of phototrophs (e.g., **Figure 1**; Hawksworth et al., 1995; Honegger, 1996). The latter are commonly shared in a single fungal individual, where green algae dominate and auxiliary cyanobacteria colonize specialized gall-like compartments, called cephalodia (Büdel and Scheidegger, 1996). A few species may even develop differently shaped morphologies (photosymbiodemes) with either of the photobiont types (Nash, 2008), which allow them to establish in different environmental situations (Honegger, 1996; Stenroos et al., 2003).

Where Do Lichens Occur?

Lichens can grow on a broad range of substrates, yet, they are most common on bare rocks, on the bark of trees, or on compacted soil. Their habitats can be much more hostile than those favorable for higher plants (Kershaw, 1985; Walser, 2004). Despite being less productive, lichens can occur in rocky deserts or even at altitudes of up to 7400 m. Lichens can also form a major component of cool and humid habitats (e.g., Arctic tundra, boreal forest floors, lava fields, antarctic habitats, or rock surfaces in high altitudes or at the coast with tidal inundation). In certain ecosystems (e.g., tropical montane forests or temperate wet forests), epiphytic lichen biomass may exceed several hundred kilograms per hectare (Kershaw, 1985; Boucher and Stone, 1992; Coxson and Nadkarni, 1995). Yet, despite the outstanding tolerance of many species for desiccation, radiation, and extremes of temperatures in hostile environments, lichens are known as bioindicators of specific ecological situations. Apparently, lichens are very sensitive to ecological conditions when they are metabolically active, but they may tolerate extreme conditions when dry and metabolically inactive.

Special Peculiarities of the Lichen Symbiosis

Morphology

Lichens develop a wide range of morphological shapes and growth styles including brushy, crust-like, or leaf-like forms, to name just the most important ones. Crustose thalli attach directly to the substrate with their entire lower surface, yet many species can also develop within the substrate (e.g., in sandstone,

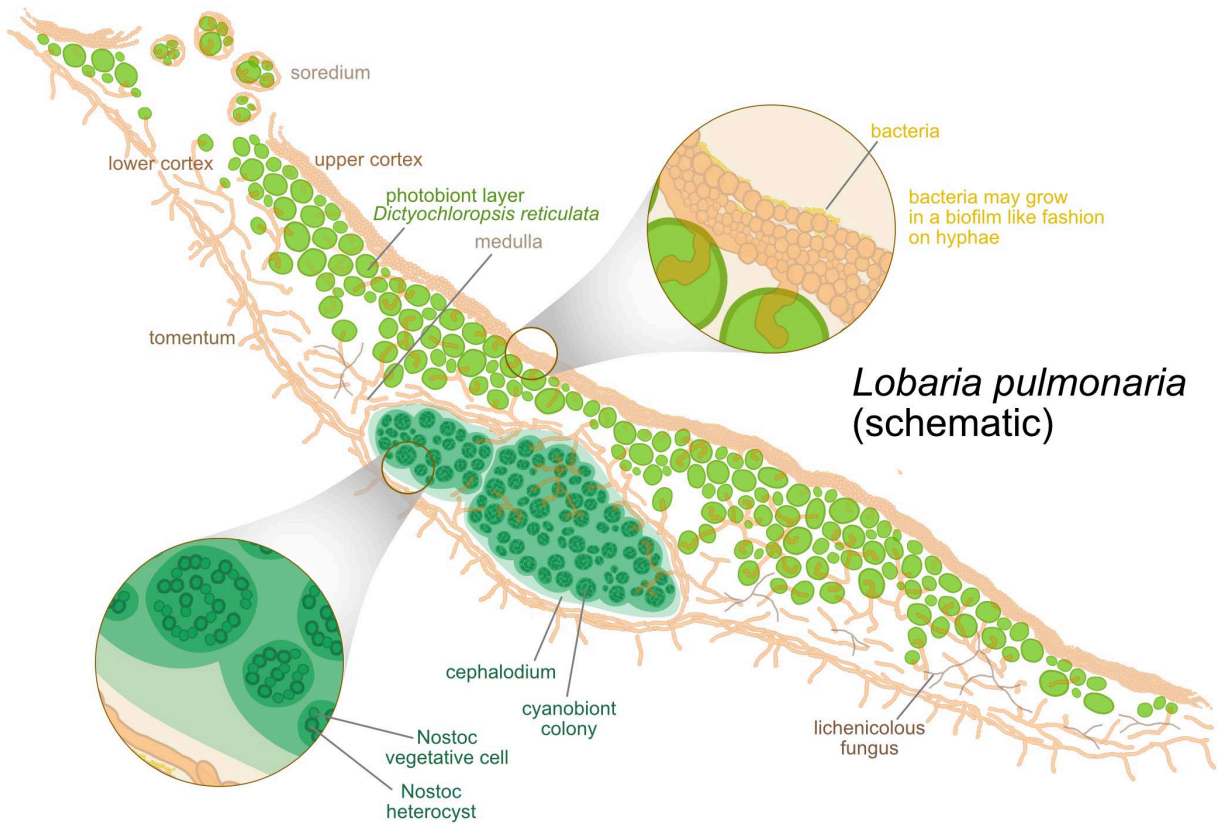


FIGURE 1 | Schematic anatomical structure of lichens, with *Lobaria pulmonaria* as an example. Algae are hosted in a layered fungal scaffold structure delimited by an upper and a lower cortex. Green algae form a layer under the upper cortex. Below the algal layer, a hydrophobic medulla facilitates gas exchange within the thallus. As a tripartite lichen, *L. pulmonaria* hosts clusters of cyanobacteria inside the thallus in addition to the algae.

calcareous rocks, or bark). Surface-detached thalli often develop specific holdfast structures. In contrast to crustose forms, lichens with leaf-like growth styles are often devoid of degrading algal cell wall remnants in their upper fungal cortex layers. The apparent lack of expressing algal cell wall digestive functions in these species possibly gives their thalli structures higher hygroscopic flexibility and structural stability. For a long time, lichen systematists classified lichens exclusively according to their thallus morphologies or by details of reproductive structures, until molecular data revealed the phylogenetic relationships of those forms in better detail. Since then, molecular studies uncovered multiple cases of convergent evolution of similar growth styles, and the repeated evolution of surface-detached forms from crustose ancestors (Grube and Hawksworth, 2007).

The proper development of the vegetative lichen thallus with specific algal strains is also generally necessary but not always sufficient for sexual reproduction by fruitbodies of the mycobiont partner and/or for production of asexual/mitotic structures for joint propagation of both symbionts.

Most of the lichen thalli are rather brittle when dry, but rather flexible when wet. Some of those containing cyanobacteria may then have a rather gelatinous consistency as well (Büdel and Scheidegger, 1996).

The formation of tight, yet swellable fungal layers to wrap algal colonies, is apparently one of the key evolutionary innovations enabling lichen thallus evolution. The processes leading to gluing fungal hyphae together by their outer cell walls might be related to those involved in the formation of fruit body structures in ascomycetes. The interaction of the fungi with appropriate photobionts is required for the production of the characteristic thallus morphologies, but also for production of specialized metabolites, which accumulate - often at substantial amounts - as crystallized lichen compounds in the intercellular spaces of the thalli.

What Holds the Lichen Together?

Hydrophilic, extracellular substances (EPS; **Figure 1**; Spribille et al., 2020) achieve the gluing together of short, branched and anastomosing fungal hyphae. In many lichens, the protecting conglutinated fungal layer not only contains mycobiont hyphae, but also bacteria and basidiomycete yeasts. Due to its well-developed EPS and multiorganismal composition, this layer can thus be considered as a complex biofilm or as an extracellular interaction matrix (EIM; Spribille, 2018; Tuovinen et al., 2019). The EIM not only functions as a sort of exoskeleton for stabilization but also as a receptacle for mineral nutrients, a development zone for secondary metabolites and as medium of intercell recognition and signaling. As far as is known, the EIM of lichens contains different polysaccharides, which typically consist of D-glucose, D-galactose and D-mannose. The different polysaccharides, such as β- and α-glucans, α-mannans or heteroglycans, found in lichen mycobionts are discussed in several reviews (e.g., Olafsdottir and Ingólfsdottir, 2001; Spribille et al., 2020).

The Basidiomycete genus *Tremella* produces glucuronoxylomannans as exopolysaccharide capsule material (GXM; Gorin and Barreto-Bergter, 1983; De Baets and Vandamme, 2001; Martinez and Casadevall, 2015). No GXM is currently known from lichens, but *Tremella* yeasts are known from several lichens such as *Letharia vulpina* (Tuovinen et al., 2019). Therefore, Spribille et al. (2020) assume that GXM occurs also in lichens. This would agree with observations of Grube and De Los Rios (2001) who observed acidic polysaccharides on the outer cell walls of the lichen-associated *Tremella* relative *Biatoropsis usnearum*. Beside polysaccharides found in the EIM and secreted by fungal partners, additional ones are isolated from lichen algal photobionts, like amylose, β-xylan (Cordeiro et al., 2003), β-(1→5)-galactofuranan (Cordeiro et al., 2005, 2008), rhamnogalactofuranan (Cordeiro et al., 2013), mannogalactan (Cordeiro et al., 2010), cellulose or 4-linked mannan (Carbonero et al., 2005; Centeno et al., 2016). Additionally, some cyanobacterial EPS are known, such as β-(1→4)-xylan and a complex polysaccharide formed of β-linked L-arabinose and D-xylose (Ruthes et al., 2010).

The role of lichen-associated bacteria in EIMs will be discussed in sections "Uptake and supply of nutrients, essential compounds and trace elements" and "Microbiome Acquisition and Shaping."

Poikilohydry: Life With Fluctuating Water Supply

Lichens are poikilohydric organisms meaning that they lack the ability to maintain and/or actively regulate their water content. Rather, their water status varies passively with the surrounding environmental conditions. In this regard, lichens differ from higher plants, where stomata and the cuticula help to manage the water balance. Therefore, lichens do not have a clear-cut separation between exo- and endosphere. As poikilohydric organisms, many lichens evolved extraordinary tolerance to desiccation. This includes antioxidant and photoprotective mechanisms (Kranner et al., 2005, 2008), and strategies enabling the lichen to cope with mechanical stress under changing hydration conditions. In dry conditions, the fungal layer consists of a dense EPS matrix and compressed hyphae. During lichen hydrating, the extracellular matrix expands by multiple times of its desiccated volume (Spribille et al., 2020). A more or less coherent layer that can flexibly respond to shrinking and swelling of the algal cells under variable conditions of hydration represents a main principle of the vegetative mycelium. The adaptation of some lichens to habitats with different water regimes also includes the choice of the photobiont. Green algae in lichens can activate their photosynthesis in a humid/moist environment whereas cyanobacteria in lichens usually require liquid water (Richardson, 2002). This explains why cyanolichens are more commonly found under conditions characterized by run-off water. Generally, the light and desiccation tolerance is facilitated by symbiotic interaction, thus ameliorating the stress faced by separated partners (Walser, 2004; Kranner et al., 2005; Jüriado and Liira, 2009; see also section "What **H**olds the **L**ichen **T**ogether?").

Reproduction

Sexual reproduction of the algal partner is generally suppressed, but the fungal partner sexually reproduces commonly by production of meiospores in fruit-bodies. Fruit-bodies are usually perennial and can produce spores for many years under suitable

circumstances, unlike those of many non-lichenized ascomycetes. Asexual, mitotic reproduction of the fungal partner is also known (e.g., thallospores, conidia). However, the fungal spores need to re-establish the symbiosis with a specific photobiont species, and despite the partners having the potential to grow autonomously (as they can be cultivated separately and are found by environmental sequencing), only the encounter of suitable photobiont initiates the ontogenetic processes to produce the characteristic thallus morphology. Therefore, lichens evolved also very diverse forms of asexual symbiotic propagules apart from the dispersal of fungal propagules alone (**Figure 1**). These structures range from dust-like or grainy microscopic particles containing few algal-encaging fungal hyphae (soredia) to more complex, stratified structures of different shapes and sizes (blastidia, isidia, schizidia, phyllidia, etc.) or small thallus fragments (folioles, squamules, etc.) that easily detach from the parental lichen thallus (Bowler and Rundel, 1975; Scheidegger, 1995).

Lichen Tolerance to Toxic Compounds

A remarkable feature of lichens (and many other macroscopic fungi) is that they are valuable and sensitive bioindicators of environmental and industrial pollution. Most lichens are sensitive to sulfur dioxide, which disrupts photosynthesis. Some lichens, however, have the ability to reduce the uptake of acidic precipitation containing sulfur dioxide. Hauck et al. (2008) pointed out that surface hydrophobicity is the main factor controlling the tolerance of these lichens for sulfur dioxide. Hydrophobicity could also explain that sulfur dioxide tolerant lichen species show a general toxin tolerance. As lichens also take up and tolerate metals in the thallus, they are used for environmental monitoring of industrial pollution (Garty, 2001). The production of metal oxalates (e.g., hydrated copper oxalate, manganese oxalate or lead oxalate) is a common adaptive biological mechanism for metal detoxification in lichens (reviewed by Purvis, 2014). Superficial oxalates on the thallus surface may help deflect the amount of light reaching the lichen photobiont and therefore help lichens to survive in extreme environments. An additional detoxification response to heavy metals is found in *Lecanora polytropa*. Phytochelatin (cysteine-rich peptides derived from glutathione) is produced to chelate these metals intracellularly (reviewed by Purvis, 2014).

Symbiotic Partnership

Mycobiont: Home Sweet Home

The lichen-forming fungus develops the characteristic morphology of the lichen. It exposes the photobiont to controlled levels of sunlight during physiologically active stages, because a too strong light intensity can damage the photobiont (**Figure 1**; Larson, 1987).

A highly hydrophobic cell wall surface layer of mycobiont origin can be formed at the contact of the growing hyphae with a photobiont cell. This layer spreads over the wall surface of the entire algal cell, which seals the apoplastic space and channels the metabolic flux among symbiotic partners. Proteins, lipids and fungal derived phenolic secondary compounds form this hydrophobic coat. These secondary metabolites crystallize on and within the cell wall surface layer (Honegger, 1984, 1991; Scherrer et al., 2000).

The mycobiont is able to shift the position of the algal cells over short distances to secure adequate illumination and most efficient photosynthesis (Honegger, 1987, 1990). The hydration state of the cortical layer influences the light absorption and transmission. Additionally, the thickness of the cortical layer or the presence of insoluble mineral complexes, such as oxalates of calcium, copper, magnesium or manganese, and fungal-derived crystalline secondary compounds may affect the amount and spectral composition of incoming light (Honegger, 1991). Furthermore, the cortical layer has to facilitate the gas exchange of its algal photobiont (Honegger, 1990), which benefits from the position of the photobiont cells at the periphery of the gas-filled thalline interior underneath the fungal cortical layer. The cortex allows CO_2 diffusion when the thalline water content is moderate, while water-supersaturated conditions may limit net photosynthesis rates significantly. As an adaptation to adequate level of CO_2 absorption, a distinct proportion of the thalline volume [e.g., 30-50% in *Parmelia sulcata* (Fiechter, 1990)] acts as gas-filled intercellular space in the medullary and algal layers where mycobiont-derived respiratory CO_2 can be stored (Honegger, 1991).

High light intensity can damage the photobiont (e.g., Demmig-Adams et al., 1990; Gauslaa and Solhaug, 1996, 1999, 2000). Depending on the long-term level of solar radiation, some lichenized fungi also produce melanic compounds in the outer layer of the upper cortex as a sunscreen. The melanic compounds reduce UV-B and UV-A wavelengths, but also visible wavelengths, reaching the photobiont layer. The melanisation leads to a browning of the cortex. This browning of the cortex is a physiologically active process, which occurs only in hydrated lichen thalli (Gauslaa and Solhaug, 2000, 2001; Solhaug et al., 2003; Antoine and McCune, 2004; Gauslaa and McEvoy, 2005; McEvoy et al., 2007; Nybakken et al., 2007; Matee et al., 2016; Mafole et al., 2017). As a side effect, melanins increase the absorbance of solar energy for the whole thallus resulting in a temperature increase of up to 3°C, which could suggest quicker water loss than in other species, which instead use crystallized secondary metabolites (e.g., atranorin) as light shields. Secondary metabolites appear as effective as melanin in reducing the transmission of photosynthetic active radiation, but reflect rather than absorb this radiation. Thus, these compounds have less effect on the heat balance of lichens (McEvoy et al., 2007; Solhaug et al., 2010; Mafole et al., 2017). Beside the production of melanic compounds or secondary metabolites, curling of the thalli during drying represents an additional strategy against serious photo-damaging thalli of *L. pulmonaria* during high light exposure (Barták et al., 2006).

Photobiont: The Breadwinner

The photobiont produces and secretes mobile energy-rich carbohydrates, which are provided to the fungal partner as sugar alcohols, such as polyols including ribitol, erythritol or sorbitol (Richardson et al., 1968; Hill, 1972; Brown and Hellebust, 1978; Smith and Douglas, 1987; Eisenreich et al., 2011; Eymann et al., 2017). The photobiont's cell walls are permeable to

carbohydrates in the lichenized state. Therefore, ribitol reaches the lichen fungus via diffusion (Richardson et al., 1968; Hill, 1976). The photosynthate release is probably stimulated by the synthesis and metabolism of taurine (Wang and Douglas, 1997). Taurine catabolism dioxygenase, which is an indicator for sulfonate utilization and metabolism of taurine, is identified in the proteome of the mycobiont of *L. pulmonaria* (Eymann et al., 2017). For storage, the mycobiont partly converts the carbohydrates provided by the photobiont into mannitol via the pentose phosphate pathway (Smith, 1980; Lines et al., 1989; Eisenreich et al., 2011). Polyols cannot be regarded as safe storage products because they can be washed out of the thallus during rewetting after drought stress (MacFarlane and Kershaw, 1985; Dudley and Lechowicz, 1987). Polyols more likely protect enzyme systems during stress events and act as osmoregulators during the wetting and drying cycles. Additionally, they can be substituted for water and stabilize proteins and membranes under dry conditions (Bewley and Krochko, 1982; Farrar, 1988; Jennings and Lysek, 1996). This raises the interesting hypothesis that the green algae originally synthesize the sugar alcohols only as compatible solutes mainly just to keep water in the cells and to protect their proteins under dry stress conditions. Rapid rewetting, e.g., by rain, could force the green algae to export polyols very rapidly to prevent uncontrolled water influx and cell damage. Involuntarily released sugar alcohols could thus possibly played a role in early stages of lichen evolution.

When the lichen thallus is drying the water from the apoplastic space between the cell walls of the symbiotic partners is lost at first. Afterward, cellular water is partially lost, leading to drastic but reversible cell shrinkage. The mycobiont and photobiont cells are able to tolerate dramatic fluctuations in cellular water contents between saturation (> 150% water content in relation to dry weight) and desiccation (< 20% water content in relation to dry weight). Soluble compounds are passively released into the apoplastic space due to reversible leaching from the cell during extreme drought. Lichen photobionts reversibly inactivate their photosystem II during desiccation. Water flows passively back and forth within the apoplastic space during the regularly occurring de- and rehydration of the thallus. Thereby, not only dissolved mineral nutrients, but also other passively and actively released metabolites from the symbiotic partners are translocated (MacFarlane and Kershaw, 1985; Brown et al., 1987; Dudley and Lechowicz, 1987; Honegger, 1991, 1997; Scheidegger, 1994; Heber et al., 2010).

Eymann et al. (2017) assign beta-catenin to the proteome of the algal photobiont of *L. pulmonaria*. It is important for the signaling during cell-cell adhesion of unicellular organisms (Abedin and King, 2010), in the case of lichens between cells of the mycobiont and photobiont or among the photobiont cells and allows a rapid exchange of signals and substrates.

Cyanobiont: Grabbing Nitrogen

In tripartite lichens, the cyanobiont is predominantly responsible for nitrogen fixation (Millbank and Kershaw, 1970). This is supported by the identification of glutamate synthase and molybdenum nitrogenase in the proteome of the cyanobiont of *L. pulmonaria* (Eymann et al., 2017). In other lichens, such as *Peltigera aphthosa*, alternative nitrogenases, which use vanadium or iron instead of molybdenum at the active site, might also play an important role in biological nitrogen fixation lichens (Darnajoux et al., 2017). Because oxygen is inhibiting denitrogenase, the fungal partner creates microaerobic conditions and accumulates the cyanobiont cells in gall-like structures, also known as cephalodia (Jordan, 1970; Hawksworth and Hill, 1984; Jahns, 1988; Honegger, 1993; Büdel and Scheidegger, 1996; Cornejo and Scheidegger, 2013). Nitrogen fixation of the cyanobionts of lichens is substantial for nitrogen cycling in nutrient-poor environments where nitrogen leaks from growing and degrading lichens (Nash, 1996). In tripartite lichens, the cyanobacterial heterocyst frequency is increased. In bipartite lichens, the heterocyst frequency varies between 2% and 8%, whereas in tripartite lichens, it varies between 10% and 55%. Nevertheless, the relative number of cyanobacteria is kept lower than that of the algal photobiont. Thus, the lichen fitness increases through specialization of the cyanobiont on nitrogen fixation (Hyvärinen et al., 2002).

Although labor partition favors the role of nitrogen fixation in cephalodiate cyanobionts, the cyanobiont is also partly responsible for carbon fixation and thus, involved in photosynthesis. Cyanobacteria as well as red algae have a light-harvesting complex, which is different to that of green algae, the phycobilisome. This complex is built up of phycobilins, like phycoerythrin and phycocyanin. The phycobilisome absorbs light between 450 nm and 650 nm and sometimes beyond 700 nm. Chlorophyll a and b absorb light between 400 and 480 (blue light) and between 550 and 700 (yellow to red light). Therefore, green light is rarely absorbed by the green algal photobiont, whereby tripartite lichens and chlorolichens appear greenish (Kadereit et al., 2014; Adir et al., 2019). The existence of phycobilisome proteins is reported in the proteome of the cyanobiont of *L. pulmonaria* (Grube and Berg, 2009; Eymann et al., 2017).

Mycobiome: The Recycler, Probably?

The longevity and slow growth of lichen structures may also foster the colonization of lichens by more or less specific additional microorganisms. Lichenicolous (= lichen colonizing) fungi (**Figure 1**) were actually recognized even before the symbiotic nature of lichens was discovered in the second half of the 19th century. About 1800 lichenicolous fungi are known today and most of these are characterized by their morphological structures[1] (Lawrey and Diederich, 2003; Diederich et al., 2018). Each lichen can harbor a wide range of additional fungal components, which constitute an associated mycobiome (Fernandéz-Mendoza et al., 2017; Muggia and Grube, 2018). The majority of the lichenicolous fungi only cause local infections in the host thallus or are more or less commensalic. The commensalic behavior usually correlates with a preference for the algal photobiont of the host lichen. Recent evidence suggests that commensalism also applies to yeasts resident in fungal layers of the lichen. Spribille et al. (2016) assumed that commensalic basidiomycete yeasts residing in the fungal upper

[1] www.lichenicolous.net

cortex maintain close associations with specific lichen species over large spatial distances. This view was questioned by recent evidence of low host specificity of yeast stages compared to the known mycelial lichenicolous fungi. According to Mark et al. (2020), various epiphytic lichen species growing on the same tree trunk consistently harbor specific *Trebouxia* lineages as photobionts, while genotypes of the cystobasidiomycetous yeasts were irregularly distributed among the species.

Lichenicolous fungi contact the cells of their hosts using specific structures. De Los Rios and Grube (2000) described infectious hyphal structures, including simple or complex haustoria with projections into the host hyphae. Other species were found to use haustoria to penetrate the algal photobiont of their hosts (*Zwackhiomyces*; Grube and Hafellner, 1990). Furthermore, some lichen-associated fungi have necrotic to saprobic life styles, preferring decaying parts of lichens (Aptroot and Alstrup, 1999). Other lichenicolous fungi can cause hypertrophic deformations (galls) in lichen thalli, which usually contain hyphae of both fungal species. These galls also provide a microhabitat for additional fungi, including the yeast genus *Cyphobasidium* (Millanes et al., 2016).

Only a small fraction of lichenicolous species actually cause dramatic damage to their hosts and may rapidly erase lichen coverage of substrates (including the facultative lichen pathogen *Athelia arachnoidea*).

Lichenicolous fungi, which use structures of the host to establish their own lichenized thalli are called lichenicolous lichens (Poelt, 1990). Their parasitic thalli develop on the surface or as internal structures of the host (Grube, 2018), and they often also take up the algae of the hosts. Because the lichenicolous lichens can also be infected by lichenicolous fungi, lichens also provide interesting examples of hyperparasitism. For example, the lichenicolous lichen *Rhizocarpon diploschistidina* parasitizes the lichen *Diploschistes muscorum*, which usually grows as a juvenile parasite of *Cladonia* species (Lumbsch et al., 2011).

Microbiome: The New Teammate

Lichen-associated bacteria were initially isolated in the first half of the 20th century (Cengia Sambo, 1926; Henckel and Yuzhakova, 1936; Honegger, 1990). These characterizations already indicated a possible role in nitrogen fixation for some of these lichen-associated bacteria. Evidence for the presence of bacteria (other than cyanobacteria) in lichens is provided by a series of studies that are based on morphological evidence using cultivable isolates of bacteria in lichens (e.g., Panosyan and Nikogosyan, 1966; Henkel and Plotnikova, 1973). In the early 1980s, Lenova and Blum (1983) estimated that millions of bacterial cells per gram could colonize a lichen thallus. More than 20 years later, diversity and specificity was studied in more detail by culture-independent sequencing approaches, and later by more sophisticated omics technologies, which demonstrated that lichens are furnished with a complex bacterial microbiome (**Figure 1**) (e.g., González et al., 2005; Cardinale et al., 2006; Liba et al., 2006; Grube et al., 2009; Selbmann et al., 2010; Bates et al., 2011; Hodkinson et al., 2012; Aschenbrenner et al., 2014). The lichen microbiome is identified as a surprisingly abundant and structurally integrated element of the classical lichen symbiosis (e.g., Gauslaa and Solhaug, 1996, 1999; Aschenbrenner et al., 2016; Leiva et al., 2021). These studies reveal that lichen-associated bacterial communities are not merely a simple extension of the prokaryotic community of the lichen-surrounding environment. The lichen microbiome consists of two parts. The first part is a core microbial community. Therefore, the microbiome is stable to a certain extent. The second part of the microbiome is a specific microbial community, even in close spatial proximity or when lichens are reshaped by parasitic invasion of one lichen into the other (Wedin et al., 2016). Thus, the lichen species is probably the best predictor of its microbiome composition (Bates et al., 2011). These findings led to reconsideration of the lichen symbiosis including the microbiome as an additional component (Hawksworth and Grube, 2020).

Lichen-Associated Viruses

Reports of lichen-associated viruses are found in recent studies. In several lichens, single and double-stranded RNA viruses, similar to those of plants were found (Petrzik et al., 2014, 2016). Yet, lichen viruses are not only of apparent algal origin. Petrzik et al. (2019) detected novel dsRNA viruses in the lichens *Chrysothrix chlorina* (Chrysothrix chrysovirus 1; CcCV1) and *Lepraria incana* (Lepraria chrysovirus 1; LiCV1) and classified them to the genus *Alphachrysovirus,* with a relationship to chrysoviridae from filamentous ascomycetous fungi. However, the authors showed that CcCV1 was not found in the lichen mycobiont but in the accompanying endolichenic fungus *Penicillium citreosulfuratum*. Using dsRNA-seq technology, Urayama et al. (2020) characterized the total dsRNA viral community of a lichen species (species name not indicated, but apparently belonging to the *Cladonia pyxidata* complex according to their **Figure 1**), and revealed that partitiviruses were dominant and active. Sequences found in this study were classified into two genera, which include both plant- and fungi-infecting partitiviruses. Apparently, each of the lichen partners may harbor several virus species independently and simultaneously because CaMV and the capsid protein gene of ApMV were detected in the photobiont of *Xanthoria parietina* and both, the plant cytorhabdovirus and the ApMV, were detected in *U. chaetophora* (Petrzik et al., 2014, 2015). Also, proteins assigned to rhabdoviruses and betaflexiviruses were found in the metaproteome of *L. pulmonaria* (Eymann et al., 2017), and Grube et al. (2015) found bacteriophage sequences in the metagenome of the same lichen. Presence of bacteriophages in lichens is confirmed by occurrences of bacteriophage proteins assigned to the families Myoviridae and Siphoviridae which infect Bacteria and Archaea (Eymann et al., 2017).

THE PROKARYOTIC MICROBIOME OF LICHENS

Bacteria Living With Lichens

More than 800 types of bacteria can contribute to the bacterial microbiome of a single lichen individual. In many so far studied lichens, Alphaproteobacteria form the largest and metabolically most active bacterial class

(Cardinale et al., 2006, 2008; Grube and Berg, 2009; Grube et al., 2009, 2015; Bates et al., 2011; Schneider et al., 2011). Within the Alphaproteobacteria, the Rhizobiales make up the majority. In chlorolichens, Rhodospirillales are often co-dominant contrary to cyanolichens where Sphingomonadales normally co-dominate. However, Alphaproteobacteria are not always dominant. The rock-inhabiting lichen *Ophioparma* is dominated by Acidobacteria (Hodkinson et al., 2012), and marine lichens (e.g., *Lichina pygmaea*) differ by a dominance of Bacteroidetes beside Chloroflexi and Thermi (West et al., 2018). Due to their preference to form communities with fungi and in addition with Alphaproteobacteria on plant surfaces, it is not surprising to find also *Paenibacillus* and *Burkholderia* phylotypes within the microbial community of terrestrial lichens (Berg and Hallmann, 2006; Cardinale et al., 2006; Grube and Berg, 2009; Partida-Martinez et al., 2007). Other well-known bacterial lineages can be found in lichens such as Firmicutes, Bacteroidetes, Verrumicrobia, Acidobacteraceae, Acetobacteraceae, Brucellaceae or Chloroflexi (González et al., 2005; Grube et al., 2009; Bates et al., 2011; Schneider et al., 2011; Hodkinson et al., 2012; Garg et al., 2016).

So far, most microbiome studies focused on lichens belonging to Lecanoromycetes. One of them is the lung lichen *L. pulmonaria* L. Hoffm. (**Figure 2**) and is currently under intense investigation. One third of the overall bacteria in *L. pulmonaria* belong to the Rhizobiales (in particular to the families Methylobacteriaceae, Bradyrhizobiaceae, and Rhizobiaceae), which are well known partners in plant-microbe interactions (Erlacher et al., 2015). Actinobacteria, Betaproteobacteria, Firmicutes or Deinococcus (Cardinale et al., 2006; Hodkinson and Lutzoni, 2009) and Archaea are complementing the *L. pulmonaria* microbiome (Schneider et al., 2011; Eymann et al., 2017). *L. pulmonaria* is an interesting model to investigate genotypic and phenotypic traits that enable the lichen to adapt to changing climate conditions. In the following paragraphs, we will present a more detailed look at *L. pulmonaria* and its microbiome together with the microbiome of other lichen species. Moreover, we will introduce important techniques for studying composition and function of lichen microbiomes.

The Model Lichen *L. pulmonaria*

Lobaria pulmonaria is a large foliose lichen (**Figure 2A**). Besides a myco- and a photobiont, *L. pulmonaria* contains a cyanobiont, a cyanobacterium belonging to the genus *Nostoc*. The green algal photobiont *Dictyochloropsis reticulata* dominates 90% of the subcortical layer of the thallus (Honegger, 1991; Myllys et al., 2007; **Figure 2B**).

Lobaria pulmonaria is distributed over an area in the Northern Hemisphere from the boreal to the meridional zone (**Figure 3**, GBIF.org 2019). In Eurasia as well as in Northern America, it has a Western and an Eastern subarea, excluding regions influenced by a strong and dry continental climate with precipitation less than (450)-500 mm. Furthermore, *L. pulmonaria* occurs in the montane belt of East Africa, on Madagascar, the Mascarene archipelago and in South Africa (**Figure 3**). Occurrences in Central America still merit further investigation and need to be validated. The geographic range reflects the ability of *L. pulmonaria* to inhabit regions with different overall conditions. The mean annual temperature at the Northernmost locations in Scandinavia is about 1°C and the mean annual precipitation about 500 mm, on the Azores archipelago about 18°C and 1.500 mm, respectively, and in Siberia at the Baikal lake −0,9°C and about 500 mm, respectively (Worldclim 2.0). Consequently, *L. pulmonaria* occurs in a wide spectrum of habitats mostly on tree trunks, e.g., *Fagus sylvatica*, *Quercus robur* or *Acer platanoides* (Hakulinen, 1964; Wirth, 1968; Hallingbäck and Martinsson, 1987; Rose, 1992; Wolseley and James, 2000; Kalwij et al., 2005; Jüriado and Liira, 2009; Smith et al., 2009; Wirth et al., 2013), but it can also colonize dwarf scrubs (e.g., *Calluna*), mossy rocks and soil (Ingólfsdóttir et al., 1998; Scheidegger and Goward, 2002; Grube et al., 2015). The current distribution of *L. pulmonaria* is the result of several factors. Air pollution by sulfur dioxide (e.g., Hawksworth and Rose, 1970; Hawksworth et al., 1973; Hallingback and Olsson, 1987; Farmer et al., 1991), and later, by nitrogen containing air pollutants (e.g., Hauck and Wirth, 2010) led to extinction or a reduced frequency in many regions of the original distribution area. The forestry practices strongly influenced *L. pulmonaria* in different respects for a long time. Clear cutting and afforestation with trees not suitable to the location (e.g., spruce, pine) led directly to the loss of habitats, other forestry measures to negative changes of the habitat conditions (e.g., Edman et al., 2008; Jüriado et al., 2011; Wirth et al., 2011; Schiefelbein and Thell, 2018). In conclusion, *L. pulmonaria* occurs mostly in undisturbed natural forests, with long ecological continuity due to a stable environment in terms of light, moisture and temperature, which are little influenced by air pollution, agriculture and forestry practices.

Under suitable conditions, *L. pulmonaria* may have a long life (acc. to estimates up to 200 years) (Scheidegger et al., 1998) and is described as a 'patchtracking' organism (Snäll et al., 2003), colonizing tree boles, where it may persist until the tree dies or microclimatic conditions change. During the life cycle of *L. pulmonaria*, estimated to an average of 35 years, the dispersal via vegetative diaspores (soredia, isidioid soredia) or thallus fragments predominates (Scheidegger et al., 1998; Scheidegger and Goward, 2002). Molecular and empirical studies show that *L. pulmonaria* can efficiently propagate by their vegetative propagules up to 75 m (Walser et al., 2001; Öckinger et al., 2005; Werth et al., 2006), but the majority of *L. pulmonaria* vegetative propagules are detected at a very short spatial scale, i.e., < 40 m (Werth et al., 2006). Under proper conditions (which usually agrees with larger thallus sizes), fruitbodies and ascospores develop (Scheidegger and Goward, 2002; Martínez et al., 2012; Wirth et al., 2013) and facilitate the long-distance dispersal of the mycobiont (Werth et al., 2006). With up to 5 mm annual tip growth, *L. pulmonaria* is one of the fastest growing lichens in Europe (Phillips, 1969). Microsatellite analysis by Werth and Scheidegger (2012) reveal dispersal via symbiotic propagules as the key factor shaping the genetic structure of this species. The highest growth rates and shortest generation times, with reproductive maturity are achieved within around five to ten years, as Eaton and Ellis (2014) observe in an oceanic hazelwood in Western Scotland. Thus, in oceanic environments,

FIGURE 2 | *Lobaria pulmonaria*. **(A)** Specimen growing on *Fagus sylvatica* (photographed in May 2016 near Viborg, Denmark); **(B)** Microscopic cross section. The upper cortex forms the habitat of the microbiome. Image produced with dry, sectioned material using a Keyence Digital VHX-5000 microscope with automated image stacking.

e.g., along the west coast of Scotland (Seaward, 1998; Eaton and Ellis, 2014), *L. pulmonaria* is very common. The difference in abundance between oceanic and more continental environments possibly reflects an interaction between the macroclimate and an organisms' microhabitat specificity (Lisewski and Ellis, 2010), such that a species may become increasingly restricted to a limited suite of buffered microclimatic niches under sub-optimal macroclimatic conditions (Doering and Coxson, 2010), and therefore rarer.

Because of its specific features and threats, *L. pulmonaria* is extinct or at risk in many regions of its distribution area (e.g., Türk and Hafellner, 1999; Scheidegger and Clerc, 2002; Bardunov and Novikov, 2008; Randlane et al., 2008; Farkas and Lõkös, 2009; Aptroot et al., 2012; Wirth et al., 2011).

This lichen species is used as a model lichen for studies on the lichen microbiome mainly due to its relatively fast growth and its ecological significance.

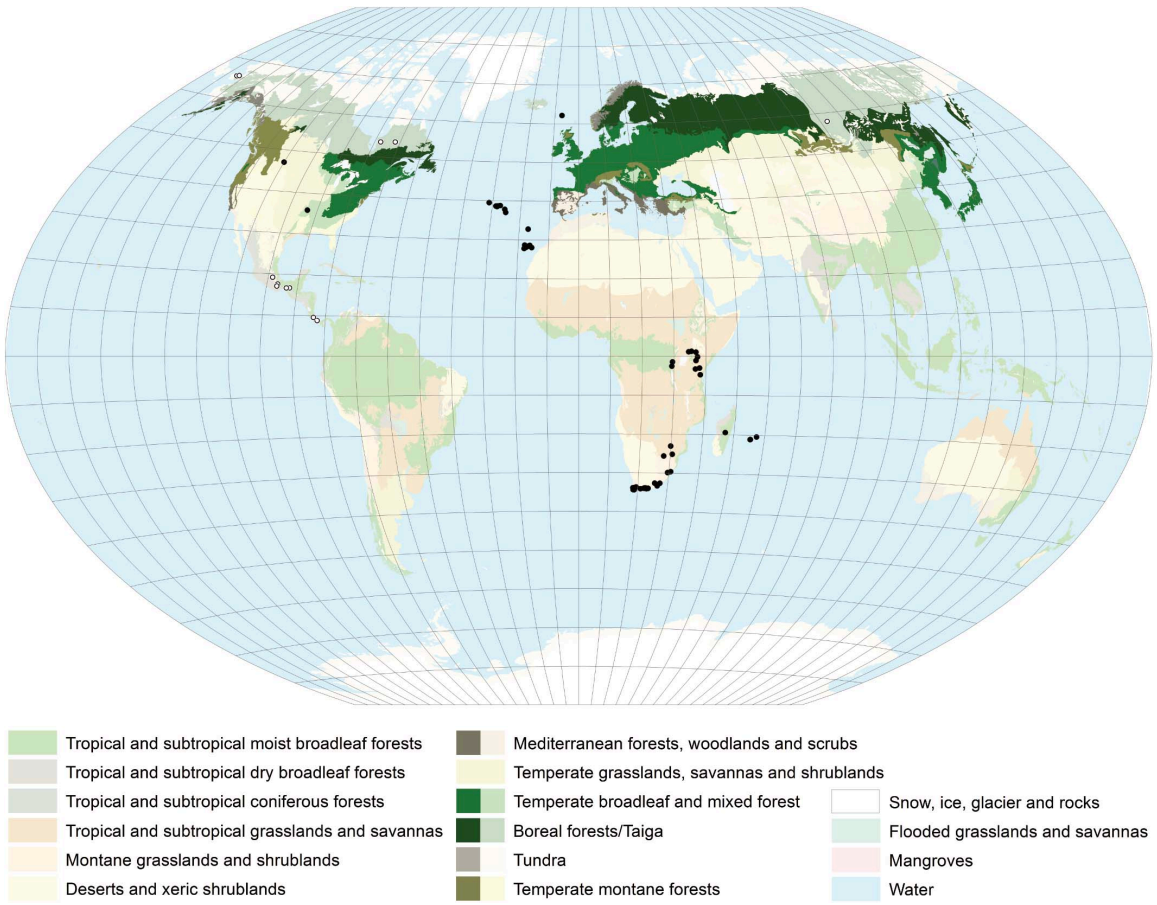


FIGURE 3 | Distribution map of *L. pulmonaria* created with ArcGIS 10. Colors indicate the different vegetation zones. Strongly colored areas – general distribution regions. Solid dots – single occurrence spots. Open dots – occurrence not clarified. *L. pulmonaria* has a worldwide distribution mainly occurring in the Northern Hemisphere.

Molecular Tools to Investigate Lichen-Associated Microorganisms

The Rise of 'Omics' Technologies

Lichen-associated bacteria were initially identified and investigated describing bacteria by their phenotypical and physiological characterizations (Cengia Sambo, 1926; Iskina, 1938; Panosyan and Nikogosyan, 1966; Henkel and Plotnikova, 1973; Lenova and Blum, 1983). Later, molecular techniques using bacterial isolates were conducted (González et al., 2005; Cardinale et al., 2006; Liba et al., 2006; Selbmann et al., 2010). However, culture-dependent methods generally capture only a minor fraction of the bacterial diversity of environmental samples (Amann et al., 1995). Therefore, new techniques were elaborated and the first culture-independent investigations, such as fingerprinting methods (Cardinale et al., 2006, 2012a; Grube et al., 2009; Bjelland et al., 2011; Mushegian et al., 2011) or molecular cloning approaches (Hodkinson and Lutzoni, 2009), were started to generate microbial community profiles. A further advantage of these methods is the possibility to analyze many samples in parallel and to compare their profiles consistently. Using the pyrosequencing method, several DNA-based studies described lichen-associated bacterial communities (Bates et al., 2011; Grube et al., 2012; Hodkinson et al., 2012; Aschenbrenner et al., 2014).

By improvement of the sequencing methods and bioinformatic tools, research focus shifted to a more detailed and holistic view on lichen microbial communities. In this respect, the lichen microbiome can be studied using a diverse spectrum of 'omics' technologies (**Figure 4**). Despite metagenomic analyses (e.g., Grube et al., 2015; Cernava et al., 2015; Aschenbrenner et al., 2017; Cernava et al., 2018; Graham et al., 2018), many studies applied metatranscriptomic (e.g., Cernava et al., 2017, 2019) and metaproteomic (e.g., Berg et al., 2011; Schneider et al., 2011; Grube et al., 2015; Cernava et al., 2017; Eymann et al., 2017; Ullah et al., 2020) approaches to study lichen symbioses.

(Meta)Genomics: Accessing Life's Blueprint

The total genetic information (encoded by DNA) of an organism is defined as its genome and all genomes within a habitat form the habitat's metagenome. Despite long-term evolutionary processes the environment has only minor influence on the DNA structure and information content (**Figure 4**). Due to DNA's stability and its

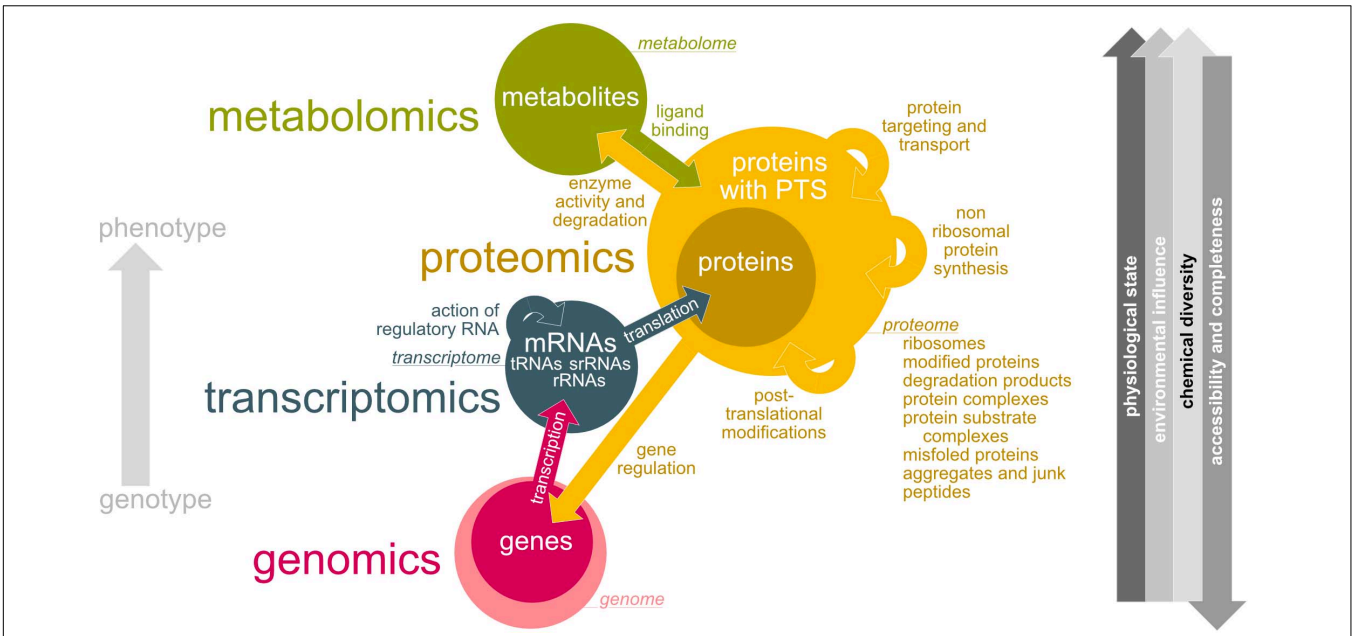


FIGURE 4 | Overview about '-omics' technologies used for lichen microbiome research. Green – metabolomics, yellow – proteomics, blue – transcriptomics, pink – genomics. Colored arrows indicate interactions between metabolome, proteome, transcriptome and genome and how they are affecting each other. Circle sizes illustrate estimated complexity. Gray arrow direction (right hand side) implies an increase.

low chemical diversity, methods for extraction, preparation and sequencing became routinely applicable (**Figure 4**). Genomics reads, assembles, stores and interpretes the DNA-encoded information and is important to understand the organization, structure and function of genes of an organism.

Metagenomic analyses of lichen microbiomes (Cernava et al., 2015; Aschenbrenner et al., 2017; Cernava et al., 2018; Graham et al., 2018) identify (gene)sequences covering a wide range of potential bacterial functions with possible impact on the symbiotic system and their assignments to the taxa which are present. However, metagenomics does not provide information whether these functions are specifically expressed by any of these bacteria under the given environmental conditions. However, this knowledge is required to understand how the microbiome responds to changing conditions in the lichens natural habitats.

Metagenomic analyses are currently dominated by high throughput sequencing of relatively short reads, but they are shorter than most genes and thus, read-based methods cannot provide sufficient information about the genomic organization of genes. Huge amounts of previously uncharacterized genomic diversity can be unveiled by creating metagenome-assembled genomes (MAGs; Parks et al., 2017; Maguire et al., 2020). Thereby, contigs are assigned to clusters or so-called "bins" based on their similarities in relative abundance and sequence composition parameters such as frequencies of nucleotide tuples (di-, tri-nucleotides). The creation of MAGs including tools are reviewed by Sangwan et al. (2016). The MAG approach has been successfully applied to a wide range of environments, e.g., aquatic habits (Narasingarao et al., 2012). For the first time, Wicaksono et al. (2020) reconstructed 29 bacterial MAGs (contamination < 10%) from metagenomes of lichens (17 MAGs: *L. pulmonaria*, 7 MAGs: *C. furcata* and 5 MAGs: *P. polydactylon*). Most of the created MAGs were assigned to Proteobacteria. This study indicates that creating MAGs can be crucial for further exploration of the ecological role of bacterial symbionts in lichens. The comparison of metagenomic data with those derived from other 'omics' techniques reveal that only a fraction of bacteria is active in lichens at the same time (Grube et al., 2015). Furthermore, MAGs could help analyze the utilization potential of complex carbohydrates by bacteria (Hassa et al., 2018).

(Meta)Transcriptomics: Determining Active Genes

The transcriptome is the total RNA transcribed from the genome of an organism at a given time point. Thus, the composition of the transcriptome directly reflects the entirety of external environmental stimuli on gene expression level (**Figure 4**). Due to the lack of available complete (meta)genomes only RNASeq (the determination of short cDNA sequence reads) is applicable for metatranscriptome studies of more complex biological systems such as lichens. Nevertheless, the availability of reference genomes would dramatically support the differentiation of transcripts between host and microbial RNA (Aguiar-Pulido et al., 2016).

Environmental metatranscriptomics focuses on mRNA to estimate the expressed genes in a given community and thus, helps to identify active metabolic pathways functioning under specific environmental conditions.

Cernava et al. (2017) analyzed the functional diversification of the microbiome of *L. pulmonaria* using a meta-omics approach. By using metatranscriptomics in addition to metagenomics and -proteomics facilitate the detection of so far unknown bacterial

species in the lichen symbiosis. Metatranscriptomics as well as metagenomics revealed various strategies of lichen-associated bacteria to survive under stress conditions. The data derived from metaproteomic analyses can validate and corroborate metagenomic or -proteomic results.

Recently, Cernava et al. (2019) studied the role of lichen-associated microorganisms (*L. pulmonaria*) in enduring dehydration and drought using a metaproteomics approach. They revealed that the microbiome is well-adapted to dehydration by stress protection and additionally changes of the metabolism. Furthermore, an interplay in holobiont functioning under drought stress is indicated by their results.

The high abundance of ribosomal RNA in mRNA preparations may reduce the RNASeq coverage and the instability of mRNA in general has to be considered appropriately in metatranscriptomics.

(Meta)Proteomics: Seeing the Blueprint at Work

Proteins are synthesized by translating the available and intact mRNAs (**Figure 4**). By this reason, only the complement of active genes (mRNAs) also occurs on protein level. According to varying sequence length, the protein composition from 20 amino acids recombines the amino acid's chemical properties. After translation, proteins may undergo wanted but also arbitrary chemical modifications such as phosphorylations and many others, site-specific cleavage, aggregation with other proteins or prosthetic groups. Transport processes may remove proteins from the analyzed sample. Unstable proteins will be rapidly degraded while proteins with long half lifes will last from several days until years. This results in protein-to-protein concentration differences covering several orders of magnitude. All of this impacts single protein molecules but also the composition of the protein pool at all. This explains why the proteome - the entirety of all proteins of a biological system at a given time point at a defined developmental stage under defined environmental conditions - is one of the most complex and dynamic biological entities under investigation we may need to handle. Covering all proteins in one experimental setup for the analysis of (meta)proteomes is not possible. Especially less abundant proteins below detection threshold and proteins tending to form complexes, to precipitate, or to bind to surfaces of sample particles or lab equipment may escape analysis or make an enrichment or adaptations of sample preparation protocols necessary.

For liquid chromatography mass spectrometry (LCMS) based proteomics, extracted proteins are enzymatic cleaved for generating peptides which subsequently can be ionized and mass-analyzed (Karpievitch et al., 2010). With tandem MS (MSMS) devices sequencing of higher abundant peptides is performed. The determined masses, sequence tags and signal strengths of peptides are then assigned to protein sequences.

For correctly assigning peptide data to proteins, a suitable bioinformatic workflow including the selection of an appropriate database is of fundamental importance (Heyer et al., 2017). Such databases are made from a collection of protein sequences, which are expected to be found in the analyzed sample. Usually one considers protein sequences from taxonomic and metagenomic analyses of comparable samples (Kunath et al., 2019). Including sequenced mRNA in the database allows us to focus on the active part of the genes/community (Hassa et al., 2018; Kunath et al., 2019). If only sequences of model organisms or any other biased data were used for database construction the peptide masses of unknown taxa will produce incorrect sequence hits or no hits at all.

After assignment of measured mass signals and sequence tags to peptides and peptides to proteins, the total quantities of single proteins by summing up all peptide signals is possible. The final result per sample will have determined the quantitative distribution of proteins. Species specific and functional protein assignments may reveal, which constituents of an analyzed sample are specialized to fulfill specific functions (Eymann et al., 2017). Per taxon quantification of all assigned proteins may show, to which extent each taxon contributes to the whole system on protein level (Eymann et al., 2017).

Putting the data of a set of samples together may allow binary sample-to-sample comparisons or the extraction of protein expression profiles along spatial or other environmental gradients. Such kind of differential metaproteome analysis could help for understanding the responses of microbial communities as an entirety to changing conditions and can thus provide information about community resistance and resilience under environmental stress. Schiebenhoefer et al. (2019) discuss appropriate bioinformatic tools for metaproteomic analysis.

More recent developments which are based on differential detection of peptides with different stable isotope composition offer fascinating opportunities to elucidate metabolically active taxa and metabolite fluxes within microbial communities (for an outlook see Kleiner, 2019). For stable isotope probing (SIP) labeled substrates such as sugars and carbon compounds or amino acids containing ^{13}C, ^{15}N, ^{18}O or ^{36}S respectively are applied. Active cells take up the labeled substrates, which can be later detected within the proteins via MS. The rate of incorporation in defined taxa of the investigated sample community can be used for assessing their metabolic activity or their preferences for the applied substrate (Seifert et al., 2012; Von Bergen et al., 2013).

Stable isotope fingerprinting (SIF) is an approach similar in principle, but circumvents the artificial application of labeled substrates. Due to naturally occuring carbon isotope discriminative effects against ^{13}C in C1 pathways (e.g., for assimilation of bicarbonate, CO_2, or methane), metabolites and biomass originating from these pathways show an altered carbon isotope ($^{13}C/^{12}C$, commonly called δC) ratio that can be used to reveal chemical fluxes and dependencies within metaproteome samples (Jehmlich et al., 2016). The software Calis-p supporting the direct extraction of species-specific δCs from standard metaproteome datasets was presented and made available (Kleiner et al., 2018). To our knowledge, SIP as well as SIF were not applied to lichens until now.

Metabolomics: Analyzing Enzyme's Action

Proteins interact with each other resulting in a complex metabolic system of an organism. Changes in the proteome lead to changes in the metabolome. Thus, the metabolome is highly dynamic and

complex (**Figure 4**). Changes can develop in fractions of seconds making metabolome analysis and sample preparation a very challenging task. Metabolites can additionally act as cofactors, signaling molecules, substrates or stabilizing agents for proteins. Metabolomics determines a sample's profile of metabolites at a defined time point under certain environmental conditions (**Figure 4**). Metabolomics may play a role to examine the available metabolites of the lichen and its microbiome.

Antibiotic effects of a number of lichen metabolites against gram-positive bacteria is known since long (reviewed, e.g., in Shrestha and Clair, 2013), and presumably, such compounds could regulate bacterial growth in lichens. A disadvantage of metabolomics in contrast to metaproteomics is the fact that it is challenging to assign metabolites to specific taxa, due to the lack of taxon-specific signatures for metabolites.

While for homogenous microbial cultures, protocols for rapid sample processing for immediate metabolome access are available, this needs still to be established for complex systems such as lichens. With current protocols at hand only stable metabolites such as secondary metabolites including those of bacteria (Boustie and Grube, 2005), polymeric compounds or compounds terminating metabolic build-up pathways may be reliably accessed and quantified.

One method to study metabolic pathways and fluxes of lichens *in vivo* is based on the utilization of stable isotope labeling. They are based on the supply of the labeled tracer to the growth medium (Kuhn et al., 2019). The positional labeling patterns can reveal the biosynthetic history of the studied products (Kinoshita et al., 2015). Labeling studies of intact lichens are challenging, because the symbiosis between photo- and mycobionts could trigger the metabolism of both (Calcott et al., 2018), but several projects engaged in this subject. Experimentation with ^{14}C was popular in lichen physiology mainly for studying carbohydrate transfer (e.g., Richardson et al., 1968), but declined since then, while more recent studies favor the use of ^{13}C in experiments. Lines et al. (1989) conducted ^{13}C-NMR analysis and revealed the transport of ^{13}C-photosynthate from the photo- into the mycobiont of *X. calciola* for mannitol biosynthesis. Sundberg et al. (1999) showed that there may be significant differences in carbon transfer rates and partitioning of carbon between the symbionts in different lichen (e.g., *L. pulmonaria*) by using ^{13}C-NMR spectroscopy in combination with $^{13}CO_2$ labeling. Aubert et al. (2007) identified 30 metabolites in *X. elegans* using ^{13}C- and ^{31}P-NMR spectroscopy and with that indicated that metabolite composition is affected by stress conditions. An additional study reveals that cyano- and tripartite lichens have a strong respiratory response to glucose by conducting carbon dioxide flux measurements and phospholipid acid analysis with experimental application of $^{13}C_6$ (Campbell et al., 2013). Recently, Kuhn et al. (2019) performed *in vivo* labeling experiments with *Usnea dasypoga* using $^{13}CO_2$ or [U-$^{13}CO_6$]-glucose and reconstructed the biosynthetic pathway of usnic acid.

Non-canonical amino acids (NCAAs), or "unnatural amino acids," are identified in several microorganisms. Non-canonical D-amino acids (NCDAAs) are secreted by various bacteria as signaling molecules to aid the bacteria to cope with changing environmental conditions (Lam et al., 2009; Kolodkin-Gal et al., 2010; Leiman et al., 2013). Cava et al. (2011) indicate that NCDAAs cause biofilm dispersal in aging bacterial communities. NCAAs are additionally involved in the formation of cyanobacterial hepatotoxins. In recent studies, the hepatotoxins microcystin-LR and nodularin were frequently found in lichen cyanobionts (Oksanen et al., 2004; Kaasalainen et al., 2009, 2012). Such toxins can have grazing inhibition effects and therefore may protect the lichen against herbivory (Hrouzek, 2017). Labeling studies using NCAAs are known from several proteomic and biotechnological studies, because it benefits from the ability to enrich labeled proteins. Saleh et al. (2019) recently reviewed this method. However, to our knowledge this method was not used in lichen metabolite studies so far.

Molecular Imaging: Visualizing Interactions *in situ*

By combining the different omics approaches with imaging techniques previously overlooked participants, metabolites and the spatial occurrence of both can be found in lichens.

With fluorescence *in situ* hybridization (FISH) specific nucleic acid sequences can be detected and localized by using fluorescent probes. It can be used to uncover the taxonomical and spatial structure of bacterial communities in lichens (e.g., Cardinale et al., 2008; Muggia et al., 2013; Maier et al., 2014; Erlacher et al., 2015; Cernava et al., 2017). Levsky and Singer (2003) reviewed the methodology.

With matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS), the distribution of proteins and small molecules within biological systems can be investigated by *in situ* analysis of thin tissue sections. It can determine the distribution of hundreds of compounds in only a single measurement and enables the acquisition of cellular expression profiles without destroying the cellular and molecular integrity. Cornett et al. (2007) and Walch et al. (2008) reviewed the methodology. In lichens, IMS can be used to visualize the distribution of secondary compounds, which was first shown by Le Pogam et al. (2016). Linking imaging mass spectrometry with metagenomics can determine the localization and organization of small molecules within the microbial community of lichens. For example, an ordered layering of molecules assigned to specific lichen symbiotic partners can be revealed (Garg et al., 2016).

The Bacterial Community of *L. pulmonaria*

Where Bacteria Reside Within the Lichen Thallus

The surface of lichens, best studied in *L. pulmonaria* and *C. arbuscula*, is densely colonized by bacteria (**Figure 1**; Cardinale et al., 2008, 2012a,b; Grube et al., 2009; Schneider et al., 2011). The density of bacteria on lichen thalli (e.g., *Cladonia rangifera*: c. 10^7–10^8 cells/g) is higher in relation to surfaces of higher plants (leaf surface: c. 10^5 cells/cm^2) (Cardinale et al., 2008; Grube et al., 2009; Saleem, 2015).

The Alpha diversities (Shannon index) of bacterial communities can vary between different lichens. *L. pulmonaria* shows an index of 7.0 whereas *Solorina crocea* an index of 4.5 (Grube et al., 2012; Aschenbrenner et al., 2014).

Lichen associated bacteria colonize distinct thallus parts in various abundances and patterns due to chemically and

physiologically differences. Alphaproteobacteria are widespread on both the upper and lower surface of *L. pulmonaria* (Cardinale et al., 2012a; Grube et al., 2015). This is also demonstrated for other dorsoventrally organized lichen species, like *Umbilicaria* sp. (Grube et al., 2015). In contrast to Alphaproteobacteria, Betaproteobacteria are locally restricted on the lower surface (Grube et al., 2015). For *Cladonia* species, the highest amount of bacteria is found on the internal layer of the podetia (Cardinale et al., 2008, 2012b), whereas bacterial colonization on crustose lichens (e.g., *Lecanora* sp.) is higher in the cracks and fissures between the areoles of the crustose thalli (Grube et al., 2009), perhaps because humidity stays longer. Cardinale et al. (2008) found evidence for bacterial presence in cell walls of *C. arbuscula*, but not in the cytoplasm. Unsurprisingly, bacteria are also found on decaying lichen material of some terricolous lichens, like *Baeomyces rufus* or *C. rangiferina*, or in the lichen-substrate interface (Asta et al., 2001). In their study of the lichen genus *Xanthoparmelia*, Mushegian et al. (2011) found that the older central parts of this leaf-like lichen contain a richer and more stable bacteria community than the younger thallus periphery. This is partly confirmed by a study from Cardinale et al. (2012b) and Noh et al. (2020) for the upright thalli of *Cladonia* sp. The microbiome shows an increase in diversity from apical to basal parts and significant differences according to the vertical position within the thallus.

Microbiome Functions

Overview

The lichen microbiome may contribute multiple functions to the lichen symbiotic system (**Figure 5**) (Hunter, 2006; Grube et al., 2015; Eymann et al., 2017). This includes essential functions such as nutrient supply by uptake and/or assimilation of e.g., iron, phosphate, sulfur, amino acids and dipeptides, sugar and xylose (e.g., Sphingomonadales, Burkholderia or Acetobacteraceae), resistance against abiotic factors (e.g., toxic environmental compounds, oxidative or osmotic stress; Chtoniobacterales, Rhodospirillales, Myxococcales), growth hormone production or nitrogen fixation (**Figure 5**; Liba et al., 2006; Grube et al., 2009, 2015; Schneider et al., 2011; Cardinale et al., 2012a; Erlacher et al., 2015; Cernava et al., 2017).

Grube et al. (2015) reveal that Alphaproteobacteria covered up to 50% of the microbiome functions in *L. pulmonaria*, e.g., production of amino acids or vitamins, carbohydrate metabolism, stress response or protein degradation. Proteobacteria and Bacteroidetes are involved in iron uptake. Ton and Tol transport systems and especially TonB-dependent receptors are revealed by metagenomic and –proteomic analyses. The amount of bacterial functions serving the self-preservation of the microbes or the entire lichen has to be elucidated in more detail in the future.

Uptake and supply of nutrients, essential compounds and trace elements

Bacteria can influence the growth of their host by e.g., producing growth hormones. This is supported by the identification of genes/proteins of the bacterial microbiome of *L. pulmonaria* (Grube et al., 2009) involved in auxin biosynthesis. The mobilization and recycling of material from senescent thallus parts may facilitate growth of the young and growing thallus parts (Grube and Berg, 2009; Grube et al., 2015; Cernava et al., 2017; Eymann et al., 2017).

Granulicella and *Lichenibacter* are lichen-associated bacteria for which carbohydrate use and production are known (Pankratov and Dedysh, 2010; Pankratov et al., 2019). The first one, the genus *Granulicella*, hydrolyzes pectin, xylan, laminarin and lichenan and produces an amorphous EPS matrix composed of polysaccharides in *Cladonia* lichens (Pankratov and Dedysh, 2010). *Lichenibacter* utilizes starch and xylan (Pankratov et al., 2019).

The role of bacterial strains (e.g., *Azospirillum*, *Bradyrhizobium* and *Frankia*) for supplements of fixed nitrogen to the symbiotic partners is obvious. Creating a partial anaerobic biofilm as bacterial habitat is important for bacterial nitrogen fixation, which may augment the nitrogen budget in lichens lacking a N-compound-donating cyanobiont. Several lichen-associated bacteria, like Alphaproteobacteria (Grube et al., 2009; Almendras et al., 2018), Gammaproteobacteria (Liba et al., 2006), Firmicutes (Grube et al., 2009; Almendras et al., 2018) and Actinobacteria (Almendras et al., 2018) contain *nifH* genes. In the case of N-limiting conditions, bacterial N-fixation, by e.g., *Azotobacter,* Betaproteobacteria or Alphaproteobacteria, could be of considerable importance for the vitality of lichens (Leveau and Preston, 2008; Grube and Berg, 2009; Grube et al., 2009; Bates et al., 2011; Erlacher et al., 2015). This fits well with studies of Almendras et al. (2018) showing that Chlorolichens have a higher diversity of N-fixing bacteria than cyanolichens. However, nitrogen supply is not a problem in many parts of Europe, as the atmosphere carries massive amounts of nitrogen compounds from agriculture over large distances.

Many algae are auxotrophic for vitamin B_{12} that is often synthesized by bacteria in symbiotic communities (Croft et al., 2005). Bacteria (e.g., Chtoniobacterales, Sphingomonadales, Sphingobacteriales, Myxococcales, and Rhizobiales) may be of importance for cofactor and vitamin synthesis and supplementation of the whole symbiotic system. Bacterial enzymes are found in *L. pulmonaria*, which are involved in the synthesis of cobalamin belonging to the Vitamin B12 group (relevant for photosynthesis), biotin (Vitamin B7; important for gene regulation), folate (Vitamin B9/B11; important for C_1-metabolism), pantothenate (Vitamin B5; synthesis and degradation of carbohydrates) or pyridine (herbicide production) precursors supporting fungal growth (Erlacher et al., 2015; Grube et al., 2015).

We suggest that lichens, which can live up to hundreds of years, maintain a dynamic equilibrium of bacteria. The growing parts (alphaproteobacterial dominance) act as anabolic systems. The senescing parts might represent catabolic sinks. Cardinale et al. (2012b) assume that the bacteria, which are colonizing the older lichen parts, help to convert the old lichen biomass into simple molecules. These will then be released into the substrate or can be recycled to the growing lichen parts as it was shown previously (Ellis et al., 2005).

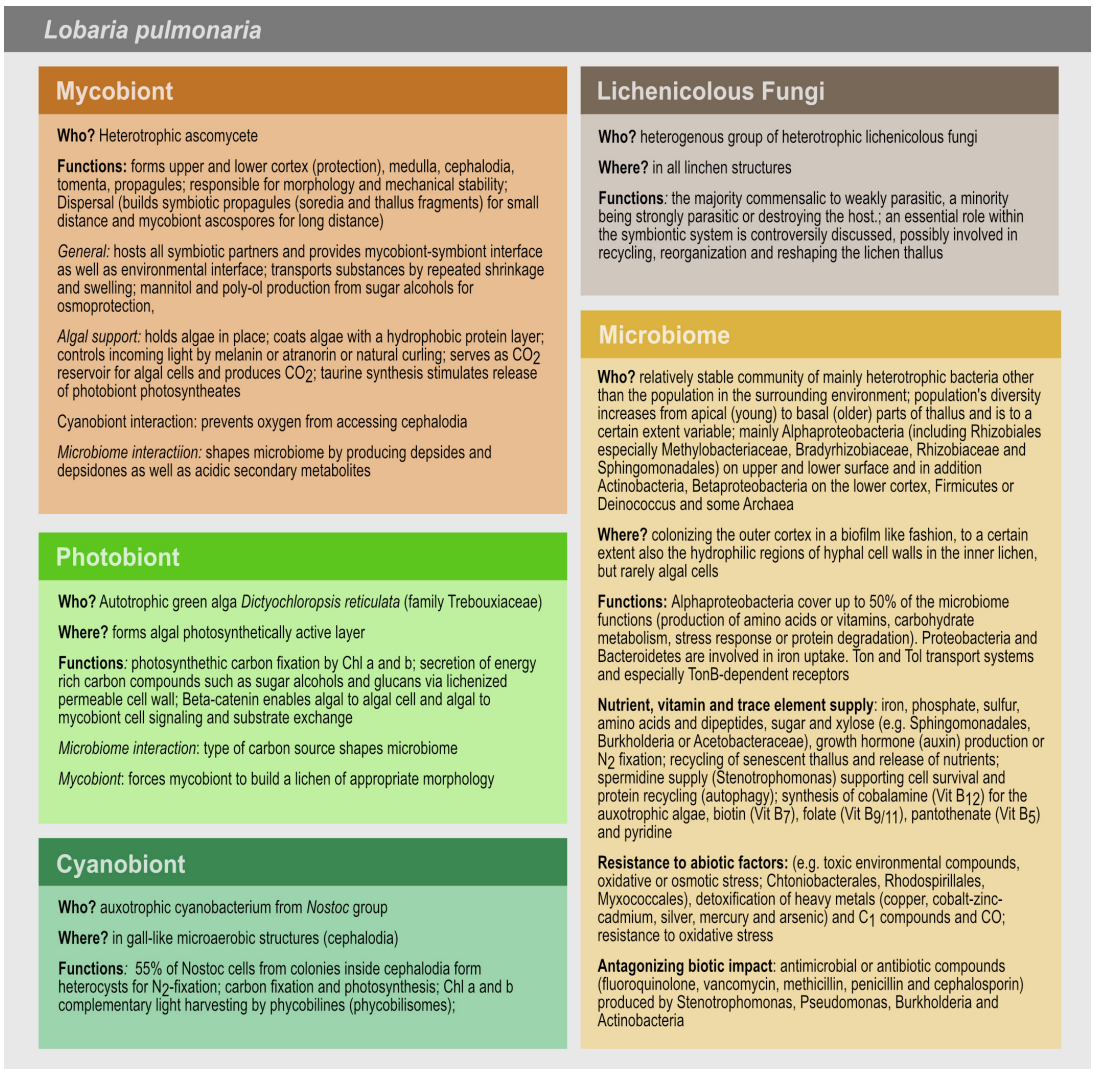


FIGURE 5 | Microbiome and symbiotic partner's functions in the lichen *L. pulmonaria*. The mycobiont is responsible for the holobionts' morphology, mechanical stability and reproduction, protects and supports the photo- and cyanobiont and shapes the microbial community. The photobiont is in charge of the photosynthetic carbon fixation. The cyanobiont is responsible for nitrogen and carbon fixation. The detailed role of the lichenicolous fungi is unclear. The microbiome plays a role in nutrient, vitamin and trace element supply, in resistance to abiotic factors and in antagonizing biotic impact.

Abiotic stress and toxic compound protection

The bacterial microbiome of *L. pulmonaria* (e.g., Chthoniobacterales, Myxococcales, Sphingomonadales, Sphingobacteriales) shows resistance against abiotic stressors (Grube et al., 2015; Cernava et al., 2017), but the detailed mechanisms remain mostly unknown. Cernava et al. (2018) show that the microbial community structures do not depend on the level of arsenic concentration at the sampling site, whereas the functional spectrum related to arsenic metabolism is enhanced. Furthermore, the amount of detoxification related genes is higher in arsenic-polluted samples. Additionally, oxidative-stress protectants to heavy metal efflux are observed (Grube et al., 2015). Besides creating anaerobic niches, EPS and thus biofilm formation may play a role in the protection against pH and metals as shown for *Rhizobium leguminosarum*, a soil bacterium that establishes symbiosis with *Trifolium* spp. (Kopycińska et al., 2018).

Besides confirming the resistance against abiotic stressors, the bacterial microbiome may help with the detoxification of methanol/C_1-metabolites. Formaldehyde-activating enzymes are highly abundant in bacterial samples, especially Methylobacteriaceae, of *L. pulmonaria*. These enzymes are involved in oxidation of methanol to carbon dioxide and formaldehyde detoxification. The assignment of carbon monoxide dehydrogenase to the same bacterial family in the metaproteomic analysis of *L. pulmonaria* indicates the existence of carbooxidotrophic bacteria or the detoxification of carbon monoxide, which competes with O_2 for cytochromoxidase of the respiration chain (Eymann et al., 2017).

Antagonists of biotic impact

Lobaria pulmonaria and potentially other lichens may be important reservoirs for bacteria acting against bacterial and fungal pathogens. In former studies, it was shown that *Rhinocladiella* sp. and *Botrytis cinerea* were inhibited by bacterial strains isolated from *L. pulmonaria* (Cernava et al., 2015). *Rhinocladiella* are black fungi that may opportunistically act as human pathogens and could for example cause cerebral phaeohyphomycosis (neurotropic fungus *R. mackenziei*) by infecting nerve cells (Jabeen et al., 2011; Didehdar et al., 2015). *Botrytis cinerea is a plant pathogen* causing the gray mold disease (Williamson et al., 2007). The defense against such pathogens is possible by secretion of protective substances such as antimicrobial or antibiotic compounds (vancomycin, penicillin and cephalosporin). *Stenotrophomonas*, *Pseudomonas*, *Burkholderia* and Actinobacteria dominate the abundant antagonistic community of *L. pulmonaria* as producers of bioactive volatiles (Cernava et al., 2015, 2017).

Microbiome Acquisition and Shaping

Little is known about the intraspecific variation of microbiome composition, and how lichens acquire their specific bacterial communities. A study of Aschenbrenner et al. (2014) indicate that the propagules of lichens contribute to a co-dispersal of lichen-associated bacteria (**Figure 6**), since the propagules (isidia) of *L. pulmonaria* share the overall bacterial community with the parental thalli at class level. This also suggests that the bacterial community structure might change over time at lower taxonomic ranks. Cardinale et al. (2012a) suppose that when propagules of *L. pulmonaria* are dispersed, the high-abundant *Alphaproteobacteria* are maintained for successful colonization of the new site. During colonization, both *Burkholderia* and nitrogen fixers will be lost, and local, better-adapted competitors may be picked up from the new environment.

The prokaryotic community may be recruited from "bacterial rain", as for example known from phyllosphere microbiomes (Mechan Llontop, 2020), or from the adjacent substrate (**Figure 6**). Uptake of cyanobacterial photobionts from mosses growing around *L. pulmonaria* thalli was indicated by a study of Aschenbrenner et al. (2014). The mosses provide the cyanobacteria to the lichen propagules and young lichen thalli respectively. Cyanobacterial uptake from neighboring mosses was also demonstrated for the lichen *Erioderma pedicellatum* (Cornejo et al., 2005). Recently, Aschenbrenner et al. (2017) indicated that mosses growing around *L. pulmonaria* thalli may generally facilitate bacterial colonization of this lichen species. Additionally, they demonstrated that the bark microbiome of the inhabited tree species shares partly the same bacterial taxa with the lichen microbiome.

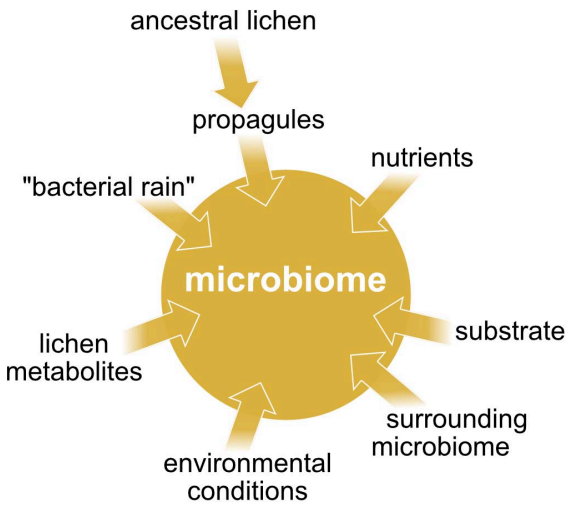


FIGURE 6 | The main factors affecting the microbiome composition in lichens.

It is shown that the composition of the microbial communities is lichen specific (Grube et al., 2009; Bates et al., 2011; Hodkinson et al., 2012). On the lichens surfaces, a mixture of both abiotic and biotic conditions possibly controls bacterial growth. The poikilohydric conditions, with recurrent desiccation, may prevent the persistence of fast growing bacterial opportunists. Furthermore, it is known that rehydration following a desiccation period causes an oxidative burst by induction of high rates of superoxide production by the mycobiont. We argue this affects the bacterial microbiome or mycobiome and could select for survival of superoxide resistant bacteria, while the killed bacteria may serve as a nutrient source to support further lichen biomass production after rehydration.

Reports about antibacterial effects of lichen compounds or extracts (Burkholder et al., 1944; Ingólfsdóttir et al., 1998; Francolini et al., 2004; Boustie and Grube, 2005) suggest a biotic lichen originated control (**Figure 6**). These compounds may have antibiotic or rather antimicrobial properties and therefore may play an active role in selecting for specific types of bacteria. Among those biorelevant compounds you can find polyphenols such as depsides and depsidones, but also lactones, anthraquinones etc. (Hodkinson et al., 2012). Lichens producing substantial amounts of acidic secondary metabolites have significantly different bacterial communities than others (Grube and Berg, 2009). *L. pulmonaria* contains lower concentrations of such lichen-specific substances than many other Peltigerineae lichens (Beckett et al., 2003). Grube et al. (2015) hypothesize that bacterial colonization is mostly regulated by the mycobiont because they found only little evidence for quorum sensing in the microbiome of *L. pulmonaria*.

Besides secondary metabolites, the availability of nutrients may affect the microbial community (**Figure 6**). There is a significant correlation between photobiont-type and bacterial community composition. This is due to the major difference between cyanolichens and chlorolichens in the availability of fixed nitrogen (Hodkinson et al., 2012). Bacteria that are associated with cyanolichens have access to fixed nitrogen whereas bacteria of chlorolichens lack this benefit. Presumably, chlorolichens enrich bacterial species, which are capable of nitrogen fixation. Details of carbon release may play an additional role in shaping the prokaryotic community. Lichen-associated green algae release fixed carbon as sugar alcohols, contrary to glucose release of cyanobacteria. The bacterial utilization of sugar alcohols requires an adapted set of enzymes such as

polyol dehydrogenases. For this reason, prokaryotic communities associated with chlorolichens consist of bacteria, which are able to synthesize sugar alcohol specific transporters and degrading enzymes while glucose degradation in cyanolichens does not need bacteria with adapted enzyme panels (Palmqvist, 2002; Adams et al., 2006; Hodkinson et al., 2012).

Metabolic processes can additionally modulate and organize complex microbial community structures by exchange of nutrients. Spatial concentration gradients of metabolites in biofilms lead to a varying nutrient availability and therefore could regulate the distribution of species (Cardinale et al., 2002; Stewart and Franklin, 2008). A diverse spectrum of chemical gradients can be established, like oxygen, nutrient or bacterial signaling compound gradients. The adaptation of the bacterial microorganisms to these gradients includes differences in gene expression and thus protein production (Stewart and Franklin, 2008). By competing for nutrients, metabolic tasks in a community can be divided. Therefore, the population can spatially differentiate in different sections such as metabolically active and inactive microbial cells (Liu et al., 2015). The exchange of nutrients is suggested to maintain genotypic diversity within naturally bacterial communities (Germerodt et al., 2016). To our best knowledge, there is no information about cross-feeding in lichen biofilms. As it is common in the microbial world (Pande et al., 2014), we suppose nutrient exchange also occurs in lichen biofilms.

Microbiome Diversity and Adaptability

Functional diversification in *L. pulmonaria*, due to a multiplayer network of the symbiotic partners supports longevity and persistence under changing environmental conditions (Grube et al., 2015). Lichen-associated bacteria seem to be influenced by climatically or geographically differences. The geographical patterning is defined by dispersal on a larger scale where host dispersal could be the limiting factor (Hodkinson et al., 2012). In *L. pulmonaria*, the diversity of Alphaproteobacteria is affected by geography. *Burkholderia* ssp. and nitrogen fixers are mostly taken up from the local environment (Cardinale et al., 2012a). Eymann et al. (2017) compared two different sampling sites of *L. pulmonaria* (Darß, Germany and Styria, Austria) and suggested the presence of a relatively stable core microbiome. There are differences in the distribution of families within the Alphaproteobacteria order Rhizobiales. Actinobacteria were more abundant in the samples collected from Darß and Acidobacteria and Planctomycetes were more abundant in the samples collected in Styria. The study indicates significant differences between the proteomes of the two lichen microbiomes in contrast to the rather stable fungal and algal protein profiles. For example, cold shock proteins were more abundant in the Styria lichen material. A study of Hodkinson et al. (2012) reveals that the major bacterial community is correlated with differences in large-scale geography. Printzen et al. (2012) found a less diverse microbial community of the lichen *Cetraria aculeata* in polar habitats. Antarctic and arctic communities are more similar to each other compared to samples from other regions such as Germany and Spain.

To understand how the extant microbiome responds to fluctuating environmental conditions in the natural habitats, Cernava et al. (2019) sampled lichens under representative hydration stages. Bacterial metatranscriptomes from *L. pulmonaria* reveal significant structural shifts and functional specialization corresponding to lichen hydration stages. The hydrated stage is correlated with upregulated transcription of transport systems, tRNA modification and various porins (Omp2b by Rhizobiales), whereas the desiccated samples suggest stress-adaptive responses. Carbohydrate metabolism is activated under both conditions, but under dry conditions, upregulation of a specialized ketone metabolism indicates a switch to lipid-based nutrition, reminiscent of 'fasting metaorganism.'

The possibility that the bacterial microbiome composition and functionality change according to ecological and climatic variations, could lead to an increase in the adaptivity of the holobiont. The adaptation of lichen populations to new habitats is presumably accompanied by changes in the bacterial communities similar to previously observed switches of the photobiont strain correlating with different ecological conditions (Blaha et al., 2006; Grube and Berg, 2009). Photobiont switches can be beneficial for the mycobiont if the locally occurring photobiont strains are better adapted to the environmental conditions at a particular site than the carried photobiont strain. In such cases the adapted photobiont strain will be incorporated into the lichen during thallus establishment (Werth and Sork, 2010; Wornik and Grube, 2010; Werth and Scheidegger, 2012).

Klarenberg et al. (2020) provide evidence of compositional shifts in individual taxa of the microbiome of *Cetraria islandica* due to climatic warming. Warming alters the abundance of the most common taxa such as *Granulicella* or *Endobacter*. The abundance of *Granulicella* or *Bryocella* is decreasing whereas the abundance of *Acidisphaera*, *Sphingomonas* and *Endobacter* is increasing. After long warming periods bacterial microbiomes can acclimatize and therefore shift back to the original composition (Bradford et al., 2008; Crowther and Bradford, 2013; Romero-Olivares et al., 2017). Warming periods also affect the amount of lichen secondary metabolites and can therefore have an effect on the composition of the lichen microbiome. Usnic acid concentration is increasing during warming and perlatolic acid concentration is reduced (Asplund et al., 2017). Usnic acid acts as an antimicrobial protectant against fungal parasites, like *Fusarium moniliforme* (Cardarelli et al., 1997), which is involved in several human and animal diseases and produces different toxins (reviewed by Nelson, 1992). Despite that, usnic acid plays an additional role in protection against indicator bacteria such as *Staphylococcus aureus* or *Enterococcus faecalis* (Lauterwein et al., 1995). Perlatolic acid is also showing antibacterial, e.g., against *S. aureus* or *Escherichia coli* (Piovano et al., 2002; Gianini et al., 2008) and antifungal activities such as against *Cladosporium sphaerospermum* (Gianini et al., 2008).

Cardinale et al. (2012b) found out that lichens (e.g., *L. pulmonaria* or *C. arbuscula*) harbor higher numbers of bacteria when growing under shaded conditions. Despite that, lichens growing on rock harbor fewer bacteria than lichens growing on soil or bark. It seems that the amount of bacteria may be associated with the humidity of the habitat.

Biotechnological Potential

Lichens tolerate extreme abiotic stressors (e.g., extreme climates or osmotic conditions) and accumulate toxic compounds, heavy metals or radionuclides. Therefore, they could be sources of biotechnologically interesting strains, compounds and enzymes (Davies et al., 2002, 2005; González et al., 2005; Grube and Berg, 2009). Most lichen secondary metabolites are of fungal origin, e.g., stictic acid as herbivory protectant (Elix and Stocker-Wörgötter, 1996), but evidence for a bacterial origin is already found. González et al. (2005) published a study focusing on lichen-associated Actinobacteria and their bioactivity. A large number of strains were obtained. Several strains belonged to the family Streptomycetaceae that is well known to produce bioactive compounds. Other actinobacterial families, which are also known for the production of bioactive compounds, such as Micromonosporaceae, Pseudonocardiaceae, and Thermomonosporaceae, were also isolated. About 30% of the strains showed antimicrobial activity against other microorganisms. The presence of some structurally identified bioactive molecules is reported for a few bacterial strains although there are many other strains of relevance (Cardinale et al., 2006; Liba et al., 2006; Grube et al., 2009; Selbmann et al., 2010; Pankratov, 2012; Kim et al., 2014; Lee et al., 2014; Sigurbjörnsdóttir et al., 2014; Cernava et al., 2015; Parrot et al., 2015). In the following paragraph, we summarize information about several lichen-associated bacteria producing already identified bioactive compounds.

An isolate of the dominant antagonistic genus *Stenotrophomonas*, found in the microbiome of *L. pulmonaria*, produces spermidine as the main bioactive compound (Cernava et al., 2015). Spermidine is a multifunctional polyamine that is a plant growth regulator, plays a critical role in plant embryo development and protects roots against stress (Al-Whaibi et al., 2012; Alavi et al., 2013). Additionally, spermidine affects biofilm formation in various bacterial species, like *Vibrio cholerae* (McGinnis et al., 2009), and acts as an antifungal biosynthesis regulator in *Lysobacter enzymogenes* (Chen et al., 2018). In eukaryotes, spermidine prolongs the life span and is known to play vital roles in cell survival, autophagy (the degradation of damaged and aggregated waste protein) and anti-aging (Eisenberg et al., 2009; Madeo et al., 2010, 2018). Tissue spermidine concentrations decline with age in model organisms (e.g., yeast, mice) as well as in humans (Scalabrino and Ferioli, 1984; Eisenberg et al., 2009; Pucciarelli et al., 2012; Gupta et al., 2013). Polyamines, like spermidine, can have procarcinogenic properties. Increased concentrations caused by enhanced biosynthesis can be found in different cancer types (Nowotarski et al., 2013). In contrast, cardiovascular diseases in humans and cancer manifestation in mice can be delayed by spermidine (Madeo et al., 2018). Dietary spermidine protects mice and probably also humans from cardiac aging, by e.g., improving the diastolic function or left ventricular elasticity (Soda et al., 2012; Eisenberg et al., 2016).

A *Streptomyces* strain, isolated from *C. uncialis*, produces the cytostatic enediyne uncialamycin, which shows strong antibacterial activity against the human pathogens *E. coli*, *B. cepacia* and *S. aureus* (Davies et al., 2005; Parrot et al., 2016). The same *Streptomyces* strain generates also the alkaloids Cladoniamides A-G that show toxicity against human breast cancer MCF-7 cells (Williams et al., 2008). Another *Streptomyces* strain produces the tetrapeptide lichostatinal that represents a cathepsin K inhibitor and is therefore of interest for the therapy of osteoporosis (Yasuda et al., 2005; Lavallée, 2011). Two other *Streptomyces* species producing novel cytotoxic compounds: chlorinated anthraquinonic angucycline (Motohashi et al., 2010) and aminocoumarines structurally closed to novobiocine (Cheenpracha et al., 2010) were isolated from lichen species. Furthermore, *S. cyaneofuscatus* synthesizes methacrylate derivates with cytotoxic effects (Parrot et al., 2016). Other isolates of this species also demonstrate with high potential to produce a variety of anthracycline family antitumor antibiotics daunorubicin, cosmomycin B, galtamycin B and the antifungal macrolactam maltophilin (Ando et al., 1985; Jakobi et al., 1996; Antal et al., 2005). *Actinoplanes* sp. ATCC55532 produces actinoplanic acids A and B. The latter inhibits farnesyl protein transferase and the farnesylation of the oncogene protein Ras. Thus, it has a potential for the treatment of colorectal carcinoma, exocrine pancreatic carcinoma and myeloid leukemia (Singh et al., 1997). Beside the discovery of novel bioactive lead compounds, direct antagonistic effects of bacteria of the lichen microbiome can be used as biological plant protection by defending plants against fungal plant pathogens, e.g., *Alternaria alternata* or *Phytophthora infestans* (Grube et al., 2009, 2012; Gasser et al., 2011; Kim et al., 2013a, 2014).

Despite the production of small metabolites, lichen-associated bacteria also show other biotechnological potentials. Many of these bacteria have the potential to produce PHA biopolymers and show high antagonistic potential against plant pathogens, like *A. alternata* (Gasser et al., 2011; Kim et al., 2013b, 2014). This is also mentioned in section "Microbiome Acquisition and Shaping."

CONCLUDING REMARKS

The morphological structure of the lichen thallus may affect the organization of the symbiotic networking. Metaorganisms such as lichens consist of highly integrated partnerships reflecting the classical dual definitions of the lichen symbiosis, and less tightly integrated partners with auxiliary functions (associated microbiome) (Hyvärinen et al., 2002; Cornejo and Scheidegger, 2013). In order to fully understand the microbiome contribution to the lichen symbiosis from a metabolic perspective, metabolomics could inform about the modes of nutrient exchange between the participating organisms. A question not yet properly addressed is, for example, whether bacteria thriving on the polysaccharides of the lichen mycobionts actively secrete certain compounds, or whether material from degrading bacteria is passively reabsorbed by the mycobiont. Degrading of bacteria might be forced by oxidative bursts, a well-known phenomenon from lichens in response to rehydration following desiccation (Minibayeva and Beckett, 2001). Selective bacterial degradation through

oxidative bursts recalls not only a similar way of nutrient acquisition described from the plant rhizosphere (Paungfoo-Lonhienne et al., 2010), but could also exert selective pressure on the bacterial lichen colonizers, favoring species, which cope particularly well with oxidative stress. However, experiments like carbon isotope labeling and comparative physiological analysis between lichens with and without the bacterial microbiota are difficult to assess due to slow metabolism and high bacterial diversity. Additionally, variation in the structure of the lichen holobiont or histochemical variation of lichen extracellular matrix due to bacterial colonization should be incorporated in the analyses of the lichen symbiotic model. Correlations between the compositions of the bacterial microbiome, climatic changes and fungal/algal genotypes would reveal new insights into the functionality of the bacterial microbiome, its acquisition and influence on the lichen holobiont.

Certainly, more work is required to understand the complex interplay between lichens and their bacterial colonizers.

AUTHOR CONTRIBUTIONS

All authors contributed to the writing of the manuscript. MGru, JB, and US created the figures.

FUNDING

The German Research Foundation (DFG) (GRK 2010) funded this work.

ACKNOWLEDGMENTS

KR acknowledges support by the German Research Foundation (DFG) funding the Research Training Group RESPONSE (GRK 2010). MGru acknowledges support by Field of Excellence "Complexity of Life in Basic Research and Innovation (COLIBRI)" at UNI Graz.

REFERENCES

Abedin, M., and King, N. (2010). Diverse evolutionary paths to cell adhesion. *Tr. Cell Biol.* 20, 734–742. doi: 10.1016/j.tcb.2010.08.002

Adams, D. G., Bergman, B., Nierzwicki-Bauer, S. A., Rai, A. N., and Schüßler, A. (2006). "Cyanobacterial-plant symbioses," in *The Prokaryotes, A Handbook on the Biology of Bacteria: Symbiotic associations, biotechnology, applied microbiology*, 3rd Edn, Vol. 1, eds M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt (New York, NY: Springer), 331–363. doi: 10.1007/0-387-30741-9_14

Adir, N., Bar-Zvi, S., and Harris, D. (2019). The amazing phycobilisome. *BBA-Bioenergetics* 2019, 002. doi: 10.1016/j.bbabio.2019.07.002

Aguiar-Pulido, V., Huang, W., Suarez-Ulloa, V., Cickovski, T., Mathee, K., and Narasimhan, G. (2016). Metagenomics, metatranscriptomics, and metabolomics approaches for microbiome analysis. *Evol. Bioinform.* 12:S1. doi: 10.4137/EBO.S36436

Alavi, P., Starcher, M., Zachow, C., Müller, H., and Berg, G. (2013). Root-microbe systems: the effect and mode of interaction of stress protecting agent (SPA) *Stenotrophomonas rhizophila* DSM14405^T. *Front. Plant Sci.* 4:141. doi: 10.3389/fpls.2013.00141

Almendras, K., García, J., Carú, M., and Orlando, J. (2018). Nitrogen-fixing bacteria associated with *Peltigera* cyanolichens and *Cladonia* chlorolichens. *Molecules* 23:3077. doi: 10.3390/molecules23123077

Al-Whaibi, M. H., Siddiqui, M. H., Sakran, A. M., Ali, H. M., and Basalah, M. O. (2012). Influence of plant growth regulators on growth performance and photosynthetic pigments status of *Eruca sativa* Mill. *J. Med. Plants Res.* 6, 1948–1954.

Amann, R. I., Ludwig, W., and Schleifer, K. H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59, 143–169. doi: 10.1128/mmbr.59.1.143-169.1995

Ando, T., Hirayama, K., Takahashi, R., Horino, I., Etoh, Y., Morioka, H., et al. (1985). The structures of anthracycline antibiotics, cosmomycins A and B. *Agric. Biol. Chem.* 49, 1207–1209. doi: 10.1271/bbb1961.49.1207

Antal, N., Fiedler, H. P., Stackebrandt, E., Beil, W., Ströch, K., and Zeeck, A. (2005). Retymicin, galtamycin B, saquayamycin Z and ribofuranosyllumichrome. Novel Secondary Metabolites from Micromonospora sp. Tü 6368. *J. Antibiot.* 58, 95–102. doi: 10.1038/ja.2005.12

Antoine, M. E., and McCune, B. (2004). Contrasting fundamental and realized ecological niches with epiphytic lichen transplants in an old-growth *Pseudotsuga* forest. *Bryologist* 107, 163–172. doi: 10.1639/0007-2745(2004)107[0163:cfaren]2.0.co;2

Aptroot, A., and Alstrup, V. (1999). Three hyphomycetes isolated from the necrotic lichen *Cladonia rangiformis*. *Cryptogam. Mycol.* 20, 189–191. doi: 10.1016/s0181-1584(00)87028-1

Aptroot, A., Van Herk, C. M., and Sparrius, L. B. (2012). Basisrapport voor de Rode Lijst korstmossen. *Buxbaumiella* 92, 1–117.

Aschenbrenner, I. A., Cardinale, M., Berg, G., and Grube, M. (2014). Microbial cargo: do bacteria on symbiotic propagules reinforce the microbiome of lichens? *Environ. Microbiol.* 16, 3743–3752. doi: 10.1111/1462-2920.12658

Aschenbrenner, I. A., Cernava, T., Berg, G., and Grube, M. (2016). Understanding microbial multi-species symbioses. *Front. Microbiol.* 7:180. doi: 10.3389/fmicb.2016.00180

Aschenbrenner, I. A., Cernava, T., Erlacher, A., Berg, G., and Grube, M. (2017). Differential sharing and distinct co-occurrence networks among spatially close bacterial microbiota of bark, mosses and lichens. *Mol. Ecol.* 26, 2826–2838. doi: 10.1111/mec.14070

Asplund, J., Siegenthaler, A., and Gauslaa, Y. (2017). Simulated global warming increases usnic acid but reduces perlatolic acid in the mat-forming terricolous lichen *Cladonia stellaris*. *Lichenologist* 49, 269–274. doi: 10.1017/s0024282917000159

Asta, J., Orry, F., Toutain, F., Souchier, B., and Villemin, G. (2001). Micromorphological and ultrastructural investigations of the lichen-soil interface. *Soil Biol. Biochem.* 33, 323–337. doi: 10.1016/s0038-0717(00)00143-7

Aubert, S., Juge, C., Boisson, A. M., Gout, E., and Bligny, R. (2007). Metabolic processes sustaining the reviviscence of lichen *Xanthoria elegans* (Link) in high mountain environments. *Planta* 226, 1287–1297. doi: 10.1007/s00425-007-0563-6

Bardunov, L. V., and Novikov, V. S. (2008). *Red Data Book of the Russian Federation (plants and fungi)*. Moscow: KMK Scientific Press Ltd.

Barták, M., Solhaug, K. A., Vráblíková, H., and Gauslaa, Y. (2006). Curling during desiccation protects the foliose lichen *Lobaria pulmonaria* against photoinhibition. *Oecol.* 149, 553–560. doi: 10.1007/s00442-006-0476-2

Bates, S. T., Cropsey, G. W., Caporaso, J. G., Knight, R., and Fierer, N. (2011). Bacterial communities associated with the lichen symbiosis. *Appl. Environ. Microbiol.* 77, 1309–1314. doi: 10.1128/aem.02257-10

Beckett, R. P., Minibayeva, F. V., Vylegzhanina, N. N., and Tolpysheva, T. (2003). High rates of extracellular superoxide production by lichens in the suborder Peltigerineae correlate with indices of high metabolic activity. *Plant, Cell Env.* 26, 1827–1837. doi: 10.1046/j.1365-3040.2003.01099.x

Berg, G., and Hallmann, J. (2006). ""Control of plant pathogenic fungi with bacterial endophytes"," in *Microbial root endophytes*, eds B. J. E. Schulz, C. J. C. Boyle, and T. N. Sieber (Berlin: Springer-Verlag), 53–69. doi: 10.1007/3-540-33526-9_4

Berg, G., Bragina, A., Cardinale, M., Zachow, C., Müller, H., Berg, C., et al. (2011). From metagenome and proteome to biocontrol: analysis of moss-and lichen-associated microbial communities. *IOBC/WPRS Bulletin* 71, 13–16.

Bewley, J. D., and Krochko, J. E. (1982). "Desiccation-tolerance," in *Physiological Plant Ecology II. Encyclopedia of Plant Physiology (New Series), Vol. 12 / B*, eds

O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler (Berlin: Springer-Verlag), 325–378.

Bjelland, T., Grube, M., Hoem, S., Jorgensen, S. L., Daae, F. L., Thorseth, I. H., et al. (2011). Microbial metacommunities in the lichen-rock habitat. *Environ. Microbiol. Rep.* 3, 434–442. doi: 10.1111/j.1758-2229.2010.00206.x

Blaha, J., Baloch, E., and Grube, M. (2006). High photobiont diversity associated with the euryoecious lichen-forming ascomycete *Lecanora rupicola* (Lecanoraceae. Ascomycota). *Biol. J. Linn. Soc.* 88, 283–293. doi: 10.1111/j.1095-8312.2006.00640.x

Boucher, V. L., and Stone, D. E. (1992). "Epiphytic lichen biomass," in *The fungal community: Its organization and role in the ecosystem, Second edition*, eds G. C. Carroll and D. T. Wicklow (New York, NY: Marcell Dekker), 583–599.

Boustie, J., and Grube, M. (2005). Lichens - a promising source of bioactive secondary metabolites. *Plant Gen. Res.* 3, 273–287. doi: 10.1079/pgr200572

Bowler, P. A., and Rundel, P. W. (1975). Reproductive strategies in lichens. *Bot. J. Linn. Soc.* 70, 325–340. doi: 10.1111/j.1095-8339.1975.tb01653.x

Bradford, M. A., Davies, C. A., Frey, S. D., Maddox, T. R., Melillo, J. M., Mohan, J. E., et al. (2008). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Let.* 11, 1316–1327. doi: 10.1111/j.1461-0248.2008.01251.x

Brown, D. H., Rapsch, S., Beckett, A., and Ascaso, C. (1987). The effect of desiccation on cell shape in the lichen *Parmelia sulcata* Taylor. *N. Phytol.* 105, 295–299. doi: 10.1111/j.1469-8137.1987.tb00867.x

Brown, L. M., and Hellebust, J. A. (1978). Sorbitol and proline as intracellular osmotic solutes in the green alga *Stichococcus bacillaris*. *Can. J. Bot.* 56, 676–679. doi: 10.1139/b78-074

Büdel, B., and Scheidegger, C. (1996). "Thallus morphology and anatomy," in *Lichen biology 2*, ed. T. H. Nash III (Cambridge, UK: Cambridge University, Press)), 40–68. doi: 10.1017/cbo9780511790478.005

Burkholder, P. R., Evans, A. W., McVeigh, I., and Thornton, H. K. (1944). Antibiotic activity of lichens. *Proc. Natl. Acad Sci. U.S.A.* 30, 250–255. doi: 10.1073/pnas.30.9.250

Calcott, M. J., Ackerley, D. F., Knight, A., Keyzers, R. A., and Owen, J. G. (2018). Secondary metabolism in the lichen symbiosis. *Chem. Soc. Rev.* 47, 1730–1760. doi: 10.1039/C7CS00431A

Campbell, J., Bengtson, P., Fredeen, A. L., Coxson, D. S., and Prescott, C. E. (2013). Does exogenous carbon extend the realized niche of canopy lichens? Evidence from sub-boreal forests in British Columbia. *Ecology* 94, 1186–1195. doi: 10.1890/12-1857.1

Carbonero, E. R., Cordeiro, L. M., Mellinger, C. G., Sassaki, G. L., Stocker-Wörgötter, E., Gorin, P. A., et al. (2005). Galactomannans with novel structures from the lichen *Roccella decipiens* Darb. *Carbohydrate Res.* 340, 1699–1705. doi: 10.1016/j.carres.2005.03.022

Cardarelli, M., Serino, G., Campanella, L., Ercole, P., Nardone, F. D. C., Alesiani, O., et al. (1997). Antimitotic effects of usnic acid on different biological systems. *Cel. Mol. Life Sci.* 53, 667–672. doi: 10.1007/s000180050086

Cardinale, B. J., Palmer, M. A., Swan, C. M., Brooks, S., and Poff, N. L. (2002). The influence of substrate heterogeneity on biofilm metabolism in a stream ecosystem. *Ecology* 83, 412–422. doi: 10.1890/0012-9658(2002)083[0412:tiosho]2.0.co;2

Cardinale, M., Grube, M., Castro, Jr., Müller, H., and Berg, G. (2012a). Bacterial taxa associated with the lung lichen *Lobaria pulmonaria* are differentially shaped by geography and habitat. *FEMS Microbiol. Lett.* 329, 111–115. doi: 10.1111/j.1574-6968.2012.02508.x

Cardinale, M., Puglia, A. M., and Grube, M. (2006). Molecular analysis of lichen-associated bacterial communities. *FEMS Microbiol. Ecol.* 57, 484–495. doi: 10.1111/j.1574-6941.2006.00133.x

Cardinale, M., Steinová, J., Rabensteiner, J., Berg, G., and Grube, M. (2012b). Age, sun and substrate: triggers of bacterial communities in lichens. *Environ. Microbiol. Rep.* 4, 23–28. doi: 10.1111/j.1758-2229.2011.00272.x

Cardinale, M., Vieira, de Castro, Jr., Müller, H., Berg, G., and Grube, M. (2008). In situ analysis of the bacterial community associated with the reindeer lichen *Cladonia arbuscula* reveals predominance of *Alphaproteobacteria*. *FEMS Microbiol. Ecol.* 66, 63–71. doi: 10.1111/j.1574-6941.2008.00546.x

Cava, F., Lam, H., De Pedro, M. A., and Waldor, M. K. (2011). Emerging knowledge of regulatory roles of D-amino acids in bacteria. *Cell. Mol. Life Sci.* 68, 817–831. doi: 10.1007/s00018-010-0571-8

Cengia Sambo, M. (1926). Ancora della polysimbiosi nei licheni ad alghe cianoficee. 1. *Batteri simbionti. Atti Soc. Ital. Sc. Nat.* 64:5.

Centeno, D. C., Hell, A. F., Braga, M. R., Del Campo, E. M., and Casano, L. M. (2016). Contrasting strategies used by lichen microalgae to cope with desiccation–rehydration stress revealed by metabolite profiling and cell wall analysis. *Environ. Microbiol.* 18, 1546–1560. doi: 10.1111/1462-2920.13249

Cernava, T., Aschenbrenner, I. A., Soh, J., Sensen, C. W., Grube, M., and Berg, G. (2019). Plasticity of a holobiont: desiccation induces fasting-like metabolism within the lichen microbiota. *ISME J.* 13, 547–556. doi: 10.1038/s41396-018-0286-7

Cernava, T., Erlacher, A., Aschenbrenner, I. A., Krug, L., Lassek, C., Riedel, K., et al. (2017). Deciphering functional diversification within the lichen microbiota by meta-omics. *Microbiome* 5:82. doi: 10.1186/s40168-017-0303-5

Cernava, T., Müller, H., Aschenbrenner, I. A., Grube, M., and Berg, G. (2015). Analyzing the antagonistic potential of the lichen microbiome against pathogens by bridging metagenomic with culture studies. *Front. Microbiol.* 6:620. doi: 10.3389/fmicb.2015.00620

Cernava, T., Vasfiu, Q., Erlacher, A., Aschenbrenner, I. A., Francesconi, K., Grube, M., et al. (2018). Adaptions of lichen microbiota functioning under persistent exposure to arsenic contamination. *Front. Microbiol.* 9:02959. doi: 10.3389/fmicb.2018.02959

Cheenpracha, S., Vidor, N. B., Yoshida, W. Y., Davies, J., and Chang, L. C. (2010). Coumabiocins A-F aminocoumarins from an organic extract of *Streptomyces* sp. *J. Nat. Prod.* 73, 880–884. doi: 10.1021/np900843b

Chen, Y., Yu, L., Liu, F., and Du, L. (2018). Spermidine-regulated biosynthesis of heat-stable antifungal factor (HSAF) in *Lysobacter enzymogenes* OH11. *Front. Microbiol.* 9:2984. doi: 10.3389/fmicb.2018.02984

Cordeiro, L. M., Beilke, F., de Fátima Reinhardt, V., Sassaki, G. L., and Iacomini, M. (2013). Rhamnogalactofuranan from the microalga *Myrmecia biatorellae*, symbiotic partner of *Lobaria linita*. *Phytochemistry* 94, 254–259. doi: 10.1016/j.phytochem.2013.06.008

Cordeiro, L. M., Carbonero, E. R., Sassaki, G. L., Reis, R. A., Stocker-Wörgötter, E., Gorin, P. A., et al. (2005). A fungus-type β-galactofuranan in the cultivated *Trebouxia* photobiont of the lichen *Ramalina gracilis*. *FEMS Microbiol. Lett.* 244, 193–198.

Cordeiro, L. M., de Oliveira, S. M., Buchi, D. F., and Iacomini, M. (2008). Galactofuranose-rich heteropolysaccharide from *Trebouxia* sp., photobiont of the lichen *Ramalina gracilis* and its effect on macrophage activation. *In. J. Biol. Macromol.* 42, 436–440. doi: 10.1016/j.ijbiomac.2008.02.002

Cordeiro, L. M., Sassaki, G. L., Gorin, P. A., and Iacomini, M. (2010). O-Methylated mannogalactan from the microalga *Coccomyxa mucigena*, symbiotic partner of the lichenized fungus *Peltigera aphthosa*. *Phytochemistry* 71, 1162–1167. doi: 10.1016/j.phytochem.2010.04.004

Cordeiro, L. M., Stocker-Wörgötter, E., Gorin, P. A., and Iacomini, M. (2003). Comparative studies of the polysaccharides from species of the genus *Ramalina*-lichenized fungi-of three distinct habitats. *Phytochemistry* 63, 967–975. doi: 10.1016/s0031-9422(03)00336-4

Cornejo, C., and Scheidegger, C. (2013). New morphological aspects of cephalodium formation in the lichen *Lobaria pulmonaria* (Lecanorales. Ascomycota). *Lichenologist* 45, 77–87. doi: 10.1017/s0024282912000631

Cornejo, C., Nelson, P. R., Stepanchikova, I., Himelbrant, D., Jørgensen, P. M., and Scheidegger, C. (2005). Contrasting pattern of photobiont diversity in the Atlantic and Pacific populations of *Erioderma pedicellatum* (Pannariaceae). *Lichenologist* 48, 275–291. doi: 10.1017/s0024282916000311

Cornett, D. S., Reyzer, M. L., Chaurand, P., and Caprioli, R. M. (2007). MALDI imaging mass spectrometry: molecular snapshots of biochemical systems. *Nat. Methods* 4, 828–833. doi: 10.1038/nmeth1094

Coxson, D. S., and Nadkarni, N. M. (1995). "Ecological Roles of Epiphytes in Nutrient Cycles of Forest Ecosystem," in *Forest Canopies*, eds M. D. Lowman and N. M. Nadkarni (SanDiego, USA: Academic Press), 495–543.

Croft, M. T., Lawrence, A. D., Raux-Deery, E., Warren, M. J., and Smith, A. G. (2005). Algae acquire vitamin B 12 through a symbiotic relationship with bacteria. *Nature* 438, 90–93. doi: 10.1038/nature04056

Crowther, T. W., and Bradford, M. A. (2013). Thermal acclimation in widespread heterotrophic soil microbes. *Ecol. Lett.* 16, 469–477. doi: 10.1111/ele.12069

Darnajoux, R., Zhang, X., McRose, D. L., Miadlikowska, J., Lutzoni, F., Kraepiel, A. M., et al. (2017). Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications

for current biological nitrogen fixation estimates. *N. Phytol.* 213, 680–689. doi: 10.1111/nph.14166

Davies, J. E., Waters, B., and Saxena, G. (2002). *Method for inhibiting eukaryotic protein kinases. U.S. Patent No. 6,455,270*. Washington, DC: U.S. Patent and Trademark Office.

Davies, J., Wang, H., Taylor, T., Warabi, K., Huang, X. H., and Andersen, R. J. (2005). Uncialamycin, a new enediyne antibiotic. *Org. Lett.* 7, 5233–5236. doi: 10.1021/ol052081f

De Baets, S., and Vandamme, E. J. (2001). Extracellular *Tremella* polysaccharides: structure, properties and applications. *Biotechnol. Lett.* 23, 1361–1366.

De Los Rios, A., and Grube, M. (2000). Host parasite interfaces of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). *Mycol. Res.* 104, 1348–1353. doi: 10.1017/s0953756200002951

Demmig-Adams, B., Máguas, C., Adams, W. W., Meyer, A., Kilian, E., and Lange, O. L. (1990). Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. *Planta* 180, 400–409. doi: 10.1007/bf01160396

Didehdar, M., Gokanian, A., Sofian, M., Mohammadi, S., Mohammadi, R., Aslani, N., et al. (2015). First fatal cerebral phaeohyphomycosis due to *Rhinocladiella mackenziei* in Iran, based on ITS rDNA. *J. Mycol. Medicale* 25, 81–86. doi: 10.1016/j.mycmed.2014.11.003

Diederich, P., Lawrey, J. D., and Ertz, D. (2018). The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. *Bryologist* 121, 340–425. doi: 10.1639/0007-2745-121.3.340

Doering, M., and Coxson, D. (2010). Riparian alder ecosystems as epiphytic lichen refugia in sub-boreal spruce forests of British Columbia. *Botany* 88, 144–157. doi: 10.1139/b09-096

Dudley, S. A., and Lechowicz, M. J. (1987). Losses of polyol through leaching in subarctic lichens. *Plant Physiol.* 83, 813–815. doi: 10.1104/pp.83.4.813

Eaton, S., and Ellis, C. J. (2014). High demographic rates of the model epiphyte *Lobaria pulmonaria* in an oceanic hazelwood (western Scotland). *Fung. Ecol.* 11, 60–70. doi: 10.1016/j.funeco.2014.03.007

Edman, M., Eriksson, A. M., and Villard, M. A. (2008). Effects of selection cutting on the abundance and fertility of indicator lichens *Lobaria pulmonaria* and *L. quercizans*. *J. Appl. Ecol.* 45, 26–33. doi: 10.1111/j.1365-2664.2007.01354.x

Eisenberg, T., Abdellatif, M., Schroeder, S., Primessnig, U., Stekovic, S., Pendl, T., et al. (2016). Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat. Med.* 22:1428.

Eisenberg, T., Knauer, H., Schauer, A., Büttner, S., Ruckenstuhl, C., Carmona-Gutierrez, D., et al. (2009). Induction of autophagy by spermidine promotes longevity. *Nat. Cell Biol.* 11, 1305–1314.

Eisenreich, W., Knispel, N., and Beck, A. (2011). Advanced methods for the study of the chemistry and the metabolism of lichens. *Phytochem. Rev.* 10, 445–456. doi: 10.1007/s11101-011-9215-3

Elix, J. A., and Stocker-Wörgötter, E. (1996). ""Biochemistry and secondary metabolites,"," in *Lichen biology, Cambridge*, ed. T. H. Nash III (UK: Cambridge University Press), 154–180.

Ellis, C. J., Crittenden, P. D., Scrimgeour, C. M., and Ashcroft, C. J. (2005). Translocation of ^{15}N indicates nitrogen recycling in the mat-forming lichen *Cladonia portentosa*. *N. Phytol.* 168, 423–434. doi: 10.1111/j.1469-8137.2005.01524.x

Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C. W., Grube, M., et al. (2015). Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Front. Microbiol.* 6:53. doi: 10.3389/fmicb.2015.00053

Eymann, C., Lassek, C., Wegner, U., Bernhardt, J., Fritsch, O. A., Fuchs, S., et al. (2017). Symbiotic interplay of fungi, algae, and bacteria within the lung lichen *Lobaria pulmonaria* L. Hoffm. as Assessed by State-of-the-Art Metaproteomics. *J. Proteome Res.* 16, 2160–2173. doi: 10.1021/acs.jproteome.6b00974

Farkas, E., and Lökös, L. (2009). *Lobaria pulmonaria* (lichen-forming fungi) in Hungary. *Mikológiai Közlemények, Clusiana* 48, 11–18.

Farmer, A. M., Bates, J. W., and Bell, J. N. B. (1991). Comparisons of three woodland sites in NW Britain differing in richness of the epiphytic Lobarion-pulmonariae community and levels of wet acidic deposition. *Holarct. Ecol.* 19, 85–91. doi: 10.1111/j.1600-0587.1991.tb00637.x

Farrar, J. F. (1988). "Physiological buffering," in *Handbook of Lichenology*, Vol. 11, ed. M. Galun (Boca Raton, FL: CRC Press), 101–105.

Fernandéz-Mendoza, F., Fleischhacker, A., Kopun, T., Grube, M., and Muggia, L. (2017). ITS1 metabarcoding highlights low specificity of lichen mycobiomes at a local scale. *Mol. Ecol.* 26, 4811–4830. doi: 10.1111/mec.14244

Fiechter, E. (1990). *Thallusdifferenzierung und intrathalline Sekundärstoffverteilung bei Parmeliaceae (Lecanorales, lichenisierte Ascomyceten)*. doctoral dissertation, Zürich (Switzerland)]: Universität Zürich.

Francolini, I., Norris, P., Piozzi, A., Donelli, G., and Stoodley, P. (2004). Usnic acid, a natural antimicrobial agent able to inhibit bacterial biofilm formation on polymer surfaces. *Antimicrob. Agents Chemoth.* 48, 4360–4365. doi: 10.1128/aac.48.11.4360-4365.2004

Garg, N., Zeng, Y., Edlund, A., Melnik, A. V., Sanchez, L. M., Mohimani, H., et al. (2016). Spatial molecular architecture of the microbial community of a *Peltigera* lichen. *Nov. Syst. Biol. Tech.* 1:6. doi: 10.1128/mSystems.00139-16

Garty, J. (2001). Biomonitoring atmospheric heavy metals with lichens: theory and application. *Crit. Rev. Plant Sci.* 20, 309–371. doi: 10.1016/s0735-2689(01)80040-x

Gasser, I., Cardinale, M., Müller, H., Heller, S., Eberl, L., Lindenkamp, N., et al. (2011). Analysis of the endophytic lifestyle and plant growth promotion of *Burkholderia terricola* ZR2-12. *Plant Soil* 347, 125–136. doi: 10.1007/s11104-011-0833-8

Gauslaa, Y., and McEvoy, M. (2005). Seasonal changes in solar radiation drive acclimation of the sun-screening compound parietin in the lichen *Xanthoria parietina*. *Basic Appl. Ecol.* 6, 75–82. doi: 10.1016/j.baae.2004.10.003

Gauslaa, Y., and Solhaug, K. A. (1996). Differences in the susceptibility to light stress between epiphytic lichens of ancient and young boreal forest stands. *Funct. Ecol.* 10, 344–354. doi: 10.2307/2390282

Gauslaa, Y., and Solhaug, K. A. (1999). High-light damage in air-dry thalli of the old forest lichen *Lobaria pulmonaria* - interactions of irradiance, exposure duration and high temperature. *J. Exp. Bot.* 50, 697–705. doi: 10.1093/jexbot/50.334.697

Gauslaa, Y., and Solhaug, K. A. (2000). High-light-intensity damage to the foliose lichen *Lobaria pulmonaria* within natural forest: the applicability of chlorophyll fluorescence methods. *Lichenologist* 32, 271–289. doi: 10.1006/lich.1999.0265

Gauslaa, Y., and Solhaug, K. A. (2001). Fungal melanins as a sun screen for symbiotic green algae in the lichen *Lobaria pulmonaria*. *Oecol.* 126, 462–471. doi: 10.1007/s004420000541

Germerodt, S., Bohl, K., Lück, A., Pande, S., Schröter, A., Kaleta, C., et al. (2016). Pervasive selection for cooperative cross-feeding in bacterial communities. *PLoS Comput. Biol.* 12:6. doi: 10.1371/journal.pcbi.1004986

Gianini, A. S., Marques, M. R., Carvalho, N. C. P., and Honda, N. K. (2008). Activities of 2,4-Dihydroxy-6-*n*-pentylbenzoic Acid Derivates. *Z. Naturf.* 63, 29–34. doi: 10.1515/znc-2008-1-206

González, I., Ayuso-Sacido, A., Anderson, A., and Genilloud, O. (2005). *Actinomycetes* isolated from lichens: evaluation of their diversity and detection of biosynthetic gene sequences. *FEMS Microbiol. Ecol.* 54, 401–415. doi: 10.1016/j.femsec.2005.05.004

Gorin, P. A. J., and Barreto-Bergter, E. (1983). "The chemistry of polysaccharides of fungi and lichens," in *The Polysaccharides*, Vol. 2, ed. F. O. Aspinall (New York: Academic Press), 365–409. doi: 10.1016/b978-0-12-065602-8.50011-x

Graham, L. E., Trest, M. T., Will-Wolf, S., Miicke, N. S., Atonio, L. M., Piotrowski, M. J., et al. (2018). Microscopic and metagenomic analyses of *Peltigera ponojensis* (Peltigerales, Ascomycota). *Int. J. Plant Sci.* 179, 241–255. doi: 10.1086/696534

Grube, M. (2018). The lichen thallus as a microbial habitat. *Biosyst. Ecol. Ser.* 34, 528–545.

Grube, M., and Berg, G. (2009). Microbial consortia of bacteria and fungi with focus on the lichen symbiosis. *Fung. Biol. Rev.* 23, 72–85. doi: 10.1016/j.fbr.2009.10.001

Grube, M., and De Los Rios, A. (2001). Observations in *Biatoropsis usnearum* and other gall forming lichenicolous fungi using different microscopic techniques. *Mycol. Res.* 105, 1116–1122. doi: 10.1016/s0953-7562(08)61975-9

Grube, M., and Hafellner, J. (1990). Studien an flechtenbewohnenden Pilzen der Sammelgattung *Didymella* (Ascomycetes, Dothideales). *Nova Hedwigia* 51, 283–360.

Grube, M., and Hawksworth, D. L. (2007). Trouble with lichen: the re-evaluation and re-interpretation of thallus form and fruit body types in the molecular era. *Mycol. Res.* 111, 1116–1132. doi: 10.1016/j.mycres.2007.04.008

Grube, M., Cardinale, M., de Castro, J. V., Müller, H., and Berg, G. (2009). Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *ISME J.* 3, 1105–1115. doi: 10.1038/ismej.2009.63

Grube, M., Cardinale, M., de Castro, J. V., Müller, H., and Berg, G. (2012). Host-parasite interaction and microbiome response: effects of fungal infections on the bacterial community of the Alpine lichen *Solorina crocea*. *FEMS Microbiol. Ecol.* 82, 472–481. doi: 10.1111/j.1574-6941.2012.01425.x

Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., et al. (2015). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *ISME J.* 9, 412–424. doi: 10.1038/ismej.2014.138

Gupta, V. K., Scheunemann, L., Eisenberg, T., Mertel, S., Bhukel, A., Koemans, T. S., et al. (2013). Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. *Nat. Neurosci.* 16, 1453–1460. doi: 10.1038/nn.3512

Hakulinen, R. (1964). Die Flechtengattung *Lobaria* Schreb. in Ostfennoskandien. *Ann. Bot. Fen.* 1, 202–213.

Hallingbäck, T., and Martinsson, P.-O. (1987). The retreat of two lichens, *Lobaria pulmonaria* and *L. scrobiculata* in the district of Gäsene (SW Sweden). *Windahlia* 17, 27–32. doi: 10.14492/hokmj/1381758488

Hallingback, T., and Olsson, K. (1987). Lunglavens tillbakagang i Skane. *Sv. Bot. Tidskr.* 81, 103–108.

Hassa, J., Maus, I., Off, S., Pühler, A., Scherer, P., Klocke, M., et al. (2018). Metagenome, metatranscriptome, and metaproteome approaches unraveled compositions and functional relationships of microbial communities residing in biogas plants. *Appl. Microbiol. Biotechnol.* 102, 5045–5063. doi: 10.1007/s00253-018-8976-7

Hauck, M., and Wirth, V. (2010). Preference of lichens for shady habitats is correlated with intolerance to high nitrogen levels. *Lichenologist* 42, 475–484. doi: 10.1017/s0024282910000046

Hauck, M., Jürgens, S. R., Brinkmann, M., and Herminghaus, S. (2008). Surface hydrophobicity causes SO2 tolerance in lichens. *Ann. Bot.* 101, 531–539. doi: 10.1093/aob/mcm306

Hawksworth, D. L., and Grube, M. (2020). Lichens redefined as complex ecosystems. *N. Phytol.* 227, 1281–1283. doi: 10.1111/nph.16630

Hawksworth, D. L., and Hill, D. J. (1984). *The lichen-forming fungi*. Glasgow & London: Blackie.

Hawksworth, D. L., and Rose, F. (1970). Qualitative scale for estimating sulphur dioxide air pollution in England and Wales using epiphytic lichens. *Nature* 227, 145–148. doi: 10.1038/227145a0

Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. (1995). *Dictionary of the Fungi*, 8th Edn. Wallingford: CAB International.

Hawksworth, D. L., Rose, F., and Coppins, B. J. (1973). "Changes in the lichen flora of England and Wales attributable to pollution of the air by sulphur dioxide," in *Air Pollution and Lichens*, eds B. W. Ferry, M. S. Baddeley, and D. L. Hawksworth (London: Athlone Press), 330–367.

Heber, U., Bilger, W., Türk, R., and Lange, O. L. (2010). Photoprotection of reaction centres in photosynthetic organisms: mechanisms of thermal energy dissipation in desiccated thalli of the lichen *Lobaria pulmonaria*. *N. Phytol.* 185, 459–470. doi: 10.1111/j.1469-8137.2009.03064.x

Henckel, P. A., and Yuzhakova, L. A. (1936). On the role of *Azotobacter* in the lichen symbiosis. *Bull. Perm Biol. Res. Inst.* 10:315.

Henkel, P. A., and Plotnikova, T. T. (1973). Nitrogen-fixing bacteria in lichens. *Izv. Akad. Nauk. Ser. Biol.* 1973, 807–813.

Heyer, R., Schallert, K., Zoun, R., Becher, B., Saake, G., and Benndorf, D. (2017). Challenges and perspectives of metaproteomic data analysis. *J. Biotech.* 261, 24–36. doi: 10.1016/j.jbiotec.2017.06.1201

Hill, D. J. (1972). The movement of carbohydrate from the alga to the fungus in the lichen *Peltigera polydactyla*. *N. Phytol.* 71, 31–39. doi: 10.1111/j.1469-8137.1972.tb04807.x

Hill, D. J. (1976). "The physiology of lichen symbiosis," in *Lichenology: Progress and Problems*, eds D. H. Brown, D. L. Hawksworth, and R. H. Bailey (San Francisco: Academic Press), 457–496.

Hodkinson, B. P., and Lutzoni, F. (2009). A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49, 163–180. doi: 10.1007/s13199-009-0049-3

Hodkinson, B. P., Gottel, N. R., Schadt, C. W., and Lutzoni, F. (2012). Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome. *Environ. Microbiol.* 14, 147–161. doi: 10.1111/j.1462-2920.2011.02560.x

Honegger, R. (1984). Cytological aspects of the mycobiont–phycobiont relationship in lichens: haustorial types, phycobiont cell wall types, and the ultrastructure of the cell surface layers in some cultured and symbiotic myco-and phycobionts. *Lichenologist* 16, 111–127. doi: 10.1017/s0024282984000293

Honegger, R. (1987). Questions about pattern formation in the algal layer of lichens with stratified (heteromerous) thalli. *Biblioth. Lichenol.* 25, 59–71.

Honegger, R. (1990). "Surface interactions in lichens," in *Experimental Phycology Vol. 1: Cell Walls and Surfaces, Reproduction, Photosynthesis*, eds W. Wiessner, D. G. Robinson, and R. C. Starr (Berlin: Springer-Verlag), 40–54. doi: 10.1007/978-3-642-48652-4_3

Honegger, R. (1991). Functional aspects of the lichen symbiosis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42, 553–578. doi: 10.1146/annurev.pp.42.060191.003005

Honegger, R. (1993). Tansley Review No. 60: Developmental biology of lichens. *N. Phytol.* 125, 659–677. doi: 10.1111/j.1469-8137.1993.tb03916.x

Honegger, R. (1996). ""Morphogenesis,"," in *Lichen biology*, ed. T. H. Nash III (Cambridge, UK: Cambridge University Press), 65–87.

Honegger, R. (1997). "Metabolic interactions at the mycobiont-photobiont interface in lichens," in *The Mycota: Plant relationships Part A*, eds G. C. Carroll and P. Tudzynski (Berlin: Springer-Verlag), 209–221. doi: 10.1007/978-3-662-10370-8_12

Honegger, R., Edwards, D., and Axe, L. (2013). The earliest records of internally stratified cyanobacterial and algal lichens from the Lower Devonian of the Welsh Borderland. *N. Phytol.* 197, 264–275. doi: 10.1111/nph.12009

Hrouzek, P. (2017). "Secondary metabolites produced by cyanobacteria in symbiotic associations," in *Algal and cyanobacteria symbioses*, eds M. Grube, J. Seckbach, and L. Muggia (Singapore: World Scientific), 611–626. doi: 10.1142/9781786340580_0019

Hunter, P. (2006). Entente cordiale. *EMBO Rep.* 7, 861–864.

Hyvärinen, M., Härdling, R., and Tuomi, J. (2002). Cyanobacterial lichen symbiosis: the fungal partner as an optimal harvester. *Oikos* 98, 498–504. doi: 10.1034/j.1600-0706.2002.980314.x

Ingólfsdóttir, K., Chung, G. A., Skúlason, V. G., Gissurarson, S. R., and Vilhelmsdóttir, M. (1998). Antimycobacterial activity of lichen metabolites in vitro. *Europ. J. Pharma. Sci.* 6, 141–144. doi: 10.1016/s0928-0987(97)00078-x

Iskina, R. Y. (1938). On nitrogen fixing bacteria in lichens. *Isv. Biol. Inst. Permsk.* 11, 133–139.

Jabeen, K., Farooqi, J., Zafar, A., Jamil, B., Faisal Mahmood, S., Ali, F., et al. (2011). *Rhinocladiella mackenziei* as an emerging cause of cerebral phaeohyphomycosis in Pakistan: a case series. *Clin. Infect. Dis.* 52, 213–217. doi: 10.1093/cid/ciq114

Jahns, H. M. (1988). ""The lichen thallus","in *CRC Handbook of Lichenology*, Vol. 1, ed. M. Galun (Baca Raton, FL: CRC Press), 95–143. doi: 10.1201/9780429291784-4

Jakobi, M., Winkelmann, G., Kaiser, D., Kempter, C., Jung, G., Berg, G., et al. (1996). Maltophilin: A new antifungal compound produced by *Stenotrophomonas maltophilia* R3089. *J. Antibiot.* 49, 1101–1104. doi: 10.7164/antibiotics.49.1101

Jehmlich, N., Vogt, C., Lünsmann, V., Richnow, H. H., and von Bergen, M. (2016). Protein-SIP in environmental studies. *Curr. Op. Biotechnol.* 41, 26–33. doi: 10.1016/j.copbio.2016.04.010

Jennings, D. H., and Lysek, G. (1996). *Fungal biology: understanding the fungal lifestyle*. London: Bios Scientific Publishers Ltd.

Jordan, W. P. (1970). The internal cephalodia of the genus *Lobaria*. *Bryologist* 73, 669–681. doi: 10.2307/3241279

Jüriado, I., and Liira, J. (2009). Distribution and habitat ecology of the threatened forest lichen *Lobaria pulmonaria* in Estonia. *Fol. Cryptog. Est.* 46, 55–65.

Jüriado, I., Liira, J., Csencsics, D., Widmer, I., Adolf, C., Kohv, K., et al. (2011). Dispersal ecology of the endangered woodland lichen *Lobaria pulmonaria* in managed hemiboreal forest landscape. *Biodiv. Conserv.* 20, 1803–1819. doi: 10.1007/s10531-011-0062-8

Kaasalainen, U., Fewer, D. P., Jokela, J., Wahlsten, M., Sivonen, K., and Rikkinen, J. (2012). Cyanobacteria produce a high variety of hepatotoxic peptides in lichen symbiosis. *Proc. Natl. Acad Sci. U.S.A* 109, 5886–5891. doi: 10.1073/pnas.1200279109

Kaasalainen, U., Jokela, J., Fewer, D. P., Sivonen, K., and Rikkinen, J. (2009). Microcystin production in the tripartite cyanolichen *Peltigera leucophlebia*. *Mol. Plant-Microbe Interact.* 22, 695–702. doi: 10.1094/mpmi-22-6-0695

Kadereit, J. W., Körner, C., Kost, B., and Sonnewald, U. (2014). *Strasburger-Lehrbuch der Pflanzenwissenschaften*. Berlin: Springer-Verlag.

Kalwij, J. M., Wagner, H. H., and Scheidegger, C. (2005). Effects of stand-level disturbance events on the spatial distribution of a lichen indicator of forest conservation value. *Ecol. Appl.* 15, 2015–2024. doi: 10.1890/04-1912

Karpievitch, Y. V., Polpitiya, A. D., Anderson, G. A., Smith, R. D., and Dabney, A. R. (2010). Liquid chromatography mass spectrometry-based proteomics: biological and technological aspects. *Ann. Appl. Stat.* 4:4. doi: 10.1214/10-AOAS341

Kershaw, K. A. (1985). *Physiological ecology of lichens*. Cambridge: Cambridge University Press.

Kim, M. K., Oh, T. J., and Park, H. (2013b). Antimicrobial properties of the bacterial associates of the Arctic lichen *Stereocaulon* sp. *Afr. J. Microbiol. Res.* 7, 3651–3657.

Kim, M. K., Park, H., and Oh, T. J. (2013a). Antioxidant properties of various microorganisms isolated from arctic lichen *Stereocaulon* spp. *Microbiol. Biotech. Lett.* 41, 350–357. doi: 10.4014/kjmb.1303.03004

Kim, M. K., Park, H., and Oh, T. J. (2014). Antibacterial and antioxidant capacity of polar microorganisms isolated from Arctic lichen *Ochrolechia* sp. *Pol. J. Microbiol.* 63, 317–322. doi: 10.33073/pjm-2014-042

Kinoshita, K., Fukumaru, M., Yamamoto, Y., Koyama, K., and Takahashi, K. (2015). Biosynthesis of panaefluoroline B from the cultured mycobiont of *Amygdalaria panaeola*. *J. Nat. Prod.* 78, 1745–1747. doi: 10.1021/acs.jnatprod.5b00055

Klarenberg, I. J., Keuschnig, C., Warshan, D., Jónsdóttir, I. S., and Vilhelmsson, O. (2020). *The total and active bacterial community of the chlorolichen Cetraria islandica and its response to long-term warming in sub-Arctic tundra*. Available online at: https://www.biorxiv.org/content/10.1101/2020.03.04.976944v1.abstract (accessed August 11, 2020).

Kleiner, M. (2019). Metaproteomics: much more than measuring gene expression in microbial communities. *mSystems* 4:3. doi: 10.1128/mSystems.00115-19

Kleiner, M., Dong, X., Hinzke, T., Wippler, J., Thorson, E., Mayer, B., et al. (2018). Metaproteomics method to determine carbon sources and assimilation pathways of species in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.* 115:24. doi: 10.1073/pnas.1722325115

Kolodkin-Gal, I., Romero, D., Cao, S., Clardy, J., Kolter, R., and Losick, R. (2010). D-amino acids trigger biofilm disassembly. *Science* 328, 627–629. doi: 10.1126/science.1188628

Kopycińska, M., Lipa, P., Cieśla, J., Kozieł, M., and Janczarek, M. (2018). Extracellular polysaccharide protects *Rhizobium leguminosarum* cells against zinc stress in vitro and during symbiosis with clover. *Environ. Microbiol. Rep.* 10, 355–368. doi: 10.1111/1758-2229.12646

Kranner, I., Cram, W. J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., et al. (2005). Antioxidants and photoprotection in a lichen as compared to its isolated symbiotic partners. *Proc. Natl. Acad. Sci. U.S.A.* 102, 3141–3146. doi: 10.1073/pnas.0407716102

Kranner, I., Cram, W. J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., et al. (2008). Desiccation-tolerance in lichens: a review. *Bryologist* 111, 576–593. doi: 10.1639/0007-2745-111.4.576

Kuhn, V., Geisberger, T., Huber, C., Beck, A., and Eisenreich, W. (2019). A facile in vivo procedure to analyze metabolic pathways in intact lichens. *N. Phytol.* 224, 1657–1667. doi: 10.1111/nph.15968

Kunath, B. J., Minniti, G., Skaugen, M., Hagen, L. H., Vaaje-Kolstad, G., Eijsink, V. G., et al. (2019). ""Metaproteomics: sample preparation and methodological considerations,"," in *Emerging Sample Treatments in Proteomics. Advances in Experimental Medicine and Biology*, Vol. 1073, ed. J. L. Capelo-Martínez (Berlin: Springer), 187–215. doi: 10.1007/978-3-030-12298-0_8

Lam, H., Oh, D. C., Cava, F., Takacs, C. N., Clardy, J., de Pedro, M. A., et al. (2009). D-amino acids govern stationary phase cell wall remodeling in bacteria. *Science* 325, 1552–1555. doi: 10.1126/science.1178123

Larson, D. W. (1987). The absorption and release of water by lichens. *Biblioth. Lichenol.* 25, 1–360.

Lauterwein, M., Oethinger, M., Belsner, K., Peters, T., and Marre, R. (1995). In vitro activities of the lichen secondary metabolites vulpinic acid, (+)-usnic acid, and (-)-usnic acid against aerobic and anaerobic microorganisms. *Antimicrob. Ag. Chem.* 39, 2541–2543. doi: 10.1128/aac.39.11.2541

Lavallée, V. P. (2011). *Antipain and its analogues, natural product inhibitors of Cathepsin K isolated from Streptomyces*. doctoral dissertation, Vancouver (Canada): University of British Columbia.

Lawrey, J. D., and Diederich, P. (2003). Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist* 106, 80–120. doi: 10.1639/0007-2745(2003)106[0080:lfieab]2.0.co;2

Le Pogam, P., Legouin, B., Geairon, A., Rogniaux, H., Lohézic-Le Dévéhat, F., Obermayer, et al. (2016). Spatial mapping of lichen specialized metabolites using LDI-MSI: chemical ecology issues for *Ophioparma ventosa*. *Sci. Rep.* 6:1. doi: 10.1038/srep37807

Lee, Y. M., Kim, E. H., Lee, H. K., and Hong, S. G. (2014). Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World J. Microb. Biot.* 30, 2711–2721. doi: 10.1007/s11274-014-1695-z

Leiman, S. A., May, J. M., Lebar, M. D., Kahne, D., Kolter, R., and Losick, R. (2013). D-amino acids indirectly inhibit biofilm formation in *Bacillus subtilis* by interfering with protein synthesis. *J. Bacteriol.* 195, 5391–5395. doi: 10.1128/jb.00975-13

Leiva, D., Fernández-Mendoza, F., Acevedo, J., Carú, M., Grube, M., and Orlando, J. (2021). The Bacterial community of the foliose macro-lichen *Peltigera frigida* is more than a mere extension of the microbiota of the subjacent substrate. *Microb. Ecol.* 1:12. doi: 10.1007/s00248-020-01662-y

Lenova, L. I., and Blum, O. (1983). To the question on the third component of lichens. *Bot. J.* 68, 21–28.

Leveau, J. H. J., and Preston, G. M. (2008). Bacterial mycophagy: definition and diagnosis of a unique bacterial–fungal interaction. *N. Phytol.* 177, 859–876. doi: 10.1111/j.1469-8137.2007.02325.x

Levsky, J. M., and Singer, R. H. (2003). Fluorescence in situ hybridization: past, present and future. *J. Cell Sci.* 116, 2833–2838. doi: 10.1242/jcs.00633

Liba, C. M., Ferrara, F. I. S., Manfio, G. P., Fantinatti-Garboggini, F., Albuquerque, R. C., Pavan, C., et al. (2006). Nitrogen-fixing chemo-organotrophic bacteria isolated from cyanobacteria-deprived lichens and their ability to solubilize phosphate and to release amino acids and phytohormones. *J. Appl. Microbiol.* 101, 1076–1086. doi: 10.1111/j.1365-2672.2006.03010.x

Lines, C. E. M., Ratcliffe, R. G., Rees, T. A. V., and Southon, T. E. (1989). A ^{13}C NMR study of photosynthate transport and metabolism in the lichen *Xanthoria calcicola* Oxner. *N. Phytol.* 111, 447–456. doi: 10.1111/j.1469-8137.1989.tb00707.x

Lisewski, V., and Ellis, C. J. (2010). Epiphyte sensitivity to a cross-scale interaction between habitat quality and macroclimate: an opportunity for range-edge conservation. *Biodiv. Conserv.* 19, 3935–3949. doi: 10.1007/s10531-010-9938-2

Liu, J., Prindle, A., Humphries, J., Gabalda-Sagarra, M., Asally, M., Dong-yeon, D. L., et al. (2015). Metabolic co-dependence gives rise to collective oscillations within biofilms. *Nature* 523, 550–554. doi: 10.1038/nature14660

Lumbsch, H. T., Ahti, T., Altermann, S., Paz, A. D. G., Aptroot, A., Arup, U., et al. (2011). One hundred new species of lichenized fungi: a signature of undiscovered global diversity. *Phytotaxa* 18, 1–127.

MacFarlane, J. D., and Kershaw, K. A. (1985). "Some aspects of carbohydrate metabolism in lichens," in *Lichen Physiology and Cell Biology*, ed. D. H. Brown (Boston, MA: Springer), 1–8. doi: 10.1007/978-1-4613-2527-7_1

Madeo, F., Eisenberg, T., Büttner, S., Ruckenstuhl, C., and Kroemer, G. (2010). Spermidine: A novel autophagy inducer and longevity elixir. *Autophagy* 6, 160–162. doi: 10.4161/auto.6.1.10600

Madeo, F., Eisenberg, T., Pietrocola, F., and Kroemer, G. (2018). Spermidine in health and disease. *Science* 359:6374. doi: 10.1126/science.aan2788

Mafole, T. C., Chiang, C., Solhaug, K. A., and Beckett, R. P. (2017). Melanisation in the old forest lichen *Lobaria pulmonaria* reduces the efficiency of photosynthesis. *Fung. Ecol.* 29, 103–110. doi: 10.1016/j.funeco.2017.07.004

Maguire, F., Jia, B., Gray, K. L., Lau, W. Y. V., Beiko, R. G., and Brinkman, F. S. (2020). Metagenome-assembled genome binning methods with short reads disproportionately fail for plasmids and genomic islands. *Microb. Genomics* 6:10. doi: 10.1099/mgen.0.000436

Maier, S., Schmidt, T. S., Zheng, L., Peer, T., Wagner, V., and Grube, M. (2014). Analyses of dryland biological soil crusts highlight lichens as an important regulator of microbial communities. *Biodiv. Conserv.* 23, 1735–1755. doi: 10.1007/s10531-014-0719-1

Mark, K., Laanisto, L., Bueno, C. G., Niinemets, Ü, Keller, C., and Scheidegger, C. (2020). Contrasting co-occurrence patterns of photobiont and cystobasidiomycete yeast associated with common epiphytic lichen species. *N. Phytol.* 2020:16475. doi: 10.1111/nph.16475

Martínez, I., Flores, T., Otálora, M. A., Belinchón, R., Prieto, M., Aragón, G., et al. (2012). Multiple-scale environmental modulation of lichen reproduction. *Fung. Biol.* 116, 1192–1201. doi: 10.1016/j.funbio.2012.09.005

Martinez, L. R., and Casadevall, A. (2015). Biofilm formation by *Cryptococcus neoformans*. *Microbiol. Spectr.* 3:3. doi: 10.1128/9781555817466.ch7

Matee, L. P., Beckett, R. P., Solhaug, K. A., and Minibayeva, F. V. (2016). Characterization and role of tyrosinases in the lichen *Lobaria pulmonaria* (L.) Hoffm. *Lichenologist* 48, 311–322. doi: 10.1017/s0024282916000293

McEvoy, M., Gauslaa, Y., and Solhaug, K. A. (2007). Changes in pools of depsidones and melanins, and their function, during growth and acclimation under contrasting natural light in the lichen *Lobaria pulmonaria*. *N. Phytol.* 175, 271–282. doi: 10.1111/j.1469-8137.2007.02096.x

McGinnis, M. W., Parker, Z. M., Walter, N. E., Rutkovsky, A. C., Cartaya-Marin, C., and Karatan, E. (2009). Spermidine regulates *Vibrio cholera* biofilm formation via transport and signaling pathways. *FEMS Microbiol. Lett.* 299, 166–174. doi: 10.1111/j.1574-6968.2009.01744.x

Mechan Llontop, M. E. (2020). *Identification, characterization, and use of precipitation-borne and plant-associated bacteria*. Dissertation, Blacksburg (VA): Virginia Polytechnic Institute and State University.

Millanes, A. M., Diederich, P., and Wedin, M. (2016). *Cyphobasidium* gen. nov., a new lichen-inhabiting lineage in the Cystobasidiomycetes (Pucciniomycotina. Basidiomycota, Fungi). *Fung. Biol.* 120, 1468–1477. doi: 10.1016/j.funbio.2015.12.003

Millbank, J. W., and Kershaw, K. A. (1970). Nitrogen metabolism in lichens. *N. Phytol.* 69, 595–597. doi: 10.1111/j.1469-8137.1970.tb07611.x

Minibayeva, F., and Beckett, R. P. (2001). High rates of extracellular superoxide production in bryophytes and lichens, and an oxidative burst in response to rehydration following desiccation. *N. Phytol.* 152, 333–341. doi: 10.1046/j.0028-646x.2001.00256.x

Motohashi, K., Takagi, M., Yamamura, H., Hayakawa, M., and Shin-Ya, K. (2010). A new angucycline and a new butenolide isolated from lichen-derived *Streptomyces* sp. *J. Antibiot.* 63, 545–548. doi: 10.1038/ja.2010.94

Muggia, L., and Grube, M. (2018). Fungal diversity in lichens: from extremotolerance to interactions with algae. *Life* 8:15. doi: 10.3390/life8020015

Muggia, L., Klug, B., Berg, G., and Grube, M. (2013). Localization of bacteria in lichens from Alpine soil crusts by fluorescence in situ hybridization. *Appl. Soil Ecol.* 68, 20–25. doi: 10.1016/j.apsoil.2013.03.008

Mushegian, A. A., Peterson, C. N., Baker, C. C., and Pringle, A. (2011). Bacterial diversity across individual lichens. *Appl. Environ. Microbiol.* 77, 4249–4252. doi: 10.1128/aem.02850-10

Myllys, L., Stenroos, S., Thell, A., and Kuusinen, M. (2007). High cyanobiont selectivity of epiphytic lichens in old growth boreal forest of Finland. *N. Phytol.* 173, 621–629. doi: 10.1111/j.1469-8137.2006.01944.x

Narasingarao, P., Podell, S., Ugalde, J. A., Brochier-Armanet, C., Emerson, J. B., Brocks, J. J., et al. (2012). De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. *ISME J.* 6, 81–93. doi: 10.1038/ismej.2011.78

Nash, T. H. III (2008). "Nitrogen, its metabolism and potential contribution to ecosystems," in *Lichen biology*, 2nd Edn, ed. T. H. Nash III (Cambridge: Cambridge University Press), 216–233. doi: 10.1017/cbo9780511790478.012

Nash, T. H. III (ed.) (1996). *Lichen biology*. Cambridge: Cambridge University Press.

Nelson, P. E. (1992). Taxonomy and biology of *Fusarium moniliforme*. *Mycopathol.* 117, 29–36. doi: 10.1007/bf00497276

Noh, H. J., Lee, Y. M., Park, C. H., Lee, H. K., Cho, J. C., and Hong, S. G. (2020). Microbiome in *Cladonia squamosa* is vertically stratified according to microclimatic conditions. *Front. Microbiol.* 11:268. doi: 10.3389/fmicb.2020.00268

Nowotarski, S. L., Woster, P. M., and Casero, Jr. (2013). Polyamines and cancer: implications for chemotherapy and chemoprevention. *Exp. Rev. Molec. Med.* 15:3. doi: 10.1017/erm.2013.3

Nybakken, L., Asplund, J., Solhaug, K. A., and Gauslaa, Y. (2007). Forest successional stage affects the cortical secondary chemistry of three old forest lichens. *J. Chem. Ecol.* 33, 1607–1618. doi: 10.1007/s10886-007-9339-5

Öckinger, E., Niklasson, M., and Nilsson, S. G. (2005). Is local distribution of the epiphytic lichen *Lobaria pulmonaria* limited by dispersal capacity or habitat quality? *Biodiv. Conserv.* 14, 759–773. doi: 10.1007/s10531-004-4535-x

Oksanen, I., Jokela, J., Fewer, D. P., Wahlsten, M., Rikkinen, J., and Sivonen, K. (2004). Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium *Nostoc* sp. strain IO-102-I. *Appl. Environ. Microbiol.* 70, 5756–5763. doi: 10.1128/aem.70.10.5756-5763.2004

Olafsdottir, E. S., and Ingólfsdottir, K. (2001). Polysaccharides from lichens: structural characteristics and biological activity. *Planta Med.* 67, 199–208. doi: 10.1055/s-2001-12012

Palmqvist, K. (2002). "Cyanolichens: carbon metabolism," in *Cyanobacteria in symbiosis*, eds A. N. Rai, B. Bergman, and U. Rasmussen (Berlin: Springer-Verlag), 73–96. doi: 10.1007/0-306-48005-0_5

Pande, S., Merker, H., Bohl, K., Reichelt, M., Schuster, S., De Figueiredo, L. F., et al. (2014). Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria. *ISME J.* 8, 953–962. doi: 10.1038/ismej.2013.211

Pankratov, T. A. (2012). Acidobacteria in microbial communities of the bog and tundra lichens. *Microbiol.* 81, 51–58. doi: 10.1134/s0026261711060166

Pankratov, T. A., and Dedysh, S. N. (2010). *Granulicella paludicola* gen. nov., sp. nov., *Granulicella pectinivorans* sp. nov., *Granulicella aggregans* sp. nov. and *Granulicella rosea* sp. nov., acidophilic, polymer-degrading acidobacteria from *Sphagnum* peat bogs. *Int. J. Syst. Evol. Microbiol.* 60, 2951–2959. doi: 10.1099/ijs.0.021824-0

Pankratov, T. A., Grouzdev, D. S., Patutina, E. O., Kolganova, T. V., Suzina, N. E., and Berestovskaya, J. J. (2019). *Lichenibacterium ramalinae* gen. nov, sp. nov., *Lichenibacterium minor* sp. nov., the first endophytic, beta-carotene producing bacterial representatives from lichen thalli and the proposal of the new family Lichenibacteriaceae within the order Rhizobiales. *Antonie van Leeuwenhoek* 113, 477–489. doi: 10.1007/s10482-019-01357-6

Panosyan, A. K., and Nikogosyan, V. G. (1966). The presence of *Azotobacter* in lichens. *Akad. Nauk. Armenian SSR, Biol. Zhurn. Armen.* 19, 3–11.

Parks, D. H., Rinke, C., Chuvochina, M., Chaumeil, P. A., Woodcroft, B. J., Evans, P. N., et al. (2017). Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. *Nat. Microbiol.* 2, 1533–1542. doi: 10.1038/s41564-017-0012-7

Parrot, D., Antony-Babu, S., Intertaglia, L., Grube, M., Tomasi, S., and Suzuki, M. T. (2015). Littoral lichens as a novel source of potentially bioactive Actinobacteria. *Sci. Rep.* 5:15839. doi: 10.1038/srep15839

Parrot, D., Legrave, N., Delmail, D., Grube, M., Suzuki, M. T., and Tomasi, S. (2016). Review - lichen-associated bacteria as a hot spot of chemodiversity: focus on uncialamycin, a promising compound for future medicinal applications. *Planta Med.* 82, 1143–1152. doi: 10.1055/s-0042-105571

Partida-Martinez, L. P., Monajembashi, S., Greulich, K. O., and Hertweck, C. (2007). Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. *Curr. Biol.* 17, 773–777. doi: 10.1016/j.cub.2007.03.039

Paungfoo-Lonhienne, C., Rentsch, D., Robatzek, S., Webb, R. I., Sagulenko, E., Näsholm, T., et al. (2010). Turning the table: plants consume microbes as a source of nutrients. *PLoS One* 5:7. doi: 10.1371/journal.pone.0011915

Petrzik, K., Koloniuk, I., Sarkisová, T., and Ěíhal, L. (2016). Detection of herbaceous-plant pararetrovirus in lichen herbarium samples. *Acta Virol.* 60, 196–200. doi: 10.4149/av_2016_02_196

Petrzik, K., Koloniuk, I., Sehadová, H., and Sarkisova, T. (2019). Chrysoviruses inhabited symbiotic fungi of lichens. *Viruses* 11:12. doi: 10.3390/v11121120

Petrzik, K., Vondrák, J., Barták, M., Peksa, O., and Kubešová, O. (2014). Lichens-a new source or yet unknown host of herbaceous plant viruses? *Eur. J. Plant Pathol.* 138, 549–559. doi: 10.1007/s10658-013-0246-z

Petrzik, K., Vondrák, J., Kvíderová, J., and Lukavskı, J. (2015). Platinum anniversary: virus and lichen alga together more than 70 years. *PloS One* 10:3. doi: 10.1371/journal.pone.0120768

Phillips, H. C. (1969). Annual growth rates of three species of foliose lichens determined photographically. *Bull. Torrey Bot. Club* 96, 202–206. doi: 10.2307/2483333

Piovano, M., Garbarino, J. A., Giannini, F. A., Correche, E. R., Feresin, G., Tapia, A., et al. (2002). Evaluation of antifungal and antibacterial activities of aromatic metabolites from lichens. *Bol. Soc. Chi. Química* 47, 235–240.

Poelt, J. (1990). Parasitische Arten der Flechtengattung *Rhizocarpon* - eine weitere Übersicht. *Mitt. Bot. Staatssamml. München* 29, 515–538.

Printzen, C., Fernández-Mendoza, F., Muggia, L., Berg, G., and Grube, M. (2012). Alphaproteobacterial communities in geographically distant populations of the lichen *Cetraria aculeata*. *FEMS Microbiol. Ecol.* 82, 316–325. doi: 10.1111/j.1574-6941.2012.01358.x

Pucciarelli, S., Moreschini, B., Micozzi, D., De Fronzo, G. S., Carpi, F. M., Polzonetti, V., et al. (2012). Spermidine and spermine are enriched in whole blood of nona/centenarians. *Rejuv. Res.* 15, 590–595. doi: 10.1089/rej.2012.1349

Purvis, O. W. (2014). Adaptation and interaction of saxicolous crustose lichens with metals. *Bot. Stud.* 55, 1–14.

Randlane, T., Jüriado, I., Suija, A., Lõhmus, P., and Leppik, E. (2008). Lichens in the new red data list of Estonia. *Fol. Cryptog. Estonica* 44, 113–120.

Richardson, D. H. S. (2002). Reflections on lichenology: achievements over the last 40 years and challenges for the future. *Canad. J. Bot.* 80, 101–113. doi: 10.1139/b02-011

Richardson, D. H. S., Hill, D. J., and Smith, D. C. (1968). Lichen physiology: XI. The role of the alga in determining the pattern of carbohydrate movement between lichen symbionts. *N. Phytol.* 67, 469–486. doi: 10.1111/j.1469-8137.1968.tb05476.x

Romero-Olivares, A. L., Allison, S. D., and Treseder, K. K. (2017). Soil microbes and their response to experimental warming over time: a meta-analysis of field studies. *Soil Biol. Biochem.* 107, 32–40. doi: 10.1016/j.soilbio.2016.12.026

Rose, F. (1992). "Temperate forest management: its effect on bryophyte and lichen floras and habitats," in *Bryophytes and lichens in a changing environment*, eds J. W. Bates and A. M. Farmer (Oxford: Clarendon Press), 211–233.

Ruthes, A. C., Komura, D. L., Carbonero, E. R., Sassaki, G. L., Gorin, P. A., and Iacomini, M. (2010). Structural characterization of the uncommon polysaccharides obtained from *Peltigera canina* photobiont *Nostoc muscorum*. *Carbohydr. Polym.* 81, 29–34. doi: 10.1016/j.carbpol.2010.01.050

Saleem, M. (2015). "Microbiome ecosystem ecology: unseen majority in an anthropogenic ecosystem," in *Microbiome Community Ecology*, ed. S. Muhammad (Cham: Springer), 1–11. doi: 10.1007/978-3-319-11665-5_1

Saleh, A. M., Wilding, K. M., Calve, S., Bundy, B. C., and Kinzer-Ursem, T. L. (2019). Non-canonical amino acid labeling in proteomics and biotechnology. *J. Med. Biol. Eng.* 13:43. doi: 10.1186/s13036-019-0166-3

Sangwan, N., Xia, F., and Gilbert, J. A. (2016). Recovering complete and draft population genomes from metagenome datasets. *Microbiome* 4:8. doi: 10.1186/s40168-016-0154-5

Scalabrino, G., and Ferioli, M. E. (1984). Polyamines in mammalian ageing: an oncological problem, too? *A Rev. Mech. Ageing Dev.* 26, 149–164. doi: 10.1016/0047-6374(84)90090-3

Scheidegger, C. (1994). Low-temperature scanning electron microscopy: the localization of free and perturbed water and its role in the morphology of the lichen symbionts. *Cryptog. Bot.* 4, 290–290.

Scheidegger, C. (1995). Early development of transplanted Isidioid soredia of *Lobaria pulmonaria* in an endangered population. *Lichenologist* 27, 361–374. doi: 10.1017/s0024282995000478

Scheidegger, C., and Clerc, P. (2002). Rote Liste der gefährdeten Arten der Schweiz: Baum- und erdbewohnende Flechten. Bundesamt für Umwelt, Wald und Landschaft BUWAL, Bern, und Eidgenössische Forschungsanstalt WSL, Birmensdorf, und Conservatoire et Jardin botaniques de la Ville de Genève CJBG. *BUWAL-Reihe Vollzug Umwelt* 2002:124.

Scheidegger, C., and Goward, T. (2002). ""Monitoring lichens for conservation: red lists and conservation action plans,"," in *Monitoring with Lichens – Monitoring Lichens*, eds P. L. Nimis, C. Scheidegger, and P. A. Wolseley (Berlin: Springer, Kluwer Academic Publisher), 163–181. doi: 10.1007/978-94-010-0423-7_12

Scheidegger, C., Frey, B., and Walser, J. C. (1998). *"Reintroduction and augmentation of populations of the endangered Lobaria pulmonaria*: methods and concepts," in Lobarion Lichens as Indicators of Primeval Forests of Eastern Carpathians, eds S. Kondratyuk and B. Coppins. Kostrino: Darwin International Workshop, 33–52.

Scherrer, S., De Vries, O. M., Dudler, R., Wessels, J. G., and Honegger, R. (2000). Interfacial self-assembly of fungal hydrophobins of the lichen-forming ascomycetes *Xanthoria parietina* and *X. ectaneoides*. *Fung. Genet. Biol.* 30, 81–93. doi: 10.1006/fgbi.2000.1205

Schiebenhoefer, H., Van Den Bossche, T., Fuchs, S., Renard, B. Y., Muth, T., and Martens, L. (2019). Challenges and promise at the interface of metaproteomics and genomics: an overview of recent progress in metaproteogenomic data analysis. *Expert Rev. Proteomics* 16, 375–390. doi: 10.1080/14789450.2019.1609944

Schiefelbein, U., and Thell, A. (2018). Current state of *Lobaria pulmonaria* in southernmost Sweden. *Graphis Scripta* 30, 94–104.

Schneider, T., Schmid, E., de Castro, Jr., Cardinale, M., Eberl, L., Grube, M., et al. (2011). Structure and function of the symbiosis partners of the lung lichen (*Lobaria pulmonaria* L. Hoffm.) analyzed by metaproteomics. *Proteomics* 11, 2752–2756. doi: 10.1002/pmic.201000679

Seaward, M. R. D. (1998). *Lichen Atlas of the British Isles: Fascicle 3: The Foliose Physciaceae (Anaptychia, Heterodermia, Hyperphyscia, Phaeophyscia, Physcia, Physconia, Tornabea*), *Arctomia, Lobaria, Massalongia, Pseudocyphellaria, Psoroma, Solorina, Sticta, Teloschistes*. London: British Lichen Society.

Seifert, J., Taubert, M., Jehmlich, N., Schmidt, F., Völker, U., Vogt, C., et al. (2012). Protein-based stable isotope probing (protein-SIP) in functional metaproteomics. *Mass Spectr. Rev.* 31, 683–697. doi: 10.1002/mas.21346

Selbmann, L., Zucconi, L., Ruisi, S., Grube, M., Cardinale, M., and Onofri, S. (2010). Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. *Polar Biol.* 33, 71–83. doi: 10.1007/s00300-009-0686-2

Shrestha, G., and Clair, L. L. (2013). Lichens: a promising source of antibiotic and anticancer drugs. *Phytochem. Rev.* 12, 229–244. doi: 10.1007/s11101-013-9283-7

Sigurbjörnsdóttir, M. A., Heiðmarsson, S., Jónsdóttir, A. R., and Vilhelmsson, O. (2014). Novel bacteria associated with Arctic seashore lichens have potential roles in nutrient scavenging. *Can. J. Microbiol.* 60, 307–317. doi: 10.1139/cjm-2013-0888

Singh, S. B., Garrity, G. M., Genillourd, O., Lingham, R. B., Martin, I., Nallin-Omstead, M., et al. (1997). *Inhibitor compounds of farnesyl-protein transferase and chemotherapeutic compositions containing the same, produced by strain ATCC 55532*. U.S. Patent No. 5,627,057. Washington, DC: U.S. Patent and Trademark Office.

Smith, C. W., Aptroot, A., Coppins, B. J., Fletcher, A., Gilbert, O. L., James, P. W., et al. (2009). *The lichens of Great Britain and Ireland*. London: British Lichen Society.

Smith, D. C. (1980). "Mechanisms of nutrient movement between the lichen symbionts," in *Cellular Interactions in Symbiosis and Parasitism*, eds C. B. Cook, P. W. Pappas, and E. D. Rudolph (Ohio: Ohio State University Press), 197–227.

Smith, D. C., and Douglas, A. E. (1987). *The biology of symbiosis*. London: Edward Arnold Publishers.

Snäll, T., Ribeiro, Jr., and Rydin, H. (2003). Spatial occurrence and colonisations in patch-tracking metapopulations: local conditions versus dispersal. *Oikos* 103, 566–578. doi: 10.1034/j.1600-0706.2003.12551.x

Soda, K., Kano, Y., and Chiba, F. (2012). Food polyamine and cardiovascular disease- an epidemiological study. *Glob. J. Health Sci.* 4, 170–178.

Solhaug, K. A., Gauslaa, Y., Nybakken, L., and Bilger, W. (2003). UV-induction of sun-screening pigments in lichens. *N. Phytol.* 158, 91–100. doi: 10.1046/j.1469-8137.2003.00708.x

Solhaug, K. A., Larsson, P., and Gauslaa, Y. (2010). Light screening in lichen cortices can be quantified by chlorophyll fluorescence techniques for both reflecting and absorbing pigments. *Planta* 231, 1003–1011. doi: 10.1007/s00425-010-1103-3

Spribille, T. (2018). Relative symbiont input and the lichen symbiotic outcome. *Curr. Op. Pl. Biol.* 44, 57–63. doi: 10.1016/j.pbi.2018.02.007

Spribille, T., Tagirdzhanova, G., Goyette, S., Tuovinen, V., Case, R., and Zandberg, W. F. (2020). 3D biofilms: in search of the polysaccharides holding together lichen symbioses. *FEMS Microbiol. Lett.* 367:5. doi: 10.1093/femsle/fnaa023

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M. C., et al. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353, 488–492. doi: 10.1126/science.aaf8287

Stenroos, S., Stocker-Wörgötter, E., Yoshimura, I., Myllys, L., Thell, A., and Hyvönen, J. (2003). Culture experiments and DNA sequence data confirm the identity of *Lobaria* photomorphs. *Canad. J. Bot.* 81, 232–247. doi: 10.1139/b03-027

Stewart, P. S., and Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nat. Rev. Microbiol.* 6, 199–210. doi: 10.1038/nrmicro1838

Sundberg, B., Lundberg, P., Ekblad, A., and Palmqvist, K. (1999). *"In vivo* ^{13}C *NMR spectroscopy of carbon fluxes in four diverse lichens," in Physiological ecology of lichen growth, B. Sundberg,* Paper VI. Umea (Sweden): Umea University. dissertation.

Tuovinen, V., Ekman, S., Thor, G., Vanderpool, D., Spribille, T., and Johannesson, H. (2019). Two basidiomycete fungi in the cortex of wolf lichens. *Curr. Biol.* 29, 476–483. doi: 10.1016/j.cub.2018.12.022

Türk, R., and Hafellner, J. (1999). "Rote Liste gefährdeter Flechten (Lichenes) Österreichs," in *Rote Listen gefährdeter Pflanzen Österreichs. – Grüne Reihe des Bundesministeriums für Umwelt, Jugend und Familie, 2. Fassung*, ed. H. Niklfeld (Graz: Austria Medien Service), 187–228. doi: 10.1007/978-3-642-49916-6_8

Ullah, J., Khanum, Z., Khan, I. A., Khalid, A. N., Musharraf, S. G., and Ali, A. (2020). Metaproteomics reveals the structural and functional diversity of

Dermatocarpon miniatum (L.) W. Mann. *Microbiota. Fung. Biol.* 125, 32–38. doi: 10.1016/j.funbio.2020.10.001

Urayama, S. I., Kondo, F., Chiba, Y., Takaki, Y., Hirai, M., Minegishi, Y., et al. (2020). Diverged and active partitiviruses in lichen. *Front. Microbiol.* 11:2576.

Von Bergen, M., Jehmlich, N., Taubert, M., Vogt, C., Bastida, F., Herbst, F. A., et al. (2013). Insights from quantitative metaproteomics and protein-stable isotope probing into microbial ecology. *ISME J.* 7, 1877–1885. doi: 10.1038/ismej.2013.78

Walch, A., Rauser, S., Deininger, S. O., and Höfler, H. (2008). MALDI imaging mass spectrometry for direct tissue analysis: a new frontier for molecular histology. *Histochem. Cell Biol.* 130, 421–434. doi: 10.1007/s00418-008-0469-9

Walser, J. C. (2004). Molecular evidence for limited dispersal of vegetative propagules in the epiphytic lichen *Lobaria pulmonaria*. *Am. J. Bot.* 9, 1273–1276. doi: 10.3732/ajb.91.8.1273

Walser, J. C., Zoller, S., Büchler, U., and Scheidegger, C. (2001). Species-specific detection of *Lobaria pulmonaria* (lichenized ascomycete) diaspores in litter samples trapped in snow cover. *Mol. Ecol.* 10, 2129–2138. doi: 10.1046/j.1365-294x.2001.01353.x

Wang, J.-T., and Douglas, A. E. (1997). Nutrients, signals, and photosynthate release by symbiotic algae (the impact of taurine on the dinoflagellate alga *Symbiodinium* from the sea anemone *Aiptasia pulchella*). *Plant Physiol.* 114, 631–636. doi: 10.1104/pp.114.2.631

Wedin, M., Maier, S., Fernandez-Brime, S., Cronholm, B., Westberg, M., and Grube, M. (2016). Microbiome change by symbiotic invasion in lichens. *Environ. Microbiol.* 18, 1428–1439. doi: 10.1111/1462-2920.13032

Werth, S., and Scheidegger, C. (2012). Congruent genetic structure in the lichen-forming fungus *Lobaria pulmonaria* and its green-algal photobiont. *Mol. Plant-Microbe Interact.* 25, 220–230. doi: 10.1094/mpmi-03-11-0081

Werth, S., and Sork, V. L. (2010). Identity and genetic structure of the photobiont of the epiphytic lichen *Ramalina menziesii* on three oak species in southern California. *Am. J. Bot.* 97, 821–830. doi: 10.3732/ajb.0900276

Werth, S., Wagner, H. H., Gugerli, F., Holderegger, R., Csencsics, D., Kalwij, J. M., et al. (2006). Quantifying dispersal and establishment limitation in a population of an epiphytic lichen. *Ecology* 87, 2037–2046. doi: 10.1890/0012-9658(2006)87[2037:qdaeli]2.0.co;2

West, N. J., Parrot, D., Fayet, C., Grube, M., Tomasi, S., and Suzuki, M. T. (2018). Marine cyanolichens from different littoral zones are associated with distinct bacterial communities. *PeerJ.* 6:5208. doi: 10.7717/peerj.5208

Wicaksono, W. A., Cernava, T., Grube, M., and Berg, G. (2020). Assembly of bacterial genomes from the metagenomes of three lichen species. *Microbiol. Res. Announc.* 9:38. doi: 10.1128/MRA.00622-20

Williams, D. E., Davies, J., Patrick, B. O., Bottriell, H., Tarling, T., Roberge, M., et al. (2008). Cladoniamides A-G tryptophan-derived alkaloids produced in Culture by *Streptomyces uncialis*. *Org. Lett.* 10, 3501–3504. doi: 10.1021/ol801274c

Williamson, B., Tudzynski, B., Tudzynski, P., and Van Kan, J. A. (2007). *Botrytis cinerea*: the cause of grey mould disease. *Mol. Plant Pathol.* 8, 561–580. doi: 10.1111/j.1364-3703.2007.00417.x

Wirth, V. (1968). Soziologie, Standortsökologie und Areal des *Lobarion pulmonariae* im Südschwarzwald. *Bot. Jahrbücher.* 88, 317–305.

Wirth, V., Hauck, M., and Schultz, M. (2013). *Die Flechten Deutschlands*. Stuttgart: Eugen Ulmer KG, 2.

Wirth, V., Hauck, M., Brackel, W. V., Cezanne, R., De Bruyn, U., Dürhammer, O., et al. (2011). Rote Liste und Artenverzeichnis der Flechten und flechtenbewohnenden Pilze Deutschlands. *Naturschutz und Biologische Vielfalt* 70, 7–122.

Wolseley, P., and James, P. (2000). Factors affecting changes in species of *Lobaria* in sites across Britain. *For. Snow Landsc. Res.* 75, 319–338.

Wornik, S., and Grube, M. (2010). Joint dispersal does not imply maintenance of partnerships in lichen symbioses. *Microb. Ecol.* 59, 150–157. doi: 10.1007/s00248-009-9584-y

Yasuda, Y., Kaleta, J., and Brömme, D. (2005). The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. *Adv. Drug Deliv. Rev.* 57, 973–993. doi: 10.1016/j.addr.2004.12.013

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

REVIEW
published: 03 May 2021
doi: 10.3389/fmicb.2021.667864

Towards a Systems Biology Approach to Understanding the Lichen Symbiosis: Opportunities and Challenges of Implementing Network Modelling

Hadi Nazem-Bokaee[1,2,3], Erik F. Y. Hom[4], Andrew C. Warden[2], Sarah Mathews[5] and Cécile Gueidan[1]**

[1] CSIRO Australian National Herbarium, Centre for Australian National Biodiversity Research, National Research Collections Australia, NCMI, Canberra, ACT, Australia, [2] CSIRO Land and Water, Canberra, ACT, Australia, [3] CSIRO Synthetic Biology Future Science Platform, Canberra, ACT, Australia, [4] Department of Biology and Center for Biodiversity and Conservation Research, The University of Mississippi, University City, MS, United States, [5] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, United States

Lichen associations, a classic model for successful and sustainable interactions between micro-organisms, have been studied for many years. However, there are significant gaps in our understanding about how the lichen symbiosis operates at the molecular level. This review addresses opportunities for expanding current knowledge on signalling and metabolic interplays in the lichen symbiosis using the tools and approaches of systems biology, particularly network modelling. The largely unexplored nature of symbiont recognition and metabolic interdependency in lichens could benefit from applying a holistic approach to understand underlying molecular mechanisms and processes. Together with 'omics' approaches, the application of signalling and metabolic network modelling could provide predictive means to gain insights into lichen signalling and metabolic pathways. First, we review the major signalling and recognition modalities in the lichen symbioses studied to date, and then describe how modelling signalling networks could enhance our understanding of symbiont recognition, particularly leveraging omics techniques. Next, we highlight the current state of knowledge on lichen metabolism. We also discuss metabolic network modelling as a tool to simulate flux distribution in lichen metabolic pathways and to analyse the co-dependence between symbionts. This is especially important given the growing number of lichen genomes now available and improved computational tools for reconstructing such models. We highlight the benefits and possible bottlenecks for implementing different types of network models as applied to the study of lichens.

Keywords: systems biology, network modelling, signalling, metabolic model, lichen symbiosis

OPEN ACCESS

Edited by:
Enrica Pessione, University of Turin, Italy

Reviewed by:
Tomislav Cernava, Graz University of Technology, Austria
Christoph Scheidegger, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Switzerland

****Correspondence:***
Hadi Nazem-Bokaee
Hadi.Nazem-Bokaee@csiro.au
Cécile Gueidan
Cecile.Gueidan@csiro.au

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *14 February 2021*
Accepted: *09 April 2021*
Published: *03 May 2021*

Citation:
Nazem-Bokaee H, Hom EFY, Warden AC, Mathews S and Gueidan C (2021) Towards a Systems Biology Approach to Understanding the Lichen Symbiosis: Opportunities and Challenges of Implementing Network Modelling. Front. Microbiol. 12:667864. doi: 10.3389/fmicb.2021.667864

INTRODUCTION

Lichens are often seen as a typical example of successful and sustainable symbiotic interactions between micro-organisms (Ahmadjian, 1993; Honegger, 1998). With the long evolutionary history of these fungal-algal associations (Gueidan et al., 2011; Prieto and Wedin, 2013; Lutzoni et al., 2018; Nelsen et al., 2019) and their multiple origins within the evolution of fungi (Gueidan et al., 2008; Schoch et al., 2009; Nelsen et al., 2020), lichens have colonised and diversified greatly in most terrestrial and some aquatic environments, including the most inhospitable niches (Kappen, 2000; Sadowsky and Ott, 2016). They are a discrete but inherent part of most of our landscapes, including both natural and man-made. This success stems from their ability to act as self-sustainable ecosystems, for which an evolutionary modularity (i.e., selection of the most fitted partners for a particular environment) has allowed adaptation to a broad range of habitats. Because of their slow growth, they particularly excel in colonising harsh habitats in which competition with faster growing micro-organisms is low. As such, they have adapted to surviving on nutrient-poor substrates and under drastically fluctuating environmental conditions, and play key roles in their ecosystems. In the future, lichen adaptations and their natural ecological flexibility may prove to be key to the successful responses of lichens to climate change.

The lichen symbiosis is no longer perceived to be the simple union of a fungal partner (i.e., mycobiont) and a microalgal partner (i.e., photobiont), either an alga (i.e., chlorolichen) or a cyanobacterium (i.e., cyanolichen). Instead, previous studies have shown that lichens harbour a diverse microbiome (e.g., Petrini et al., 1990; Hofstetter et al., 2007; Grube et al., 2009; Hodkinson and Lutzoni, 2009), and more recent studies corroborate lichens as multi-symbioses, i.e., complex multi-species associations including bacteria and other fungi or algae (Spribille et al., 2016; Onut-Brannstrom et al., 2018; Tuovinen et al., 2019; Smith et al., 2020; Leiva et al., 2021). In such symbioses, each partner contributes to the association: the primary mycobiont provides shelter and minerals to the photobiont, while the photobiont provides organic carbon fixed from atmospheric CO_2 *via* photosynthesis (Nash, 2008a) as well as nitrogen if it is a cyanobacteria. Additional bacteria, algae, and/or fungi have also been shown to serve certain functions in the lichen symbiosis (Cernava et al., 2017; Smith et al., 2020; Tagirdzhanova et al., 2021), although much more remains to be explored. Additionally, the levels of dependence and specificity of some of these microbes to the symbiosis are still debated (Grube et al., 2015; Kono et al., 2017; Jenkins and Richards, 2019; Lendemer et al., 2019; Smith et al., 2020). Lichens demonstrate unique physiological properties and ecosystem functions (Porada et al., 2014). All lichens contribute to atmospheric carbon fixation, with global net carbon uptake by both lichens and bryophytes predicted to be 0.34–3.3 Gt carbon per year (Palmqvist, 1995; Green et al., 2008; Palmqvist et al., 2008; Porada et al., 2013). Cyanolichens are capable of both carbon and nitrogen fixation (Dahlman et al., 2004; Nash, 2008b; Porada et al., 2017). Lichens grow on various substrates (including rocks, trees, and soil), can survive extreme temperatures, tolerate desiccation (poikilohydric) and high levels of UV radiation, and form morphologically diverse structures (Beckett et al., 2008; Kranner et al., 2008). Many lichens produce unique specialised/secondary metabolites, including depsides, xanthones and dibenzofurans, some of which have been shown to have medicinal properties (Fahselt, 1994; Elix and Stocker-Worgotter, 2008; Calcott et al., 2018).

The establishment of the lichen symbiosis, or "lichenisation," has been described as a four-stage process (Ahmadjian et al., 1978): (A) a pre-contact phase (chemical interactions between symbionts but no physical contact), (B) a post-contact phase (with chemical and physical interactions), (C) a phase of growth characterised by an un-differentiated mass, and (D) a phase of differentiation that leads to a stratified thallus (**Figure 1**). Because mycobionts grow relatively slowly, the application of classical experimental microbiology techniques and co-culture/resynthesis experiments to the understanding of the development and functioning of the lichen symbiosis has lagged. Despite some recent studies focusing on early stages of lichenisation (Joneson et al., 2011; Armaleo et al., 2019; Kono et al., 2020), the molecular basis of fungal-algal interactions during lichenisation remains mostly uncharacterised, and processes involved in signalling and metabolic interplays between the symbionts are poorly understood. Contemporary systems biology approaches may facilitate tackling long-standing questions about the lichen symbiosis.

Systems biology is the study of living systems through the joint application of advanced high-data-volume generating technologies (e.g., 'omics') and computational tools (e.g., multi-scale or constraint-based modelling) to gain a more holistic understanding of the inter-dependencies of system components and underlying system complexity. Hypotheses are generally tested using iterative cycles of 'wet' (lab-based) and 'dry' (simulation-based) experiments, by which systems-level data are generated, analysed, and then used to inspire new insights and hypotheses about the biological system at hand (Kitano, 2002a,b). For instance, applying systems- and genome-level approaches to the legume-rhizobium symbiosis has greatly enhanced the knowledge on the underlying mechanisms of symbiotic interactions at molecular level, moving us one step closer to improving agricultural crop yields through the development of more efficient symbiotic N_2 fixation processes (diCenzo et al., 2019). A similar systems biology approach has not yet been applied to the study of the lichen symbiosis.

In this review, we summarise the current knowledgebase of signalling and recognition mechanisms in the lichen symbiosis. We then discuss the modelling of signalling networks as a tool to extend our understanding of such mechanisms in lichens. We review the literature on lichen metabolism and propose that modelling fluxes in metabolic networks could be a powerful tool for providing insights into lichen metabolism in particular, and the metabolic interplays between symbiotic partners in general. We provide a broad overview of metabolic network models and their applications in addition to a review of some of the symbiotic systems that have been studied through the lens of metabolic

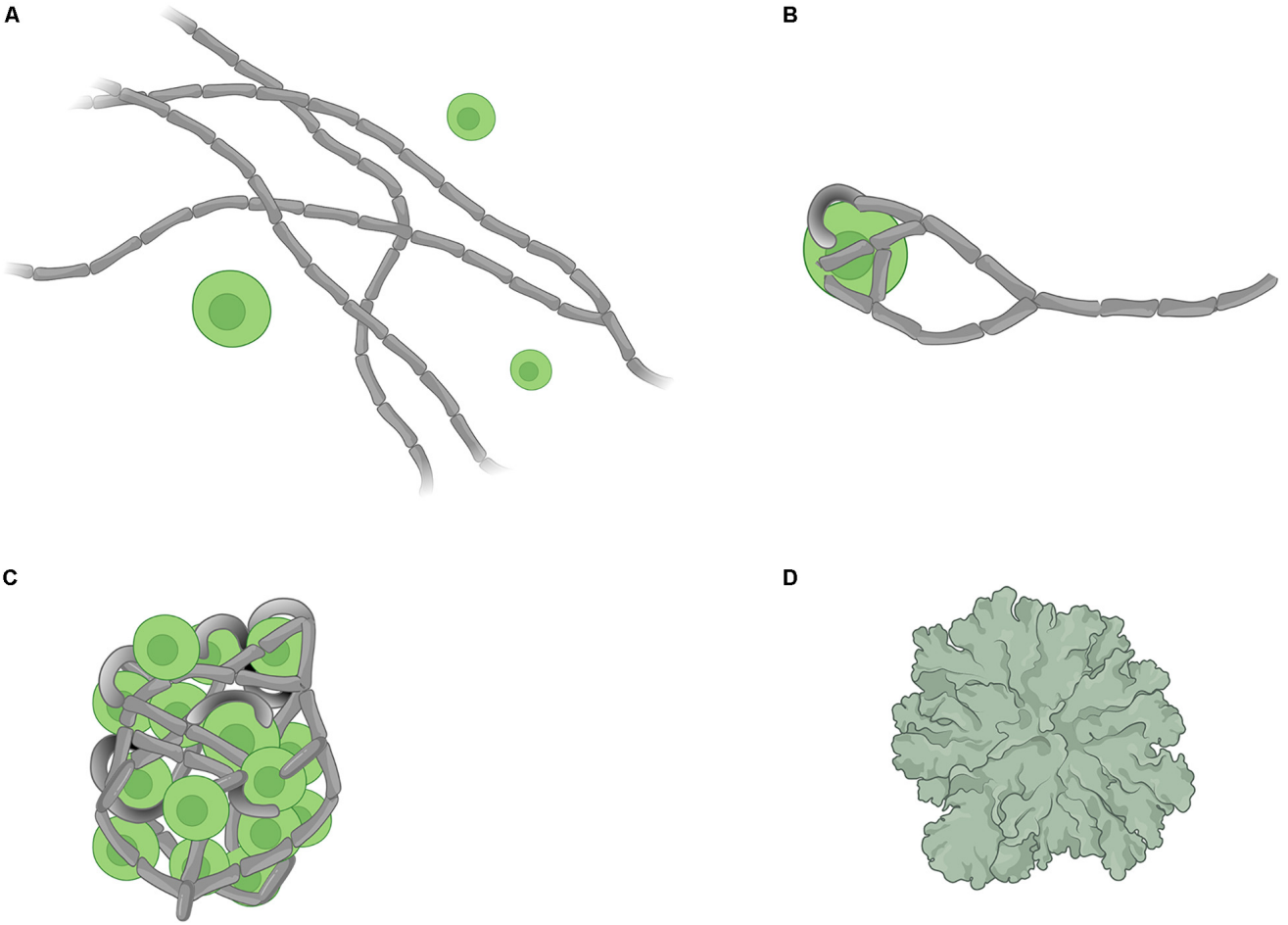


FIGURE 1 | Schematic representation of the four stages of lichen formation. **(A)** pre-contact where the symbionts are located at the proximity of each other but not in physical contact, **(B)** post-contact which marks the initiation of physical contacts between symbionts, **(C)** growth of an undifferentiated mass consisting of fungal hyphae and algal cells, and **(D)** formation of a differentiated thallus. (Image created with BioRender.com).

models. Finally, the opportunities and challenges of modelling both signalling networks and metabolic fluxes are discussed.

SIGNALLING AND RECOGNITION PATHWAYS IN THE LICHEN SYMBIOSIS

Distinct small molecules are produced by lichen symbionts during symbiosis that are absent when mycobiont and photobiont are grown separately (Green and Smith, 1974; Elshobary et al., 2015). Whether symbiont signalling and recognition processes in lichens are driven initially by those small molecules, or whether recognition processes are initiated by other regulatory mechanisms is not known. The available data for molecules with potential roles in signalling and/or recognition mechanisms during lichen symbiosis are summarised in **Table 1**. So far, there is no direct evidence confirming the production of compounds with a potential role in signalling and/or recognition during lichenisation by inhabiting fungi or bacteria. Several studies have shown that signalling between lichen symbionts can be initiated as early as the pre-contact stage of lichenisation (Joneson et al., 2011; Meessen and Ott, 2013; Piercey-Normore and Athukorala, 2017; Armaleo et al., 2019). At present and for a few reasons, it is difficult to propose universal signalling models that initiate lichen symbiosis. Firstly, there is no single signalling molecule with a known or proposed role that has been studied across different lichens. Secondly, signalling pathways of those molecules with putative recognition roles have not been elucidated. Thirdly, lichens have likely evolved independently in several fungal lineages (Gueidan et al., 2008; Schoch et al., 2009), suggesting that the nature of these signalling pathways might differ depending on the species of interest. Nonetheless, owing to advances in genetic and analytical tools, several studies have begun to uncover mechanistic details underlying partner signalling and recognition at various stages of lichenisation (Meessen et al., 2013; Meessen and Ott, 2013; Athukorala et al., 2014; Athukorala and Piercey-Normore, 2015).

Lectin-Ligand Signalling in Lichens

Lectins are glycoproteins that occur ubiquitously across all domains of life (Kennedy et al., 1995). Lectins have also been isolated and characterised from both chlorolichens and

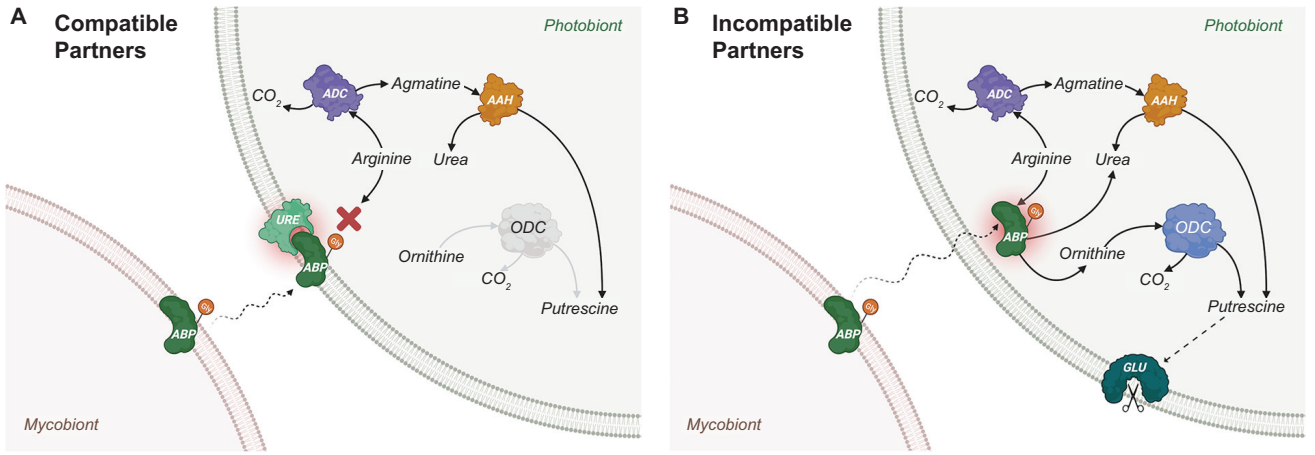


FIGURE 2 | Proposed lectin-ligand recognition and signalling for *Xanthoria parietina.* **(A)** For compatible partners. The process starts by the production and positioning of algal binding protein (ABP) on the cell wall of the mycobiont. ABP is a lectin ("receptor") which is glycosylated and secreted into the intercellular space (Molina et al., 1993; Molina and Vicente, 1995) to bind specific cognate ligands of a photobiont, with which a potential lichenisation could be established. It is speculated that ABP is part of a group of "recognition lectins" that is responsible for engaging the mycobiont with a specific photobiont, and another group of "recruitment lectins" that play a role in recruiting the photobiont cells to mycobiont hyphae (Sacristán et al., 2007). In the lichen *X. parietina*, a recruitment lectin was identified as a secreted arginase of the *Xanthoria* mycobiont (SAX). Although both ABP (recognition) and SAX (recruitment) mycobiont lectins were shown to bind to the ligand URE, a glycosylated urease located on the compatible photobiont cell wall (Millanes et al., 2004), it is not clear whether both must attach to urease for lichenisation to proceed. Both ABP and SAX lectins possess identical peptide sequences and Mn^{2+}-dependent arginase activity, hydrolysing arginine to produce urea and ornithine (Legaz et al., 2004). However, the glycosidic moiety of SAX contains galactose and glucose, whereas that of ABP contains glucose and N-acetyl-glucosamine (Molina et al., 1993). Upon binding, enzymatic activities of ABP (recognition lectin and receptor) and URE (ligand) are inhibited. As a result, putrescine concentrations in a compatible photobiont are kept at relatively low endogenous levels because it is only produced through arginine decarboxylase (ADC) and agmatine amidinohydrolase (AAH). **(B)** For incompatible partners. The binding selectivity of ABP (receptor on mycobiont) for URE (ligand on photobiont) is the key in compatible partner recognition. For example, the ABP lectin receptor of *E. prunastri* can only bind to a single galactosylated ligand (Diaz et al., 2016c). Thus, an incompatible photobiont lacking galactosylated URE would not be recognised, resulting in an internalisation of ABP by the photobiont. Without URE ligand-induced inhibition, ABP goes on to hydrolyse cytoplasmic arginine stores in the photobiont (ABP has a higher affinity for arginine than that of ADC), producing urea and ornithine and a reaction cascade (involving both ornithine decarboxylase (ODC) and AAH) that results in increased cytoplasmic putrescine levels. High intracellular putrescine concentrations lead to the activation of glucanase (GLU), which results in cell wall disruptions and, ultimately, death of the incompatible photobiont cells (Molina et al., 1998). (Image created with BioRender.com).

cyanolichens (**Table 1**). Possessing versatile carbohydrate-binding site(s), lectins can act as receptors and/or bind/agglutinate cells that may facilitate further interfacial communication between cells. The glycosidic moieties of lectins synthesised by the mycobiont may contain various combinations of carbohydrate groups that bind to specific ligands from the photobiont. In this atypical receptor-ligand system, lectins from mycobionts act as receptors for photobiont-attached ligands. A proposed mechanism of photobiont recognition and recruitment by the mycobiont is illustrated in **Figure 2**, based on the extensive studies of the lichens *Xanthoria parietina* and *Evernia prunastri* (Bubrick and Galun, 1980; Bubrick et al., 1981; Perezurria and Vicente, 1989; Vicente and Perezurria, 1989; Rodriguez and Vicente, 1991; Molina et al., 1993, 1998; Molina and Vicente, 1995, 2000; Legaz et al., 2004; Millanes et al., 2004). Lectins characterised from other lichens also have been proposed to have roles in the establishment and/or maintenance of compatible symbiotic relationships (**Table 1**).

In several lichen associations (including *X. parietina* and *E. prunastri* shown in **Figure 2**), the ligand for lectin receptors has been identified as urease, which is bound to the cell wall of the photobiont (Molina et al., 1993; Millanes et al., 2004; Diaz et al., 2009). In the lichen *Cladonia rangiferina*, a urease-like recognition-related protein (RR1) was characterised and speculated to act as a ligand on the cell wall of the compatible photobiont of this lichen association (Athukorala et al., 2014; Athukorala and Piercey-Normore, 2015). Urease is produced by several lichens (presumably by the photobiont) and is secreted into the culture medium under laboratory conditions (Perezurria et al., 1989, 1993). The secretion of urease into the medium is hypothesised to be the consequence of its transfer from the photobiont to the mycobiont, depending on the nitrogen content of the mycobiont as well as the water content of the lichen thallus (Perezurria et al., 1989). However, it is not clear, whether the secreted ureases play a role similar to that of membrane-bound urease.

The lectin recognition and signalling mechanism summarised for chlorolichens in **Figure 2** can be true of all or some cyanolichens (Sacristán et al., 2007; Vivas et al., 2010; Díaz et al., 2016a). Diaz et al. (2015), Diaz et al. (2016b) showed that actin- and myosin-like proteins produced by the cyanobacterial photobiont *Nostoc* of the lichen *Peltigera canina* is involved in the chemotactic movement of photobiont cells towards the lectin of the mycobiont. The process also involves a contractile protein and ATPase of photobiont, which creates a series of contraction-relaxation steps that result in photobiont

TABLE 1 | Molecules produced by different lichen symbionts with proposed roles in symbiotic signalling and recognition.

Molecule	Chemical class	Proposed role	Mycobiont	Photobiont°	References
Produced by the mycobiont					
Algal binding protein (ABP)	Glycoprotein	Plays a role in recognition of photobiont ligand	*Xanthoria parietina*[1]	*Trebouxia sp.?*	Molina et al., 1993; Molina and Vicente, 2000
Cyanobacterium-binding protein (CBP)	Possibly a glycoprotein	Plays a role in the first step of the recognition of compatible symbionts in a cyanolichen	*Peltigera canina*[2]	*Nostoc sp.*	Diaz et al., 2009
			Scytinium palmatum[4]	*Nostoc sp.*	Vivas et al., 2010
Galectin LEC-1 and LEC-2	Glycan-binding proteins	Plays a role in recognition of photobiont ligand	*Peltigera membranacea*[2]	*Nostoc sp.*	Manoharan et al., 2012; Miao et al., 2012
Nephroma laevigatum agglutinin (NLA)	Possibly a glycoprotein	Functions as a determinant of specificity at the initial stage of symbiont interaction	*Nephroma laevigatum*[3]	*Nostoc sp.*	Kardish et al., 1991
Peltigera membranacea agglutinin (PMA)	Glycoprotein	Functions in the recognition process between symbionts	*Peltigera membranacea*[2]	*Nostoc sp.*	Lehr et al., 1995
Phytohemagglutinins	Glycoprotein	May be involved in the initial stages of the symbiosis establishment	*Peltigera canina*[2]	*Nostoc sp.*	Lockhart et al., 1978
			Peltigera polydactyla[2]	*Nostoc sp.*	Lockhart et al., 1978
Phytolectin	Glycoprotein	May be involved in the recognition or initial interactions between compatible lichen symbionts	*Peltigera horizontalis*[2]	*Nostoc sp.*	Petit, 1982
			Peltigera canina var. *canina*[2]	*Nostoc sp.*	Petit et al., 1983
Secreted arginase of *Evernia* (SAE)	Hydrolytic enzyme	Plays a role in recognition of photobiont ligand (e.g., urease)	*Evernia prunastri*[5]	*Trebouxia excentrica*	Legaz et al., 2004
Secreted arginase of *Xanthoria* (SAX)			*Xanthoria parietina*[1]	*Trebouxia sp.?*	Molina et al., 1993; Molina and Vicente, 2000
			Xanthoria parietina[1]	*Pseudotrebouxia aggregata*	Legaz et al., 2004
Xanthoria-protein	Glycoprotein	May have role in initiation of lichen resynthesis and discriminate between photobionts	*Xanthoria parietina*[1]	*Trebouxia sp.*	Bubrick and Galun, 1980; Bubrick et al., 1981
			Variospora aurantia[1]	*Pseudotrebouxia sp.*	Bubrick and Galun, 1980
			Flavoplaca citrina[1]	*Pseudotrebouxia sp.*	Bubrick and Galun, 1980
Produced by the photobiont					
Chitinase	Hydrolytic enzyme	Regulates controlled parasitism between the symbionts	*Cladonia rangiferina*[6]	*Asterochloris sp.*	Athukorala and Piercey-Normore, 2015
Cyclo-L-leucyl-L-tyrosyl (CLT)	Cyclic dipeptide*	Not known	*Romjularia lurida*[8]	*Asterochloris sp.*	Meessen et al., 2013
Cyclo-L-tryptophyl-L-tryptophyl (CTT)	Cyclic dipeptide*	Promotes the germination rate of mycobiont *in vitro* after 30 days	*Gyalolechia bracteata* [1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Gyalolechia fulgens* [1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Thalloidima sedifolium* [7]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Xanthoria elegans*[1]	*Trebouxia sp.*	Meessen et al., 2013
Indole-3-carbaldehyde (ICA)	Phytohormone precursor	Decreases the germination rate of mycobiont *in vitro*	*Gyalolechia bracteata* [1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Gyalolechia fulgens* [1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Thalloidima sedifolium* [7]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
		Not known	*Xanthoria elegans*[1]	*Trebouxia sp.*	Meessen et al., 2013
Rhamnose	Deoxy sugar	Decreases the germination rate of mycobiont *in vitro*	*Gyalolechia bracteata*[1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013
Ribitol	Sugar alcohol	Acts as pre-/post-contact signal molecule	*Cladonia grayi*[6]	*Asterochloris sp.*	Joneson et al., 2011
		Overcomes the growth arrest of the mycobiont and promotes mycelium growth[#]	*Gyalolechia bracteata* [1]	*Trebouxia sp.*, Cl.1, sbgr.1	Meessen et al., 2013

(Continued)

TABLE 1 | Continued

Molecule	Chemical class	Proposed role	Mycobiont	Photobiont°	References
Urease	Hydrolytic enzyme	Serves as a ligand for different lichen lectins	*Xanthoria parietina*[1]	*Pseudotrebouxia aggregata*	Millanes et al., 2004
			Evernia prunastri[1]	*Trebouxia excentrica*	Millanes et al., 2004
Produced by the symbiosis as a whole (The experiment settings did not allow to attribute the compound to either the mycobiont or the photobiont)					
1-aminocyclopropane-l-carboxylic acid (ACC)	Phytohormone precursor	Affects differentiation and regulates interactions in the lichen thallus	*Cladonia rangiferina*[6]	Unidentified	Ott et al., 2000
		Not known	*Usnea longissima*[5]	Unidentified	Ott et al., 2000
		Not known	*Parmelia saxatilis*[5]	Unidentified	Ott et al., 2000
		Not known	*Usnea sphacelata*[5]	Unidentified	Ott et al., 2000
		Not known	*Peltigera polydactyla*[2]	Unidentified	Ott et al., 2000
		Not known	*Peltigera canina*[2]	Unidentified	Ott et al., 2000
		Not known	*Nephroma resupinatum*[3]	Unidentified	Ott et al., 2000
		Not known	*Scytinium palmatum*[4]	*Nostoc sp.*	Vivas et al., 2010
Abscisic acid (ABA)	Phytohormone	Affects differentiation and regulates interactions in the lichen thallus	*Cladonia rangiferina*[6]	Unidentified	Ott et al., 2000
		Not known	*Cladonia arbuscula*[6]	Unidentified	Ott et al., 2000
		Not known	*Cetraria islandica*[5]	Unidentified	Ott et al., 2000
Indole-3-acetic acid (IAA)	Phytohormone	Affects differentiation and regulates interactions in the lichen thallus	*Cladonia rangiferina*[6]	Unidentified	Ott et al., 2000
		Not known	*Peltigera hymenina*[2]	Unidentified	Ott et al., 2000
		Not known	*Cetraria islandica*[5]	Unidentified	Ott et al., 2000
		Not known	*Cladonia arbuscula*[6]	Unidentified	Ott et al., 2000
		Not known	*Ramalina duriaei*[7]	*Trebouxia sp.*	Epstein et al., 1986

°*Trebouxia, Pseudotrebouxia, and Asterochloris are eukaryotic algae (Chlorophyta, Trebouxiophyceae) and Nostoc is a prokaryotic alga (cyanobacteria).*
Fungal lineages are as follows: [1] Ascomycota, Lecanoromycetes, Teloschistales, Teloschistaceae; [2] Ascomycota, Lecanoromycetes, Peltigerales, Peltigeraceae; [3] Ascomycota, Lecanoromycetes, Peltigerales, Nephromataceae; [4] Ascomycota, Lecanoromycetes, Peltigerales, Collemataceae; [5] Ascomycota, Lecanoromycetes, Lecanorales, Parmeliaceae; [6] Ascomycota, Lecanoromycetes, Lecanorales, Cladoniaceae; [7] Ascomycota, Lecanoromycetes, Lecanorales, Ramalinaceae; [8] Ascomycota, Lecanoromycetes, Lecideales, Lecideaceae.
#*Ribitol in these pre-contact experiments (mycobiont and photobiont separated by a membrane) was added in concentrations of 0.05, 0.8, and 2.0%w/v to the culture media (water agar and malt yeast agar). Ribitol was not identified as photobiont exudate in these experiments, as seen in other studies (Richardson et al., 1968).*
**Belong to the class of diketopiperazines (DKPs) with potential applications as antitumor, antiviral, antifungal, and antibacterial properties.*

movement towards mycobiont lectin (Diaz et al., 2011). Upon cell contact of photobiont and mycobiont, desensitisation occurs and photobiont contractile motility stops. It is yet unknown if a similar type of chemotaxis applies to chlorolichens.

It is speculated that mycobionts of some lichens not only rely on lectin-ligand recognition mechanisms for establishing the initial photobiont contact, but that these mechanisms might also be involved in further replication and growth of young photobiont cells within the lichen thallus (Díaz et al., 2016a). The factors triggering the initiation of symbiont recognition through lectin-ligand binding and the ways in which symbionts increase the probability of association have been poorly understood, although several hypotheses have been proposed (Díaz et al., 2016a). For example, the photobiont could secrete a yet unknown diffusible compound that is sensed by a compatible mycobiont to trigger mycobiont lectin biosynthesis. Mycobionts may also produce multiple lectins with competing specificities for different photobionts, which may also be a strategy for rejecting incompatible photobionts. We could test some of these hypotheses using a systems biology approach, for example, through time-course analysis of coupled gene expression and metabolome profiles of lichen co- and mono-cultures to identify candidate genes and molecules with potential signalling roles. Armaleo et al. (2019) recently pursued a transcriptome study exploring the differential expression of genes involved in symbiosis and signalling between *Cladonia grayi* and its algal partner *Asterochloris glomerata*. While only a snapshot in time, this work provided unprecedented insights into the complexity of responses underlying lichen symbioses.

Exudates Signalling in Lichens

Carbohydrate release and translocation from photobiont to mycobiont of a lichen was first proposed in the mid-1960s by Drew and Smith, who used radioactive isotope tracing to estimate the proportion of labelled carbon in sodium [^{14}C]-bicarbonate fixed to [^{14}C]-glucose by the cyanobacterial symbiont (*Nostoc*) of *Peltigera polydactyla* compared with its free-living and cultured forms (Drew and Smith, 1967a,b). Carbohydrate movement from photobiont to mycobiont has been investigated for more than 30 additional lichens and is reviewed elsewhere (Smith et al., 1969). The results of these studies showed that glucose and

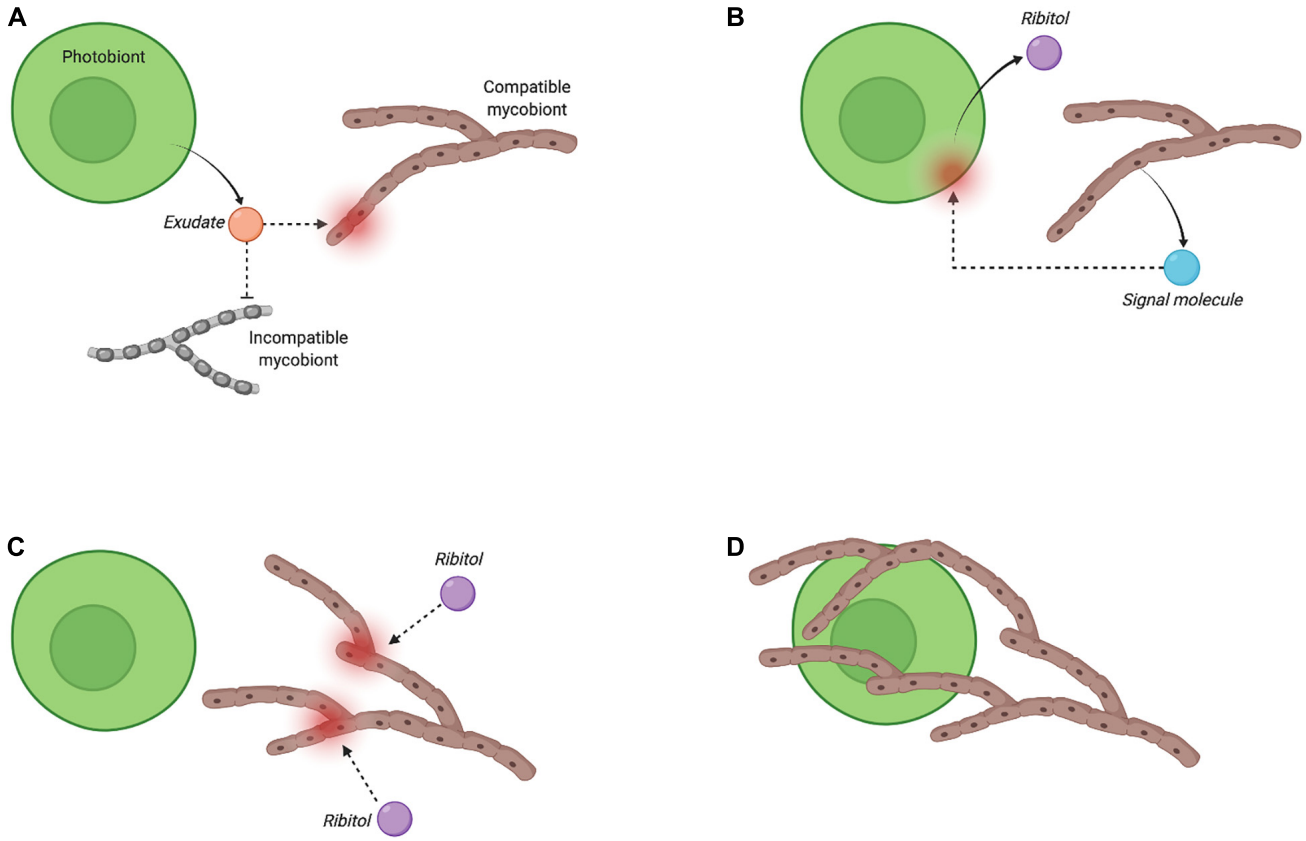


FIGURE 3 | Proposed exudate-based recognition and signalling for *Fulgensia bracteata* (adapted from Meessen et al., 2013). The bi-directional and multi-step recognition process (i.e., pre-contact stage) is initiated by a compatible photobiont through secretion of an exudate, which is speculated to be species-dependent but has yet to be identified for most lichen species **(A)**. The exudate induces the production and secretion by the mycobiont of a yet unknown signalling molecule that stimulates ribitol release by the photobiont **(B)**. Ribitol then triggers fungal growth by relieving hyphal growth arrest **(C)**, and allowing hyphal branching, and the engulfment of the photobiont cell by the mycobiont **(D)**. The two last steps may be accompanied by the secretion of a mucilage in some lichens (Meessen et al., 2013; Meessen and Ott, 2013). (Image created with BioRender.com).

sugar alcohols are the main forms of carbohydrates released by cyanobacterial and microalgal photobionts, respectively, and that they are translocated to the respective mycobionts. In the absence of a symbiotic relationship, the levels of carbohydrate released by the photobionts decline significantly or drop to zero. Following these initial studies, the importance of carbohydrate release by lichen photobionts gained a renewed interest in efforts to uncover the molecular mechanisms behind the early stage of lichenisation (Joneson et al., 2011; Meessen et al., 2013; Meessen and Ott, 2013; Athukorala et al., 2014; Athukorala and Piercey-Normore, 2014; Armaleo et al., 2019). A possible exudate signalling model based on the release and movement of ribitol is shown in **Figure 3**, and is largely based on independent studies observing ribitol release in the cultures of *Gyalolechia bracteata* (Meessen et al., 2013; Meessen and Ott, 2013) and *Cladonia grayi* (Joneson et al., 2011). Although the exact nature of the secreted molecules in this exudate-signalling model has not been fully elucidated, it is speculated that an exchange of carbon and nitrogen could be the driver for uniting symbionts in the first place. Hom and Murray (Hom and Murray, 2014) showed that co-culturing of model fungi *Saccharomyces cerevisiae, Aspergillus nidulans, or Neurospora crassa* with the alga *Chlamydomonas reinhardtii* could facilitate mutualistic interactions through exchanging carbon and nitrogen under specific growth conditions; their results also suggest that carbon released by mycobiont respiration (as CO_2) could be recaptured by the photobiont for efficient carbon recycling within the lichen symbiosis (Schwartzman, 2010). Thus, the need for nutrient exchange between species could trigger the initiation of symbiotic interaction in lichens. Signalling network modelling, discussed in the following section, is one approach to generate insights on how specific exudate compounds could play a role in the overall flow of signals through the proposed 'exudates signalling' mechanism.

Signalling Network Modelling: Challenges and Opportunities for the Lichen Symbiosis

A signalling network consists of a series of 'signals' and 'receptors' whose relationships are determined by the signal transduction mechanisms governing the network. These signals and receptors

could be any or combination of enzymes (e.g., kinases), organic substances (e.g., ATP), inorganic molecules (e.g., phosphates), or other proteins or biomolecules. Reactions connecting these molecules frame the underlying signalling mechanisms and the goal of signalling network modelling would be to predict such interactions and the emergent cascade of signalling events that can explain or predict the behaviour of the signalling network. Signalling network models are often divided into descriptive and predictive subtypes. Descriptive models are usually simpler and provide a qualitative overview of the signalling pathway structure (i.e., topology of signal molecules and reactions), whereas predictive models may capture kinetics of the signalling pathway (i.e., reaction rates) and be capable of estimating system behaviours under new perturbations. The application of diverse descriptive and predictive modelling to signalling networks has been reviewed elsewhere (Hyduke and Palsson, 2010; Morris et al., 2010; Terfve and Saez-Rodriguez, 2012; Rother et al., 2013; Lavrik and Samsonova, 2016; Antebi et al., 2017). The scope and choice of signalling network modelling approach vary with the complexity of the network being explored. For example, some of the most detailed and comprehensive predictive signalling models have been developed for complex but known signalling networks of human B-cells (Papin and Palsson, 2004), prostate cancer cells (Dasika et al., 2006; Vardi et al., 2012), and Toll-like receptors (TLRs) functioning in immune system (Li et al., 2009).

In symbiotic systems, signalling pathways have been a topic of particular focus for legumes-rhizobia and plants-root fungi (mycorrhiza) symbioses (Bonfante and Genre, 2010; Bonfante and Requena, 2011; Oldroyd, 2013; Venkateshwaran et al., 2013; Mohanta and Bae, 2015; Martin et al., 2017; Poole et al., 2018; Clear and Hom, 2019). However, modelling the signalling networks in these systems has not received much attention, perhaps due largely to the knowledge gap in certain key signalling steps. For example, in the common symbiotic signalling "SYM" pathway, which shares similar signalling steps between arbuscular mycorrhizal and rhizobial symbioses, it remains unclear how symbiosis receptor kinases (SYMRK) transmit signals to downstream cation channelling proteins (i.e., CASTOR/POLLUX) located in the nucleus (Huisman and Geurts, 2020). Moreover, the precise mechanisms for how plants discriminate between arbuscular mycorrhiza and rhizobia symbionts are still unknown, although signalling pathways functioning in parallel to the SYM seem likely to be involved. Modelling signalling networks could represent a complementary approach to fill such gaps by simulating system behaviours with proposed/candidate mechanisms implemented by which symbionts transduce signals and communicate.

Faced with the paucity of detailed mechanistic knowledge on signalling networks in lichens (despite several potential signal molecules identified; see **Table 1**), the modelling of signalling networks in lichens suffers from similar challenges as those of other symbiotic systems and no models have yet been reported. Nevertheless, given the recent availability of 'omics' data for a variety of lichens (Mittermeier et al., 2015; Wang et al., 2015; Armaleo et al., 2019), there are now new opportunities to develop signalling models of lichens. For instance, a proteomics approach could enable measuring lectin and urease levels of lichen cultures at pre- and post-contact stages informing the relative abundances of these proteins. The proteome profile of such cultures could also indicate the presence/absence of other specific proteins at the corresponding stages of lichenisation that may correlate with lectin/urease activity levels and provide deeper insights into how the recognition process initiates. A signalling pathway model could be developed to explore the link between putrescine biosynthesis and lectin production in repression of cell wall disruption of compatible photobiont as described in **Figure 3**.

METABOLIC INTERPLAY IN THE LICHEN SYMBIOSIS

The literature on lichen metabolism has been largely focused on understanding the exchange of key nutrients between symbionts (Lines et al., 1989; Kono et al., 2020; ten Veldhuis et al., 2020) and identifying lichen secondary metabolites and their biosynthetic pathways (i.e., metabolite profiling) (Fahselt, 1994; Aubert et al., 2007; Elix and Stocker-Worgotter, 2008; Mittermeier et al., 2015; Bertrand et al., 2018b; Brakni et al., 2018; Calcott et al., 2018; Kuhn et al., 2019; Goga et al., 2020; **Figure 4**). In the 1960s, observations of carbohydrate storage and translocation between the symbionts of *Peltigera polydactyla* (Smith and Drew, 1965; Drew and Smith, 1967a,b) together with a series of similar studies on other lichens (Smith et al., 1969) established the foundations for studying the metabolic interplay in lichens. The primary aim of those studies was to identify the form of carbon translocated between lichen symbionts, as explained in the previous sections. Next to nothing is known about the metabolic program and gene expression in lichen symbionts following carbohydrate exchange and assimilation. Most metabolic studies in lichens have concentrated on understanding the overall carbon and nitrogen economy in lichens, mainly with respect to overall carbon fixation, carbon sinks, lichen growth, and nitrogen fixation by cyanolichens (Honegger et al., 1993; Dahlman et al., 2004; Nash, 2008b; Palmqvist et al., 2008). Eisenreich and colleagues (Eisenreich et al., 2011) suggested that using 'omics' methods together with isotope labelling experiments (increasingly referred to as 'fluxomics') could enhance our understanding of lichen metabolic pathways, although this has yet to be fully realised to study lichen metabolism at a systems-level.

Thus, despite of being broadly recognised that carbohydrates and inorganic molecules are exchanged between lichen symbionts, a systems-level molecular understanding of metabolism is still lacking for lichens, including their primary symbionts and auxiliary partners. This lack has left key features of metabolism unexplored, including, for example, central aspects of carbon metabolism with respect to lichen compartmentalisation or the role of cell wall components and biosynthesis on the growth and metabolite exchange between symbionts. A systems-level understanding of lichen metabolism will become more likely in near future in light of the recent insights on lichen microbiota composition and functions within

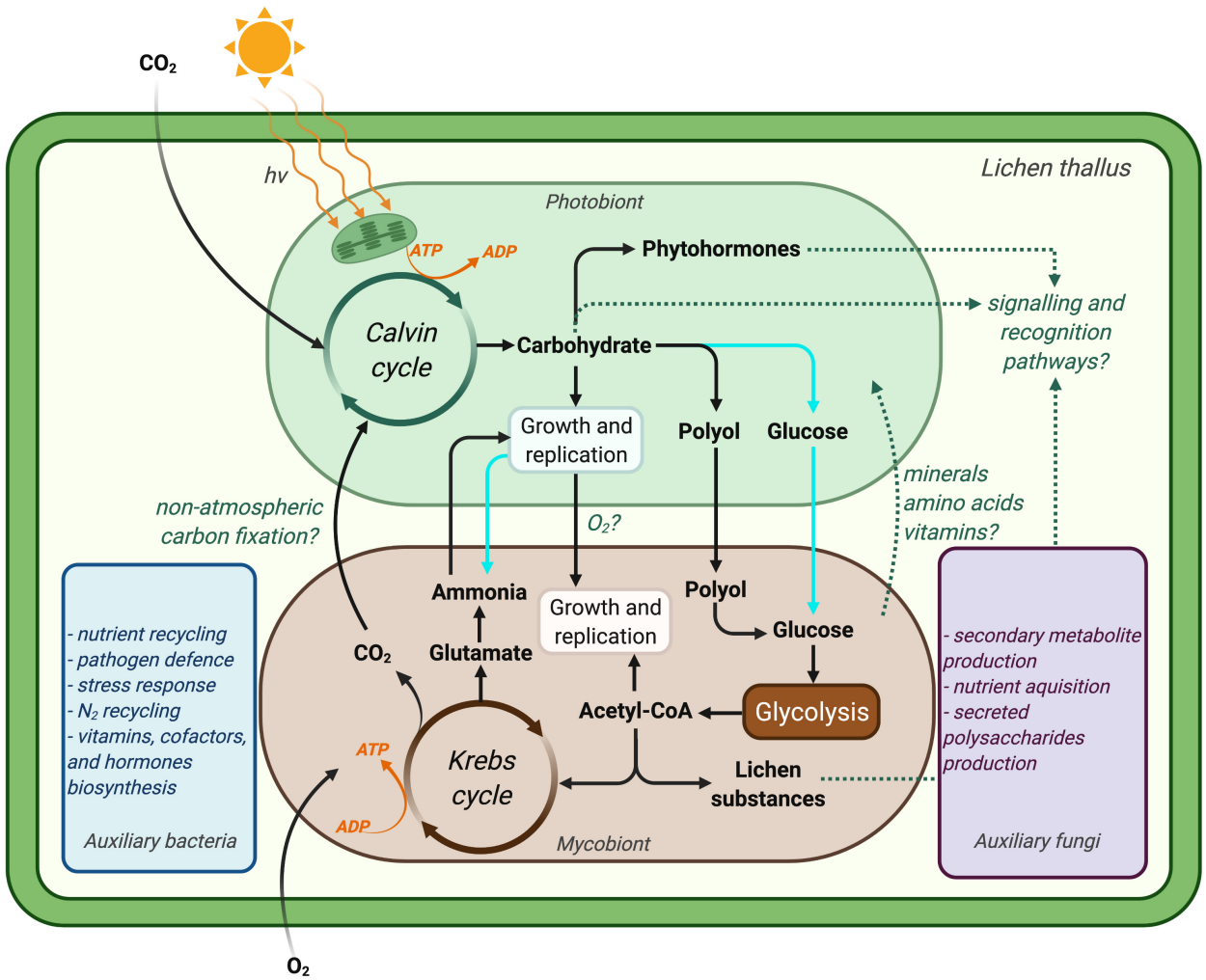


FIGURE 4 | Simplified illustration of a lichen symbiosis showing our current understanding of the metabolic interactions between lichen symbionts. Dotted line indicates knowledge gaps that may not be generalisable to all lichens. Atmospheric carbon is fixed to carbohydrates by the photobiont at the expense of ATP, which is generated by photosynthesis. Chlorolichens convert carbohydrate to polyols and transport it to their respective mycobionts, whereas cyanolichens (cyan pathways) translocate carbohydrate in the form of glucose to their mycobionts. Mycobiont metabolism is fuelled by such carbohydrate translocation, which results in the provisioning of nitrogen for the photobiont (in case for chlorolichens). Recent studies also suggest coupled metabolisms for lichen symbionts with respect to the supply of oxygen (in addition to carbohydrates) by photobionts in return for carbon dioxide from their mycobionts (Schwartzman, 2010; Hom and Murray, 2014; ten Veldhuis et al., 2020). The production of lichen substances (mainly by mycobionts) and the production of phytohormones and/or carbohydrates by photobionts are believed to play key roles in the recognition of symbionts. The proposed functions of auxiliary lichen bacteria and fungi during the symbiosis are also indicated based on comprehensive multi-omics studies (blue and purple rectangles). It is speculated that the lichen microbiome forms a biofilm on the surface of the mycobiont, through which they communicate (Image created with BioRender.com).

the lichen symbiosis (Spribille et al., 2016; Cernava et al., 2017; Smith et al., 2020).

Rhizobiales have been found to be a dominant bacterial order in the microbiome of various terrestrial or marine lichens examined to date (Grube et al., 2009; Hodkinson and Lutzoni, 2009; Hodkinson et al., 2012; Erlacher et al., 2015). Specifically, Rhodospirillales were found to be common in chlorolichens, and Sphingomonadales and Bacteroidetes in cyanolichens (Hodkinson et al., 2012; Graham et al., 2018; West et al., 2018). Several factors are believed to influence lichen-associated bacterial community composition and diversity. These include the nature of lichen secondary metabolites (driven mainly by the type of primary mycobiont), large-scale geography, growth type, and the type of primary lichen photobiont (Grube et al., 2009; Hodkinson et al., 2012; Aschenbrenner et al., 2016). Some of these auxiliary bacteria were thought to be able to fix atmospheric nitrogen and, as cyanobacterial photobionts, might play an important role as a nitrogen source for the lichen symbiosis (Hodkinson and Lutzoni, 2009). Additionally, cyanobacterial lichens, which often grow in nitrogen-limited environments, were shown to harbour a diversity of bacteria that would otherwise not grow in such nitrogen-limited environments (Hodkinson et al., 2012). Apart from nitrogen fixation, meta-omics (e.g., meta-genomics, meta-transcriptomics, and meta-proteomics) studies have revealed functional roles for the microbiome of

the lichen *Lobaria pulmonaria*, including: nutrient recycling in the decaying parts of the lichen thallus, pathogen defence, detoxification processes, protection against oxidative stress, biosynthesis of vitamins, cofactors, and hormones, activation of ketone metabolism during dehydration, and upregulated transcription of transport systems, tRNA modification and various porins during hydration (Cernava et al., 2015; Grube et al., 2015; Aschenbrenner et al., 2016; Sigurbjornsdottir et al., 2016; Cernava et al., 2017; Cernava et al., 2019). The role of these auxiliary bacteria is thus critical to the maintenance and functioning of the lichen symbiosis.

The large diversity of lichen-associated fungi has been revealed through culture-dependent methods first (Petrini et al., 1990; Arnold et al., 2009), then meta-omics data analyses (Spribille et al., 2016; Smith et al., 2020). The low biomass of these auxiliary fungi relative to the primary mycobiont and the inability to culture them have prevented a detailed analysis of their functional roles in the lichen symbiosis. However, based on the analysis of meta-genome of the lichen *Alectoria sarmentosa*, a recent study showed that auxiliary fungi (two basidiomycete yeasts) may play roles in producing secreted extracellular polysaccharides, lichen nutrient acquisition, and secondary metabolite production (Tagirdzhanova et al., 2021). They are therefore also likely to play an important role in the maintenance and functioning of the lichen symbiosis.

Although meta-omics analyses of lichen microbiomes have provided invaluable insights on the diversity and function of multi-species lichen symbioses, constraint-based metabolic modelling could potentially enable a deeper understanding of the multi-species metabolic interplay. For example, by applying a systems biology approach using genome-scale metabolic reconstructions for 773 human gut bacteria (AGORA), a more sophisticated understanding of the interactions between the host and gut microbiome was achieved, revealing how system responses depended upon the metabolic potential of each component species and the nutrients available (Magnusdottir et al., 2017). The AGORA framework confirmed that a high fibre diet (usually linked to a healthy microbiome) would result in higher proportion of commensal and mutualistic pair-wise interactions between gut microbes. This framework was able to show how the host-microbiome operates mechanistically and indicate how many positive interactions are sufficient to maintain a healthy gut community. A similar systems-level understanding of lichens could help in understanding the metabolic interdependency for symbiotic establishment and maintenance, and in predicting the role of associated lichen microbes and lichen responses to environmental changes or likely environmental niches. This would also aid in re-creating/re-synthesizing lichens *in vitro* and using them for biotechnological applications.

Genome-Scale Metabolic Flux Modelling: Challenges and Opportunities for the Lichen Symbiosis

Genome-scale metabolic network models simulate the metabolism of a living cell as a collection of hundreds to thousands of biochemical reactions (forming metabolic pathways of an organism) and enable quantitative and gene-grounded predictions of phenotypes under different growth conditions (Varma and Palsson, 1994; Covert et al., 2001). This set of reactions is framed as a set of ordinary differential equations, in which the number of variables and equations are defined by the number of metabolites and reactions, respectively. Solving this system of equations under a given set of assumptions (e.g., net zero system flux or "flux balance") allows for determining optimal fluxes for each reaction in the metabolic network. Specific constraints describing the physico-chemical, environmental, regulatory, and/or topological conditions of the metabolic network can be imposed to identify optimal flux distributions consistent with these assumptions (Price et al., 2004). Such constraint-based metabolic modelling enables a wide range of applications including, but not limited to, predicting cellular functions (e.g., energy production) (Edwards et al., 2001; Orth and Palsson, 2012; Bordbar et al., 2014), identifying optimal strains and culture media conditions for specific applications (Pharkya et al., 2004; Nazem-Bokaee and Senger, 2015), formulating metabolic/strain engineering strategies (Burgard et al., 2003; Chung et al., 2010; Kim and Reed, 2010; Ranganathan et al., 2010; Rocha et al., 2010; McAnulty et al., 2012; Yen et al., 2013; Kim et al., 2019), identifying drug targets (Kim et al., 2011, 2012; Angione, 2019; Gu et al., 2019), producing natural/non-natural chemicals and precursors (Yim et al., 2011; Ye et al., 2014; Nazem-Bokaee et al., 2016; Wei et al., 2017; Nazem-Bokaee and Maranas, 2018; Biz et al., 2019; Gu et al., 2019), creating knowledgebases of metabolic, genomic, and biodiversity information (Kumar et al., 2012; Pabinger et al., 2014; King et al., 2016; Nazem-Bokaee et al., 2017; Norsigian et al., 2020), and studying syntrophic/symbiotic communities (see below). **Table 2** lists select examples of two-species metabolic models that have been studied.

Techniques developed for the characterisation of metabolic interactions among members of microbial communities based on genome-scale metabolic modelling can be classified into two main groups: lumped (also called enzyme soup, mixed bag, or metagenome-scale modelling (Chan et al., 2017a)) and compartmentalised (Biggs et al., 2015; Henry et al., 2016; Zomorrodi and Segre, 2016). The analysis of interactions in a microbial community can be performed under steady-state or dynamic conditions. While an extensive description of these techniques and their implementation can be found elsewhere (Biggs et al., 2015; Zomorrodi and Segre, 2016; Chan et al., 2017a; Ang et al., 2018; Garcia-Jimenez et al., 2021) and is beyond the scope of this review, it is worth broadly covering the general aim of each technique. The lumped modelling approach seeks to find optimal conditions that benefits the whole community (e.g., mutualistic symbiosis) by neglecting boundaries between members of the community (Taffs et al., 2009; Henry et al., 2016). The compartmentalised modelling approach, on the other hand, retains boundaries between members while also allowing individual members to share a compartment and transfer metabolites. For example, the compartmentalised modelling approach enables considering a member-level objective towards achieving a community-level

TABLE 2 | Select two-species metabolic network models that have been constructed and analysed[1].

Partners/symbionts[2]	Community modelling approach[3]	Key outcomes of the study	References
Desulfovibrio vulgaris (r: 89) *Methanococcus maripaludi* (r: 82)	Compartmentalised; steady-state	This is the first study on modelling mutualistic interactions between a sulphate-reducing bacterium and a methanogen using a compartmentalised approach. Using relatively small metabolic networks of the two microbes, a syntrophic methanogenesis was simulated when *D. vulgaris* produced hydrogen, carbon dioxide, and acetate, which were utilised by the methanogen.	Stolyar et al., 2007
Geobacter sulfurreducens (c: 2, g: 588, r: 727) *Rhodoferax ferrireducens* (c: 2, g: 744, r: 762)	Compartmentalised; dynamic	This work analysed the dynamics of growth between two bacteria competing for uranium bioremediation.	Zhuang et al., 2011
Scheffersomyces stipites (c: 3, g: 814, r: 1371) *Saccharomyces cerevisiae* (c: 8, g: 904, r: 1412)	Lumped; dynamic (s: 3588)	In this study a co-culture simulating lignocellulosic feed breakdown for biofuel production was analysed using metabolic models of *S. cerevisiae* converting hexose and *S. stipites* converting pentose part of the synthetic feed into ethanol.	Hanly and Henson, 2013
Geobacter metallireducens (c: 2, g: 987, r: 1284) *Geobacter sulfurreducens* (c: 2, g: 837, r: 1085)	Compartmentalised; steady-state (t: 36)	A multi-omics approach was used in this study to understand electron flow mechanisms between the two bacteria. Results suggested that while *G. metallireducens* could respond only to syntrophic changes at transcriptomic level, *G. sulfurreducens* responded at both transcriptomic and genomic levels.	Nagarajan et al., 2013
Bifidobacterium adolescentis (g: 452, r: 699) *Faecalibacterium prausnitzii* (g: 484, r: 713)	Compartmentalised, steady-state	This study demonstrated that through modelling only two representatives of human gut microbiome, *B. adolescentis* and *F. prausnitzii*, the growth of the latter is severely affected when acetate production by the first microbe became limited.	El-Semman et al., 2014
Salmonella enterica *Escherichia coli K12 strain*	Compartmentalised; dynamic	Community modelling confirmed growth of *E. coli* on lactose minimal media was feasible only in co-culture with *S. enterica*, which received acetate and produced methionine in return.	Harcombe et al., 2014
Escherichia coli K strain (c: 3, g: 1260, r: 2073) *Escherichia coli L strain* (c: 3, g: 1260, r: 2073)	Compartmentalised; dynamic (t: 2)	Auxotrophy was studied using two mutants of *E. coli*, in which one grew with leucine and produced lysine that was assimilated by the other strain.	Zhang and Reed, 2014
Ketogulonicigenium vulgare (c: 3, g: 663, r: 2073) *Bacillus megaterium* (c: 3, g: 1055, r: 2073)	Compartmentalised; steady-state (t: 453)	In this study an artificial consortium was constructed to analyse the production of vitamin C and other metabolites (e.g., 2-keto-l-gulonic acid) during two-step fermentation process	Ye et al., 2014
Leptospirillum ferriphilum (r: 87) *Ferroplasma acidiphilum* (r: 71)	Compartmentalised; steady-state	In this work, a bacteria-archaea mixed culture was modelled to study bioleaching (oxidizing iron)	Merino et al., 2015
Chlamydomonas reinhardtii (c: 10, g: 1080, r: 2191) *Saccharomyces cerevisiae* (c: 8, g: 750, r: 1266)	Compartmentalised; dynamic (t: 2)	The goal of this study was to feed process models with metabolic models of algal-fungal co-culture for optimizing biodiesel production. The alga produced oxygen for the yeast and in return received carbon dioxide secreted by the yeast. This study is an example of creating artificial symbiosis through exchange of key metabolites between an alga and a fungus, which could lead to higher biodiesel production compared with single cultures of the alga.	Gomez et al., 2016
Thermosynechococcus elongatus BP-1 (g: 583, r: 917) *Meiothermus ruber strain A* (g: 729, r: 1163)	Lumped and compartmentalised; steady-state (s: 1707)	The lumped model showed highest overall consistency between predicted fluxes and measured gene expression data. However, this approach provided no information on the potential interactions between the two members of consortia. The gap-filled compartmentalised model provided the best performance among all models with respect to predicting key metabolites interacting between the two bacteria.	Henry et al., 2016
Medicago truncatula (c: 8, g: 3403, r: 2909) *Sinorhizobium meliloti*	Compartmentalised; steady-state (t: 20)	The community model predicted the preferred uptake of ammonia over nitrate when both present in excess. At dark and when ammonia is limiting, the model predictions were in favour of nitrate uptake. The symbiotic model predicted amino acid cycling which is shown to be essential for nitrogen fixation for some rhizobial strains.	Pfau et al., 2018
Nitrosomonas europaea (g: 578) *Nitrobacter winogradskyi* (g: 579)	Compartmentalised; dynamic (t: 25)	Aerobic co-culture of two model nitrifying bacteria was used to study the dynamics of nitrification in agricultural settings	Mellbye et al., 2018
Phaeodactylum tricornutum (c: 6, g: 1027, r: 4456) *Pseudoalteromonas haloplanktis* (c: 2, g: 721, r: 1322)	Lumped; dynamic (s: 3588)	This work demonstrates the advantages of using metabolic models to simulate a diatom-bacteria co-culture to study the effect of changes in growth parameters on the co-culture to represent ocean food ecosystem. Using a linear community-level biomass objective function, a multi-compartment model was built, and then, converted into a dynamic, constraint-based, model of co-culture. Simulating this synthetic ecosystem revealed that the growth of the diatom was negatively affected by the growth of the bacterium due to the shortage of phosphate and sulphate.	Fondi and Di Patti, 2019

[1] Community metabolic models developed to study interactions among more than two organisms in any microbiota was excluded in this table for simplicity. For further information on larger communities of microbes the reader is referred to the text and these reviews (Zomorrodi and Segre, 2016; Ang et al., 2018; Chan et al., 2017a; Gu et al., 2019).

[2] Numbers in parenthesis indicate the number of compartments (c), genes (g), and reactions (r), if available, captured in the respective metabolic model of the symbiont.

[3] Numbers in parenthesis indicate the number of inter-species transporters (t) or shared reactions (s), when available, captured in the respective community metabolic model.

objective by imposing a constant growth rate across all members for a community to ensure co-existence and stability (Chan et al., 2017b). Although computationally more expensive, the compartmentalised modelling approach also allows for the study of different types of species-species interactions (e.g., parasitism) (Zomorrodi and Maranas, 2012). A dynamic modelling approach enables predictions of changes in metabolites and biomass over time within the community and relies on kinetic data of uptake reactions. The dynamic approach has been extended to enable spatial analysis of communities, as in the COMETS (Computation Of Microbial Ecosystems in Time and Space) framework, which coupled metabolic with diffusion modelling and was applied to understand metabolite exchange within a three-member microbial community (Harcombe et al., 2014).

To our knowledge, no genome-scale metabolic network model has yet been constructed for any lichen association or its symbionts. With the first genomes of mycobionts (Park et al., 2013a,b, 2014a,b; Armstrong et al., 2018; Bertrand et al., 2018a; Wang et al., 2018) and photobionts (Armaleo et al., 2019) of several lichens assembled and more foreseen to come, it is a timely opportunity to understand the lichen symbiosis through the lens of genome-scale metabolic models. Since little is known about the metabolic response of lichens to different environmental conditions (e.g., light intensity, water content, nutrient availability, etc.), developing a metabolic network model could shed invaluable insights on symbiosis at the molecular level. Furthermore, the available computational tools for modelling community interactions could allow for predicting the role of a specific symbiont on the performance of a lichen under a known environmental perturbation (e.g., nutrient limitation). A lichen metabolic model could be used as the framework for the integration of 'omics' data obtained for lichens to test multiple hypotheses including, for example, the regulatory effect of different carbohydrates on the growth and exchange of metabolites between lichen symbiont. Since *in vitro* lichen re-synthesis is still hampered by the complexity of the lichenisation process, metabolic modelling could highlight potential metabolites that may need to be exchanged between symbionts as well as the metabolic pathways that may lead to successful differentiation and growth. Moreover, metabolic modelling could be used to examine the potential for symbiosis between various combinations of mycobionts and photobionts, and provide insights into the evolution of the lichen symbiosis. Validating predictions of flux distribution by community metabolic models could be a challenge, due to multi-compartmental nature of lichen symbiosis and difficulties in measuring fluxes through each compartment *in vivo*. However, recent advances in the field of metabolic flux analysis now make it possible to resolve fluxes by carefully designing the isotope labels and tracing them across different compartments (Schwechheimer et al., 2018). Another practical challenge for the development of lichen metabolic models may pertain to the characterisation of the cellular composition of individual lichen symbionts. For example, many lichen mycobionts grow slowly, making it experimentally difficult to obtain sufficient cell mass needed to formulate a 'biomass' reaction in a metabolic model representing cellular growth. Moreover, due to the lack of data specific to the metabolic pathways of lichens, the model curation process may be patchy, with irreconcilable gaps and network disconnects. However, metabolic models for lichens could be reconstructed by leveraging the ever-increasing number of high-quality metabolic models becoming available for not-too-distantly related filamentous fungi, microalgae, or cyanobacteria (Brandl and Andersen, 2015; Gomez et al., 2016; Santos-Merino et al., 2019).

CONCLUSION AND FUTURE PERSPECTIVES

Lichens, although historically well-known and iconic symbioses, still bear a sense of mystery as our understanding of the signalling networks and pathways responsible for their symbiotic establishment and maintenance is still in its infancy. Two signalling mechanisms were reviewed in this article but many more could be explored with the aid of techniques such as untargeted metabolomics. Signalling/metabolic network modelling approaches could support the field of experimental lichenology by providing insights into: (1) the signalling molecules and the roles they play at different stages of lichenisation, (2) how lichen symbionts benefit from the symbiosis with regards to carbon, nitrogen, and other limiting nutrients or environmental conditions, (3) which conditions allow lichens to produce secondary metabolites and the genes that are involved, and (4) how lichens manage to accumulate and tolerate high levels of toxic metals. Advances in DNA sequencing technologies in recent years have significantly reduced the cost of generating genome sequences. At the same time, improvements in high performance computing and development of more biologist-friendly tools for modelling and analysing 'genome-scale' metabolic networks have enabled the exploration of metabolically-coupled microbial communities. Combining these genome resources and systems biology tools could open up a whole new era for the study of the lichen symbiosis.

AUTHOR CONTRIBUTIONS

HN-B and CG conceptualised and wrote the manuscript. HN-B and CG designed and created figures. EFYH, ACW, and SM revised the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by funding from the CSIRO Synthetic Biology Future Science Platform (FSP), Grant # OD-206013. EFYH was funded in part by NSF grants #1541538 and #1846376.

ACKNOWLEDGMENTS

We would like to thank Colin Scott (CSIRO) as well as the reviewers for critical reading and suggesting constructive improvements to the manuscript.

REFERENCES

Ahmadjian, V. (1993). *The Lichen Symbiosis*. Hoboken, NJ: John Wiley & Sons.

Ahmadjian, V., Jacobs, J. B., and Russell, L. A. (1978). Scanning electron-microscope study of early lichen synthesis. *Science* 200, 1062–1064. doi: 10.1126/science.200.4345.1062

Ang, K. S., Lakshmanan, M., Lee, N. R., and Lee, D. Y. (2018). Metabolic modeling of microbial community interactions for health, environmental and biotechnological applications. *Curr. Genom.* 19, 712–722. doi: 10.2174/1389202919666180911144055

Angione, C. (2019). Human systems biology and metabolic modelling: a reviewfrom disease metabolism to precision medicine. *Biomed. Res. Intern.* 2019:8304260.

Antebi, Y. E., Nandagopal, N., and Elowitz, M. B. (2017). An operational view of intercellular signaling pathways. *Curr. Opin. Syst. Biol.* 1, 16–24. doi: 10.1016/j.coisb.2016.12.003

Armaleo, D., Muller, O., Lutzoni, F., Andresson, O. S., Blanc, G., Bode, H. B., et al. (2019). The lichen symbiosis re-viewed through the genomes of *Cladonia grayi* and its algal partner *Asterochloris glomerata*. *BMC Genom.* 20:605. doi: 10.1186/s12864-019-5629-x

Armstrong, E. E., Prost, S., Ertz, D., Westberg, M., Frisch, A., and Bendiksby, M. (2018). Draft genome sequence and annotation of the lichen-forming fungus *Arthonia radiata*. *Genome Announc.* 6:e0281-18.

Arnold, A. E., Miadlikowska, J., Higgins, K. L., Sarvate, S. D., Gugger, P., Way, A., et al. (2009). A phylogenetic estimation of trophic transition networks for Ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Syst. Biol.* 58, 283–297. doi: 10.1093/sysbio/syp001

Aschenbrenner, I. A., Cernava, T., Berg, G., and Grube, M. (2016). Understanding microbial multi-species symbioses. *Front. Microbiol.* 7:180. doi: 10.3389/fmicb.2016.00180

Athukorala, S. N. P., Huebner, E., and Piercey-Normore, M. D. (2014). Identification and comparison of the 3 early stages of resynthesis for the lichen *Cladonia rangiferina*. *Can. J. Microbiol.* 60, 41–52. doi: 10.1139/cjm-2013-0313

Athukorala, S. N. P., and Piercey-Normore, M. D. (2014). Recognition between *Cladonia rangiferina* and the algal partner during resynthesis. *Bot. Botaniq.* 92, 640–640.

Athukorala, S. N. P., and Piercey-Normore, M. D. (2015). Recognition- and defense-related gene expression at 3 resynthesis stages in lichen symbionts. *Can. J. Microbiol.* 61, 1–12. doi: 10.1139/cjm-2014-0470

Aubert, S., Juge, C., Boisson, A. M., Gout, E., and Bligny, R. (2007). Metabolic processes sustaining the reviviscence of lichen *Xanthoria elegans* (Link) in high mountain environments. *Planta* 226, 1287–1297. doi: 10.1007/s00425-007-0563-6

Beckett, R. P., Kranner, I., and Minibayeva, F. V. (2008). "Stress physiology and the symbiosis," in *Lichen Biology*, 2nd Edn, ed. T. H. Nash (Cambridge: Cambridge University Press), 134–151. doi: 10.1017/cbo9780511790478.009

Bertrand, R. L., Abdel-Hameed, M., and Sorensen, J. L. (2018a). Lichen biosynthetic gene clusters. Part I. Genome sequencing reveals a rich biosynthetic potential. *J. Nat. Prod.* 81, 723–731. doi: 10.1021/acs.jnatprod.7b00769

Bertrand, R. L., Abdel-Hameed, M., and Sorensen, J. L. (2018b). Lichen biosynthetic gene clusters Part II: Homology mapping suggests a functional diversity. *J. Nat. Prod.* 81, 732–748. doi: 10.1021/acs.jnatprod.7b00770

Biggs, M. B., Medlock, G. L., Kolling, G. L., and Papin, J. A. (2015). Metabolic network modeling of microbial communities. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 7, 317–334. doi: 10.1002/wsbm.1308

Biz, A., Proulx, S., Xu, Z., Siddartha, K., Mulet Indrayanti, A., and Mahadevan, R. (2019). Systems biology based metabolic engineering for non-natural chemicals. *Biotechnol. Adv.* 37:107379. doi: 10.1016/j.biotechadv.2019.04.001

Bonfante, P., and Genre, A. (2010). Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. *Nat. Commun.* 1:48.

Bonfante, P., and Requena, N. (2011). Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 14, 451–457. doi: 10.1016/j.pbi.2011.03.014

Bordbar, A., Monk, J. M., King, Z. A., and Palsson, B. O. (2014). Constraint-based models predict metabolic and associated cellular functions. *Nat. Rev. Genet.* 15, 107–120. doi: 10.1038/nrg3643

Brakni, R., Ahmed, M. A., Burger, P., Schwing, A., Michel, G., Pomares, C., et al. (2018). UHPLC-HRMS/MS based profiling of algerian lichens and their antimicrobial activities. *Chem. Biodiver.* 15:e1800031. doi: 10.1002/cbdv.201800031

Brandl, J., and Andersen, M. R. (2015). Current state of genome-scale modeling in filamentous fungi. *Biotechnol. Lett.* 37, 1131–1139. doi: 10.1007/s10529-015-1782-8

Bubrick, P., and Galun, M. (1980). Proteins from the lichen *Xanthoria parietina* which bind to phycobiont cell walls correlation between binding patterns and cell wall Cyto Chemistry. *Protoplasma* 104, 167–173. doi: 10.1007/bf01279379

Bubrick, P., Galun, M., and Frensdorff, A. (1981). Proteins from the lichen *Xanthoria parietina* which bind to phycobiont cell walls localization in the intact lichen and cultured mycobiont. *Protoplasma* 105, 207–211. doi: 10.1007/bf01279219

Burgard, A. P., Pharkya, P., and Maranas, C. D. (2003). OptKnock: a bilevel programming framework for identifying gene knockout strategies for microbial strain optimization. *Biotechnol. Bioeng.* 84, 647–657. doi: 10.1002/bit.10803

Calcott, M. J., Ackerley, D. F., Knight, A., Keyzers, R. A., and Owen, J. G. (2018). Secondary metabolism in the lichen symbiosis. *Chem. Soc. Rev.* 47, 1730–1760. doi: 10.1039/c7cs00431a

Cernava, T., Aschenbrenner, I. A., Soh, J., Sensen, C. W., Grube, M., and Berg, G. (2019). Plasticity of a holobiont: desiccation induces fasting-like metabolism within the lichen microbiota. *ISME J.* 13, 547–556. doi: 10.1038/s41396-018-0286-7

Cernava, T., Erlacher, A., Aschenbrenner, I. A., Krug, L., Lassek, C., Riedel, K., et al. (2017). Deciphering functional diversification within the lichen microbiota by meta-omics. *Microbiome* 5:82.

Cernava, T., Muller, H., Aschenbrenner, I. A., Grube, M., and Berg, G. (2015). Analyzing the antagonistic potential of the lichen microbiome against pathogens by bridging metagenomic with culture studies. *Front. Microbiol.* 6:620. doi: 10.3389/fmicb.2015.00620

Chan, S. H. J., Simons, M., and Maranas, C. D. (2017a). Computational modeling of microbial communities. *Syst. Biol.* 163–189. doi: 10.1002/9783527696130.ch6

Chan, S. H. J., Simons, M. N., and Maranas, C. D. (2017b). SteadyCom: predicting microbial abundances while ensuring community stability. *PLoS Comput. Biol.* 13:e1005539. doi: 10.1371/journal.pcbi.1005539

Chung, B. K., Selvarasu, S., Andrea, C., Ryu, J., Lee, H., Ahn, J., et al. (2010). Genome-scale metabolic reconstruction and in silico analysis of methylotrophic yeast *Pichia pastoris* for strain improvement. *Microb. Cell Fact.* 9:50. doi: 10.1186/1475-2859-9-50

Clear, M. R., and Hom, E. F. (2019). The evolution of symbiotic plant–microbe signalling. *Annu. Plant Rev.* 2:684.

Covert, M. W., Schilling, C. H., Famili, I., Edwards, J. S., Goryanin, I. I., Selkov, E., et al. (2001). Metabolic modeling of microbial strains in silico. *Trends Biochem. Sci.* 26, 179–186. doi: 10.1016/s0968-0004(00)01754-0

Dahlman, L., Persson, J., Palmqvist, K., and Nasholm, T. (2004). Organic and inorganic nitrogen uptake in lichens. *Planta* 219, 459–467.

Dasika, M. S., Burgard, A., and Maranas, C. D. (2006). A computational framework for the topological analysis and targeted disruption of signal transduction networks. *Biophys. J.* 91, 382–398. doi: 10.1529/biophysj.105.069724

Díaz, E., Sánchez Elordi, E., Santiago, R., Vicente Córdoba, C., and Legaz González, M. E. (2016a). Algal-fungal mutualism: cell recognition and maintenance of the symbiotic status of lichens. *J. Vet. Med. Res.* 3:1052.

Diaz, E. M., Ampe, C., Van Troys, M., Vicente-Manzanares, M., Legaz, M. E., and Vicente, C. (2016b). An actomyosin-like cytoskeleton in the cyanobiont (*Nosctoc* sp.) of *Peltigera canina*. *Phytochem. Lett.* 16, 249–256. doi: 10.1016/j.phytol.2016.05.005

Diaz, E. M., Cutrona, C., Sanchez-Elordi, E., Legaz, M. E., and Vicente, C. (2016c). Direct and cross-recognition of lichenized *Trebouxia puymaly* (Chlorophyta, Trebouxiophyceae) and *Nostoc* Vaucher ex Bornet (Cyanobacteria, Cyanophyceae) by their homologous and heterologous fungal lectins. *Braz. J. Bot.* 39, 507–518. doi: 10.1007/s40415-016-0268-9

Diaz, E. M., Sacristan, M., Legaz, M. E., and Vicente, C. (2009). Isolation and characterization of a cyanobacterium-binding protein and its cell wall receptor in the lichen *Peltigera canina*. *Plant Signal. Behav.* 4, 598–603. doi: 10.4161/psb.4.7.9164

Diaz, E. M., Vicente-Manzanares, M., Legaz, M. E., and Vicente, C. (2015). A cyanobacterial beta-actin-like protein, responsible for lichenized *Nostoc* sp motility towards a fungal lectin. *Acta Physiol. Plant.* 37:249.

Diaz, E. M., Vicente-Manzanares, M., Sacristan, M., Vicente, C., and Legaz, M. E. (2011). Fungal lectin of *Peltigera canina* induces chemotropism of compatible

Nostoc cells by constriction-relaxation pulses of cyanobiont cytoskeleton. *Plant Signal. Behav.* 6, 1525–1536. doi: 10.4161/psb.6.10.16687

diCenzo, G. C., Zamani, M., Checcucci, A., Fondi, M., Griffitts, J. S., Finan, T. M., et al. (2019). Multidisciplinary approaches for studying rhizobium-legume symbioses. *Can. J. Microbiol.* 65, 1–33. doi: 10.1139/cjm-2018-0377

Drew, E. A., and Smith, D. C. (1967a). Studies in physiology of Lichens 7. Physiology of *Nostoc* Symbiont of *Peltigera polydactyla* compared with cultured and free-living forms. *New Phytol.* 66, 379–388. doi: 10.1111/j.1469-8137.1967.tb06017.x

Drew, E. A., and Smith, D. C. (1967b). Studies in physiology of Lichens.8. Movement of glucose from alga to fungus during photosynthesis in thallus of *Peltigera polydactyla*. *New Phytol.* 66, 389–389. doi: 10.1111/j.1469-8137.1967.tb06018.x

Edwards, J. S., Ibarra, R. U., and Palsson, B. O. (2001). In silico predictions of *Escherichia coli* metabolic capabilities are consistent with experimental data. *Nat. Biotechnol.* 19, 125–130.

Eisenreich, W., Knispel, N., and Beck, A. (2011). Advanced methods for the study of the chemistry and the metabolism of lichens. *Phytochem. Rev.* 10, 445–456. doi: 10.1007/s11101-011-9215-3

Elix, J. A., and Stocker-Worgotter, E. (2008). "Biochemistry and secondary metabolites," in *Lichen Biology*, 2nd Edn, ed. T. H. Nash III (Cambridge: Cambridge University Press), 104–133. doi: 10.1017/cbo9780511790478.008

El-Semman, I. E., Karlsson, F. H., Shoaie, S., Nookaew, I., Soliman, T. H., and Nielsen, J. (2014). Genome-scale metabolic reconstructions of *Bifidobacterium adolescentis* L2-32 and *Faecalibacterium prausnitzii* A2-165 and their interaction. *BMC Syst. Biol.* 8:41. doi: 10.1186/1752-0509-8-41

Elshobary, M. E., Osman, M. E. H., Abushady, A. M., and Piercey-Normore, M. D. (2015). Comparison of lichen-forming cyanobacterial and green algal photobionts with free-living algae. *Cryptogam. Algol.* 36, 81–100. doi: 10.7872/crya.v36.iss1.2015.81

Epstein, E., Sagee, O., Cohen, J. D., and Garty, J. (1986). Endogenous auxin and ethylene in the lichen Ramalina duriaei. *Plant Physiol.* 82, 1122–1125. doi: 10.1104/pp.82.4.1122

Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C. W., Grube, M., et al. (2015). Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Front. Microbiol.* 6:53. doi: 10.3389/fmicb.2015.00053

Fahselt, D. (1994). Secondary biochemistry of lichens. *Symbiosis* 16, 117–165.

Fondi, M., and Di Patti, F. (2019). A synthetic ecosystem for the multi-level modelling of heterotroph-phototroph metabolic interactions. *Ecol. Model.* 399, 13–22. doi: 10.1016/j.ecolmodel.2019.02.012

Garcia-Jimenez, B., Torres-Bacete, J., and Nogales, J. (2021). Metabolic modelling approaches for describing and engineering microbial communities. *Comput. Struct. Biotechnol. J.* 19, 226–246. doi: 10.1016/j.csbj.2020.12.003

Goga, M., Eleèko, J., Marcinèinová, M., Ruèová, D., Baèkorová, M., and Baèkor, M. (2020). "Lichen metabolites: an overview of some secondary metabolites and their biological potential," in *Co-Evolution of Secondary Metabolites*, eds J. M. Merillon and K. Ramawat (Cham: Springer), 175–209. doi: 10.1007/978-3-030-16814-8_6

Gomez, J. A., Hoffner, K., and Barton, P. I. (2016). From sugars to biodiesel using microalgae and yeast. *Green Chem.* 18, 461–475. doi: 10.1039/c5gc01843a

Graham, L. E., Trest, M. T., Will-Wolf, S., Miicke, N. S., Atonio, L. M., Piotrowski, M. J., et al. (2018). Microscopic and metagenomic analyses of *Peltigera ponojensis* (Peltigerales, Ascomycota). *Intern. J. Plant Sci.* 179, 241–255. doi: 10.1086/696534

Green, T. G. A., Nash, T. H., and Lange, O. L. (2008). "Physiological ecology of carbon dioxide exchange," in *Lichen Biology*, 2nd Edn, eds T. G. A. Green, T. H. Nash, and O. L. Lange (Cambridge: Cambridge University Press), 152–181. doi: 10.1017/cbo9780511790478.010

Green, T. G. A., and Smith, D. C. (1974). Lichen Physiology XIV. Differences between lichen algae in symbiosis and in isolation. *New Phytol.* 73, 753–766. doi: 10.1111/j.1469-8137.1974.tb01303.x

Grube, M., Cardinale, M., De Castro, J. V., Muller, H., and Berg, G. (2009). Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *ISME J.* 3, 1105–1115. doi: 10.1038/ismej.2009.63

Grube, M., Cernava, T., Soh, J., Fuchs, S., Aschenbrenner, I., Lassek, C., et al. (2015). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *ISME J.* 9, 412–424. doi: 10.1038/ismej.2014.138

Gu, C., Kim, G. B., Kim, W. J., Kim, H. U., and Lee, S. Y. (2019). Current status and applications of genome-scale metabolic models. *Genome Biol.* 20:121.

Gueidan, C., Ruibal, C., De Hoog, G. S., and Schneider, H. (2011). Rock-inhabiting fungi originated during periods of dry climate in the late devonian and middle triassic. *Fungal Biol.* 115, 987–996. doi: 10.1016/j.funbio.2011.04.002

Gueidan, C., Villasenor, C. R., De Hoog, G. S., Gorbushina, A. A., Untereiner, W. A., and Lutzoni, F. (2008). A rock-inhabiting ancestor for mutualistic and pathogen-rich fungal lineages. *Stud. Mycol.* 61, 111–119. doi: 10.3114/sim.2008.61.11

Hanly, T. J., and Henson, M. A. (2013). Dynamic metabolic modeling of a microaerobic yeast co-culture: predicting and optimizing ethanol production from glucose/xylose mixtures. *Biotechnol. Biofuels* 6:44. doi: 10.1186/1754-6834-6-44

Harcombe, W. R., Riehl, W. J., Dukovski, I., Granger, B. R., Betts, A., Lang, A. H., et al. (2014). Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics. *Cell Rep.* 7, 1104–1115. doi: 10.1016/j.celrep.2014.03.070

Henry, C. S., Bernstein, H. C., Weisenhorn, P., Taylor, R. C., Lee, J. Y., Zucker, J., et al. (2016). Microbial community metabolic modeling: a community data-driven network reconstruction. *J. Cell. Physiol.* 231, 2339–2345. doi: 10.1002/jcp.25428

Hodkinson, B. P., Gottel, N. R., Schadt, C. W., and Lutzoni, F. (2012). Photoautotrophic symbiont and geography are major factors affecting highly structured and diverse bacterial communities in the lichen microbiome. *Environ. Microbiol.* 14, 147–161. doi: 10.1111/j.1462-2920.2011.02560.x

Hodkinson, B. P., and Lutzoni, F. (2009). A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49, 163–180. doi: 10.1007/s13199-009-0049-3

Hofstetter, V., Miadlikowska, J., Kauff, F., and Lutzoni, F. (2007) Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the Lecanoromycetes (Ascomycota). *Mol. Phylogenet Evol.* 44, 412–426. doi: 10.1016/j.ympev.2006.10.016

Hom, E. F. Y., and Murray, A. W. (2014). Niche engineering demonstrates a latent capacity for fungal-algal mutualism. *Science* 345, 94–98. doi: 10.1126/science.1253320

Honegger, R. (1998). The Lichen symbiosis - What is so spectacular about it? *Lichenologist* 30, 193–212. doi: 10.1017/s002428299200015x

Honegger, R., Kutasi, V., and Ruffner, H. P. (1993). Polyol patterns in 11 species of aposymbiotically cultured lichen mycobionts. *Mycol. Res.* 97, 35–39. doi: 10.1016/s0953-7562(09)81109-x

Huisman, R., and Geurts, R. (2020). A roadmap toward engineered nitrogen-fixing nodule symbiosis. *Plant Commun.* 1:100019. doi: 10.1016/j.xplc.2019.100019

Hyduke, D. R., and Palsson, B. O. (2010). Towards genome-scale signalling network reconstructions. *Nat. Rev. Genet.* 11, 297–307. doi: 10.1038/nrg2750

Jenkins, B., and Richards, T. A. (2019). Symbiosis: wolf lichens harbour a choir of fungi. *Curr. Biol.* 29, R88–R90.

Joneson, S., Armaleo, D., and Lutzoni, F. (2011). Fungal and algal gene expression in early developmental stages of lichen-symbiosis. *Mycologia* 103, 291–306. doi: 10.3852/10-064

Kappen, L. (2000). Some aspects of the great success of lichens in Antarctica. *Antarct. Sci.* 12, 314–324. doi: 10.1017/s0954102000000377

Kardish, N., Silberstein, L., Fleminger, G., and Galun, M. (1991). Lectin from the lichen *Nephroma laevigatum* Ach localization and function. *Symbiosis* 11, 47–62.

Kennedy, J. F., Palva, P. M. G., Corella, M. T. S., Cavalcanti, M. S. M., and Coelho, L. C. B. B. (1995). Lectins, versatile proteins of recognition - a review. *Carbohydr. Polym.* 26, 219–230. doi: 10.1016/0144-8617(94)00091-7

Kim, H. U., Kim, S. Y., Jeong, H., Kim, T. Y., Kim, J. J., Choy, H. E., et al. (2011). Integrative genome-scale metabolic analysis of *Vibrio vulnificus* for drug targeting and discovery. *Mol. Syst. Biol.* 7:460. doi: 10.1038/msb.2010.115

Kim, H. U., Sohn, S. B., and Lee, S. Y. (2012). Metabolic network modeling and simulation for drug targeting and discovery. *Biotechnol. J.* 7, 330–342. doi: 10.1002/biot.201100159

Kim, J., and Reed, J. L. (2010). OptORF: optimal metabolic and regulatory perturbations for metabolic engineering of microbial strains. *BMC Syst. Biol.* 4:53. doi: 10.1186/1752-0509-4-53

Kim, M., Park, B. G., Kim, E. J., Kim, J., and Kim, B. G. (2019). In silico identification of metabolic engineering strategies for improved lipid production in *Vibrio vulnificus* by genome-scale metabolic modeling. *Biotechnol. Biofuels* 12:187.

King, Z. A., Lu, J., Drager, A., Miller, P., Federowicz, S., Lerman, J. A., et al. (2016). BiGG Models: a platform for integrating, standardizing and sharing genome-scale models. *Nucleic Acids Res.* 44, D515–D522.

Kitano, H. (2002a). Computational systems biology. *Nature* 420, 206–210.

Kitano, H. (2002b). Systems biology: a brief overview. *Science* 295, 1662–1664. doi: 10.1126/science.1069492

Kono, M., Kon, Y., Ohmura, Y., Satta, Y., and Terai, Y. (2020). In vitro resynthesis of lichenization reveals the genetic background of symbiosis-specific fungal-algal interaction in *Usnea hakonensis*. *BMC Genom.* 21:671. doi: 10.1186/s12864-020-07086-9

Kono, M., Tanabe, H., Ohmura, Y., Satta, Y., and Terai, Y. (2017). Physical contact and carbon transfer between a lichen-forming *Trebouxia* alga and a novel *Alphaproteobacterium*. *Microbiol. SGM* 163, 678–691. doi: 10.1099/mic.0.000461

Kranner, I., Beckett, R., Hochman, A., and Nash, T. H. (2008). Desiccation-tolerance in lichens: a review. *Bryologist* 111, 576–593. doi: 10.1639/0007-2745-111.4.576

Kuhn, V., Geisberger, T., Huber, C., Beck, A., and Eisenreich, W. (2019). A facile in vivo procedure to analyze metabolic pathways in intact lichens. *New Phytol.* 224, 1657–1667. doi: 10.1111/nph.15968

Kumar, A., Suthers, P. F., and Maranas, C. D. (2012). MetRxn: a knowledgebase of metabolites and reactions spanning metabolic models and databases. *BMC Bioinform.* 13:6. doi: 10.1186/1471-2105-13-

Lavrik, I. N. S., and Samsonova, M. G. (2016). The systems biology of signaling pathways. *Biophysics* 61, 78–84.

Legaz, M. E., Fontaniella, B., Millanes, A. M., and Vicente, C. (2004). Secreted arginases from phylogenetically far-related lichen species act as cross-recognition factors for two different algal cells. *Eur. J. Cell Biol.* 83, 435–446. doi: 10.1078/0171-9335-00384

Lehr, H., Fleminger, G., and Galun, M. (1995). Lectin from the lichen *Peltigera membranacea* (Ach) Nyl - characterization and function. *Symbiosis* 18, 1–13.

Leiva, D., Fernandez-Mendoza, F., Acevedo, J., Caru, M., Grube, M., and Orlando, J. (2021). The bacterial community of the foliose macro-lichen *Peltigera frigida* is more than a mere extension of the microbiota of the subjacent substrate. *Microb. Ecol.* doi: 10.1007/s00248-020-01662-y. Epub ahead of print.

Lendemer, J. C., Keepers, K. G., Tripp, E. A., Pogoda, C. S., Mccain, C. M., and Kane, N. C. (2019). A taxonomically broad metagenomic survey of 339 species spanning 57 families suggests cystobasidiomycete yeasts are not ubiquitous across all lichens. *Am. J. Bot.* 106, 1090–1095.

Li, F., Thiele, I., Jamshidi, N., and Palsson, B. O. (2009). Identification of potential pathway mediation targets in toll-like receptor signaling. *PLoS Computat. Biol.* 5:e1000292. doi: 10.1371/journal.pcbi.1000292

Lines, C. E. M., Ratcliffe, R. G., Rees, T. A. V., and Southon, T. E. (1989). A C-13 Nmr-study of photosynthate transport and metabolism in the lichen *Xanthoria calcicola* Oxner. *New Phytol.* 111, 447–456. doi: 10.1111/j.1469-8137.1989.tb00707.x

Lockhart, C. M., Rowell, P., and Stewart, W. D. P. (1978). Phytohemagglutinins from nitrogen-fixing lichens *Peltigera canina* and *Peltigera polydactyla*. *FEMS Microbiol. Lett.* 3, 127–130.

Lutzoni, F., Nowak, M. D., Alfaro, M. E., Reeb, V., Miadlikowska, J., Krug, M., et al. (2018). Contemporaneous radiations of fungi and plants linked to symbiosis. *Nat. Commun.* 9:5489.

Magnusdottir, S., Heinken, A., Kutt, L., Ravcheev, D. A., Bauer, E., Noronha, A., et al. (2017). Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat. Biotechnol.* 35, 81–89. doi: 10.1038/nbt.3703

Manoharan, S. S., Miao, V. P., and Andresson, O. S. (2012). LEC-2, a highly variable lectin in the lichen *Peltigera membranacea*. *Symbiosis* 58, 91–98. doi: 10.1007/s13199-012-0206-y

Martin, F. M., Uroz, S., and Barker, D. G. (2017). Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science* 356:eaad4501. doi: 10.1126/science.aad4501

McAnulty, M. J., Yen, J. Y., Freedman, B. G., and Senger, R. S. (2012). Genome-scale modeling using flux ratio constraints to enable metabolic engineering of clostridial metabolism in silico. *BMC Syst. Biol.* 6:42. doi: 10.1186/1752-0509-6-42

Meessen, J., Eppenstein, S., and Ott, S. (2013). Recognition mechanisms during the pre-contact state of lichens: II. Influence of algal exudates and ribitol on the response of the mycobiont of *Fulgensia bracteata*. *Symbiosis* 59, 131–143. doi: 10.1007/s13199-012-0219-6

Meessen, J., and Ott, S. (2013). Recognition mechanisms during the pre-contact state of lichens: I. Mycobiont-photobiont interactions of the mycobiont of *Fulgensia bracteata*. *Symbiosis* 59, 121–130. doi: 10.1007/s13199-013-0232-4

Mellbye, B. L., Giguere, A. T., Murthy, G. S., Bottomley, P. J., Sayavedra-Soto, L. A., and Chaplen, F. W. R. (2018). Genome-scale, constraint-based modeling of nitrogen oxide fluxes during coculture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi*. *mSystems* 3:e0170-17.

Merino, M. P., Andrews, B. A., and Asenjo, J. A. (2015). Stoichiometric model and flux balance analysis for a mixed culture of *Leptospirillum ferriphilum* and *Ferroplasma acidiphilum*. *Biotechnol. Prog.* 31, 307–315. doi: 10.1002/btpr.2028

Miao, V. P. W., Manoharan, S. S., Snaebjarnarson, V., and Andresson, O. S. (2012). Expression of lec-1, a mycobiont gene encoding a galectin-like protein in the lichen *Peltigera membranacea*. *Symbiosis* 57, 23–31. doi: 10.1007/s13199-012-0175-1

Millanes, A. M., Fontaniella, B., Garcia, M. L., Solas, M. T., Vicente, C., and Legaz, M. E. (2004). Cytochemical location of urease in the cell wall of two different lichen phycobionts. *Tissue Cell* 36, 373–377. doi: 10.1016/j.tice.2004.06.007

Mittermeier, V. K., Schmitt, N., Volk, L. P. M., Suarez, J. P., Beck, A., and Eisenreich, W. (2015). Metabolic profiling of alpine and ecuadorian lichens. *Molecules* 20, 18047–18065. doi: 10.3390/molecules201018047

Mohanta, T. K., and Bae, H. (2015). Functional genomics and signaling events in mycorrhizal symbiosis. *J. Plant Interact.* 10, 21–40. doi: 10.1080/17429145.2015.1005180

Molina, M. C., Stocker-Worgotter, E., Turk, R., Bajon, C., and Vicente, C. (1998). Secreted, glycosylated arginase from *Xanthoria parietina* thallus induces loss of cytoplasmic material from *Xanthoria* photobionts. *Cell Adhesion Commun.* 6, 481–490. doi: 10.3109/15419069809010796

Molina, M. C., and Vicente, C. (1995). Correlationships between enzymatic-activity of lectins, putrescine content and chloroplast damage in *Xanthoria parietina* phycobionts. *Cell Adhesion Commun.* 3, 1–12. doi: 10.3109/15419069509081274

Molina, M. C., and Vicente, C. (2000). Purification and characterization of two isolectins with arginase activity from the lichen *Xanthoria parietina*. *J. Biochem. Mol. Biol.* 33, 300–307.

Molina, M. D., Muniz, E., and Vicente, C. (1993). Enzymatic-activities of algal-binding protein and its algal cell-wall receptor in the lichen *Xanthoria parietina* - an approach to the parasitic basis of mutualism. *Plant Physiol. Biochem.* 31, 131–142.

Morris, M. K., Saez-Rodriguez, J., Sorger, P. K., and Lauffenburger, D. A. (2010). Logic-based models for the analysis of cell signaling networks. *Biochemistry* 49, 3216–3224. doi: 10.1021/bi902202q

Nagarajan, H., Embree, M., Rotaru, A. E., Shrestha, P. M., Feist, A. M., Palsson, B. O., et al. (2013). Characterization and modelling of interspecies electron transfer mechanisms and microbial community dynamics of a syntrophic association. *Nat. Commun.* 4:2809.

Nash, T. H. (2008a). *Lichen Biology*. Cambridge: Cambridge University Press.

Nash, T. H. (2008b). "Nitrogen, its metabolism and potential contribution to ecosystems," in *Lichen Biology*, 2nd Edn, ed. T. H. Nash (Cambridge: Cambridge University Press), 216–233. doi: 10.1017/cbo9780511790478.012

Nazem-Bokaee, H., Gopalakrishnan, S., Ferry, J. G., Wood, T. K., and Maranas, C. D. (2016). Assessing methanotrophy and carbon fixation for biofuel production by *Methanosarcina acetivorans*. *Microb. Cell Fact.* 15:10.

Nazem-Bokaee, H., and Maranas, C. D. (2018). A prospective study on the fermentation landscape of gaseous substrates to biorenewables using *Methanosarcina acetivorans* metabolic model. *Front. Microbiol.* 9:1855. doi: 10.3389/fmicb.2018.01855

Nazem-Bokaee, H., and Senger, R. S. (2015). ToMI-FBA: a genome-scale metabolic flux based algorithm to select optimum hosts and media formulations for expressing pathways of interest. *Aims Bioeng.* 2, 335–374. doi: 10.3934/bioeng.2015.4.335

Nazem-Bokaee, H., Yen, J. Y., Athamneh, A. I. M., Apte, A. A., Mcanulty, M. J., and Senger, R. S. (2017). SyM-GEM: a pathway builder for genome-scale models. *Adv. Biochem. Biotechnol.* 2:141. doi: 10.29011/2574-7258.000041

Nelsen, M. P., Lucking, R., Boyce, C. K., Lumbsch, H. T., and Ree, R. H. (2019). No support for the emergence of lichens prior to the evolution of vascular plants. *Geobiology* 18, 3–13. doi: 10.1111/gbi.12369

Nelsen, M. P., Lucking, R., Boyce, C. K., Lumbsch, H. T., and Ree, R. H. (2020). The macroevolutionary dynamics of symbiotic and phenotypic diversification in lichens. *Proc. Natl. Acad. Sci. U.S.A.* 117, 21495–21503. doi: 10.1073/pnas.2001913117

Norsigian, C. J., Pusarla, N., Mcconn, J. L., Yurkovich, J. T., Drager, A., Palsson, B. O., et al. (2020). BiGG models 2020: multi-strain genome-scale models and expansion across the phylogenetic tree. *Nucleic Acids Res.* 48, D402–D406.

Oldroyd, G. E. D. (2013). Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat. Rev. Microbiol.* 11, 252–263. doi: 10.1038/nrmicro2990

Onut-Brannstrom, I., Benjamin, M., Scofield, D. G., Heidmarsson, S., Andersson, M. G. I., Lindstrom, E. S., et al. (2018). Sharing of photobionts in sympatric populations of *Thamnolia* and *Cetraria* lichens: evidence from high-throughput sequencing. *Sci. Rep.* 8:4406.

Orth, J. D., and Palsson, B. (2012). Gap-filling analysis of the iJO1366 *Escherichia coli* metabolic network reconstruction for discovery of metabolic functions. *BMC Syst. Biol.* 6:30. doi: 10.1186/1752-0509-6-30

Ott, S., Krieg, T., Spanier, U., and Schieleit, P. (2000). Phytohormones in lichens with emphasis on ethylene biosynthesis and functional aspects on lichen symbiosis. *Phyton Annale. Rei Bot.* 40, 83–94.

Pabinger, S., Snajder, R., Hardiman, T., Willi, M., Dander, A., and Trajanoski, Z. (2014). MEMOSys 2.0: an update of the bioinformatics database for genome-scale models and genomic data. *Datab. J. Biol. Datab. Curat.* 2014:bau004.

Palmqvist, K. (1995). Uptake and fixation of CO_2 in lichen photobionts. *Symbiosis* 18, 95–109.

Palmqvist, K., Dahlman, L., Jonsson, A., and Nash, T. H. (2008). "The carbon economy of lichens," in *Lichen Biology*, 2nd Edn, ed. T. H. Nash (Cambridge: Cambridge University Press), 182–215. doi: 10.1017/cbo9780511790478.011

Papin, J. A., and Palsson, B. O. (2004). The JAK-STAT signaling network in the human B-cell: an extreme signaling pathway analysis. *Biophys. J.* 87, 37–46. doi: 10.1529/biophysj.103.029884

Park, S. Y., Choi, J., Kim, J. A., Jeong, M. H., Kim, S., Lee, Y. H., et al. (2013a). Draft genome sequence of *Cladonia macilenta* KoLRI003786, a lichen-forming fungus producing biruloquinone. *Genome Announc.* 1:e0695-13.

Park, S. Y., Choi, J., Kim, J. A., Yu, N. H., Kim, S., Kondratyuk, S. Y., et al. (2013b). Draft genome sequence of lichen-forming fungus *Caloplaca flavorubescens* Strain KoLRI002931. *Genome Announc.* 1:e0678-13.

Park, S. Y., Choi, J., Lee, G. W., Kim, J. A., Oh, S. O., Jeong, M. H., et al. (2014a). Draft genome sequence of lichen-forming fungus *Cladonia metacorallifera* Strain KoLRI002260. *Genome Announc.* 2:e01065-13.

Park, S. Y., Choi, J., Lee, G. W., Park, C. H., Kim, J. A., Oh, S. O., et al. (2014b). Draft genome sequence of *Endocarpon pusillum* strain KoLRILF000583. *Genome Announc.* 2:e0452-14.

Perezurria, E., Avalos, A., Guzman, G., and Vicente, C. (1993). Urease production and secretion by 3 antarctic lichen species. *Endocytobios. Cell Res.* 9, 239–243.

Perezurria, E., and Vicente, C. (1989). Purification and some properties of a secreted urease from *Evernia prunastri* thallus. *J. Plant Physiol.* 133, 692–695. doi: 10.1016/s0176-1617(89)80074-4

Perezurria, E., Vicente, C., and Filho, L. X. (1989). Screening of urease production and secretion by 7 species of finnish lichens. *Biochem. Syst. Ecol.* 17, 359–363. doi: 10.1016/0305-1978(89)90048-3

Petit, P. (1982). Phytolectins from the nitrogen-fixing lichen *Peltigera horizontalis* - the binding pattern of primary-protein extract. *New Phytol.* 91, 705–710. doi: 10.1111/j.1469-8137.1982.tb03349.x

Petit, P., Lallemant, R., and Savoye, D. (1983). Purified phytolectin from the lichen *Peltigera canina var canina* which binds to the phycobiont cell-walls and its use as cytochemical marker insitu. *New Phytol.* 94, 103–110. doi: 10.1111/j.1469-8137.1983.tb02726.x

Petrini, O., Hake, U., and Dreyfuss, M. M. (1990). An analysis of fungal communities isolated from fruticose lichens. *Mycologia* 82, 444–451. doi: 10.2307/3760015

Pfau, T., Christian, N., Masakapalli, S. K., Sweetlove, L. J., Poolman, M. G., and Ebenhoh, O. (2018). The intertwined metabolism during symbiotic nitrogen fixation elucidated by metabolic modelling. *Sci. Rep.* 8:12504.

Pharkya, P., Burgard, A. P., and Maranas, C. D. (2004). OptStrain: a computational framework for redesign of microbial production systems. *Genome Res.* 14, 2367–2376. doi: 10.1101/gr.2872004

Piercey-Normore, M. D., and Athukorala, S. N. P. (2017). Interface between fungi and green algae in lichen associations. *Botany* 95, 1005–1014. doi: 10.1139/cjb-2017-0037

Poole, P., Ramachandran, V., and Terpolilli, J. (2018). Rhizobia: from saprophytes to endosymbionts. *Nat. Rev. Microbiol.* 16, 291–303. doi: 10.1038/nrmicro.2017.171

Porada, P., Poschl, U., Kleidon, A., Beer, C., and Weber, B. (2017). Estimating global nitrous oxide emissions by lichens and bryophytes with a process-based productivity model. *Biogeosciences* 14, 1593–1602. doi: 10.5194/bg-14-1593-2017

Porada, P., Weber, B., Elbert, W., Poschl, U., and Kleidon, A. (2013). Estimating global carbon uptake by lichens and bryophytes with a process-based model. *Biogeosciences* 10, 6989–7033. doi: 10.5194/bg-10-6989-2013

Porada, P., Weber, B., Elbert, W., Poschl, U., and Kleidon, A. (2014). Estimating impacts of lichens and bryophytes on global biogeochemical cycles. *Glob. Biogeochem. Cycles* 28, 71–85. doi: 10.1002/2013gb004705

Price, N. D., Reed, J. L., and Palsson, B. O. (2004). Genome-scale models of microbial cells: evaluating the consequences of constraints. *Nat. Rev. Microbiol.* 2, 886–897. doi: 10.1038/nrmicro1023

Prieto, M., and Wedin, M. (2013). Dating the diversification of the major lineages of ascomycota (Fungi). *PLoS One* 8:e65576. doi: 10.1371/journal.pone.0065576

Ranganathan, S., Suthers, P. F., and Maranas, C. D. (2010). OptForce: an optimization procedure for identifying all genetic manipulations leading to targeted overproductions. *PLoS Computat. Biol.* 6:e1000744. doi: 10.1371/journal.pcbi.1000744

Richardson, D. H., Hill, D. J., and Smith, D. C. (1968). Lichen physiology XI. Role of alga in determining pattern of carbohydrate movement between lichen symbionts. *New Phytol.* 67, 469–486. doi: 10.1111/j.1469-8137.1968.tb05476.x

Rocha, I., Maia, P., Evangelista, P., Vilaca, P., Soares, S., Pinto, J. P., et al. (2010). OptFlux: an open-source software platform for in silico metabolic engineering. *BMC Syst. Biol.* 4:45. doi: 10.1186/1752-0509-4-45

Rodriguez, M., and Vicente, C. (1991). Water status and urease secretion from 2 ecotypes of *Xanthoria parietina*. *Symbiosis* 11, 255–262.

Rother, M., Munzner, U., Thieme, S., and Krantz, M. (2013). Information content and scalability in signal transduction network reconstruction formats. *Mol. Biosyst.* 9, 1993–2004. doi: 10.1039/c3mb00005b

Sacristán, M., Vivas, M., Millanes, A. M., Fontaniella, B., Vicente, C., and Legaz, M. E. (2007). "The recognition pattern of green algae by lichenized fungi can be extended to lichens containing a cyanobacterium as photobiont," in *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, Vol. 1, ed. A. M. éndez-Vilas (Cham: Springer), 213–219.

Sadowsky, A., and Ott, S. (2016). Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* photosystem II in relation to lichen pigmentation. *Polar Biol.* 39, 139–151. doi: 10.1007/s00300-015-1677-0

Santos-Merino, M., Singh, A. K., and Ducat, D. C. (2019). New applications of synthetic biology tools for cyanobacterial metabolic engineering. *Front. Bioeng. Biotechnol.* 7:33. doi: 10.3389/fbioe.2019.00033

Schoch, C. L., Sung, G. H., Lopez-Giraldez, F., Townsend, J. P., Miadlikowska, J., Hofstetter, V., et al. (2009). The ascomycota tree of life: a phylum-wide phylogeny clarifies the origin and evolution of fundamental reproductive and ecological traits. *Syst. Biol.* 58, 224–239.

Schwartzman, D. W. (2010). Was the origin of the lichen symbiosis triggered by declining atmospheric carbon dioxide levels? *Biol. Lichens Symbios.* 105, 191–196.

Schwechheimer, S. K., Becker, J., and Wittmann, C. (2018). Towards better understanding of industrial cell factories: novel approaches for (13)C metabolic flux analysis in complex nutrient environments. *Curr. Opin. Biotechnol.* 54, 128–137. doi: 10.1016/j.copbio.2018.07.001

Sigurbjornsdottir, M. A., Andresson, O. S., and Vilhelmsson, O. (2016). Nutrient scavenging activity and antagonistic factors of non-photobiont lichen-associated bacteria: a review. *World J. Microbiol. Biotechnol.* 32:68.

Smith, D., Muscatine, L., and Lewis, D. (1969). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biol. Rev.* 44, 17–85. doi: 10.1111/j.1469-185x.1969.tb00821.x

Smith, D. C., and Drew, E. A. (1965). Studies in physiology of lichens v. translocation from algal layer to medulla in *Peltigera polydactyla*. *New Phytol.* 64, 195–200. doi: 10.1111/j.1469-8137.1965.tb05390.x

Smith, H. B., Dal Grande, F., Muggia, L., Keuler, R., Divakar, P. K., Grewe, F., et al. (2020). Metagenomic data reveal diverse fungal and algal communities associated with the lichen symbiosis. *Symbiosis* 82, 133–147. doi: 10.1007/s13199-020-00699-4

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M. C., et al. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* 353, 488–492. doi: 10.1126/science.aaf8287

Stolyar, S., Van Dien, S., Hillesland, K. L., Pinel, N., Lie, T. J., Leigh, J. A., et al. (2007). Metabolic modeling of a mutualistic microbial community. *Mol. Syst. Biol.* 3:92. doi: 10.1038/msb4100131

Taffs, R., Aston, J. E., Brileya, K., Jay, Z., Klatt, C. G., Mcglynn, S., et al. (2009). In silico approaches to study mass and energy flows in microbial consortia: a syntrophic case study. *BMC Syst. Biol.* 3:114. doi: 10.1186/1752-0509-3-114

Tagirdzhanova, G., Saary, P., Tingley, J. P., Diaz-Escandon, D., Abbott, D. W., Finn, R. D., et al. (2021). Predicted input of uncultured fungal symbionts to a lichen symbiosis from metagenome-assembled genomes. *Genome Biol. Evol.* 2021:evab047.

ten Veldhuis, M. C., Ananyev, G., and Dismukes, G. C. (2020). Symbiosis extended: exchange of photosynthetic O-2 and fungal-respired CO_2 mutually power metabolism of lichen symbionts. *Photosynthes. Res.* 143, 287–299. doi: 10.1007/s11120-019-00702-0

Terfve, C., and Saez-Rodriguez, J. (2012). Modeling signaling networks using high-throughput phospho-proteomics. *Adv. Exp. Med. Biol.* 736, 19–57. doi: 10.1007/978-1-4419-7210-1_2

Tuovinen, V., Ekman, S., Thor, G., Vanderpool, D., Spribille, T., and Johannesson, H. (2019). Two basidiomycete fungi in the cortex of wolf lichens. *Curr. Biol.* 29, 476–483. doi: 10.1016/j.cub.2018.12.022

Vardi, L., Ruppin, E., and Sharan, R. (2012). A linearized constraint-based approach for modeling signaling networks. *J. Comput. Biol.* 19, 232–240. doi: 10.1089/cmb.2011.0277

Varma, A., and Palsson, B. O. (1994). Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type *Escherichia coli* W3110. *Appl. Environ. Microbiol.* 60, 3724–3731. doi: 10.1128/aem.60.10.3724-3731.1994

Venkateshwaran, M., Volkening, J. D., Sussman, M. R., and Ane, J. M. (2013). Symbiosis and the social network of higher plants. *Curr. Opin. Plant Biol.* 16, 118–127. doi: 10.1016/j.pbi.2012.11.007

Vicente, C., and Perezurria, E. (1989). Production and secretion of urease by *Evernia prunastri* thallus and its symbionts. *Endocytobios. Cell Res.* 6, 87–97.

Vivas, M., Sacristan, M., Legaz, M. E., and Vicente, C. (2010). The cell recognition model in chlorolichens involving a fungal Lectin binding to an algal ligand can be extended to cyanolichens. *Plant Biol.* 12, 615–621.

Wang, Y., Yuan, X., Chen, L., Wang, X., and Li, C. (2018). Draft genome sequence of the lichen-forming fungus *Ramalina intermedia* strain YAF0013. *Genome Announc.* 6:e0478-18.

Wang, Y., Zhang, X., Zhou, Q., Zhang, X., and Wei, J. (2015). Comparative transcriptome analysis of the lichen-forming fungus *Endocarpon pusillum* elucidates its drought adaptation mechanisms. *Sci. China Life Sci.* 58, 89–100. doi: 10.1007/s11427-014-4760-9

Wei, S. S., Jian, X. X., Chen, J., Zhang, C., and Hua, Q. (2017). Reconstruction of genome-scale metabolic model of *Yarrowia lipolytica* and its application in overproduction of triacylglycerol. *Bioresourc. Bioprocess.* 4:51.

West, N. J., Parrot, D., Fayet, C., Grube, M., Tomasi, S., and Suzuki, M. T. (2018). Marine cyanolichens from different littoral zones are associated with distinct bacterial communities. *PeerJ* 6:e5208. doi: 10.7717/peerj.5208

Ye, C., Zou, W., Xu, N., and Liu, L. (2014). Metabolic model reconstruction and analysis of an artificial microbial ecosystem for vitamin C production. *J. Biotechnol.* 182–183, 61–67. doi: 10.1016/j.jbiotec.2014.04.027

Yen, J. Y., Nazem-Bokaee, H., Freedman, B. G., Athamneh, A. I. M., and Senger, R. S. (2013). Deriving metabolic engineering strategies from genome-scale modeling with flux ratio constraints. *Biotechnol. J.* 8, 581–594. doi: 10.1002/biot.201200234

Yim, H., Haselbeck, R., Niu, W., Pujol-Baxley, C., Burgard, A., Boldt, J., et al. (2011). Metabolic engineering of *Escherichia coli* for direct production of 1,4-butanediol. *Nat. Chem. Biol.* 7, 445–452.

Zhang, X., and Reed, J. L. (2014). Adaptive evolution of synthetic cooperating communities improves growth performance. *PLoS One* 9:e108297. doi: 10.1371/journal.pone.0108297

Zhuang, K., Izallalen, M., Mouser, P., Richter, H., Risso, C., Mahadevan, R., et al. (2011). Genome-scale dynamic modeling of the competition between *Rhodoferax* and *Geobacter* in anoxic subsurface environments. *ISME J.* 5, 305–316. doi: 10.1038/ismej.2010.117

Zomorrodi, A. R., and Maranas, C. D. (2012). OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities. *PLoS Computat. Biol.* 8:e1002363. doi: 10.1371/journal.pcbi.1002363

Zomorrodi, A. R., and Segre, D. (2016). Synthetic ecology of microbes: mathematical models and applications. *J. Mol. Biol.* 428, 837–861. doi: 10.1016/j.jmb.2015.10.019

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Microbiology

ORIGINAL RESEARCH
published: 06 August 2021
doi: 10.3389/fmicb.2021.668274

Disentangling the Possible Drivers of *Indri indri* Microbiome: A Threatened Lemur Species of Madagascar

Federico Correa[1], Valeria Torti[2], Caterina Spiezio[3], Alice Checcucci[1], Monica Modesto[1], Luigimaria Borruso[4], Luciano Cavani[1], Tanja Mimmo[4], Stefano Cesco[4], Diana Luise[1], Rose M. Randrianarison[5,6], Marco Gamba[2], Nianja J. Rarojoson[7], Maurizio Sanguinetti[8,9], Maura Di Vito[8], Francesca Bugli[8,9], Paola Mattarelli[1]*, Paolo Trevisi[1], Cristina Giacoma[2] and Camillo Sandri[1,3]*

[1] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy, [2] Department of Life Sciences and Systems Biology, University of Torino, Turin, Italy, [3] Department of Animal Health Care and Management, Parco Natura Viva – Garda Zoological Park, Verona, Italy, [4] Faculty of Science and Technology, Free University of Bolzano-Bozen, Bolzano, Italy, [5] Groupe d'Étude et de Recherche sur les Primates de Madagascar, Antananarivo, Madagascar, [6] Mention d'Anthropobiologie et de Deiveloppement Durable, Université de Antananarivo, Antananarivo, Madagascar, [7] Laboratoire de Pédologie, FOFIFA à Tsimbazaza, Antananarivo, Madagascar, [8] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy, [9] Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Research on the gut microbiome may help with increasing our understanding of primate health with species' ecology, evolution, and behavior. In particular, microbiome-related information has the potential to clarify ecology issues, providing knowledge in support of wild primates conservation and their associated habitats. Indri (*Indri indri*) is the largest extant living lemur of Madagascar. This species is classified as "critically endangered" by the IUCN Red List of Threatened Species, representing one of the world's 25 most endangered primates. Indris diet is mainly folivorous, but these primates frequently and voluntarily engage in geophagy. Indris have never been successfully bred under human care, suggesting that some behavioral and/or ecological factors are still not considered from the *ex situ* conservation protocols. Here, we explored gut microbiome composition of 18 indris belonging to 5 different family groups. The most represented phyla were Proteobacteria 40.1 ± 9.5%, Bacteroidetes 28.7 ± 2.8%, Synergistetes 16.7 ± 4.5%, and Firmicutes 11.1 ± 1.9%. Further, our results revealed that bacterial alpha and beta diversity were influenced by indri family group and sex. In addition, we investigated the chemical composition of geophagic soil to explore the possible ecological value of soil as a nutrient supply. The quite acidic pH and high levels of secondary oxide-hydroxides of the soils could play a role in the folivorous diet's gut detoxification activity. In addition, the high contents of iron and manganese found the soils could act as micronutrients in the indris' diet. Nevertheless, the concentration of a few elements (i.e., calcium, sulfur, boron, nickel, sodium, and chromium) was higher in non-geophagic than in geophagic soils. In conclusion, the data presented herein provide a baseline for outlining some possible drivers responsible for the gut microbiome diversity in indris, thus laying the foundations for developing further strategies involved in indris' conservation.

Keywords: gut microbiome, soil quality, non-human primate, animal ecology, endangered species, geophagy, forest ecology

OPEN ACCESS

Edited by:
Nathalie Connil,
Université de Rouen, France

Reviewed by:
Christopher Gaulke,
University of Illinois at Urbana-Champaign, United States
Huan Li,
Lanzhou University, China

****Correspondence:***
Luigimaria Borruso
luigimaria.borruso@unibz.it
Paola Mattarelli
paola.mattarelli@unibo.it

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: *15 February 2021*
Accepted: *08 July 2021*
Published: *06 August 2021*

Citation:
Correa F, Torti V, Spiezio C, Checcucci A, Modesto M, Borruso L, Cavani L, Mimmo T, Cesco S, Luise D, Randrianarison RM, Gamba M, Rarojoson NJ, Sanguinetti M, Di Vito M, Bugli F, Mattarelli P, Trevisi P, Giacoma C and Sandri C (2021) Disentangling the Possible Drivers of Indri indri Microbiome: A Threatened Lemur Species of Madagascar. Front. Microbiol. 12:668274. doi: 10.3389/fmicb.2021.668274

INTRODUCTION

Studies on human and animal microbiome have provided compelling evidence that gut microbial diversity is fundamental in shaping metabolic and regulatory networks involved in the maintenance of host healthy status, as well as in a spectrum of disease states (Shreiner et al., 2015; Sandri et al., 2020). Indeed, the mammalian gut microbiome plays a crucial role in host physiology, supporting vitamin synthesis, helping in complex carbohydrates digestion, toxins metabolism, pathogens antagonism, and immune system modulation (Cresci and Bawden, 2015). Factors influencing the differences in mammalian gut microbiome are debated: host behaviors and environments, biogeography, and host genetic effects (e.g., gastrointestinal tract morphology) are of great importance (Lankau et al., 2012; Moeller et al., 2013; Amato et al., 2016). Previous studies showed that frequent social networks are positively associated with high similarity in gut microbial diversity (Tung et al., 2015; Perofsky et al., 2019). Vertical transmission from parent to offspring is the first driver for gut microbiome development, but horizontal transmission from the environment provides a crucial microbial colonization route. Even if microbial transmission due to sociality has traditionally been viewed as a risk for pathogen exposure, it may also be essential to host health. Therefore, it can avoid bottleneck-induced extinctions that could occur when the transmission of microorganisms is strict from parent to offspring. Indeed, it can allow the acquisition of beneficial microbes, particularly those that might not be gained through vertical transmission (Lombardo, 2008; Amaral et al., 2017). Moeller et al. (2013) underlined that gut microbial populations' social inheritance might be fundamental for preserving microbial diversity over evolutionary time scales.

The lemurs harbored species-specific and/or populations specific microbiomes, which are mainly influenced by their dietary specificity, even on a seasonal basis (Fogel, 2015; Greene et al., 2020). Globally, host habitat is one of the most important factors for gut microbiome modulation, and recently, increasing attention has been devoted to the soil. Indeed, a recent study (Grieneisen et al., 2019) on the gut microbiome of terrestrially living baboons showed that bare soil exceeds 15 times the predictive ability of host genetics in shaping the gut microbiome. Studies in mice (Li et al., 2016; Zhou et al., 2018) confirmed that the effect of soil on gut microbiome composition is comparable to that exerted by diet. Therefore, these studies suggest that contact/ingestion of soil components is beneficial for a healthy gut microbiome.

Indri indri is the largest extant living lemur (**Figure 1** and **Supplementary Video 1**). It is mainly arboreal and is the only lemur that communicates using songs. Indris songs mediate both intra- and inter-group communication (Torti et al., 2013) and relay information regarding individual features (i.e., sex and age) (De Gregorio et al., 2019, 2021). This species has never successfully been kept in a controlled environment and it is considered one of the Malagasy most critically endangered lemurs according to the IUCN Red List of Threatened Species (King et al., 2020), representing one of the world's 25 most endangered primates (Torti et al., 2019). This species is also listed in Appendix I of CITES (Heinen and Mehta, 1999). Indris are territorial, socially primates living in small family groups (Pollock, 1979; Bonadonna et al., 2019), generally consisting of an adult male and female with their related offspring (2–6 individuals) (Torti et al., 2013; Gamba et al., 2016).

Non-human primates are characterized by many dietary specializations (Campbell, 2017). In particular, the ability to consume leaves is typical of new world monkeys (e.g., howler monkeys), old world monkeys (e.g., colobines), apes (e.g., gorillas), and also prosimians (e.g., indris, bamboo lemurs, and sportive lemurs). Indri is the most specialized folivorous among lemurs and, as such, has the highest degree of morphological specialization for leaves' consumption and digestion. Leaves contain carbohydrates, including cellulose and hemicellulose, and secondary metabolites, including toxic ones such as tannins and phenolics (Norconk et al., 2009). Indris are characterized by the typical morphology and anatomical specializations of folivorous primates, such as hypertrophic salivary glands, voluminous stomachs, sacculated caeca, and looped colons that facilitate efficient fermentation of leaf matter (Greene et al., 2020). The species shows a preference for immature leaves (72%) with a reduced emphasis on fruit seeds/whole fruits (16%) and flowers (7%) (Powzyk, 1997). Leaves and fruit seeds could contain toxic compounds varying in percentage depending on the season, maturity, etc. (Pebsworth et al., 2019). In addition, indris perform geophagy by consuming soil intentionally (Britt et al., 2002; Borruso et al., 2021). Some evidence suggests that geophagy is an adaptive behavior to protect from ingested toxic compounds and mineral supplementation as it facilitates consumption of plants binding toxic plant secondary compounds (PSCs) (Pebsworth et al., 2019). As a result of metabolic activity, plants with relevant antioxidant properties produce primary and secondary compounds. Nevertheless, several metabolites are universally distributed in many plant species; some are unique to individual plant cultivars and fill essential functions (Geilfus, 2019).

Studies regarding geophagy across non-human primates revealed that they eat items high in PSCs. Furthermore, they consume soil more often than sympatric populations, suggesting a decrease in gastrointestinal distress caused by PSCs. Geophagy can help the utilization of dietary resources high in PSCs, expanding the range of dietary components (Overdorff, 1993; Bocian, 1997; Powzyk and Mowry, 2003; Dew, 2005; Pebsworth et al., 2019). In addition to dietary toxins, mineral deficiencies, diarrhea, and altered gut pH were reported to cause geophagy (Krishnamani and Mahaney, 2000; Ferrari et al., 2008; Young et al., 2011). As these processes are not necessarily mutually exclusive, geophagy can play different functions, such as rare element supplementation, detoxification, and protection (Davies and Baillie, 1988; Huffman et al., 1997; Krishnamani and Mahaney, 2000; Pebsworth et al., 2019). Interestingly, geophagic soil could also be a reservoir for microbial species affecting indris' gut microbiome (Borruso et al., 2021). The highly specialized diet, physiology, and morphology of indri's gut may contribute to their susceptibility in a human-controlled environment. This is in analogy for what has been described for other endangered folivorous primate whose breeding was unsuccessful (Hale et al., 2018, 2019).

FIGURE 1 | Distribution of the territories occupied by the indri family groups sampled (main figure, **d**) and composition of each single group. Both adult and youngster indris, both sexes, feed on leaves **(a,b)** and perform geophagic behavior, eating soil in specific sites **(c)**.

Understanding the drivers of the gut indris microbiome and their relationship to the soil could be essential for planning strategies to conserve, monitor, and promote their health. Whether the gut microbiome facilitates the use of these hard-to-digest food items, it would be crucial to characterize the bacterial gut microbiome's shaping factors. Therefore, our work aimed to analyze: (1) the gut microbiome composition of wild indris belonging to five different familiar groups in Maromizaha, eastern Madagascar; (2) the potential drivers affecting host-microbial diversity, including sex, family group, and age class (3) the chemical composition of geophagic and non-geophagic soil, to unravel the possible adaptive ecological value as nutrient supply.

MATERIALS AND METHODS

Fecal and Soil Samples Collection

Fecal and soil samples were collected in a very narrow temporal window (between December 4th and 6th, 2018) to avoid confounding potential seasonal effects. Individual fecal samples were obtained from 18 indris (fecal material) belonging to 5 different social family groups (**Table 1** and **Figure 1**) (latitude 18°57′S and 19°00′S, longitude 48°26′E and 48°31′E, Madagascar). The samples were collected immediately after defecation, when only one animal, recognized using natural marks (Torti et al., 2013), was present. This procedure was essential to avoid individual misidentification during the sampling process (Bonadonna et al., 2019). Approximately 5 g of fecal samples were collected from each of the 18 individuals (**Table 1**) following the procedure described in Borruso et al. (2021).

Each sample was classified according to the following categories: sex, family group, and age class (Adult > 6 years and Juvenile < 6 years) (**Table 1** and **Figure 1**). In addition, soil samples were collected from seven geophagic and seven non-geophagic (control) sites. All the geophagic sites were at the bases of trees uprooted by wind or rainfall, with the lower soil horizons exposed. We noted the location (waypoint) during soil-eating events, and we followed behaviors before and after the geophagy event. Control sites were selected from areas with the same characteristics (slope, vegetation, etc.) and located at less than 20 m from geophagic sites after removing the superficial soil layer to sample the same soil layer of the geophagic sites. The presence of the superficial layer together with debris proved that the groups have never used the control locations to consume soil. All samples were maintained in a portable cooler with ice packs before arrival at the lab.

Soil Characterization

Soil samples were air-dried, milled, and sieved at 2 mm for soil analysis in agreement with Soil Science Society of America (SSSA) methods (Sparks et al., 1996). Briefly, pH was determined in water (1:2.5, m/V), total carbon (C), and total nitrogen (N) using an elemental analyzer (CHNS-O Elemental Analyzer 1110, Thermo Scientific GmbH, Germany). Pseudo total element concentrations were determined after acid mineralization with aqua regia and hydrogen peroxide in an Ethos TC microwave lab station (Milestone, Italy) using an inductively coupled plasma

TABLE 1 | Description of each indri individual including sex, class age (Adult, >6 years; Juvenile, <6 years; NA*, not available) and family group, bacterial observed richness, and bacterial Shannon index values.

Samples ID	Sex	Class age	Family group	Observed richness	Shannon
L	Female	Adult	1MZ	44	2.87
M	Female	Juvenile	1MZ	41	2.55
O2	Male	Adult	1MZ	43	2.11
R	Female	Juvenile	2MZ	44	2.55
N2	NA*	Juvenile	2MZ	44	2.76
P	Male	Adult	2MZ	46	2.67
Q	Female	Adult	2MZ	47	2.90
G	Female	Juvenile	3MZ	35	2.55
H	Male	Juvenile	3MZ	37	1.95
I	Female	Adult	3MZ	35	2.41
C	Female	Adult	4MZ	38	2.70
E2	Male	Adult	4MZ	45	2.40
K	Female	Adult	6MZ	47	2.75
S	Male	Adult	6MZ	39	2.59
A2	Female	Adult	8MZ	55	2.94
B2	Male	Juvenile	8MZ	58	2.69
D2	Male	Adult	8MZ	55	2.62
F2	Male	Adult	8MZ	58	2.89

optical emission spectrometer (ICP-OES, Ametek Spectro, Arcos, Germany). Available metals were determined by ICP-OES after extraction for 2 h with 1 mol L^{-1} ammonium nitrate (NH_4NO_3) solution (1:2.5, m/V).

DNA Extraction and NGS Sequencing

Total DNA was isolated and extracted from indri fecal samples with DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) with slight modifications. Briefly, the lysis step was enhanced using a bead-beater (FastPrep 24G, MP Biomedicals, France), in which the "Powerbead" tubes containing the pellets (250 mg of fecal sample) and 800 μL of CD1 solution were subjected to two cycles of bead-beating at a speed of 4 m/s for 60 s with 45 s pause between cycles. The final elution volume was 100 μL in water. DNA was checked for purity (absorbance ratio 260/280 and 260/230) by spectrophotometry using NanoDrop (Fisher Scientific, 13 Schwerte, Germany) and quantified with the fluorometer Qubit® 2.0 (Invitrogen, Italy). Next, the DNA concentration of each sample was normalized to 1 ng μL^{-1}. The PCR was performed amplifying the V3–V4 region of the 16S rRNA gene (∼460 bp) with the primers Pro341F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG-3′) and Pro805R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC-3′) (Takahashi et al., 2014), using Platinum Taq DNA Polymerase High Fidelity (Thermo Fisher Scientific, Italy). The thermal cycling protocol consisted of the following conditions: initial denaturation at 94°C for 1′, followed by 25 cycles of denaturation at 94°C for 30″, annealing at 55°C for 30″, and extension 65°C for 45″, ending with 1 cycle at 68°C for 7′. Further, PCR samples were sent to BMR-Genomics Ltd., that according to the standard protocols carried out the other steps of the workflow and finally sequenced the libraries using a MiSq platform (300 × 2 bp) (Illumina Inc., San Diego, CA, United States).

The raw reads obtained are publicly available at the Sequence Read Archive (SRA) under the accession number: PRJNA701813.

Bioinformatic Analysis

Sequencing data analysis was performed using DADA2 1.14.0 (Callahan et al., 2016) running on R 3.6.2 (R Core Team, 2021). The forward and reverse reads were trimmed to remove low-quality nucleotides and primers sequences using the filterAndTrim function with the following parameters: truncLen = c(290, 220), trimLeft = c(50, 55), and maxN = 0, truncQ = 2. The amplicon sequence variants were inferred using the DADA2 core sample inference algorithm with default parameters. Forward and reverse reads were merged and reads with mismatches were removed. Chimeras were identified using the removeBimeraDenovo function and removed. Further, the SILVA database release 132 (Quast et al., 2013) was used for the taxonomic assignment. Finally, the AVSs table was rarefied to 25,181 reads per sample.

Statistical Analysis

Statistical analyses were carried out using Phyloseq 1.32 (McMurdie and Holmes, 2013) and Vegan 2.5 (Dixon, 2003) packages. The differences between the geophagic and non-geophagic control soil composition were tested via Mann–Whitney *U*-test. Alpha diversity was explored considering the Shannon index and Observed richness calculated from the rarefied AVSs table (25,181 reads). Both indices values were checked for normality using the Shapiro–Wilk test. The possible effects of sex, age class, and family group on alpha diversity indices were evaluated with a Linear Model (ANOVA type III). Beta dispersion was calculated to test if the groups, classified

FIGURE 2 | Visualization of **(A)** a research guide recording the location of a geophagic site (GPS waypoint); **(B)** an indri (*Indri indri*) performing geophagic behavior, eating soil in a specific site; **(C)** a geophagic site under a fallen tree; **(D)** soil horizon eaten in the geophagic site; **(E)** a control site with the upper surface untouched; and **(F)** enlargement of the soil sampled in the control site, under the surface, in the horizon normally eaten by indri. Soil is collected free of debris (grass, leaves, stones, roots).

according to sex, age class, and family group, had the same centroids and heterogeneity. Permutational multivariate analysis of variance (PERMANOVA) was applied to test the possible effect of sex, age class, and family group on the bacterial communities. In addition, the Constrained Analysis of Principal Coordinates (CAP) based on Bray Curtis was used to generate the ordination plots.

Linear discriminant analysis effect size (LEfSe) algorithm (LDA score ≥ 2 and p-value < 0.05) was applied to detect the biomarker taxa for each category (Segata et al., 2011). We excluded from the LEfSe analysis the family groups with less than three individuals (i.e., 4MZ and 6MZ).

RESULTS

Geophagy Site Characterization

Indris were observed to eat soil in sites at the bases of trees uprooted by wind and/or by rainfall, with the lower soil horizons exposed (**Figure 2** and **Supplementary Video 1**). Geophagic and non-geophagic soil samples were characterized by an acidic pH and rich content in total C and N. With regards to the pseudo-total metals, soil samples showed poor content in Calcium (Ca), Phosphorus (P), Sulfur (S), and higher content in Iron (Fe). Manganese (Mn) and Fe were the most extractable in ammonium nitrate in the case of available metals (**Supplementary Table 1**).

Some differences were found between geophagic and non-geophagic sites. Specifically, the concentration of Ca, S, sodium (Na), chromium (Cr), boron (B), and available Nickel (Ni) resulted in being higher in non-geophagic than in geophagic soil samples (p-value < 0.01) (**Figure 3**). On the other hand, for all the other parameters, including pH, total C, total N, the remaining pseudo-total elements, and metals extractable in ammonium nitrate, no statistically significant differences were observed (**Supplementary Table 1**).

Bacterial Taxonomic Community Composition

After quality checking and filtering, 645,297 reads (including non-bacterial reads) were generated from the MiSeq run. The reads assigned as Bacteria were 616,269 resulting in 131 amplicon sequence variants (**Supplementary Tables 2, 3**).

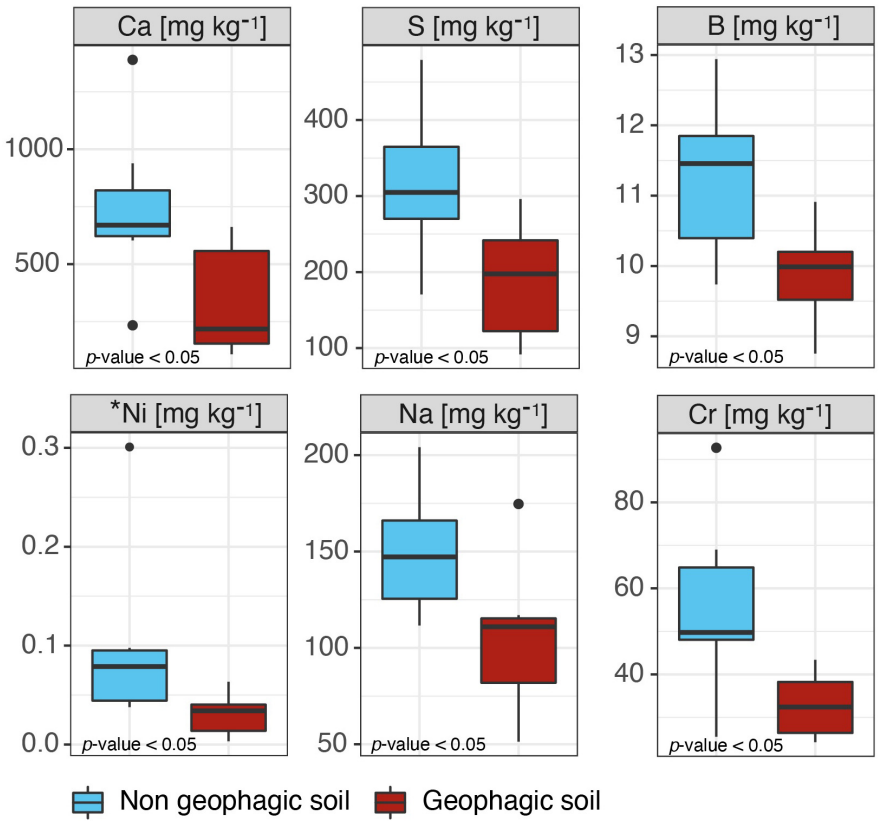


FIGURE 3 | Boxplots representing the chemical parameters resulted statistically different (p-value < 0.05) between the geophagic and non-geophagic soils. *Available metal.

Rarefaction curves showed that all the samples nearly reached the plateau (**Supplementary Figure 1**). All the samples were identified at phylum level: Proteobacteria 40.1 ± 9.5%, Bacteroidetes 28.7 ± 2.8%, Synergistetes 16.7 ± 4.5%, Firmicutes 11.1 ± 1.9%, Verrucomicrobia 2.0 ± 1.2%, Actinobacteria 1.2 ± 0.6%, and Cyanobacteria 0.2 ± 0.3% (**Figure 4A**). At family level the most abundant groups were: Succinivibrionaceae 39.6 ± 11.6%, Prevotellaceae 26.4 ± 3.2%, Synergistaceae 16.7 ± 4.5%, Ruminococcaceae 6.6 ± 2.7%, Acidaminococcaceae 3.3 ± 1.2%, and Puniceicoccaceae 2.0 ± 1.2% (**Figure 4B**). At a finer taxonomic level, the prevalent genera identified were: *Anaerobiospirillum* 39.3 ± 11.9% and Prevotellaceae NK3B31 group 19.8 ± 3.8%, *Cloacibacillus* 8.2% ± 7.2%, *Ruminococcus* 1, 5.0 ± 2.8%, *Jonquetella* 4.24% ± 2.8%, *Pyramidobacter* 4.0 ± 2.8%, *Phascolarctobacterium* 2.6 ± 1.2%, and *Cerasicoccus* 2.0 ± 1.2% (**Figure 4C**).

Effect of Family Group, Sex, and Age Class on Indri Bacterial Diversity

Considering all the individuals, the mean Shannon diversity was 2.61 ± 0.26, whereas the Observed richness's value was 45 ± 7. The values for each individual are reported in **Table 1**. Shannon diversity and Observed richness data resulted to be normally distributed (Shapiro–Wilk normality test: Observed richness, $W = 0.92$, p-value = 0.14; Shannon diversity, $W = 0.91$, p-value = 0.07).

The Linear Model revealed that Observed richness was influenced by family group ($F = 17.69$, p-value = 0.0002), whereas Shannon diversity was affected by both family group ($F = 4.37$, p-value = 0.02) and sex ($F = 10.02$, p-value = 0.01). In particular, females showed higher alpha diversity values if compared to males. Finally, no significant effect was detected according to the age class (**Supplementary Table 4**).

Beta-dispersion of bacterial communities revealed that the samples had homogeneous dispersion (Sex, $F = 1.24$ and p-value = 0.31; family group, $F = 1.21$ and p-value = 0.43; age class $F = 0.002$ and p-value = 0.98). PERMANOVA analysis showed that sex ($F = 7.43$, p-value = 0.001) and family group ($F = 7.4707$, p-value = 0.001) resulted to significantly affect the bacterial communities's beta-diversity, differently from age class ($F = 0.89$, p-value = 0.51). Further, CAP analysis, confirming the results obtained with the PERMANONVA, found that among all the tested possible drivers, sex, and family group influenced the bacterial community's structure (com ∼ family group + Sex; $F = 5.94$ p-value = 0.001) (**Figure 5**).

Linear discriminant analysis effect size algorithm found 15 ASVs biomarkers for the group 1MZ, 17 ASVs with 2MZ, 11 ASVs with 3MZ, and 25 with 8MZ (**Supplementary Table 5**). At phylotype level, Proteobacteria, mainly with the

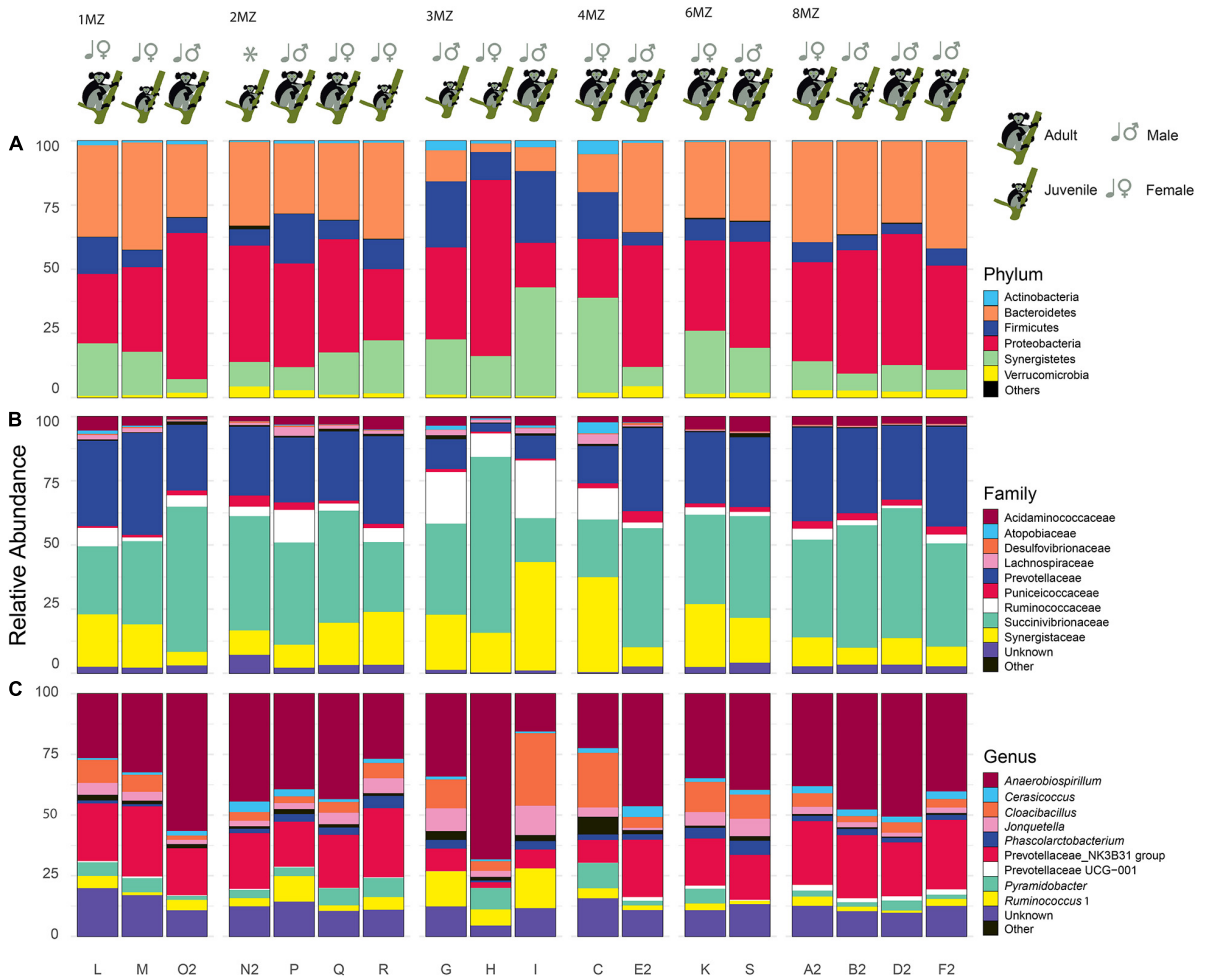


FIGURE 4 | Bar plots of each individual representing the most abundant taxa (average > 1%). Phyum **(A)**, family **(B)**, and genus **(C)**. "Unclassified" represents ASV not classified for the considered taxonomic level. The taxa with a relative average abundance < 1% are collapsed in "Others". In addition, information regarding family group, class age, and sex is reported on the top of each plot.

genus *Desulfovibrio*, characterized the group 2MZ, whereas Actinobacteria with *Atopobium* and Firmicutes with *Tyzzerella* 3 were biomarkers of 3MZ (**Figure 6A**). Further, Bacteroidetes with Prevotellaceae UCG001 and Verrucomicrobia with *Cerasicoccus* were more abundant in the group 8MZ (**Figure 6A**).

Concerning sex, four AVSs biomarkers were found for females and two AVSs males (**Supplementary Table 6**). Moreover, Firmicutes and Synergistetes with the genera *Cloacibacillus* and *Jonquetella* were more abundant in females; differently, Verrucomicrobia with the genus *Cerasicoccus* and Proteobacteria with the genus *Anaerobiospirillum* were mainly present in males (**Figure 6B**).

DISCUSSION

Indris Gut Microbiome Diversity

Although in different proportions, the most abundant phyla found in indris' gut (i.e., Proteobacteria, Bacteroides, and Firmicutes) are consistent with those found in other studies involving primates (Aivelo et al., 2016). On the other hand, the relative abundance of Proteobacteria found in our study was almost five times higher than that found in other lemurs species, such as *Lemur catta* (Umanets et al., 2018), *Eulemur rufifrons*, and *E. rubriventer* (Bennett et al., 2016; **Table 2**). Nevertheless, Greene et al. (2020) investigating wild indris' gut microbiome diversity found a higher abundance of Proteobacteria compared to the other three lemur species (i.e., *L. catta*, *E. rufifrons*, and *E. rubriventer*) (Bennett et al., 2016; Umanets et al., 2018), but still lower than what we found in our work (**Table 2**). With this regard, the high relative abundance of Proteobacteria present in our samples and found in Greene et al. (2020) could represent the typical composition of the gut microbiome of healthy individuals. Differently, in humans, an increased prevalence of Proteobacteria has been observed as a potential signature of dysbiosis (Illiano et al., 2020). Specifically, altered homeostasis, caused by environmental or host factors, such as a low-fiber diet and acute or chronic inflammation, could be a selection driver

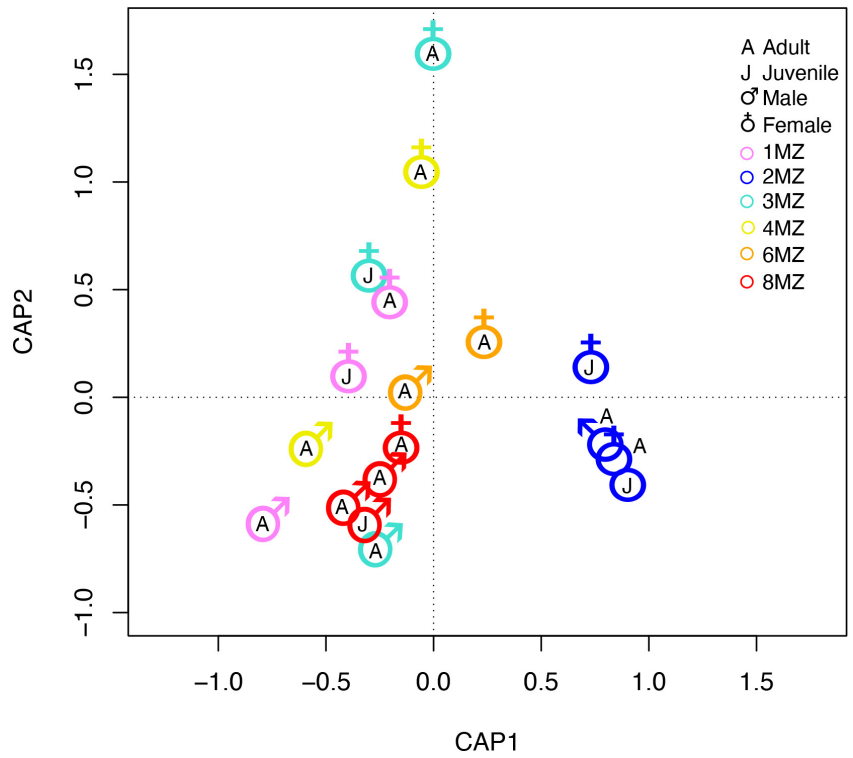


FIGURE 5 | Constrained analysis of principal coordinate ordination plot on bacterial communities of indris fecal samples.

and cause dysbiosis with an increased number of Proteobacteria in the gut. For what concerns the indris, their diet is based on fiber due to its folivores' habitus, with usual consumption of soil as integration. Plant leaves and soil could most likely be an important source of Proteobacteria; in fact, plant leaves, and soil contain about 62 and 36.5% of Proteobacteria, respectively (Shin et al., 2015). Proteobacteria could play a key role in cinnamates degradation and hydroxycinnamates and hydroxycinnamic acids utilization for energy recovery (Greene et al., 2020). Further, indris might rely primarily on Proteobacteria, and secondly on Bacteroidetes and Firmicutes (e.g., *Prevotella* and *Ruminobacter*) for fiber digestion (Biddle et al., 2013). Indeed, Firmicutes members such as Lachnospiraceae and Ruminococcaceae, with some Bacteroidetes, have known fiber fermenting abilities. Interestingly, they have been associated with the production of the appreciated colonocyte nutrient butyrate (Biddle et al., 2013; Meehan and Beiko, 2014). The presence of functionally redundant taxa might support functional stability during ordinary life and possible life disturbance (Vital et al., 2017).

Regarding the factors driving microbial diversity, this study showed the crucial role of social groups in shaping the indris microbiome for the first time. Differences among social groups may be related to feeding and social interactions like grooming, which provide close contact between subjects of the same group (Bennett et al., 2016; Raulo et al., 2018). These mechanisms were identified as relevant factors influencing the microbiome composition of baboons and chimpanzees (Degnan et al., 2012; Tung et al., 2015). A study that analyzed the dynamics of the composition of 10 wild groups in the Maromizaha NAP, comprising the groups sampled in this work, found evidence of only one immigrant female and one immigrant male out of 68 indris over 12 years (Rolle et al., 2021 in press). This very low rate of intergroup mobility limits the number of social partners that indri can have in their lives and, consequently, the intergroup transmission of microorganisms and parasites. In addition, sex was another factor that significantly influenced the microbiome alpha and beta-diversity. Particularly, the higher bacterial Shannon diversity found in females than males could be due to the sex hormones that play a crucial role in sex dimorphism (Haro et al., 2016). Moreover, females showed a higher abundance of *Cloacibacillus* and *Jonquetella*, both belonging to the novel phylum Synergistetes, that inhabits the mammalian gastrointestinal tract typically (Jumas-Bilak et al., 2007; Looft et al., 2013). Differently, males had a higher abundance of bacteria from the *Anaerobiospirillum* genus. This difference can be explained by the fact that females and males differ in nutritional and energetic demands for growth, development, and reproduction. Moreover, sex-specific traits influence the ecological structure of the gut microbiome, maintaining sex differences in physiology and behavior throughout life (Jašarević et al., 2016).

Geophagy in Indris

Typical Oxisols with a reddish color characterized geophagic and non-geophagic sampling sites. Some inherent characteristics of the Oxisols, such as the quite acidic pH, the richness of

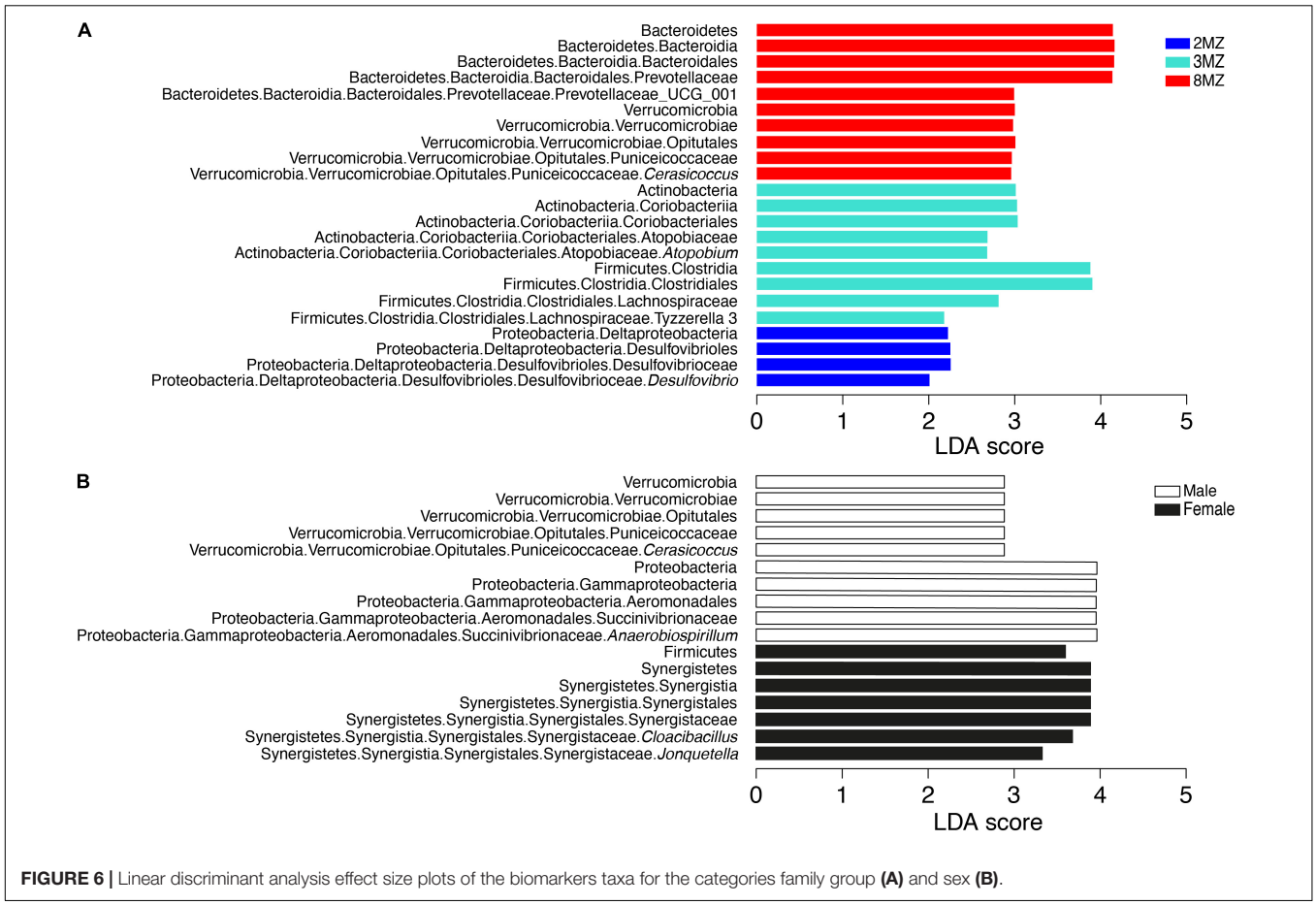


FIGURE 6 | Linear discriminant analysis effect size plots of the biomarkers taxa for the categories family group **(A)** and sex **(B)**.

TABLE 2 | Percentage of the three top bacterial phyla found in this study and other studies.

Lemurs species	References	Firmicutes (%)	Bacteroidetes (%)	Proteobacteria (%)
Lemur catta	Umanets et al., 2018	51.57 ± 0.11	15.81 ± 0.11	5.21 ± 0.11
Eulemur rufifrons and *E. rubriventer*	Bennett et al., 2016	43.3 ± 0.064	30.3 ± 0.053	7.4 ± 0.031
I. indri	Greene et al., 2020	19.70	47.70	20.50
I. indri	This study	11.1 ± 1.9	28.7 ± 2.8	40.1 ± 9.5

secondary oxide-hydroxides and highly weathered clays, seem more important for geophagy than the content in pseudo-total or available elements (Vågen et al., 2006; Borruso et al., 2021). According to the adaptive hypothesis of geophagy, the soil ingested by indri could play a crucial role in micronutrient supplementation and detoxification (i.e., adsorption functions via oxyhydroxides and clays) (Pebsworth et al., 2019). Indeed, indris are folivorous, consuming mainly immature leaves rich in potentially toxic compounds such as tannins, terpenes, and cyanogenic glycosides derived (Hemingway, 1998); thus, the geophagic soil could be involved in the plant's toxin adsorption derived from the diet (de Souza et al., 2002; Pebsworth et al., 2019).

However, the reason behind the selection of one site instead of another one remains unclear. The choice of the sites characterized by the exposition of lower soil horizons could be a strategy to limit the energy expended in obtaining soil from the intact ground. Nevertheless, some elements (i.e., Ca, S, Na, Cr, B, and available Ni) were present at lower concentrations in geophagic than in non-geophagic soil. Although we cannot directly explain these differences, they could indicate that other soil quality traits could orientate the selection of a specific soil.

In conclusion, studies on different species suggested that geophagic sites are required to maintain individual and population health (Pebsworth et al., 2019). Accordingly, preserving the geophagic sites is crucial in wildlife conservation policy.

Microbial Ecology and Indri Conservation

Microbial ecology offers valuable perspectives to investigate primate health and improve conservation efforts. Understanding the drivers affecting the microbiome associated with the host (e.g., indri) is critical for conservation biology. It is well known that the microbial gut communities profoundly affect host health,

nutrition, physiology, and immune systems (Sandri et al., 2020). For instance, our study is fundamental to document the typical composition of healthy individuals considering sex and group influence (Amato et al., 2020). Therefore, many studies have been conducted on the human microbiome where microbial biomarkers of health have been shown, such as the presence of *Faecalibacterium prausnitzii* (Manor et al., 2020). The acquisition of new information about animal gut microbiomes can help identify biomarkers for animal health. In addition, microbial gut communities are sensitive to environmental alterations and their diversity seems to be correlated with habitat quality and, thus, with possible health implications (Scotti et al., 2017). The application of gut microbiome analyses to wildlife conservation of endangered species is currently in its infancy but holds enormous potential. To date, no conservation policy or legislation includes microbiome assessments. Integrating a new understanding of the patterns of microbial diversity and early signs of impending microbial disruption offer valuable tools for informing conservation strategies and monitoring and promoting primate health (Stumpf et al., 2016). The present study represents a first insight toward understanding the overall diversity and ecology of indris microbiome in different familiar groups and a sex-dependent baseline that can be tracked over time as a component of efforts to help animal conservation.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Sequence Read Archive (SRA) BioProject ID: PRJNA701813.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because. The non-invasive methods used for fecal collections of wild indris adhere to the International Primatological Society (IPS) "Principles for the Ethical Treatment of Non-Human Primates." Field data collection protocols were reviewed and approved by Madagascar's Ministere de l'Environnement, de l'Ecologie et des Forêts (Permit 2018: N° 91/18/MEEF/SG/DGF/DSAP/SCB.Re). Field data collection protocols were also approved by Groupe d'Étude et de Recherche sur les Primates de Madagascar (GERP), the association governing research in the Maromizaha New Protected Area.

AUTHOR CONTRIBUTIONS

CSa CSp, CG, PM, PT, and LB conceived and designed the experiments. VT and RR supervised the collection of the fecal and soil samples. FC, AC, DL, LC, and MM carried out the experiments. FC, LB, DL, MM, VT, LC, MS, TM, AC, MD, and FB analyzed the data. FC, AC, LB, CSa, CSp, CG, VT, SC, TM, and PM wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Open Access Publishing Fund of the Free University of Bozen-Bolzano.

ACKNOWLEDGMENTS

We are grateful to the Botanist, Rakotozafy M. Andriatsarafara for having contributed to the qualitative study of the soil, Matteo Moretti for the graphics design and Manuela Dasser of Marameolab for graphical illustrations. We thank Chiara De Gregorio and the research guides in Maromizaha (Boto Zafison, Ranaivomanana Jean, and Kotoarisoa Gilbert), for helping in collecting fecal and soil samples and for helping us with the filming. We would also thank Cesare Avesani Zaborra, CEO and Scientific Director of Parco Natura Viva for his continuous support and help to the conservation of Maromizaha Forest fauna and flora.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.668274/full#supplementary-material

REFERENCES

Aivelo, T., Laakkonen, J., and Jernvall, J. (2016). Population- and individual-level dynamics of the intestinal microbiota of a small primate. *Appl. Environ. Microbiol.* 82, 3537–3545. doi: 10.1128/AEM.00559-16

Amaral, W. Z., Lubach, G. R., Proctor, A., Lyte, M., Phillips, G. J., and Coe, C. L. (2017). Social influences on *Prevotella* and the gut microbiome of young monkeys. *Psychosom. Med.* 79, 888–897. doi: 10.1097/PSY.0000000000000454

Amato, K. R., Kuthyar, S., Ekanayake-Weber, M., Salmi, R., Snyder-Mackler, N., Wijayathunga, L., et al. (2020). Gut microbiome, diet, and conservation of endangered langurs in Sri Lanka. *Biotropica* 52, 981–990. doi: 10.1111/btp.12805

Amato, K. R., Martinez-Mota, R., Righini, N., Raguet-Schofield, M., Corcione, F. P., Marini, E., et al. (2016). Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia* 180, 717–733. doi: 10.1007/s00442-015-3507-z

Bennett, G., Malone, M., Sauther, M. L., Cuozzo, F. P., White, B., Nelson, K. E., et al. (2016). Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *Am. J. Primatol.* 78, 883–892. doi: 10.1002/ajp.22555

Biddle, A., Stewart, L., Blanchard, J., and Leschine, S. (2013). Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and Ruminococcaceae in diverse gut communities. *Diversity* 5, 627–640. doi: 10.3390/d5030627

Bocian, C. (1997). *Niche separation of Black-and-White Colobus monkeys (Colobus angolensis and C. guereza) in the Ituri Forest*. Available online at: https://www.semanticscholar.org/paper/Niche-separation-of-Black-and-White-Colobus-monkeys-Bocian/83d202c81a4b27697ab2d6f763edac13b534330d (accessed February 13, 2021).

Bonadonna, G., Torti, V., Gregorio, C. D., Valente, D., Randrianarison, R. M., Pozzi, L., et al. (2019). Evidence of genetic monogamy in the lemur Indri (*Indri indri*). *Am. J. Primatol.* 81:e22993. doi: 10.1002/ajp.22993

Borruso, L., Checcucci, A., Torti, V., Correa, F., Sandri, C., Luise, D., et al. (2021). I like the way you eat it: Lemur (*Indri indri*) gut mycobiome and geophagy. *Microb. Ecol.* [Epub ahead of print]. doi: 10.1007/s00248-020-01677-5

Britt, A., Randriamandratonirina, N. J., Glasscock, K. D., and Iambana, B. R. (2002). Diet and feeding behaviour of *Indri indri* in a low-altitude rain forest. *Folia Primatol.* 73, 225–239. doi: 10.1159/000067455

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869

Campbell, C. J. (2017). "Primates in perspective," in *The International Encyclopedia of Primatology*. ed. C. J. Campbell (Hoboken, NJ: Wiley).

Cresci, G. A., and Bawden, E. (2015). Gut Microbiome: what we do and don't know. *Nutr. Clin. Pract.* 30, 734–746. doi: 10.1177/0884533615609899

Davies, A. G., and Baillie, I. C. (1988). Soil-eating by red leaf monkeys (*Presbytis rubicunda*) in Sabah, Northern Borneo. *Biotropica* 20, 252–258. doi: 10.2307/2388241

De Gregorio, C., Carugati, F., Estienne, V., Valente, D., Raimondi, T., Torti, V., et al. (2021). Born to sing! Song development in a singing primate. *Curr. Zool.* [Epub ahead of print]. doi: 10.1093/cz/zoab018

De Gregorio, C., Valente, D., Torti, V., Zanoli, A., Colaci Cirillo, D., Bazzano, G., et al. (2019). Song structure and sex specific features in the indris. *Proc. Meet. Acoust.* 36:010002. doi: 10.1121/2.0001021

de Souza, L. L., Ferrari, S. F., da Costa, M. L., and Kern, D. C. (2002). Geophagy as a correlate of folivory in red-handed howler monkeys (*Alouatta belzebul*) from eastern Brazilian Amazonia. *J. Chem. Ecol.* 28, 1613–1621. doi: 10.1023/A:1019928529879

Degnan, P. H., Pusey, A. E., Lonsdorf, E. V., Goodall, J., Wroblewski, E. E., Wilson, M. L., et al. (2012). Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. *Proc. Natl. Acad. Sci. U.S.A.* 109, 13034–13039. doi: 10.1073/pnas.1110994109

Dew, J. L. (2005). Foraging, food choice, and food processing by sympatric ripe-fruit specialists: *Lagothrix lagotricha poeppigii* and *Ateles belzebuth belzebuth*. *Int. J. Primatol.* 26, 1107–1135. doi: 10.1007/s10764-005-6461-5

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* 14, 927–930. doi: 10.1111/j.1654-1103.2003.tb02228.x

Ferrari, S. F., Veiga, L. M., and Urbani, B. (2008). Geophagy in new world monkeys (*Platyrrhini*): ecological and geographic patterns. *Folia Primatol.* 79, 402–415. doi: 10.1159/000141901

Fogel, A. T. (2015). The gut microbiome of wild lemurs: a comparison of sympatric *Lemur catta* and *Propithecus verreauxi*. *Folia Primatol.* 86, 85–95. doi: 10.1159/000369971

Gamba, M., Torti, V., Estienne, V., Randrianarison, R. M., Valente, D., Rovara, P., et al. (2016). The *Indris* have got rhythm! timing and pitch variation of a primate song examined between sexes and age classes. *Front. Neurosci.* 10:249. doi: 10.3389/fnins.2016.00249

Geilfus, C.-M. (2019). "Plant secondary compounds," in *Controlled Environment Horticulture: Improving Quality of Vegetables and Medicinal Plants*, ed. C.-M. Geilfus (Cham: Springer International Publishing), 19–33. doi: 10.1007/978-3-030-23197-2_3

Greene, L. K., Williams, C. V., Junge, R. E., Mahefarisoa, K. L., Rajaonarivelo, T., Rakotondrainibe, H., et al. (2020). A role for gut microbiota in host niche differentiation. *ISME J.* 14, 1675–1687. doi: 10.1038/s41396-020-0640-4

Grieneisen, L. E., Charpentier, M. J. E., Alberts, S. C., Blekhman, R., Bradburd, G., Tung, J., et al. (2019). Genes, geology and germs: gut microbiota across a primate hybrid zone are explained by site soil properties, not host species. *Proc. R. Soc. B Biol. Sci.* 286:20190431. doi: 10.1098/rspb.2019.0431

Hale, V. L., Tan, C. L., Niu, K., Yang, Y., Knight, R., Zhang, Q., et al. (2018). Diet versus phylogeny: a comparison of gut microbiota in captive colobine monkey species. *Microb. Ecol.* 75, 515–527. doi: 10.1007/s00248-017-1041-8

Hale, V. L., Tan, C. L., Niu, K., Yang, Y., Zhang, Q., Knight, R., et al. (2019). Gut microbiota in wild and captive Guizhou snub-nosed monkeys, *Rhinopithecus brelichi*. *Am. J. Primatol.* 81:e22989. doi: 10.1002/ajp.22989

Haro, C., Rangel-Zúñiga, O. A., Alcalá-Díaz, J. F., Gómez-Delgado, F., Pérez-Martínez, P., Delgado-Lista, J., et al. (2016). Intestinal microbiota is influenced by gender and body mass index. *PLoS One* 11:e0154090. doi: 10.1371/journal.pone.0154090

Heinen, J., and Mehta, J. (1999). Conceptual and legal issues in the designation and management of conservation areas in Nepal. *Environ. Conserv.* 26, 21–29. doi: 10.1017/S0376892999000053

Hemingway, C. A. (1998). Selectivity and variability in the diet of Milne-Edwards' sifakas (*Propithecus diadema edwardsi*): implications for folivory and seed-eating. *Int. J. Primatol.* 19, 355–377. doi: 10.1023/A:1020344018670

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M., and Yoshida, K. (1997). Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates* 38, 111–125. doi: 10.1007/BF02382002

Illiano, P., Brambilla, R., and Parolini, C. (2020). The mutual interplay of gut microbiota, diet and human disease. *FEBS J.* 287, 833–855. doi: 10.1111/febs.15217

Jašarević, E., Morrison, K. E., and Bale, T. L. (2016). Sex differences in the gut microbiome–brain axis across the lifespan. *Philos. Trans. R. Soc. B. Biol. Sci.* 371:20150122. doi: 10.1098/rstb.2015.0122

Jumas-Bilak, E., Carlier, J.-P., Jean-Pierre, H., Citron, D., Bernard, K., Damay, A., et al. (2007). *Jonquetella anthropi* gen. nov., sp. nov., the first member of the candidate phylum "Synergistetes" isolated from man. *Int. J. Syst. Evol. Microbiol.* 57, 2743–2748. doi: 10.1099/ijs.0.65213-0

King, T., Dolch, R., Randriahaingo, H. N. T., Randrianarimanana, L., and Ravaloharimanitra, M. (2020). *IUCN Red List of Threatened Species: Indri indri. IUCN Red List Threat. Species*. Available online at: https://dx.doi.org/10.2305/IUCN.UK.2020-2.RLTS.T10826A115565566.en (accessed June 19, 2021).

Krishnamani, R., and Mahaney, W. C. (2000). Geophagy among primates: adaptive significance and ecological consequences. *Anim. Behav.* 59, 899–915. doi: 10.1006/anbe.1999.1376

Lankau, E. W., Hong, P.-Y., and Mackie, R. I. (2012). Ecological drift and local exposures drive enteric bacterial community differences within species of *Galápagos iguanas*. *Mol. Ecol.* 21, 1779–1788. doi: 10.1111/j.1365-294X.2012.05502.x

Li, H., Li, T., Yao, M., Li, J., Zhang, S., Wirth, S., et al. (2016). Pika gut may select for rare but diverse environmental bacteria. *Front. Microbiol.* 7:1269. doi: 10.3389/fmicb.2016.01269

Lombardo, M. P. (2008). Access to mutualistic endosymbiotic microbes: an underappreciated benefit of group living. *Behav. Ecol. Sociobiol.* 62, 479–497. doi: 10.1007/s00265-007-0428-9

Looft, T., Levine, U. Y., and Stanton, T. B. (2013). *Cloacibacillus porcorum* sp. nov., a mucin-degrading bacterium from the swine intestinal tract and emended description of the genus *Cloacibacillus*. *Int. J. Syst. Evol. Microbiol.* 63, 1960–1966. doi: 10.1099/ijs.0.044719-0

Manor, O., Dai, C. L., Kornilov, S. A., Smith, B., Price, N. D., Lovejoy, J. C., et al. (2020). Health and disease markers correlate with gut microbiome composition across thousands of people. *Nat. Commun.* 11:5206. doi: 10.1038/s41467-020-18871-1

McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217

Meehan, C. J., and Beiko, R. G. (2014). A phylogenomic view of ecological specialization in the *Lachnospiraceae*, a family of digestive tract-associated bacteria. *Genome Biol. Evol.* 6, 703–713. doi: 10.1093/gbe/evu050

Moeller, A. H., Peeters, M., Ndjango, J.-B., Li, Y., Hahn, B. H., and Ochman, H. (2013). Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. *Genome Res.* 23, 1715–1720. doi: 10.1101/gr.154773.113

Norconk, M. A., Wright, B. W., Conklin-Brittain, N. L., and Vinyard, C. J. (2009). "Mechanical and nutritional properties of food as factors in platyrrhine dietary adaptations," in *South American Primates: Comparative Perspectives in the Study of Behavior, Ecology, and Conservation Developments in Primatology: Progress and Prospects*, eds P. A. Garber, A. Estrada, J. C. Bicca-Marques, E. W. Heymann, and K. B. Strier (New York, NY: Springer), 279–319. doi: 10.1007/978-0-387-78705-3_11

Overdorff, D. J. (1993). Similarities, differences, and seasonal patterns in the diets of *Eulemur rubriventer* and *Eulemur fulvus rufus* in the Ranomafana National Park, Madagascar. *Int. J. Primatol.* 14, 721–753. doi: 10.1007/BF02192188

Pebsworth, P. A., Huffman, M. A., Lambert, J. E., and Young, S. L. (2019). Geophagy among nonhuman primates: a systematic review of current

knowledge and suggestions for future directions. *Am. J. Phys. Anthropol.* 168, 164–194. doi: 10.1002/ajpa.23724

Perofsky, A. C., Lewis, R. J., and Meyers, L. A. (2019). Terrestriality and bacterial transfer: a comparative study of gut microbiomes in sympatric Malagasy mammals. *ISME J.* 13, 50–63. doi: 10.1038/s41396-018-0251-5

Pollock, J. I. (1979). Female dominance in *Indri indri*. *Folia Primatol.* 31, 143–164. doi: 10.1159/000155877

Powzyk, J. A. (1997). *The Socio-Ecology Of Two Sympatric Indriids: Propithecus Diadema Diadema And Indri indri, A Comparison Of Feeding Strategies And Their Possible Repercussions On Species-Specific Behaviors*. Ph.D. thesis. Durham: Duke University.

Powzyk, J. A., and Mowry, C. B. (2003). Dietary and feeding differences between sympatric *Propithecus diadema diadema* and *Indri indri*. *Int. J. Primatol.* 24, 1143–1162. doi: 10.1023/B:IJOP.0000005984.36518.94

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596. doi: 10.1093/nar/gks1219

R Core Team (2021). *R: A Language And Environment For Statistical Computing*.Vienna, VIE: R Foundation for Statistical Computing.

Raulo, A., Ruokolainen, L., Lane, A., Amato, K., Knight, R., Leigh, S., et al. (2018). Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): in search of the role of immunity in the evolution of sociality. *J. Anim. Ecol.* 87, 388–399. doi: 10.1111/1365-2656.12781

Rolle, F., Torti, V., Valente, D., De Gregorio, C., Giacoma, C., and von Hardenberg, A. (2021). Sex and age-specific survival and life expectancy in a free ranging population of *Indri indri* (Gmelin, 1788). *Eur. Zool. J.* [Epub ahead of print]. doi: 10.1080/24750263.2021.1947398

Sandri, C., Correa, F., Spiezio, C., Trevisi, P., Luise, D., Modesto, M., et al. (2020). Fecal microbiota characterization of seychelles giant tortoises (*Aldabrachelys gigantea*) living in both wild and controlled environments. *Front. Microbiol.* 11:569249. doi: 10.3389/fmicb.2020.569249

Scotti, E., Boué, S., Sasso, G. L., Zanetti, F., Belcastro, V., Poussin, C., et al. (2017). Exploring the microbiome in health and disease: implications for toxicology. *Toxicol. Res. Appl.* 1:2397847317741884. doi: 10.1177/2397847317741884

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., et al. (2011). Metagenomic biomarker discovery and explanation. *Genome Biol.* 12:R60. doi: 10.1186/gb-2011-12-6-r60

Shin, N.-R., Whon, T. W., and Bae, J.-W. (2015). *Proteobacteria*: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol.* 33, 496–503. doi: 10.1016/j.tibtech.2015.06.011

Shreiner, A. B., Kao, J. Y., and Young, V. B. (2015). The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* 31, 69–75. doi: 10.1097/MOG.0000000000000139

Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., et al. (1996). *Methods of Soil Analysis: Part 3*. Madison, MDN: Soil Science Society of America.

Stumpf, R. M., Gomez, A., Amato, K. R., Yeoman, C. J., Polk, J. D., Wilson, B. A., et al. (2016). Microbiomes, metagenomics, and primate conservation: new strategies, tools, and applications. *Biol. Conserv.* 199, 56–66. doi: 10.1016/j.biocon.2016.03.035

Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., and Nishijima, M. (2014). Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. *PLoS One* 9:e105592. doi: 10.1371/journal.pone.0105592

Torti, V., Miaretsoa, L., Valente, D., De Gregorio, C., Bonadonna, C., Randrianarison, R. M., (2019). "Indri indri (Gmelin, 1788)," in *Primates in Peril: The World's 25 Most Endangered Primates 2018–2020*, eds C. Schwitzer, R. A. Mittermeier, A. B. Rylands, F. Chiozza, E. A. Williamson, D. Byler, S. Wich, T. Humle, C. Johnson, H. Mynott, and G. McCabe (Washington, DC: IUCN SSC Primate Specialist Group, International Primatological Society, Global Wildlife Conservation, and Bristol Zoological Society), 24–27.

Torti, V., Gamba, M., Rabemananjara, Z. H., and Giacoma, C. (2013). The songs of the indris (Mammalia: Primates: Indridae): contextual variation in the long-distance calls of a lemur. *Ital. J. Zool.* 80, 596–607. doi: 10.1080/11250003.2013.845261

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J.-C., Lynch, J., Grieneisen, L. E., et al. (2015). Social networks predict gut microbiome composition in wild baboons. *eLife* 4:e05224. doi: 10.7554/eLife.05224

Umanets, A., de Winter, I., IJdema, F., Ramiro-Garcia, J., van Hooft, P., Heitkönig, I. M. A., et al. (2018). Occupancy strongly influences faecal microbial composition of wild lemurs. *FEMS Microbiol. Ecol.* 94:fiy017. doi: 10.1093/femsec/fiy017

Vågen, T.-G., Shepherd, K. D., and Walsh, M. G. (2006). Sensing landscape level change in soil fertility following deforestation and conversion in the highlands of Madagascar using Vis-NIR spectroscopy. *Geoderma* 133, 281–294. doi: 10.1016/j.geoderma.2005.07.014

Vital, M., Karch, A., and Pieper, D. H. (2017). Colonic butyrate-producing communities in humans: an overview using omics data. *mSystems* 2:e00130-17. doi: 10.1128/mSystems.00130-17

Young, S. L., Sherman, P. W., Lucks, J. B., and Pelto, G. H. (2011). Why on earth?: evaluating hypotheses about the physiological functions of human geophagy. *Q. Rev. Biol.* 86, 97–120. doi: 10.1086/659884

Zhou, D., Bai, Z., Zhang, H., Li, N., Bai, Z., Cheng, F., et al. (2018). Soil is a key factor influencing gut microbiota and its effect is comparable to that exerted by diet for mice. *F1000Research* 7:1588. doi: 10.12688/f1000research.15297.1

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Microbiology

REVIEW
published: 06 October 2021
doi: 10.3389/fmicb.2021.690942

Inter-Kingdom Signaling of Stress Hormones: Sensing, Transport and Modulation of Bacterial Physiology

Amine Mohamed Boukerb[1†], Melyssa Cambronel[1†], Sophie Rodrigues[2], Ouiza Mesguida[1], Rikki Knowlton[3], Marc G. J. Feuilloley[1], Mohamed Zommiti[1] and Nathalie Connil[1]*

[1] Laboratoire de Microbiologie Signaux et Microenvironnement EA 4312, Université de Rouen, Normandie Université, Évreux, France, [2] EA 3884, LBCM, IUEM, Université de Bretagne-Sud, Lorient, France, [3] MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

OPEN ACCESS

Edited by:
Qi Zhao,
University of Science and Technology Liaoning, China

Reviewed by:
Ting Qin,
Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, China
Balbina J. Plotkin,
Midwestern University, United States

***Correspondence:**
Nathalie Connil
nathalie.connil@univ-rouen.fr

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Systems Microbiology, a section of the journal Frontiers in Microbiology

Received: 04 April 2021
Accepted: 06 August 2021
Published: 06 October 2021

Citation:
Boukerb AM, Cambronel M, Rodrigues S, Mesguida O, Knowlton R, Feuilloley MGJ, Zommiti M and Connil N (2021) Inter-Kingdom Signaling of Stress Hormones: Sensing, Transport and Modulation of Bacterial Physiology. Front. Microbiol. 12:690942. doi: 10.3389/fmicb.2021.690942

Prokaryotes and eukaryotes have coexisted for millions of years. The hormonal communication between microorganisms and their hosts, dubbed inter-kingdom signaling, is a recent field of research. Eukaryotic signals such as hormones, neurotransmitters or immune system molecules have been shown to modulate bacterial physiology. Among them, catecholamines hormones epinephrine/norepinephrine, released during stress and physical effort, or used therapeutically as inotropes have been described to affect bacterial behaviors (i.e., motility, biofilm formation, virulence) of various Gram-negative bacteria (e.g., *Escherichia coli, Salmonella enterica* serovar Typhimurium, *Pseudomonas aeruginosa, Vibrio* sp.). More recently, these molecules were also shown to influence the physiology of some Gram-positive bacteria like *Enterococcus faecalis.* In *E. coli* and *S. enterica*, the stress-associated mammalian hormones epinephrine and norepinephrine trigger a signaling cascade by interacting with the QseC histidine sensor kinase protein. No catecholamine sensors have been well described yet in other bacteria. This review aims to provide an up to date report on catecholamine sensors in eukaryotes and prokaryotes, their transport, and known effects on bacteria.

Keywords: stress hormones, catecholamines, sensing, transport, bacterial physiology

INTRODUCTION

Stress is a complex event that impacts the homeostasis of the whole organism. In cases of stress, the concentration/release of stress hormones/catecholamines may be raised by 20–40 times the physiological values and can reach 0.17–0.54 μg per minute. In some organs, such as the spleen or the gut, they can lead to local concentrations of up to 0.1–1 mM (Felten and Olschowka, 1987; Bergquist et al., 1998). This is caused by discharge from synaptic vesicles at noradrenergic nerve endings. Catecholamines (dopamine, norepinephrine, and epinephrine) are found throughout the plant and animal kingdoms (Akula and Mukherjee, 2020). In mammals, they can stimulate lipolysis and glycogenolysis and mobilize energy more rapidly than cortisol, as the latter's activity requires transcription of genes.

Catecholamines are organic nitrogen compounds derived from the amino acid L-tyrosine (**Figure 1**). The first step in the biosynthesis of these molecules is the hydroxylation of L-tyrosine

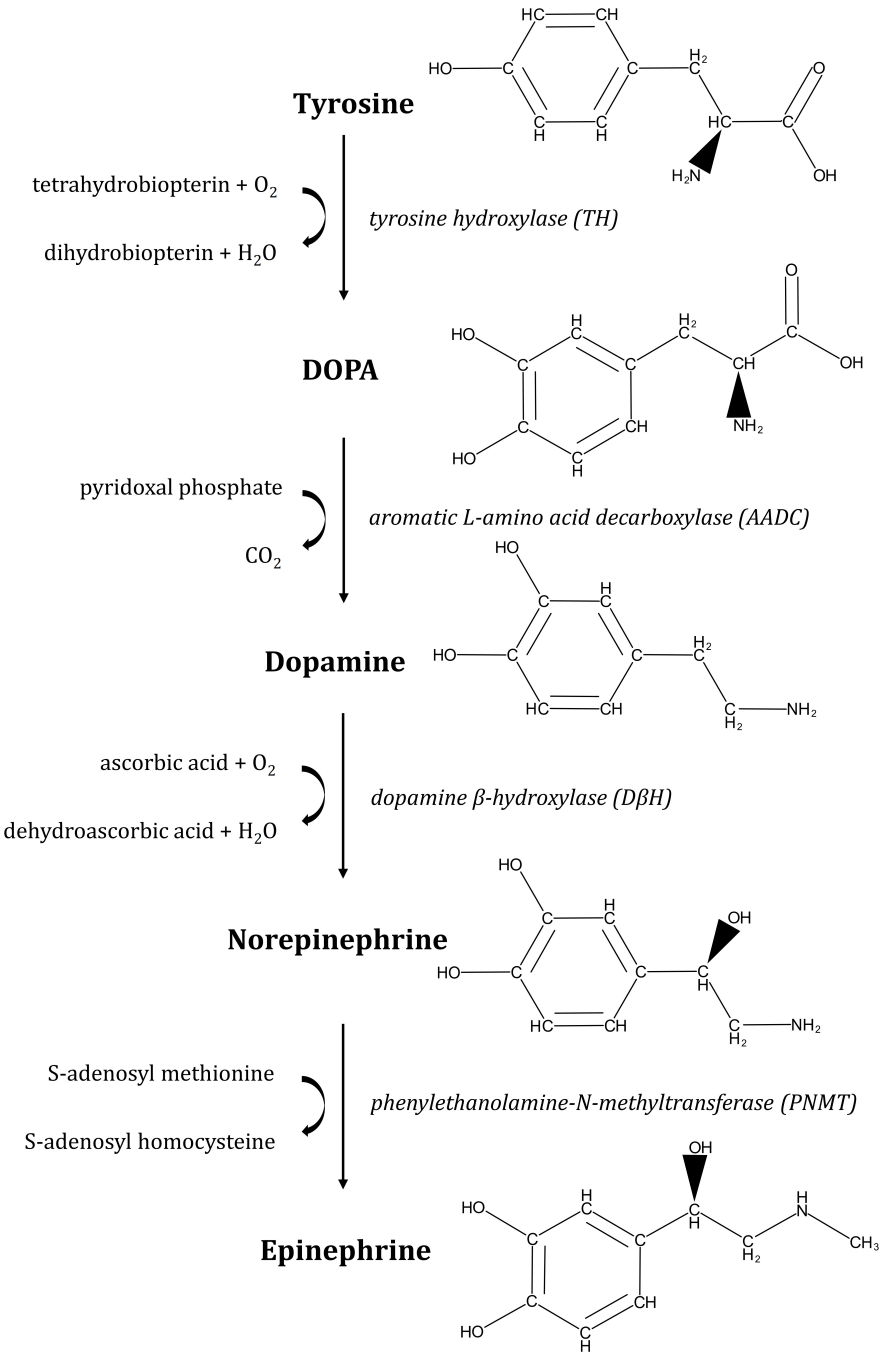


FIGURE 1 | Pathway of epinephrine and norepinephrine biosynthesis. Synthesis of catecholamines starts with conversion of L-tyrosine to L-dopa by tyrosine hydroxylase (TH). Then, L-dopa is processed to dopamine by L-aromatic amino acid decarboxylase (AADC), from where norepinephrine is formed by dopamine-β-hydroxylase (DβH). Finally, epinephrine is synthesized by addition of a methyl group to norepinephrine by phenylethanolamine-*N*-methyltransferase (PNMT). In mammals, catecholamines are synthesized from L-Dopa, obtained from dietary sources (the amino acids tyrosine and phenylalanine).

to L-dopa (L-dihydroxy-phenylalanine) by tyrosine hydroxylase. L-dopa is then decarboxylated to dopamine by Dopa-decarboxylase. This hormone is successively converted to norepinephrine (NE) and then epinephrine (Epi) by dopamine β-hydroxylase and phenylethanolamine-*N*-methyltransferase, respectively.

Intestinal expression of tyrosine hydroxylase was found upregulated in response to surgical perforation of the bowel and gut-derived sepsis in rats (Zhou et al., 2004). Subsequently, high levels of NE were detected in their fecal pellets. Catecholamines can act as hormones or neurotransmitters. They are synthesized by the cells of the adrenal medulla and by the postganglion

neurons of the orthosympathetic nervous system. Epi acts as a neurotransmitter in the central nervous system and as a hormone in the bloodstream. NE is primarily a neurotransmitter in the peripheral sympathetic nervous system but is also found in the blood. Dopamine is an essential neurotransmitter in the motivation and reward system.

In the human body, catecholamines act on almost all tissues and exert numerous activities at the cardiovascular, metabolic, endocrine, and neuronal levels. They also affect the intestinal barrier and immunity (Lymperopoulos et al., 2008). The action of catecholamines takes place after binding to specific receptors located on the cell membranes of target tissues: the α- and β-adrenergic receptors. The effects of catecholamines have long been studied only in humans without considering their possible impact on the microbiota. However, prokaryotes and eukaryotes have been intimately cohabiting for a very long time. In the gut and other tissues with contact to the external world via epithelial surfaces, catecholamines can cross the epithelial border and interact with microorganisms living in those ecological niches. In the colon, NE can reach a concentration of about 50 ng/g luminal content. Thus, bacteria have been in contact with the host hormones and have been able to develop complex interactions. In 1992, Lyte and Ernst were the pioneers who evaluated the effects of stress hormones on bacterial growth and introduced for the first time the concept of microbial endocrinology, a bi-directional interaction between human neuroendocrine factors and microorganisms (Lyte and Ernst, 1992; Sharaff and Freestone, 2011). It was subsequently shown that Epi/NE stimulates the growth of Gram-negative enteric pathogens of various genera (e.g., *Salmonella*, *Shigella*, *Yersinia*, *Vibrio,* and *Campylobacter*) as well as pathotypes of *E. coli* such as EHEC (Enterohemorrhagic *E. coli*) and ETEC (Enterotoxigenic *E. coli*). This new concept may help to understand how stress influences susceptibility to infection (Freestone et al., 2008). Since then, many studies have shown that catecholamines can have various stimulating effects on numerous Gram-negative and Gram-positive bacterial pathogens (i.e., growth, motility, biofilm formation, adhesion, cytotoxicity/virulence), and sensor systems allowing to perceive these molecules have been discovered for some bacterial species.

SENSING OF CATECHOLAMINES

Eukaryotic (Human) Sensors of Catecholamines: Adrenergic Receptors

Pharmacological classification of adrenergic receptors (AR) as α- and β-adrenergic receptors (**Table 1**) was first described in 1948 (Ahlquist, 1948). This classification was established according to their pharmacological properties and physiological effects (Perez, 2006). The α-adrenergic receptors are mainly involved in excitatory functions (vasoconstriction, uterine contraction, contraction of the nictitating membrane, pupillary dilation) whereas β-receptors are more commonly related to inhibitory responses (vasodilatation, inhibition of uterine contraction, myocardial stimulation). The α-receptor group is subdivided in two sub-groups: α-1 and α-2 (Berthelsen and Pettinger, 1977). Each of them is composed of three subtypes, α-1A, α-1B, and α-1D (Ford et al., 1994), or α-2A/D, α-2B, and α-2C (Bylund, 1988; Hieble et al., 1995). β-receptors are also subdivided in three subtypes: β-1, β-2, and β-3 (Lands et al., 1967a,b; Emorine et al., 1989). The first cloned and characterized drug receptor was the β-2 AR (ADRB2) that binds Epi (Dixon et al., 1986). NE has relatively higher binding affinity for α- ARs and β-1/3 ARs, but a lower affinity for β-2 AR. All these receptors belong to the group of G Protein-Coupled Receptors (GPCRs), the largest family of cell-surface proteins involved in signal transduction (Rosenbaum et al., 2009). They

TABLE 1 | Adrenergic receptors and physiological effects.

Subtypes	G proteins	Intracellular messengers	Target organs	Physiological effects
α1	Gq	Increase in PLC and IP3	Uterine	Contraction
			Vascular smooth muscles	Contraction
			Blood vessels	Constriction
			GI sphincter	Increase in tone
			Urinary sphincter	Increase in tone
			Pupillary radial muscle	Contraction (mydriasis)
			Pilomotor smooth muscle	Contracts (erects hair)
α2	Gi	Decrease in cAMP	Presynaptic nerves	
			Adrenergic and cholinergic nerves terminals	Inhibit transmitter release
			Platelets	Stimulate aggregation
			Some vascular smooth muscle	Contraction
			Fat cells	Inhibit lipolysis
			Pancreatic B cells	Inhibit insuline release
			Ciliary epithelium	Reduction of humor secretion
β1	Gs	Increase in cAMP	Heart	Stimulates rate and force
			Kidney	Stimulates renin release
β2	Gs	Increase in cAMP	Liver	Stimulates glycogenolysis
			Pancreatic B cells	Stimulates insulin release
			Skeletal muscle	Contraction
			Heart	Stimulates rate and force
			Ciliary epithelium	Increases of humor secretion
			Airways, uterine and vascular smooth muscle	Relaxes
			Uterine	Inhibit contraction
β3	Gs	Increase in cAMP	Adipose tissues (Fat cells)	Stimulates lipolysis

contain seven transmembrane domains (Strosberg, 1993), and are linked to a GTP-binding regulatory G protein. The G protein is composed of three subunits: α, β, and γ. The α subunit determines the signal transduction pathway which will modulate the activity of second messengers (Marinissen and Gutkind, 2001). Generally, G proteins can be divided into Gi, Gs, Gq/11, and G12/13 subfamilies according to their α subunits. Catecholamine receptors have been defined using highly selective receptor agonists and antagonists in functional pharmacological investigations, delivering information on their affinities (Kd, dissociation constant). However, such studies do not reflect the biological activity of the ligand-receptor interaction, which is an entity functionally coupled to intracellular signal transduction pathways. Thus, new strategies are now used on isolated cells and transgenic animals based on molecular cloning and structure-function analysis to encounter such limitations, providing precious data on selective antagonism, rank order of ligand potency and stereospecificity. Catecholamines activate various cellular signal transduction by binding to these receptors, activating the G proteins which can then modulate effectors such as adenylate cyclase or phospholipase C (PLC) (**Figure 2**). The α1-receptor is coupled with G_q protein, allowing activation of kinase protein C (PKC) and increase of intracellular concentration of Ca^{2+}, through the triphosphate inositol (IP3)/diacylglycerol (DAG) pathway. Activation of this pathway results from the cleavage of phosphatidylinositol-4,5-bisphosphate (PIP2) in IP3 and DAG thanks to phospholipase C (PLC). α-2 and β-receptors are coupled to G_i and G_s, respectively. In both cases, cAMP (cyclic adenosine monophosphate) is increased or decreased depending on the stimulation (G_s) or inhibition (G_i) of adenylate cyclase (AC), leading to the activation of kinase protein A (PKA). Variations in the concentrations of the intracellular secondary messengers lead to physiological modifications (e.g., vasoconstriction, uterine contraction or pupillary dilatation) (**Table 1**).

Several antagonists can bind to the adrenergic receptors with a high affinity, and competitively block the effects of ligands. For example, α-1 and β receptors can be respectively inhibited by phentolamine and propranolol. The latter is used in medical settings for hypertension as a β-blocker with an antihypertensive role (Lewis, 1976).

Prokaryotic Sensors of Catecholamines

As aforementioned, the study of microbiota response to eukaryotic signaling molecules and resulting behavior of the host is known as microbial endocrinology, highlighted by Lyte and Ernst (1992), but first evidence of interaction between bacterial pathogens and the host were described as early as 1930 (Renaud and Miget, 1930) when the syringe used to inject Epi was not correctly sterilized, leading to gas gangrene. A similar case was also described by Cooper (1946). Following these discoveries, many studies were carried out to understand the effect of Epi on bacteria. Various physiological effects of catecholamines were observed (see below) and sometimes the presence of a bacterial sensor for these molecules has been proposed.

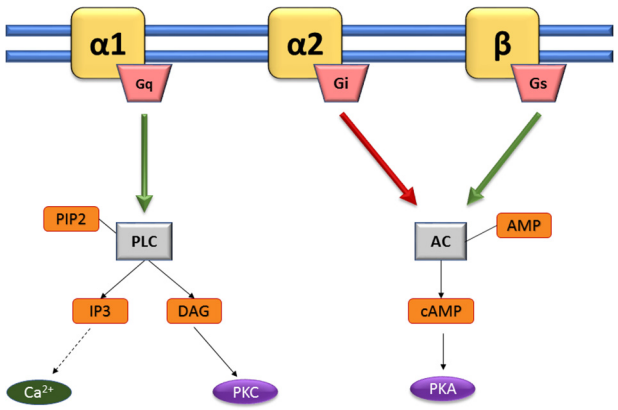


FIGURE 2 | Activating pathways of adrenergic sensors. Catecholamines activate various cellular signal transduction by binding to α 1-, α 2-, and β-adrenoreceptors (yellow). The α1-receptor is coupled with Gq protein, allowing activation of kinase protein C (PKC) and increase of intracellular concentration of Ca2+, through the triphosphate inositol (IP3)/diacylglycerol (DAG) pathway. Activation of this pathway results from the cleavage of phosphatidylinositol-4,5-bisphosphate (PIP2) in IP3 and DAG thanks to phospholipase C (PLC). α-2 and β-receptors are coupled to Gi and Gs, respectively. In both cases, cAMP (cyclic adenosine monophosphate) is increased or decreased depending on the stimulation (Gs) or inhibition (Gi) of adenylate cyclase (AC), leading to the activation of kinase protein A (PKA). Adapted from Andreis and Singer (2016).

The main signaling transduction systems in bacteria are the two-component systems. In these systems, the sensor for environmental cues is a histidine kinase, which upon autophosphorylation transfers its phosphate to an aspartate residue in the response regulator, which is usually a transcription factor that is activated by phosphorylation. Clarke et al. (2006) showed that the QseC sensor kinase is a bacterial receptor for the host Epi/NE (**Figure 3**). QseC is part of the QseBC system, initially described as a two-component system regulated by quorum sensing and involved in regulation of flagella and motility in EHEC. Sperandio et al. (2003) showed that Epi was sensed by EHEC serotype O157:H7 via QseC, and activated EHEC virulence regulators like *ler*, the regulator of the Locus of Enterocyte Effacement (LEE) which is a pathogenicity island that contains 41 genes. Most of them are organized in five major operons, coding for a type III secretion system (responsible for attaching and effacement lesion in the large intestine cells), in addition to an adhesin (intimin) and its receptor. QseC was found to activate the LEE operons through KdpE and QseF-regulated sRNAs, and suggested to be the sensor for LuxS-dependent autoinducer-3 (AI-3) and Epi (**Figure 3**). Rasko et al. (2008) showed that exposure of EHEC strain 86-24 to 50 μM Epi increased the expression of the *ler* gene by 1.5-fold. Recently, the description of the complete structure of AI-3 uncovered the most active molecule within AI-3 family being a new pyrazinone-type of metabolite (Kim et al., 2020). QseC possesses a periplasmic domain with a sensor domain, two transmembrane domains and a kinase domain, while the response regulator QseB contains a receptor domain as well as a helix-turn-helix (HTH) domain. Activation of QseC

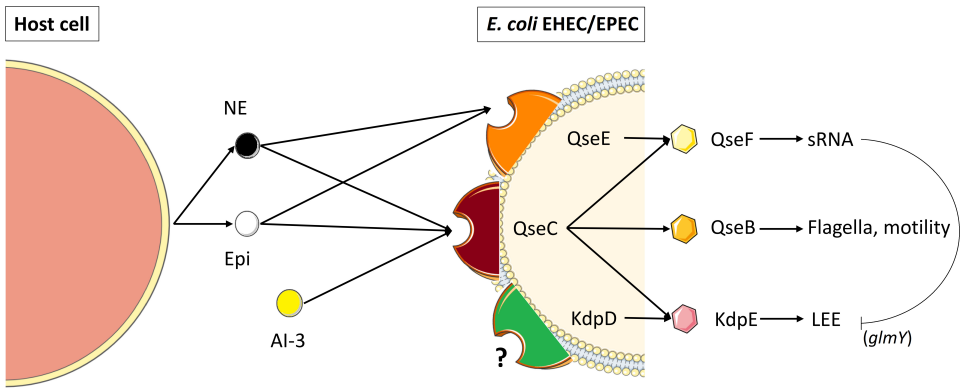


FIGURE 3 | Catecholamine sensing and signal transduction in enterohemorrhagic and enteropathogenic *E. coli* (EHEC/EPEC). QseC is an adrenergic sensor kinase that autophosphorylates on detection of NE, Epi and AI-3 and transfers the phosphate moiety to its cognate response regulator QseB, thereby activating transcription of the flagellar regulon (Clarke et al., 2006). Transcription of genes encoding a second two-component system (QseEF) is sensitive to NE and Epi and is implicated in small RNAs expression (Reading et al., 2009). Kinase activity in QseC is promiscuous and can activate two additional non-cognate response regulators, KdpE and QseF. QseC also activates the locus of enterocyte effacement (LEE), through KdpE, which is inhibited through sRNAs (*glmY*) that are modulated by QseF. (Adapted from Ellermann and Sperandio, 2020). KdpD, a transmembrane protein showing partial homology with QseC may also act as a catecholamine receptor (Borrel et al., 2019).

leads to the transfer of phosphate to the response regulator QseB, which then regulates its own transcription, as well as the transcription of flagella and motility genes (Clarke and Sperandio, 2005a,b). In addition, an osmosensitive K^+ channel histidine kinase, named KdpD, was found sharing partial homology with QseC, suggesting its possible role as a catecholamine receptor, which needs to be elucidated (**Figure 3**). Multiple studies using *E. coli* EHEC and UPEC (uropathogenic) isolates have demonstrated that *qseC* mutants have decreased motility (Sperandio et al., 2003; Hughes et al., 2009; Kostakioti et al., 2009; Hadjifrangiskou et al., 2011; Guckes et al., 2013). An *E. coli* O157:H7 *qseC* mutant presented pleiotropic phenotypes including virulence attenuation, metabolic dysregulation and decreased motility (Kostakioti et al., 2009; Hadjifrangiskou et al., 2011). Interestingly, an *E. coli* O157:H7 *qseBC* mutant has a similar motility phenotype compared to wild-type O157:H7 (Sharma and Casey, 2014a,b). However, in response to 50 μM NE, they observed a significant increase in the motility of *E. coli* O157:H7 *qseC* and *qseBC* mutants. These results suggest that in addition to QseC, other regulatory systems sense and respond to Epi and NE in *E. coli*. As such, QseC seems to be involved in an inter-kingdom communication by sensing both bacterial quorum sensing molecules (i.e., AI-3) and hormones secreted by the host (i.e., Epi and NE). Another two-component system found in *E. coli* EHEC, named QseEF (**Figure 3**) was shown to belong to the signaling cascade Epi/NE/AI-3 (Reading et al., 2007). The transcription of *qseE* can be activated by the sensor QseC, after recognition of Epi. QseE can also be activated by the presence of Epi (**Figure 3**) but not by AI-2 or AI-3 (Reading et al., 2009). An *in silico* screening of the sensing-domain of QseC revealed a high degree of conservation within several bacterial species (Clarke et al., 2006), encompassing *S. enterica* serovar Typhimurium in which it may participate in Epi/NE signaling. Furthermore, it has been shown that in absence of *qseC* in this bacterium, NE was still able to activate the transcription of some genes, meaning that QseC is not the only sensor of Epi/NE in *S. enterica* serovar Typhimurium (Moreira et al., 2010). During the same period, other alternative sensor systems for Epi/NE have been described in *Salmonella* such as BasRS and CpxAR (Karavolos et al., 2008, 2011). To our knowledge, no other sensors for catecholamines have been described lately in bacteria, except two recent works on *Cutibacterium acnes* (former *Propionibacterium acnes*), and *E. faecalis* suggesting the role of KdpD and VicK (WalK), respectively, as putative adrenergic receptors (Borrel et al., 2019; Cambronel et al., 2020). The latter authors identified VicK as the closest protein to QseC with 29% identity and 46% similarity values. Structure modeling and molecular docking of VicK corroborated its possible interactions with Epi and NE, with binding energies of –4.08 and –4.49 kcal/mol, respectively.

Eukaryotic adrenergic antagonists were used in some studies and found to be able to block the physiological effects of catecholamines in bacteria (see below). LED209 [*N*-phenyl-4-(3-phenylthioureido) benzenesulfonamide], a bacterial inhibitor of QseC has also been identified through a high-throughput screening of a library of 150,000 small organic compounds and subsequent lead optimization (Rasko et al., 2008). This inhibitor has a unique mode of action by acting as a prodrug scaffold to deliver a warhead that allosterically modifies QseC, impeding virulence in several Gram-negative pathogens (Curtis et al., 2014).

TRANSPORT OF CATECHOLAMINES

Transport in Eukaryotes

The monoamines, including the catecholamines (Epi, NE, dopamine), are a group of important neurotransmitters and neurohormones (Duan and Wang, 2010) that regulates a wide array of physiological, behavioral, cognitive, and endocrine functions in central and peripheral nervous

systems (Carlsson, 1987; Greengard, 2001). The actions of released monoamine neurotransmitters are terminated by plasma membrane transporters that actively remove them from the extracellular space (Duan and Wang, 2010). Two distinct transport systems, named uptake1 and uptake2, are responsible for the clearance of monoamines in eukaryotic cells (Gründemann et al., 1998; Eisenhofer, 2001). Uptake1 consists of Na^+ and Cl^- dependent, high-affinity transporters while the uptake2 was originally characterized as a Na^+ and Cl^- independent, low-affinity, high-capacity transport system in peripheral tissues such as heart and smooth muscle cell (Iversen, 1971; Bönisch et al., 1985; Eisenhofer, 2001). The uptake2 system has also been found in various brain areas like cortex and striatum (Hendley et al., 1970; Wilson et al., 1988). In addition, studies on transport of monoamines in the brains, of serotonin (5-HT = 5-hydroxytryptamine), dopamine, and NE transporters knockout mice, demonstrated the existence of heterologous uptake of monoamines that cannot be exhaustively explained by the promiscuity of the abovementioned neuronal high-affinity monoamine transporters (Sora et al., 2001; Wayment et al., 2001; Moron et al., 2002). The plasma membrane monoamine transporter (PMAT) and organic cation transporter 3 (OCT3) are the two most prominent low-affinity, high-capacity (uptake2) transporters for catecholamines (Duan and Wang, 2010). These authors demonstrated that hPMAT (human plasma membrane monoamine transporter) is the major uptake2 transporter for serotonin and dopamine in the central nervous system, whereas hOCT3 (human organic cation transporter 3) represents the major uptake2 transporter for histamine, NE, and Epi in peripheral organs. The PMAT transports dopamine and serotonin with Km values in the high micromolar range while histamine, Epi, and NE are transported with Km values in the low millimolar range (Engel et al., 2004; Engel and Wang, 2005). The PMAT is widely distributed throughout the brain with the highest expression in the forebrain cortex, olfactory tubercle, hippocampus, and cerebellum (Dahlin et al., 2007; Vialou et al., 2007). PMAT expression has been detected in diverse populations of neurons including pyramidal neurons, interneurons, granular neurons, and Purkinje cells while no significant expression was measured in astroglial cells (Dahlin et al., 2007).

Transport in Prokaryotes

Information about putative entrance/transport of catecholamines in bacteria is scarce and not well understood. Lyte and Brown (2018) hypothesized that probiotics belonging to the genus *Lactobacillus* express uptake systems of biogenic amines, based on the observations of Yernool et al. (2004) and Yamashita et al. (2005), who demonstrated that bacterial transporters analogous to glutamate and leucine were expressed in bacteria. To test their hypothesis, Lyte and Brown have examined monoamine uptake in *Lactobacillus* biofilms using fluorescent probes for membrane amine transport (Lyte and Brown, 2018). The results of this study suggested that some lactobacilli biofilms express functional homologs of PMAT and OCT, which could take up and potentially deliver bioactive amines to nearby microbes or host cells in the intestinal tract. Bacterial analogs of biogenic amine transporters may contribute to the ability of the microbiota-gut-brain axis to influence brain function and ultimately behavior (Lyte, 2014; Sharon et al., 2016). It remains to be determined whether other bacterial species can express these biogenic amine transporters.

The putative conversion of catecholamines by bacteria has also been suggested (Toulouse et al., 2019; Reiske et al., 2020). Epi is converted by the pathogen *V. cholerae* to adrenochrome in the course of respiration. Superoxide produced by the respiratory Na^+ translocating NADH: quinone oxidoreductase (NQR) act as an electron acceptor in the oxidative conversion of Epi to adrenochrome.

MODULATION OF BACTERIAL PHYSIOLOGY

Effect on Growth and Capture of Iron

In vitro antimicrobial activity of catecholamines have been observed by Cuvas Apan et al. (2016) and Kesici et al. (2019). In fact, Cuvas Apan et al. (2016) found that saline dilutions of Epi, NE, and dopamine at clinically used concentrations decreased microbial growth of *Staphylococcus aureus, Staphylococcus epidermidis*, and *Candida albicans*, whereas *E. coli* and *P. aeruginosa* were more resistant. Despite this, most of the studies that were carried out have shown that catecholamines generally had no impact on bacterial growth when the bacteria were cultivated in rich media. On the contrary, experiments performed in minimal media or in low-iron media like serum-SAPI showed an increase of bacterial growth in presence of catecholamines for several bacteria (**Table 2**). For example, NE can enhance the growth of *S. enterica* serovar Typhimurium in serum-SAPI minimal medium (Freestone et al., 1999; Williams et al., 2006), suggesting that additional biosynthetic pathways would be modulated besides the iron utilization and transport genes. Using microarray-based transcriptional analysis on *S. enterica* serovar Typhimurium grown in serum-SAPI medium with or without 2 mM NE, Bearson et al. (2008) found an increase in transcription of genes involved in amino acid biosynthesis, cofactor biosynthesis, energy metabolism, central intermediary metabolism and synthesis of transport and binding proteins. Thus, to take advantage of the increased availability of iron provided by NE in serum-SAPI minimal medium, *S. enterica* serovar Typhimurium modulates the biosynthesis of multiple cellular pathways to increase its growth rate. In fact, iron is an essential element for almost all organisms, as an indispensable enzymatic co-factor in many cellular processes; and most bacteria require micromolar levels of bioavailable iron for optimal growth (Ratledge and Dover, 2000). During infection, a coordinated host response limits the availability of iron to microbes and restricts the replication of invading pathogens (Ganz and Nemeth, 2015). Iron sequestration is mainly due to the mammalian ferric-iron-binding proteins transferrin in serum and lactoferrin in mucosal secretions (Mietzner and Morse, 1994). A strategy that bacteria often employ to collect essential iron is the production and utilization of siderophores, that possess high affinity for ferric iron

TABLE 2 | Bacterial growth in presence of catecholamines.

Gram-negative bacteria	Catecholamines	References
Acinetobacter lwoffii, Citrobacter freundii, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Enterobacter sakazaki, Escherichia coli, Hafnia alvei, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella enterica sv Enteritidis*, Serratia marcescens, Yersinia enterocolitica, Xanthomonas maltophilia*	NE	Freestone et al., 1999, 2000, 2003, Freestone, 2013
Actinobacillus pleuropneumoniae	Epi NE Dopamine	Li et al., 2015
Aeromonas hydrophila	Epi NE Dopamine	Dong et al., 2016
Bordetella bronchiseptica	NE	Anderson and Armstrong, 2008 Armstrong et al., 2012
Burkholderia pseudomallei	Epi	Intarak et al., 2014
Campylobacter jejuni	NE Epi NE	Cogan et al., 2007 Xu et al., 2015
Escherichia coli	Epi NE Dopamine	Burton et al., 2002
Pseudomonas aeruginosa PA14	NE NE Dopamine	Hegde et al., 2009 Freestone et al., 2012
Pseudomonas fluorescens MFN1032	NE	Biaggini et al., 2015
Salmonella enterica serovar Typhimurium	NE Dopamine	Williams et al., 2006 Dichtl et al., 2019
Vibrio parahaemolyticus, Vibrio cholerae, Vibrio vulnificus, Vibrio mimicus	NE	Nakano et al., 2007a
Vibrio cholerae	Epi NE	Halang et al., 2015
Yersinia ruckeri	NE Dopamine	Torabi Delshad et al., 2019
Gram-positive bacteria	**Catecholamines**	**References**
Enterococcus faecalis, Enterococcus faecium, Listeria monocytogenes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus dysgalactiae	NE	Freestone et al., 1999, 2000, 2003, Freestone, 2013
Listeria monocytogenes	NE	Coulanges et al., 1997
Listeria monocytogenes, Listeria innocua, Listeria ivanovii, Listeria seeligeri, Listeria grayi	Epi NE Dopamine	Coulanges et al., 1998
Staphylococcus aureus	Epi NE	Belay and Sonnenfeld, 2002
Staphylococcus aureus, Staphylococcus epidermidis	NE	Mart'yanov et al., 2021
Staphylococcus epidermidis	NE	Lyte et al., 2003
Streptococcus mutans, Streptococcus mitis, Streptococcus gordonii, Streptococcus intermedius	Epi NE	Roberts et al., 2002

(Ratledge and Dover, 2000). The catechol core found in many siderophores is also present in the stress-related hormones of the catecholamine family. Thus, many studies which show the effect of catecholamines on bacterial growth, were carried out using a serum-like medium containing transferrin (Tf) and lactoferrin (Lf). This medium more appropriately mimics a stressful and bacteriostatic environment for the bacteria and would therefore more closely resemble the conditions that the bacteria might encounter within the host (Burton et al., 2002; Freestone et al., 2003; Lyte, 2004). Sandrini et al. (2010) showed that Epi, NE, and dopamine formed a complex with the ferric iron Fe (III) present in Tf and Lf. The catechol nucleus of catecholamines will then bind the iron sequestered by Tf and Lf, reducing their affinity with them. Thus, the presence of catecholamines would stimulate growth of bacteria by making the iron contained in Tf and Lf available for them. However, the exact mechanism by which iron is incorporated into the bacteria in the presence of catecholamines remains unclear. One study has implicated the porin proteins OmpA and OmpC from several enteropathogens in transferrin binding and transferrin dependent iron uptake (Sandrini et al., 2013). The authors showed that the presence of OmpA may increase

growth of bacteria in the presence of some catecholamines. The assimilation of the siderophore/iron complex through specific Ton-B dependent receptors has also been suggested (Ratledge and Dover, 2000). For example, in *P. aeruginosa*, there are at least 34 Ton-B dependent or putative receptors, including those involved in the reuptake of pyoverdine and pyochelin, the two main siderophores (Cornelis and Bodilis, 2009), and it has been shown that in presence of NE, the expression of the genes coding for the synthesis of these siderophores are decreased (Li et al., 2009). Additionally, Perraud et al. (2020) demonstrated that catecholamine neurotransmitters (dopamine, L-DOPA, Epi, and NE) can act as siderophores, chelating iron and efficiently bringing it into *P. aeruginosa* cells via the PiuA and PirA TonB-dependent transporters (TBDTs). These authors found that PiuA exhibited more pronounced specificity for dopamine uptake than for NE, Epi, and L-DOPA, whereas PirA specificity appeared to be higher for L-DOPA and NE. Similar mechanisms were suggested in other Gram-negative bacteria. Indeed, in *E. coli* (Burton et al., 2002; Freestone et al., 2003) and *Salmonella* (Williams et al., 2006; Dichtl et al., 2019) siderophore synthesis and uptake systems appeared also to be integral elements in the mechanism by which stress-related neuroendocrine hormone induce growth. *Salmonella enterica* produces enterochelin which bioavailability for bacterial iron acquisition is reduced by the mammalian siderocalin in addition to the affinity of enterochelin for lipid membranes. *Salmonella* glucosylation of enterochelin to salmochelin by IroB reduces both membrane affinity and siderocalin binding properties (Hantke et al., 2003). The increased bioavailability of salmochelin compared to enterochelin facilitates iron acquisition from transferrin to support pathogen growth. Due to their siderophore-like properties, Epi/NE accelerate bacterial iron acquisition and therefore can enhance pathogen growth in iron-limited environments. In *Bordetella bronchiseptica*, NE has been described to mediate acquisition of transferrin iron (Anderson and Armstrong, 2008; Armstrong et al., 2012). In *Actinobacillus pleuropneumoniae*, mutation of the gene encoding TonB2 protein prevents growth stimulation by catecholamines (Li et al., 2015), and in *Aeromonas hydrophila,* the increase of bacterial growth due to these molecules seems also dependent on TonB2-energy transduction system (Dong et al., 2016). Recently, Aldriwesh et al. (2019) showed that Epi and NE present in peritoneal dialysate can enhance the development of bacteria and the infection risk via transferrin iron provision. All these results showed that catecholamines can increase growth of several bacteria, and that this effect may be, in part, closely related to iron homeostasis.

Effect of Catecholamines on Chemotaxis and Motility

Chemotaxis is one way in which bacteria could react to the compounds present in their environment. It allows them to navigate in gradients of various chemicals in order to locate conditions that are beneficial for growth. Scarce studies are available on the effect of catecholamines on chemotaxis. Chet et al. (1973) have reported that the chemotactic response of *P. fluorescens* was significantly enhanced by Epi. Later, using an agarose plug chemotaxis assay, Bansal et al. (2007) found that both Epi and NE were chemo-attractants for *E. coli* O157:H7. More recently, Lopes and Sourjik (2018) showed that *E. coli* RP437 reacts with mixed responses for dopamine and NE using FRET (Fluorescence Resonance Energy Transfer) and microfluidics assay. Indeed, both hormones elicited biphasic results. Dopamine was sensed as a repellent at concentrations below 1 mM and as attractant at 10 mM. The response to NE had an inverse pattern. This molecule behaved as a weak attractant at low concentrations, but it produced a repellent response above 1 mM.

More data are available concerning catecholamines and motility tests (**Table 3**). These molecules have been found to enhance the motility of numerous bacteria, including pathogenic strains as *P. fluorescens* MFN1032 (Biaggini et al., 2015), *P. aeruginosa* PAO1/H103 (Cambronel et al., 2019), *P. aeruginosa* PA14 (Hegde et al., 2009), *S. enterica* serovar Typhimurium (Bearson and Bearson, 2008; Peterson et al., 2011), *E. coli* O157:H7 (Bansal et al., 2007), and some *Campylobacter* and *Vibrio* species (Cogan et al., 2007; Yang et al., 2021). In *V. harveyi*, the addition of the eukaryotic α-adrenergic antagonist phentolamine, or the bacterial catecholamine receptor antagonist LED209, was able to neutralize NE-induced swimming motility (Yang Q. et al., 2014). These authors found also that the dopaminergic antagonist chlorpromazine and the LED209 antagonist reduced dopamine-induced motility of this bacterium. The motility of other *Vibrio* species (*V. anguillarum*, *V. campbellii*, *V. parahaemolyticus*) can also be modulated by catecholamines (Pande et al., 2014). Chlorpromazine was able to stop the effect of dopamine in *V. anguillarum* and *V. campbellii*, and the α-adrenergic receptor antagonists phentolamine and phenoxybenzamine neutralized the effect of NE, whereas the β-adrenergic receptor antagonist propranolol had limited to no effect. This antagonist also failed to block the motility induced by NE in *Yersinia ruckeri* (Torabi Delshad et al., 2019). In *V. parahaemolyticus*, the bacterial antagonist LED209 neutralized the stimulatory effects of catecholamines on the growth and motility of the bacteria (Yang et al., 2021). In *Burkholderia pseudomallei*, phentolamine was found to reverse only partially the effect of Epi on motility (Intarak et al., 2014).

The expression of genes involved in flagellum formation and motility has been quantified in some bacteria exposed to catecholamines, and thus found to be modulated. Indeed, Yang Q. et al. (2014) showed that NE and Dopa significantly up-regulate the expression of ten genes involved in the flagella synthesis and chemotaxis of *V. harveyi*. The same result was observed for *C. jejuni* exposed to Epi and NE, in which many of the up-regulated genes were involved in flagellar assembly pathway (Xu et al., 2015). Surprisingly, in *P. aeruginosa* PA14, a treatment with 50 μM NE was found to decrease the expression of motility genes, while at 500 μM, they were up-regulated (Hegde et al., 2009), showing the influence of the concentration of stress hormones on their effects.

Thus, the presence of Epi or NE has been shown in multiple investigations to significantly enhance the motility of bacteria. However, an impact on the transcription of genes of the flagellar

or chemotaxis operons is not always apparent, probably due to the experimental conditions. In fact, gene expression assays are usually quantified using broth cultures whereas motility assays are typically performed using semi-solid agar medium. The differences in incubation conditions for growth rate and growth phase in broth and motility assays may account for a lack of congruence between transcriptional analysis and motility phenotype.

Effect on Biofilm

Biofilms are composed of cells bound to a surface and to each other and embedded within a matrix of extracellular polymeric substances they have produced (Donlan, 2002). For most of the microorganisms, the ability to form biofilms is a key factor to colonize and survive in the host environment (Costerton et al., 1999; Donlan and Costerton, 2002), promoting bacterial growth and pathogenicity (Parsek and Singh, 2003). Catecholamines were found to stimulate the biofilm in several bacteria (**Table 4**). First evidences of positive effect were obtained with utilization of the catecholamines inotropes, NE, and dobutamine, on *S. epidermidis* (Lyte et al., 2003). These authors found that incubation of this bacterium with catecholamines in the presence of human plasma resulted in a significant increase of growth and biofilm formation on both polystyrene and silicone surfaces, which was associated with extensive exopolysaccharide production. This suggested that the stimulation of bacterial proliferation and biofilm formation by these drugs may be an etiological factor in the development of intravascular catheter colonization and catheter-related infection. Since then, several other studies have shown an impact of catecholamines on adhesion and biofilm formation for various bacteria. Most of these experiments have also been conducted on abiotic surfaces. For example, Yang Q. et al. (2014) demonstrated that both NE and dopamine could increase the biofilm formation and exopolysaccharides production in *V. harveyi*, and this was blocked by the antagonists phentolamine, phenoxybenzamine, labetalol and LED209 for NE, chlorpromazine and LED209 for dopamine. Similarly, the biofilm formation of *Y. ruckeri* was found to be enhanced by NE and dopamine and antagonists were used to inhibit these effects (Torabi Delshad et al., 2019). In *E. coli* K-12 MC1000, addition of Epi/NE increased biofilm's thickness on polyvinyl chloride surface through the QseC sensor (Yang K. et al., 2014). Siderophores and ferric iron transport system also appear to play a vital role in the mechanism by which catecholamines influence biofilms formation (Feraco et al., 2016). In *P. aeruginosa* H103, a flow-cell device with glass surface was used to investigate the biofilm formation in dynamic conditions in the presence of Epi (Cambronel et al., 2019). The authors found no modification of the biofilm architecture with this catecholamine, but thicknesses and biovolume were significantly increased with 10 μM Epi compared to the untreated biofilm. Interestingly, it has been demonstrated that catecholamines had

TABLE 3 | Bacterial motility and chemotaxis in presence of catecholamines.

Bacterial species	Catecholamines (dose)	Antagonists	References
Burkholderia pseudomallei	Epi (50 μM)	Phentolamine	Intarak et al., 2014
Campylobacter jejuni NCTC11168	NE (100 μM)	n.	Cogan et al., 2007
	Epi, NE (100 μM)		Xu et al., 2015
Escherichia coli O157:H7	Epi, NE (50 μM)	n.	Bansal et al., 2007
Escherichia coli K12-MC1000	Epi, NE (50 μM)		Yang K. et al., 2014
Pseudomonas aeruginosa PAO1 (H103)	Epi (1–10 μM)	n.	Cambronel et al., 2019
Pseudomonas aeruginosa PA14	NE (50–500 μM)	n.	Hegde et al., 2009
	Epi, NE, Dopamine		Freestone et al., 2012
Pseudomonas fluorescens MFN1032	Epi (10 μM)	n.	Biaggini et al., 2015
Salmonella enterica serovar Typhimurium	NE (50 μM)	Phentolamine	Bearson and Bearson, 2008 Peterson et al., 2011
Vibrio anguillarum *Vibrio campbellii*	NE (100 μM)	Phentolamine Phenoxybenzamine Propanolol	Pande et al., 2014
	Dopamine (100 μM)	Chlorpromazine	
Vibrio harveyi (campbellii)	NE (50 μM)	Phentolamine Phenoxybenzamine Labetalol Propanolol LED209	Yang Q. et al., 2014
	Dopamine (50 μM)	Chlorpromazine LED209	
Vibrio parahaemolyticus	Epi (100 μM NE (50 μM) Dopamine (50 μM)	LED209	Yang et al., 2021
Yersinia ruckeri	NE (100 μM)	Propanolol Labetalol Phenoxybenzamine	Torabi Delshad et al., 2019
	Dopamine (100 μM)	Chlorpromazine	

n, not used in the study.

no impact on the growth of the Gram-positive *Cutibacterium acnes*, but were able to promote its biofilm formation (Borrel et al., 2019). Recently, the effect of catecholamines were also investigated in *E. faecalis* and the authors showed that Epi and NE can stimulate biofilm formation and adhesion of pathogenic but also probiotic strains of this species (Cambronel et al., 2020). On the contrary, the responses of *S. lugdunensis*, *S. epidermidis,* and *S. aureus* to Epi and dopamine were more variable, with increase or decrease of biofilm formation depending on the tested strains (Frank and Patel, 2008). Moreover, Epi and NE were found to promote the dispersion of biofilm in *Mannheimia haemolytica* (Pillai et al., 2018). Similarly, Lanter and Davies (2015) demonstrated that *C. acnes* biofilms were dispersed when challenged with NE in the presence of iron-bound transferrin or with free iron. Another study by Lanter et al. (2014) also showed that addition of NE (at 400 μM) induced dispersion of *P. aeruginosa* biofilms when grown under low iron conditions in the presence of transferrin. This dispersion is related to the release of degradation enzymes that can be harmful to the host tissue (Lanter et al., 2014).

The impact of catecholamines on mixed biofilms remains to be investigated in the future, to better understand the competition between bacteria in ecological niches. A recent publication explored for the first time the impact of NE on monospecies and dual-species biofilms of *S. epidermidis* and *S. aureus* (Mart'yanov et al., 2021). These authors showed that NE can affect the biofilm formation of both species with a strong dependence on aerobic

TABLE 4 | Bacterial biofilm in presence of catecholamines.

Bacterial species	Catecholamines (dose)	Effects on biofilms	Antagonists	References
Aeromonas hydrophila	Epi (100 μM) NE (100 μM) Dopamine (100 μM)	Increase (Crystal violet staining)	n.	Dong et al., 2016
Cutibacterium acnes	Epi (1 μM) NE (1 μM)	Increase (Crystal violet staining and CLSM analyses)	n.	Borrel et al., 2019
Cutibacterium acnes VP1	NE (400 μM)	Dispersion (Quantification of bacteria released from biofilm)	n.	Lanter and Davies, 2015
Enterococcus faecalis	Epi (1–100 μM) NE (1–100 μM)	Increase (Crystal violet staining and CLSM analyses)	n.	Cambronel et al., 2020
Escherichia coli K12-MC1000	Epi (50 μM) NE (50 μM)	Biofilm increase (Crystal violet staining, CLSM and SEM analyses)	n.	Yang K. et al., 2014
Escherichia coli O157:H7	Epi (50 μM) NE (50 μM)	Biofilm increase (Crystal violet staining, microarray)	n.	Bansal et al., 2007
Mannheimia haemolytica	Epi (50 μM) NE (50 μM)	Dispersion (Crystal violet staining, CLSM and SEM analyses)	n.	Pillai et al., 2018
Pseudomonas aeruginosa H103	Epi (1–10 μM)	Increase (CLSM analyses)	n.	Cambronel et al., 2019
Pseudomonas aeruginosa PAO1	NE (400 μM)	Dispersion (Quantification of bacteria released from biofilm)	n.	Lanter et al., 2014
Pseudomonas aeruginosa PA14	Epi (5 μM) NE (5 μM) Dopamine (5 μM)	Increase (Crystal violet staining, SEM analyses)	n.	Freestone et al., 2012
Staphylococcus epidermidis	NE (100 μM)	Increase (SEM analyses)	n.	Lyte et al., 2003
Staphylococcus lugdunensis *Staphylococcus epidermidis* *Staphylococcus aureus*	Epi (0–50 μg/mL) Dopamine (0–32 μg/mL)	Increase or decrease (Safranin staining and quantification)	n.	Frank and Patel, 2008
Staphylococcus aureus *Staphylococcus epidermidis* (dual species biofilm)	NE (10^{-7}M)	Enumeration on Luria-Bertani agar plates	n.	Mart'yanov et al., 2021
Vibrio harveyi (Campbellii)	NE (50 μM) Dopamine (50 μM)	Increase (Crystal violet staining)	Phentolamine LED209 Phenoxybenzamine Labetalol Propranolol Chlorpromazine	Yang Q. et al., 2014
Yersinia ruckeri	NE (100 μM) Dopamine (100 μM)	Increase (Crystal violet staining)	Phenoxybenzamine Labetalol Propranolol Chlorpromazine	Torabi Delshad et al., 2019

n, not used in the study; CLSM, confocal laser scanning microscope; SEM, scanning electron microscopy.

or anaerobic culture conditions. They found that *S. epidermidis* suppresses *S. aureus* growth in dual-species biofilms and that NE can accelerate this process, contributing to the competitive behavior of staphylococci.

Effect on Adhesion/Invasion, Cytotoxicity and Virulence

Several studies have investigated the impact of catecholamines on the interaction between bacteria and eukaryotic cells (**Table 5**). NE or Epi were found to be able to stimulate the adhesion potential of bacteria (e.g., *A. hydrophyla, Act. pleuropneumoniae, C. jejuni, E. faecalis, P. aeruginosa*) on lung and intestinal cells. Conversely, a decrease of adhesion has been observed for *Streptococcus pneumoniae* on A549 lung cells when the bacteria were pre-treated with NE or incubated with cells in presence of NE (Gonzales et al., 2013). The cytotoxic activity of catecholamines have been studied in a porcine lung epithelial cell line (SJPL), infected by *Act. pleuropneumoniae* (Li et al., 2012), using a CytoTox 96 LDH kit, and staining with crystal violet. Both methods showed that cytolytic activity was significantly increased by Epi but repressed by NE. The adrenergic receptor antagonists phentolamine and propanolol were found to be able to reverse significantly the effects of Epi and NE. In a study conducted by Biaggini et al. (2015), *P. fluorescens* treated with Epi led to 75% mortality of the undifferentiated Caco-2/TC7 cells, but the cytotoxicity of the untreated bacteria was almost the same with about 70% cell mortality. Nakano et al. (2007b) have shown that NE changed expression of TTSS1-related genes of *V. parahaemolyticus* and induced cytotoxic activity.

In *Campylobacter*, Cogan et al. (2007) examined invasion of Caco-2 cells with *C. jejuni* NCTC11168, and noticed that at least 10 times more bacteria were recovered within epithelial cells after 2 h, when they were pretreated with NE. Later, in 2014, the same research group investigated the invasion of other *Campylobacter* strains in T84 epithelial cells, and they found that *C. jejuni* M1

TABLE 5 | *In vitro* bacterial effects in presence of catecholamines (adhesion/invasion, cytotoxicity).

Bacterial species	Catecholamines (dose)	Cell lines	*In vitro* effects	Antagonists	References
Aeromonas hydrophyla	Epi (100 μM) NE (100 μM) Dopamine (100 μM)	HEp-2 epithelial cells	Increase of adhesion	n.	Dong et al., 2016
Actinobacillus pleuropneumoniae	Epi (50 μM) NE (50 μM)	SJPL lung cells	Adhesion induced by NE but not by Epi Cytotoxicity enhanced by Epi but repressed by NE	Phentolamine Propanolol	Li et al., 2012
Campylobacter jejuni NCTC11168	NE (100 μM)	Caco-2 intestinal cells	Increase of invasion Decrease of TEER Breakdown of tight junction (occludin) observed by CLSM	n.	Cogan et al., 2007
	Epi (100 μM) NE (100 μM)	Caco-2 intestinal cells	Increase of adhesion/invasion	n.	Xu et al., 2015
Campylobacter species	NE (100 μM)	T84 epithelial cells	Increase of invasion for *C. jejuni* M1 and *C. fetus fetus*, not for *C. jejuni* 81116 and *C. coli* 1669 Decrease of TEER breakdown of tight junction (occludin) observed by CLSM	n.	Aroori et al., 2014
Enterococcus faecalis	Epi (1 μM) NE (1 μM)	Caco-2/TC7 intestinal cells HaCaT keratinocyte cells	Increase of adhesion	n.	Cambronel et al., 2020
Salmonella enterica serovar Typhimurium	NE (50 μM)	HeLa epithelial cells	Increase of invasion	n.	Moreira and Sperandio, 2012
Streptococcus pneumoniae	NE (50 μM)	A549 lung cells	Decrease of adhesion	n.	Gonzales et al., 2013
Pseudomonas aeruginosa PAO1	Epi (1 μM)	Caco-2/TC7 intestinal cells	Increase of adhesion/invasion and translocation Decrease in TEER	n.	Biaggini, 2015
Pseudomonas aeruginosa PA14	NE (50 and 500 μM)	HCT-8 intestinal cells	Increase of adhesion/invasion	n.	Hegde et al., 2009
Pseudomonas fluorescens MFN1032	Epi (1 μM)	Caco-2/TC7 intestinal cells	No effect on cytotoxicity Decrease of TEER F-actin cytoskeleton disorganization (CLSM observation)	n.	Biaggini et al., 2015
Vibrio parahaemolyticus	NE (50 μM)	Caco-2 intestinal cells	Increase of cytotoxicity	Phentolamine Propanonol	Nakano et al., 2007b

n, not used in the study; TEER, transepithelial electrical resistance; CLSM, confocal laser scanning microscope.

TABLE 6 | *In vivo* bacterial effects in presence of catecholamines.

Bacterial species	Catecholamines (dose)	*In vivo* effects	Antagonists	References
Aeromonas hydrophyla	NE (1 mg/400 μL)	NE enhances the systemic spread of the bacteria during infection of mice	n.	Dong et al., 2016
	NE (100 μM)	Increased mortality of crucian carp	n.	Gao et al., 2019
Pseudomonas aeruginosa H103	Epi (1–10 μM)	Increased mortality of *Galleria mellonella* larvae (with 10 μM Epi)	n.	Cambronel et al., 2019
Salmonella enterica serovar Typhimurium	NE (200 mg/kg)	Oral administration of NE, but not preculture with NE alters the course of infection in pigs	n.	Pullinger et al., 2010
Vibrio campbelli BB120	NE/Dopamine (100 μM)	Increased virulence toward giant freshwater prawn larvae (*Macrobrachium rosenbergii*)	Phentolamine Chlorpromazine	Pande et al., 2014
Vibrio harveyi	NE/Dopamine (50 μM)	Increased virulence toward gnotobiotic brine shrimp larvae	Phentolamine Phenoxybenzamine Labetalol LED209 Chlorpromazine	Yang Q. et al., 2014
Yersinia ruckeri	NE/Dopamine (100 μM)	Increased mortality of rainbow trout	Phenoxybenzamine Labetalol Propanolol Chlorpromazine	Torabi Delshad et al., 2019

n, not used in the study.

and *C. fetus* subsp. *fetus* 10842 were also more invasive after 48 h pretreatment with NE, contrary to the invasion potential of *C. jejuni* subsp. *jejuni* 81116 and *C. coli* 1669 that were not modified (Aroori et al., 2014). In this study, the authors also observed that the presence of NE enhanced the reduction of transepithelial resistance (TER) of T84 cells infected by *C. jejuni* M1, *C. coli* and *C. fetus subsp. fetus* 10842; and increased breakdown of tight junctions. Similarly, experiments performed on differentiated Caco-2/TC7 cells with *P. aeruginosa* PAO1 showed that a pre-treatment of this bacterium with Epi increased its invasive and translocation potential, and the reduction of TER (Biaggini, 2015). In *Salmonella*, the presence of 50 μM Epi in a late log phase LB-culture enhanced by 1.5-fold its invasion of HeLa cells, with an evident impact of QseC and QseE on the pathogenicity island 1 (SPI-1) upregulation (increase of *sopB* and *sipA* transcription by two-fold) that is involved in the invasion phenotype (Moreira and Sperandio, 2012). Similar effect was observed with NE in the same growth conditions, where *invF* and *sopB* encoded in SPI-1 were increased by >15-fold. In contrast, Karavolos et al. (2008) indicated a downregulation of *invF* following 30 min of exposure to 50 μM Epi in similar growth conditions.

Few *in vivo* experiments were also conducted by some researchers (**Table 6**). Pande et al. (2014) showed that 100 μM dopamine and NE significantly increased the virulence of *V. campbellii* BB120 in a model of giant shrimp larvae. The survival of the larvae was found to be reduced by 15%, after 6–8 days of infection with dopamine or NE-pretreated *V. campbellii* BB120, compared to infection with untreated bacteria. In this work, a dopaminergic receptor antagonist for dopamine (chlorpromazine) and an adrenergic receptor antagonist (phentolamine), were also tested. The authors found that chlorpromazine could not neutralize the effect of dopamine, whereas the adrenergic receptor antagonist phentolamine only neutralized the effect of NE at a relatively high concentration (500 μM) and in only 2 of the 3 trials. Similarly, in *Y. ruckeri*, these catecholamines (dopamine and NE) significantly enhanced the virulence towards rainbow trout, and some antagonists were able to neutralize the effect of the stress hormones (Torabi Delshad et al., 2019). Indeed, phenoxybenzamine (the α-adrenergic antagonist) and labetolol (the α- and β-adrenergic antagonist) were able to block the increased virulence induced by NE, and chlorpromazine (the dopaminergic antagonist) could inhibit the effect of dopamine. On the contrary, propranolol did not show any antagonist ability to neutralize the effect of NE. In a mouse model, Dong et al. (2016) found that NE increased the proliferation capacity of *A. hydrophila* in the lungs. Initially administered in the intestine, the bacteria translocated through the gut and were able to disseminate into the lungs of mice. Another study conducted by Pullinger et al. (2010) found that NE augments *Salmonella enterica*-induced enteritis in pigs, in association with increased net replication but independent of the putative adrenergic sensor kinases QseC and QseE. Gao et al. (2019) found that the mortality of crucian carp challenged with *A. hydrophila* AH196 was significantly higher in the group treated with NE. In the same study, real-time PCR analyses revealed that NE notably up-regulated 13 out of 26 virulence-associated genes expression. Dowd (2007) reported that NE increased the expression levels of the shiga-toxins coding-genes *stx1* and *stx2* of *E. coli* O157:H7 during the 5 h incubation.

Other Effects

Catecholamines have been found to be able to modulate the sensitivity/resistance to antibiotics. For example, it has been shown that catecholamines can help bacteria for better growth recovery following antibiotic treatment (Freestone et al., 2012). Indeed, when *P. aeruginosa* PA14 was exposed to sub-inhibitory concentrations of tobramycin in serum-SAPI medium, the authors showed that adding NE allowed the bacteria to

have a better growth (10^3 CFU/mL at 24 h versus 5.10^5 CFU/mL with NE). This effect has also been observed in *S. epidermidis* (Freestone et al., 2016) and *A. baumannii* (Inaba et al., 2016). In *C. jejuni*, transcriptomic analysis showed that two genes involved in antimicrobial peptide resistance (Cj1583c and Cj0193c) were overexpressed in the presence of 100 μM Epi or NE (Xu et al., 2015). On the contrary, in *S. enterica* serovar Typhimurium, Epi reduced the ability of the bacteria to survive polymyxin B treatment (Karavolos et al., 2008). Using microarray analysis, the authors observed a decrease in the expression of the *pmr* regulon (*pmrHFIJKLM*, responsible for polymyxin resistance), which was mediated by the BasSR two component signal transduction system. This phenotype was reversed by the addition of the β-adrenergic blocker propranolol. Given that PmrAB activation was observed in the presence of extracellular iron for prevention of iron toxicity (Wösten et al., 2000), the binding of iron by Epi may partly explain the decreased expression of the PmrAB regulon. Karavolos et al. (2008) also showed that Epi increased oxidative stress resistance in *S. enterica* serovar Typhimurium when the bacterium was exposed to the molecule. Indeed, using transcriptomic assay and a luminescent transcriptional reporter, the authors found that the superoxide dismutase *sodA* gene was significantly upregulated by Epi. Similarly, Intarak et al. (2014) found that Epi-treated *Burkholderia pseudomallei* exhibited increased resistance to superoxide, consistent with induction of *sod*B expression.

Besides those effects, catecholamines may also regulate the production of some metabolites. At the human oral microbiome, production of volatile sulfur compounds, the major gasses responsible for bad breath (mainly hydrogen sulfide H_2S) was observed in periodontal pathogenic bacteria (i.e., *Fusobacterium nucleatum*, *Porphyromonas endodontalis*, *Prevotella intermedia,* and *Porphyromonas*) exposed to catecholamines (Roberts et al., 2002; Calil et al., 2014; de Lima et al., 2018).

Horizontal gene transfer (HGT) in bacteria is another mechanism impacted by host signals with an enhanced conjugative gene transfer observed between enteric bacteria (Peterson et al., 2011). Exposure to 5 μM NE in LB medium stimulated the transfer of a conjugative plasmid encoding multidrug resistance from a clinical *S.* Typhimurium strain to a recipient *E. coli* strain. Interestingly, a significant up-regulation of *tra* gene expression involved with plasmid transfer was observed. Treatment with the adrenergic receptor antagonists (phentolamine at 500 μM) negated the NE-enhanced conjugation frequency to baseline levels. Thus, these mediators of host stress may influence the evolution and adaptation of pathogens in the environment due to the transfer of genes that encode resistance to antibiotics and virulence factors.

Meta-Effects Studied by Transcriptomic/Proteomic Analyses

The global effects of catecholamines on bacteria have been first studied on a *luxS* enterohemorrhagic *E. coli* (EHEC) mutant by Kendall et al. (2007) using 50 μM of Epi and GeneChip microarrays (Affymetric system) for transcriptome analysis (**Table 7**). The authors observed a differential expression for 5,204 genes. In fact, Epi mainly increased the expression of the LEE regulon, which is known to play a pivotal role in EHEC virulence. The activated genes included the LEE genes, the flagellar regulon genes, the genes encoding iron uptake systems, the gene encoding the Hfq protein (a chaperone involved in small regulatory RNA post-transcriptional regulation), and genes encoding several nucleoid proteins, all reported to be involved in LEE regulation.

Another publication reported the global effects of Epi in *S. enterica* serovar Typhimurium (Karavolos et al., 2008). In this work, the transcriptomic analyses showed that approximately 0.6% of the transcriptome of the pathogen was significantly regulated by 50 μM Epi. The major feature of this bacterial adrenaline response was the upregulation of genes involved in metal homeostasis and oxidative stress. The key metal transport systems were induced within 30 min of treatment. Alterations in genes encoding proteins of unknown functions and changes in levels of regulators and signal transduction genes were also noticed.

Hegde et al. (2009) analyzed the transcriptome of *P. aeruginosa* PA14 exposed to 50 and 500 μM NE for 7 h.

TABLE 7 | Meta-effects of catecholamines on bacteria studied by transcriptomic analysis.

Bacterial species	Catecholamines (dose)	Meta-effects	References
Actinobacillus pleuropneumoniae	Epi (50 μM) NE (100 μM)	Differential expression of 158 and 105 genes, for Epi and NE, respectively. Many virulence factors. Only 18 genes regulated by both hormones	Li et al., 2012
luxS Escherichia coli EHEC mutant	Epi (50 μM)	Differential expression of 5,204 genes. Increased expression of the locus of enterocyte effacement (LEE), flagellar regulon genes, iron uptake systems	Kendall et al., 2007
Salmonella enterica serovar Typhimurium	Epi (50 μM)	Modulation of 0.6% of the transcriptome. Upregulation of genes involved in metal homeostasis and oxidative stress	Karavolos et al., 2008
Pseudomonas aeruginosa PA14	NE (50–500 μM)	Exposure to 50 μM NE altered the expression of 184 genes (128 induced, 56 repressed) Exposure to 500 μM NE induced 287 genes and repressed 50 genes. Up-regulation of virulence with 500 μM NE but not 50	Hegde et al., 2009
Campylobacter jejuni NCTC11168	Epi (100 μM) NE (100 μM)	Differential expression of 183 and 156 genes, for Epi and NE, respectively. 102 of these modulated genes were common for the two hormones (iron uptake, motility, virulence, oxidative stress response, nitrosative stress tolerance, enzyme metabolism, DNA repair and metabolism, ribosomal protein biosynthesis)	Xu et al., 2015

They found that 500 μM but not 50 μM of this molecule upregulated the genes involved in the regulation of the virulence determinants pyocyanin, elastase, and the *Pseudomonas* quinolone signal (PQS, 2-heptyl-3-hydroxy-4-quinolone).

Genome-wide Mu*dJ* transposon mutagenesis was used to study Epi- and NE-regulated genes in *S. Typhimurium* (Spencer et al., 2010). A transposon library of 10,000 *S.* Typhimurium mutants was screened and led to the identification of seven down-regulated and one up-regulated fusions in the presence of 250 μM Epi. The down-regulated genes included two virulence-related genes *virK* and *mig14* (involved in bacterial resistance to antimicrobial peptides), in addition to *iroC* (ABC transporter), *accC* (Acetyl-CoA carboxylase subunit), *nrdF* (Ribonucleotide diphosphate reductase subunit), *yedP* (Putative mannosyl-3-phosphoglycerate phosphatase), and STM3081 (Putative l-lactate/malate dehydrogenase), while the *yhaK* gene was up-regulated whose product is a putative cytoplasmic protein of unknown function. The regulation of *virK*, *mig14* and *yhaK* by 500 μM Epi and NE could be reversed in a promoter-luciferase fusion assay by addition of an α-adrenergic antagonist (phentolamine at 500 μM). In addition, exposure to 500 μM Epi or NE significantly increased sensitivity of *S.* Typhimurium to the antimicrobial peptide cathelicidin LL-37. Interestingly, a significant increase in sensitivity to LL-37 was demonstrated for the *virK* mutant in the absence of catecholamines.

Later, Li et al. (2012) investigated the global effects of catecholamines on *Act. pleuropneumoniae*, an important porcine respiratory pathogen causing significant economic loss in the global pig industry. Gene expression profiles after Epi and NE treatment were compared with untreated bacteria. The microarray results showed that 158 and 105 genes were differentially expressed in the presence of Epi and NE, respectively. These genes were assigned to various functional categories including many virulence factors, whereas only 18 genes were regulated by both catecholamines. Thus, differential regulation of gene expression suggests that this pathogen may have multiple responsive systems for the two hormones.

Transcriptomic analyses using Agilent microarrays were then performed in *C. jejuni* NCTC 11168, cultivated in iron-restricted medium, and supplemented with Epi and NE (Xu et al., 2015). The authors found that Epi and NE respectively modified the expression of 183 and 156 genes, compared to the expression in absence of these hormones, and 102 of these modulated genes were common for Epi and NE treatments. Various cellular functions were found to be modified, including iron uptake, motility, virulence, oxidative stress response, nitrosative stress tolerance, enzyme metabolism, DNA repair and metabolism, and ribosomal protein biosynthesis.

At the time of writing this review, only two proteomic analyses on the effect of catecholamines on bacteria were retrieved. The first study was done by Toulouse et al. (2019). The authors investigated the impact of Epi and adrenochrome on *V. cholerae*. They observed a proteome change in proteins involved in iron homeostasis, metabolism, signaling or translational and transcriptional control. The second study was conducted by Scardaci et al. (2021) using the probiotic strain *Enterococcus faecium* NCIMB10415. Combining a gel-free/label-free proteomic analysis, these authors evaluated the global changes induced by NE treatment in the bacterium. They found that exposure of *E. faecium* NCIMB10415 to this bioactive molecule enhanced the abundance of proteins related to stress response and to host-microbe interaction, such as moonlight proteins involved in adhesion and immune stimulation.

All these meta-effects showed that catecholamines can modulate several functions in bacteria, but it is important to notice that the results obtained may depend on the hormone (Epi or NE), their concentrations, and the medium used for the bacterial growth.

CONCLUSION AND FUTURE PROSPECT

Thirty years of studies in the field of microbial endocrinology have shown that there are multiple bi-directional interactions between bacteria and their host. In such interactions, the main actors are neurotransmitters whose mission is the transmission of information within and between living cells, giving the inter-kingdom signaling. As for the genetic code shared by all living organisms, a common class of mediator-type molecules indicates that the mechanism of communication within neurotransmitters appears to be widely recognized. The role of regulatory proteins in catecholamines sensing is an area of growing research. Though evidence exists that catecholamines exert effects on bacteria within *in vitro* culture conditions (i.e., growth, motility, adhesion, virulence). However, these effects are mainly dependent on media composition and inoculum density (growth phase), being most prominent in a minimal salt's medium (i.e., serum-SAPI) miming nutrient poor and iron-limited conditions encountered *in vivo*. The variability of results obtained in the literature concerning catecholamines can also be due to a poor stability of the molecules in solution as they need to be freshly prepared before the experiments.

Within the human microbiome, the ability of microorganisms to respond to the panoply of neuroendocrine hormones is becoming increasingly recognized as playing a pivotal role in disease pathogenesis and maintenance of homeostasis (Lyte, 2013). In the near future, new methodologies as organ-on-chip will be a promising sophisticated tool to investigate the interactions between bacteria and the host in the presence of several metabolites and molecules including hormones. This state of art technology will ensure a stable and/or variable dynamic flow of catecholamines, on bacteria and eukaryotic cells, and thus simulates more accurately what can happen *in vivo*, especially in case of a stress peak.

During their evolution, pathogenic bacteria can adapt and develop sophisticated systems allowing them to sense eukaryotic signals and to use them to their advantage to stimulate their colonization and virulence. While several data indicate that Gram-negative bacterial pathogens possess elements that specifically interact with catecholamines allowing them to sense the host environment, scarce data is available on such elements found in Gram-positive bacteria and especially in probiotic

bacteria. A better knowledge of these systems will allow to decipher how stress hormones could be involved in the colonization by microbiota and in the eubiosis/dysbiosis of the gut or other organs. This may help to develop new treatments with medical and economic interest.

AUTHOR CONTRIBUTIONS

NC supervised the project of the review. All authors participated in the redaction of the manuscript and accepted the final version.

REFERENCES

Ahlquist, R. P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.* 153, 586–600. doi: 10.1152/ajplegacy.1948.153.3.586

Akula, R., and Mukherjee, S. (2020). New insights on neurotransmitters signaling mechanisms in plants. *Plant Signal. Behav.* 15:1737450. doi: 10.1080/15592324.2020.1737450

Aldriwesh, M., Al-Dayan, N., Barratt, J., and Freestone, P. (2019). The iron biology status of peritoneal dialysis patients may be a risk factor for development of infectious peritonitis. *Perit. Dial. Int.* 39, 362–374. doi: 10.3747/pdi.2018.00052

Anderson, M. T., and Armstrong, S. K. (2008). Norepinephrine mediates acquisition of transferrin-iron in *Bordetella bronchiseptica*. *J. Bacteriol.* 190, 3940–3947. doi: 10.1128/jb.00086-08

Andreis, D. T., and Singer, M. (2016). Catecholamines for inflammatory shock: a Jekyll-and-Hyde conundrum. *Intens. Care Med.* 42, 1387–1397. doi: 10.1007/s00134-016-4249-z

Armstrong, S. K., Brickman, T. J., and Suhadolc, R. J. (2012). Involvement of multiple distinct *Bordetella* receptor proteins in the utilization of iron liberated from transferrin by host catecholamine stress hormones. *Mol. Microbiol.* 84, 446–462. doi: 10.1111/j.1365-2958.2012.08032.x

Aroori, S. V., Cogan, T. A., and Humphrey, T. J. (2014). Effect of noradrenaline on the virulence properties of *Campylobacter* species. *Int. J. Microbiol.* 2014:279075. doi: 10.1155/2014/279075

Bansal, T., Englert, D., Lee, J., Hegde, M., Wood, T. K., and Jayaraman, A. (2007). Differential effects of epinephrine, norepinephrine, and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. *Infect. Immun.* 75, 4597–4607. doi: 10.1128/iai.00630-07

Bearson, B. L., and Bearson, S. M. (2008). The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar Typhimurium. *Microb. Pathog.* 44, 271–278. doi: 10.1016/j.micpath.2007.10.001

Bearson, B. L., Bearson, S. M., Uthe, J. J., Dowd, S. E., Houghton, J. O., Lee, I., et al. (2008). Iron regulated genes of *Salmonella enterica* serovar Typhimurium in response to norepinephrine and the requirement of fepDGC for norepinephrine-enhanced growth. *Microbes Infect.* 10, 807–816. doi: 10.1016/j.micinf.2008.04.011

Belay, T., and Sonnenfeld, G. (2002). Differential effects of catecholamines on *in vitro* growth of pathogenic bacteria. *Life Sci.* 71, 447–456. doi: 10.1016/s0024-3205(02)01683-1

Bergquist, J., Tarkowski, A., Ewing, A., and Ekman, R. (1998). Catecholaminergic suppression of immunocompetent cells. *Immunol. Today* 19, 562–567. doi: 10.1016/s0167-5699(98)01367-x

Berthelsen, S., and Pettinger, W. A. (1977). A functional basis for classification of alpha-adrenergic receptors. *Life Sci.* 21, 595–606. doi: 10.1016/0024-3205(77)90066-2

Biaggini, K. (2015). *Etude du dialogue système entéro-endocrinien/microbiote intestinal*. Ph.D. thesis. Saint-Étienne-du-Rouvray: Université de Rouen.

Biaggini, K., Barbey, C., Borrel, V., Feuilloley, M., Dechelotte, P., and Connil, N. (2015). The pathogenic potential of *Pseudomonas fluorescens* MFN1032 on enterocytes can be modulated by serotonin, substance P and epinephrine. *Arch. Microbiol.* 197, 983–990. doi: 10.1007/s00203-015-1135-y

Bönisch, H., Bryan, L. J., Henseling, M., O'donnell, S. R., Stockmann, P., and Trendelenburg, U. (1985). The effect of various ions on uptake2 of catecholamines. *Naunyn Schmiedebergs Arch. Pharmacol.* 328, 407–416. doi: 10.1007/bf00692909

Borrel, V., Thomas, P., Catovic, C., Racine, P. J., Konto-Ghiorghi, Y., Lefeuvre, L., et al. (2019). Acne and Stress: impact of Catecholamines on *Cutibacterium acnes*. *Front. Med.* 6:155. doi: 10.3389/fmed.2019.00155

Burton, C. L., Chhabra, S. R., Swift, S., Baldwin, T. J., Withers, H., Hill, S. J., et al. (2002). The growth response of *Escherichia coli* to neurotransmitters and related catecholamine drugs requires a functional enterobactin biosynthesis and uptake system. *Infect. Immun.* 70, 5913–5923. doi: 10.1128/iai.70.11.5913-5923.2002

Bylund, D. B. (1988). Subtypes of alpha 2-adrenoceptors: pharmacological and molecular biological evidence converge. *Trends Pharmacol. Sci.* 9, 356–361. doi: 10.1016/0165-6147(88)90254-4

Calil, C. M., Oliveira, G. M., Cogo, K., Pereira, A. C., Marcondes, F. K., and Groppo, F. C. (2014). Effects of stress hormones on the production of volatile sulfur compounds by periodontopathogenic bacteria. *Braz. Oral Res.* 28:S1806-S83242014000100228. doi: 10.1590/1807-3107bor-2014.vol28.0008

Cambronel, M., Nilly, F., Mesguida, O., Boukerb, A. M., Racine, P. J., Baccouri, O., et al. (2020). Influence of Catecholamines (Epinephrine/Norepinephrine) on Biofilm Formation and Adhesion in Pathogenic and Probiotic Strains of *Enterococcus faecalis*. *Front. Microbiol.* 11:1501. doi: 10.3389/fmicb.2020.01501

Cambronel, M., Tortuel, D., Biaggini, K., Maillot, O., Taupin, L., Rehel, K., et al. (2019). Epinephrine affects motility, and increases adhesion, biofilm and virulence of *Pseudomonas aeruginosa* H103. *Sci. Rep.* 9:20203. doi: 10.1038/s41598-019-56666-7

Carlsson, A. (1987). Perspectives on the discovery of central monoaminergic neurotransmission. *Annu. Rev. Neurosci.* 10, 19–40. doi: 10.1146/annurev.ne.10.030187.000315

Chet, I., Henis, Y., and Mitchell, R. (1973). Effect of biogenic amines and cannabinoids on bacterial chemotaxis. *J. Bacteriol.* 115, 1215–1218. doi: 10.1128/jb.115.3.1215-1218.1973

Clarke, M. B., and Sperandio, V. (2005a). Transcriptional autoregulation by quorum sensing *Escherichia coli* regulators B and C (QseBC) in enterohaemorrhagic *E. coli* (EHEC). *Mol. Microbiol.* 58, 441–455. doi: 10.1111/j.1365-2958.2005.04819.x

Clarke, M. B., and Sperandio, V. (2005b). Transcriptional regulation of *flh*DC by QseBC and sigma (FliA) in enterohaemorrhagic *Escherichia coli*. *Mol. Microbiol.* 57, 1734–1749. doi: 10.1111/j.1365-2958.2005.04792.x

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., and Sperandio, V. (2006). The QseC sensor kinase: a bacterial adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* 103, 10420–10425. doi: 10.1073/pnas.0604343103

Cogan, T. A., Thomas, A. O., Rees, L. E., Taylor, A. H., Jepson, M. A., Williams, P. H., et al. (2007). Norepinephrine increases the pathogenic potential of *Campylobacter jejuni*. *Gut* 56, 1060–1065. doi: 10.1136/gut.2006.114926

Cooper, E. V. (1946). Gas-gangrene following injection of adrenaline. *Lancet* 1, 459–461. doi: 10.1016/s0140-6736(46)91431-6

Cornelis, P., and Bodilis, J. (2009). A survey of TonB-dependent receptors in fluorescent pseudomonads. *Environ. Microbiol. Rep.* 1, 256–262. doi: 10.1111/j.1758-2229.2009.00041.x

Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science* 284, 1318–1322. doi: 10.1126/science.284.5418.1318

Coulanges, V., Andre, P., and Vidon, D. J. (1998). Effect of siderophores, catecholamines, and catechol compounds on *Listeria* spp. Growth in iron-complexed medium. *Biochem. Biophys. Res. Commun.* 249, 526–530. doi: 10.1006/bbrc.1998.9184

Coulanges, V., Andre, P., Ziegler, O., Buchheit, L., and Vidon, D. J. (1997). Utilization of iron-catecholamine complexes involving ferric reductase activity in *Listeria monocytogenes*. *Infect. Immun.* 65, 2778–2785. doi: 10.1128/iai.65.7.2778-2785.1997

Curtis, M. M., Russell, R., Moreira, C. G., Adebesin, A. M., Wang, C., Williams, N. S., et al. (2014). QseC inhibitors as an antivirulence approach for Gram-negative pathogens. *mBio* 5:e02165-14. doi: 10.1128/mBio.02165-14

Cuvas Apan, O., Apan, T. Z., and Apan, A. (2016). *In vitro* antimicrobial activity of commonly used vasoactive drugs. *J. Clin. Anesth.* 34, 407–411. doi: 10.1016/j.jclinane.2016.05.008

Dahlin, A., Xia, L., Kong, W., Hevner, R., and Wang, J. (2007). Expression and immunolocalization of the plasma membrane monoamine transporter in the brain. *Neuroscience* 146, 1193–1211. doi: 10.1016/j.neuroscience.2007.01.072

de Lima, P. O., Nani, B. D., Almeida, B., Marcondes, F. K., Groppo, F. C., de Moraes, A. B. A., et al. (2018). Stress-related salivary proteins affect the production of volatile sulfur compounds by oral bacteria. *Oral Dis.* 24, 1358–1366. doi: 10.1111/odi.12890

Dichtl, S., Demetz, E., Haschka, D., Tymoszuk, P., Petzer, V., Nairz, M., et al. (2019). Dopamine is a siderophore-like iron chelator that promotes *Salmonella enterica* Serovar Typhimurium virulence in mice. *mBio* 10:e02624-18. doi: 10.1128/mBio.02624-18

Dixon, R. A., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., et al. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature* 321, 75–79. doi: 10.1038/321075a0

Dong, Y., Liu, J., Pang, M., Du, H., Wang, N., Awan, F., et al. (2016). Catecholamine-Stimulated Growth of *Aeromonas hydrophila* Requires the TonB2 Energy Transduction System but Is Independent of the Amonabactin Siderophore. *Front. Cell. Infect. Microbiol.* 6:183. doi: 10.3389/fcimb.2016.00183

Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerg. Infect. Dis.* 8, 881–890. doi: 10.3201/eid0809.020063

Donlan, R. M., and Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 15, 167–193. doi: 10.1128/cmr.15.2.167-193.2002

Dowd, S. E. (2007). *Escherichia coli* O157:H7 gene expression in the presence of catecholamine norepinephrine. *FEMS Microbiol. Lett.* 273, 214–223. doi: 10.1111/j.1574-6968.2007.00800.x

Duan, H., and Wang, J. (2010). Selective transport of monoamine neurotransmitters by human plasma membrane monoamine transporter and organic cation transporter 3. *J. Pharmacol. Exp. Ther.* 335, 743–753. doi: 10.1124/jpet.110.170142

Eisenhofer, G. (2001). The role of neuronal and extraneuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol. Ther.* 91, 35–62. doi: 10.1016/s0163-7258(01)00144-9

Ellermann, M., and Sperandio, V. (2020). Bacterial signaling as an antimicrobial target. *Curr. Opin. Microbiol.* 57, 78–86. doi: 10.1016/j.mib.2020.08.001

Emorine, L. J., Marullo, S., Briend-Sutren, M. M., Patey, G., Tate, K., Delavier-Klutchko, C., et al. (1989). Molecular characterization of the human beta 3-adrenergic receptor. *Science* 245, 1118–1121. doi: 10.1126/science.2570461

Engel, K., and Wang, J. (2005). Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Mol. Pharmacol.* 68, 1397–1407. doi: 10.1124/mol.105.016832

Engel, K., Zhou, M., and Wang, J. (2004). Identification and characterization of a novel monoamine transporter in the human brain. *J. Biol. Chem.* 279, 50042–50049. doi: 10.1074/jbc.m407913200

Felten, S. Y., and Olschowka, J. (1987). Noradrenergic sympathetic innervation of the spleen: II. Tyrosine hydroxylase (TH)-positive nerve terminals form synapticlike contacts on lymphocytes in the splenic white pulp. *J. Neurosci. Res.* 18, 37–48. doi: 10.1002/jnr.490180108

Feraco, D., Blaha, M., Khan, S., Green, J. M., and Plotkin, B. J. (2016). Host environmental signals and effects on biofilm formation. *Microb. Pathog.* 99, 253–263. doi: 10.1016/j.micpath.2016.08.015

Ford, A. P., Williams, T. J., Blue, D. R., and Clarke, D. E. (1994). Alpha 1-adrenoceptor classification: sharpening Occam's razor. *Trends Pharmacol. Sci.* 15, 167–170. doi: 10.1016/0165-6147(94)90136-8

Frank, K. L., and Patel, R. (2008). Intravenously administered pharmaceuticals impact biofilm formation and detachment of *Staphylococcus lugdunensis* and other staphylococci. *Diagn. Microbiol. Infect. Dis.* 60, 9–16. doi: 10.1016/j.diagmicrobio.2007.07.008

Freestone, P. (2013). Communication between Bacteria and Their Hosts. *Scientifica* 2013:361073. doi: 10.1155/2013/361073

Freestone, P. P. E., Al-Dayan, N., and Lyte, M. (2016). Staphylococci, Catecholamine Inotropes and Hospital-Acquired Infections. *Adv. Exp. Med. Biol.* 874, 183–199. doi: 10.1007/978-3-319-20215-0_8

Freestone, P. P., Haigh, R. D., Williams, P. H., and Lyte, M. (1999). Stimulation of bacterial growth by heat-stable, norepinephrine-induced autoinducers. *FEMS Microbiol. Lett.* 172, 53–60. doi: 10.1111/j.1574-6968.1999.tb13449.x

Freestone, P. P., Haigh, R. D., Williams, P. H., and Lyte, M. (2003). Involvement of enterobactin in norepinephrine-mediated iron supply from transferrin to enterohaemorrhagic *Escherichia coli*. *FEMS Microbiol. Lett.* 222, 39–43. doi: 10.1016/s0378-1097(03)00243-x

Freestone, P. P., Hirst, R. A., Sandrini, S. M., Sharaff, F., Fry, H., Hyman, S., et al. (2012). *Pseudomonas aeruginosa*-catecholamine inotrope interactions: a contributory factor in the development of ventilator-associated pneumonia? *Chest* 142, 1200–1210. doi: 10.1378/chest.11-2614

Freestone, P. P., Lyte, M., Neal, C. P., Maggs, A. F., Haigh, R. D., and Williams, P. H. (2000). The mammalian neuroendocrine hormone norepinephrine supplies iron for bacterial growth in the presence of transferrin or lactoferrin. *J. Bacteriol.* 182, 6091–6098. doi: 10.1128/JB.182.21.6091-6098.2000

Freestone, P. P., Sandrini, S. M., Haigh, R. D., and Lyte, M. (2008). Microbial endocrinology: how stress influences susceptibility to infection. *Trends Microbiol.* 16, 55–64. doi: 10.1016/j.tim.2007.11.005

Ganz, T., and Nemeth, E. (2015). Iron homeostasis in host defence and inflammation. *Nat. Rev. Immunol.* 15, 500–510. doi: 10.1038/nri3863

Gao, J., Xi, B., Chen, K., Song, R., Qin, T., Xie, J., et al. (2019). The stress hormone norepinephrine increases the growth and virulence of *Aeromonas hydrophila*. *Microbiologyopen* 8:e00664. doi: 10.1002/mbo3.664

Gonzales, X. F., Castillo-Rojas, G., Castillo-Rodal, A. I., Tuomanen, E., and López-Vidal, Y. (2013). Catecholamine norepinephrine diminishes lung epithelial cell adhesion of *Streptococcus pneumoniae* by binding iron. *Microbiology* 159, 2333–2341. doi: 10.1099/mic.0.065607-0

Greengard, P. (2001). The neurobiology of dopamine signaling. *Biosci. Rep.* 21, 247–269. doi: 10.1023/a:1013205230142

Gründemann, D., Schechinger, B., Rappold, G. A., and Schomig, E. (1998). Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat. Neurosci.* 1, 349–351. doi: 10.1038/1557

Guckes, K. R., Kostakioti, M., Breland, E. J., Gu, A. P., Shaffer, C. L., Martinez, C. R. III, et al. (2013). Strong cross-system interactions drive the activation of the QseB response regulator in the absence of its cognate sensor. *Proc. Natl. Acad. Sci. U.S.A.* 110, 16592–16597. doi: 10.1073/pnas.1315320110

Hadjifrangiskou, M., Kostakioti, M., Chen, S. L., Henderson, J. P., Greene, S. E., and Hultgren, S. J. (2011). A central metabolic circuit controlled by QseC in pathogenic *Escherichia coli*. *Mol. Microbiol.* 80, 1516–1529. doi: 10.1111/j.1365-2958.2011.07660.x

Halang, P., Toulouse, C., Geißel, B., Michel, B., Flauger, B., Müller, M., et al. (2015). Response of *Vibrio cholerae* to the catecholamine hormones epinephrine and norepinephrine. *J. Bacteriol.* 197, 3769–3778. doi: 10.1128/JB.00345-15

Hantke, K., Nicholson, G., Rabsch, W., and Winkelmann, G. (2003). Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IroN. *Proc. Natl. Acad. Sci. U.S.A.* 100, 3677–3682. doi: 10.1073/pnas.0737682100

Hegde, M., Wood, T. K., and Jayaraman, A. (2009). The neuroendocrine hormone norepinephrine increases *Pseudomonas aeruginosa* PA14 virulence through the las quorum-sensing pathway. *Appl. Microbiol. Biotechnol.* 84, 763–776. doi: 10.1007/s00253-009-2045-1

Hendley, E. D., Taylor, K. M., and Snyder, S. H. (1970). 3H-normetanephrine uptake in rat brain slices. Relationship to extraneuronal accumulation of norepinephrine. *Eur. J. Pharmacol.* 12, 167–179. doi: 10.1016/0014-2999(70)90062-2

Hieble, J. P., Bondinell, W. E., and Ruffolo, R. R. Jr. (1995). Alpha- and beta-adrenoceptors: from the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. *J. Med. Chem.* 38, 3415–3444. doi: 10.1021/jm00018a001

Hughes, D. T., Clarke, M. B., Yamamoto, K., Rasko, D. A., and Sperandio, V. (2009). The QseC adrenergic signaling cascade in Enterohemorrhagic *E. coli* (EHEC). *PLoS Pathog.* 5:e1000553. doi: 10.1371/journal.ppat.1000553

Inaba, M., Matsuda, N., Banno, H., Jin, W., Wachino, J. I., Yamada, K., et al. (2016). *In vitro* reduction of antibacterial activity of tigecycline against multidrug-resistant *Acinetobacter baumannii* with host stress hormone norepinephrine. *Int. J. Antimicrob. Agents* 48, 680–689. doi: 10.1016/j.ijantimicag.2016.09.022

Intarak, N., Muangsombut, V., Vattanaviboon, P., Stevens, M. P., and Korbsrisate, S. (2014). Growth, motility and resistance to oxidative stress of the melioidosis pathogen *Burkholderia pseudomallei* are enhanced by epinephrine. *Pathog. Dis.* 72, 24–31. doi: 10.1111/2049-632x.12181

Iversen, L. L. (1971). Role of transmitter uptake mechanisms in synaptic neurotransmission. *Br. J. Pharmacol.* 41, 571–591. doi: 10.1111/j.1476-5381.1971.tb07066.x

Karavolos, M. H., Bulmer, D. M., Spencer, H., Rampioni, G., Schmalen, I., Baker, S., et al. (2011). *Salmonella* Typhi sense host neuroendocrine stress hormones and release the toxin haemolysin E. *EMBO Rep.* 12, 252–258. doi: 10.1038/embor.2011.4

Karavolos, M. H., Spencer, H., Bulmer, D. M., Thompson, A., Winzer, K., Williams, P., et al. (2008). Adrenaline modulates the global transcriptional profile of *Salmonella* revealing a role in the antimicrobial peptide and oxidative stress resistance responses. *BMC Genomics* 9:458. doi: 10.1186/1471-2164-9-458

Kendall, M. M., Rasko, D. A., and Sperandio, V. (2007). Global effects of the cell-to-cell signaling molecules autoinducer-2, autoinducer-3, and epinephrine in a *lux*S mutant of enterohemorrhagic *Escherichia coli*. *Infect. Immun.* 75, 4875–4884. doi: 10.1128/iai.00550-07

Kesici, S., Demirci, M., and Kesici, U. (2019). Antibacterial effects of lidocaine and adrenaline. *Int. Wound J.* 16, 1190–1194. doi: 10.1111/iwj.13182

Kim, C. S., Gatsios, A., Cuesta, S., Lam, Y. C., Wei, Z., Chen, H., et al. (2020). Characterization of Autoinducer-3 Structure and Biosynthesis in *E. coli*. *ACS Cent. Sci.* 6, 197–206. doi: 10.1021/acscentsci.9b01076

Kostakioti, M., Hadjifrangiskou, M., Pinkner, J. S., and Hultgren, S. J. (2009). QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic *Escherichia coli*. *Mol. Microbiol.* 73, 1020–1031. doi: 10.1111/j.1365-2958.2009.06826.x

Lands, A. M., Arnold, A., Mcauliff, J. P., Luduena, F. P., and Brown, T. G. Jr. (1967a). Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 214, 597–598. doi: 10.1038/214597a0

Lands, A. M., Luduena, F. P., and Buzzo, H. J. (1967b). Differentiation of receptors responsive to isoproterenol. *Life Sci.* 6, 2241–2249. doi: 10.1016/0024-3205(67)90031-8

Lanter, B. B., and Davies, D. G. (2015). *Propionibacterium acnes* recovered from atherosclerotic human carotid arteries undergoes biofilm dispersion and releases lipolytic and proteolytic enzymes in response to norepinephrine challenge *in vitro*. *Infect. Immun.* 83, 3960–3971. doi: 10.1128/iai.00510-15

Lanter, B. B., Sauer, K., and Davies, D. G. (2014). Bacteria present in carotid arterial plaques are found as biofilm deposits which may contribute to enhanced risk of plaque rupture. *mBio* 5:e01206-14. doi: 10.1128/mBio.01206-14

Lewis, P. (1976). The essential action of propranolol in hypertension. *Am. J. Med.* 60, 837–852. doi: 10.1016/0002-9343(76)90904-9

Li, L., Chen, Z., Bei, W., Su, Z., Huang, Q., Zhang, L., et al. (2015). Catecholamines promote *Actinobacillus pleuropneumoniae* growth by regulating iron metabolism. *PLoS One* 10:e0121887. doi: 10.1371/journal.pone.0121887

Li, L., Xu, Z., Zhou, Y., Sun, L., Liu, Z., Chen, H., et al. (2012). Global effects of catecholamines on *Actinobacillus pleuropneumoniae* gene expression. *PLoS One* 7:e31121. doi: 10.1371/journal.pone.0031121

Li, W., Lyte, M., Freestone, P. P., Ajmal, A., Colmer-Hamood, J. A., and Hamood, A. N. (2009). Norepinephrine represses the expression of *tox*A and the siderophore genes in *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* 299, 100–109. doi: 10.1111/j.1574-6968.2009.01739.x

Lopes, J. G., and Sourjik, V. (2018). Chemotaxis of *Escherichia coli* to major hormones and polyamines present in human gut. *ISME J.* 12, 2736–2747. doi: 10.1038/s41396-018-0227-5

Lymperopoulos, A., Rengo, G., Zincarelli, C., Soltys, S., and Koch, W. J. (2008). Modulation of adrenal catecholamine secretion by *in vivo* gene transfer and manipulation of G protein-coupled receptor kinase-2 activity. *Mol. Ther.* 16, 302–307. doi: 10.1038/sj.mt.6300371

Lyte, M. (2004). Microbial endocrinology and infectious disease in the 21st century. *Trends Microbiol.* 12, 14–20. doi: 10.1016/j.tim.2003.11.004

Lyte, M. (2013). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. *PLoS Pathog.* 9:e1003726. doi: 10.1371/journal.ppat.1003726

Lyte, M. (2014). Microbial endocrinology and the microbiota-gut-brain axis. *Adv. Exp. Med. Biol.* 817, 3–24. doi: 10.1007/978-1-4939-0897-4_1

Lyte, M., and Brown, D. R. (2018). Evidence for PMAT- and OCT-like biogenic amine transporters in a probiotic strain of *Lactobacillus*: implications for interkingdom communication within the microbiota-gut-brain axis. *PLoS One* 13:e0191037. doi: 10.1371/journal.pone.0191037

Lyte, M., and Ernst, S. (1992). Catecholamine induced growth of gram negative bacteria. *Life Sci.* 50, 203–212. doi: 10.1016/0024-3205(92)90273-r

Lyte, M., Freestone, P. P., Neal, C. P., Olson, B. A., Haigh, R. D., Bayston, R., et al. (2003). Stimulation of *Staphylococcus epidermidis* growth and biofilm formation by catecholamine inotropes. *Lancet* 361, 130–135. doi: 10.1016/s0140-6736(03)12231-3

Marinissen, M. J., and Gutkind, J. S. (2001). G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol. Sci.* 22, 368–376. doi: 10.1016/s0165-6147(00)01678-3

Mart'yanov, S. V., Botchkova, E. A., Plakunov, V. K., and Gannesen, A. V. (2021). The impact of norepinephrine on mono-species and dual-species Staphylococcal biofilms. *Microorganisms* 9:820. doi: 10.3390/microorganisms9040820

Mietzner, T. A., and Morse, S. A. (1994). The role of iron-binding proteins in the survival of pathogenic bacteria. *Annu. Rev. Nutr.* 14, 471–493. doi: 10.1146/annurev.nu.14.070194.002351

Moreira, C. G., and Sperandio, V. (2012). Interplay between the QseC and QseE bacterial adrenergic sensor kinases in *Salmonella enterica* serovar Typhimurium pathogenesis. *Infect. Immun.* 80, 4344–4353. doi: 10.1128/iai.00803-12

Moreira, C. G., Weinshenker, D., and Sperandio, V. (2010). QseC mediates *Salmonella enterica* serovar Typhimurium virulence *in vitro* and *in vivo*. *Infect. Immun.* 78, 914–926. doi: 10.1128/iai.01038-09

Moron, J. A., Brockington, A., Wise, R. A., Rocha, B. A., and Hope, B. T. (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *J. Neurosci.* 22, 389–395. doi: 10.1523/jneurosci.22-02-00389.2002

Nakano, M., Takahashi, A., Sakai, Y., Kawano, M., Harada, N., Mawatari, K., et al. (2007a). Catecholamine-induced stimulation of growth in *Vibrio* species. *Lett. Appl. Microbiol.* 44, 649–653. doi: 10.1111/j.1472-765x.2007.02136.x

Nakano, M., Takahashi, A., Sakai, Y., and Nakaya, Y. (2007b). Modulation of pathogenicity with norepinephrine related to the type III secretion system of *Vibrio parahaemolyticus*. *J. Infect. Dis.* 195, 1353–1360. doi: 10.1086/513275

Pande, G. S., Suong, N. T., Bossier, P., and Defoirdt, T. (2014). The catecholamine stress hormones norepinephrine and dopamine increase the virulence of pathogenic *Vibrio anguillarum* and *Vibrio campbellii*. *FEMS Microbiol. Ecol.* 90, 761–769. doi: 10.1111/1574-6941.12432

Parsek, M. R., and Singh, P. K. (2003). Bacterial biofilms: an emerging link to disease pathogenesis. *Annu. Rev. Microbiol.* 57, 677–701. doi: 10.1146/annurev.micro.57.030502.090720

Perez, D. M. (2006). *The Adrenergic Receptors in the 21st Century*. (Totowa, NJ: Humana Press), 129–134.

Perraud, Q., Cantero, P., Roche, B., Gasser, V., Normant, V. P., Kuhn, L., et al. (2020). Phenotypic adaption of *Pseudomonas aeruginosa* by hacking siderophores produced by other microorganisms. *Mol. Cell. Proteomics* 19, 589–607. doi: 10.1074/mcp.ra119.001829

Peterson, G., Kumar, A., Gart, E., and Narayanan, S. (2011). Catecholamines increase conjugative gene transfer between enteric bacteria. *Microb. Pathog.* 51, 1–8. doi: 10.1016/j.micpath.2011.03.002

Pillai, D. K., Cha, E., and Mosier, D. (2018). Role of the stress-associated chemicals norepinephrine, epinephrine and substance P in dispersal of *Mannheimia haemolytica* from biofilms. *Vet. Microbiol.* 215, 11–17. doi: 10.1016/j.vetmic.2017.11.025

Pullinger, G. D., Van Diemen, P. M., Carnell, S. C., Davies, H., Lyte, M., and Stevens, M. P. (2010). 6-hydroxydopamine-mediated release of norepinephrine increases faecal excretion of *Salmonella enterica* serovar Typhimurium in pigs. *Vet. Res.* 41:68. doi: 10.1051/vetres/2010040

Rasko, D. A., Moreira, C. G., Li De, R., Reading, N. C., Ritchie, J. M., Waldor, M. K., et al. (2008). Targeting QseC signaling and virulence for antibiotic development. *Science* 321, 1078–1080. doi: 10.1126/science.1160354

Ratledge, C., and Dover, L. G. (2000). Iron metabolism in pathogenic bacteria. *Annu. Rev. Microbiol.* 54, 881–941. doi: 10.1146/annurev.micro.54.1.881

Reading, N. C., Rasko, D. A., Torres, A. G., and Sperandio, V. (2009). The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 5889–5894. doi: 10.1073/pnas.0811409106

Reading, N. C., Torres, A. G., Kendall, M. M., Hughes, D. T., Yamamoto, K., and Sperandio, V. (2007). A novel two-component signaling system that activates transcription of an enterohemorrhagic *Escherichia coli* effector involved in remodeling of host actin. *J. Bacteriol.* 189, 2468–2476. doi: 10.1128/jb.01848-06

Reiske, L., Schmucker, S. S., Steuber, J., Toulouse, C., Pfaffinger, B., and Stefanski, V. (2020). Interkingdom cross-talk in times of stress: *Salmonella* Typhimurium grown in the presence of catecholamines inhibits porcine immune functionality *in vitro*. *Front. Immunol.* 11:572056. doi: 10.3389/fimmu.2020.572056

Renaud, M., and Miget, A. (1930). Role favorisant des perturbations locales causees par l'adrenaline sur le developpement des infections microbiennes. *CR Seances Soc. Biol. Fil.* 103, 1052–1054.

Roberts, A., Matthews, J. B., Socransky, S. S., Freestone, P. P., Williams, P. H., and Chapple, I. L. (2002). Stress and the periodontal diseases: effects of catecholamines on the growth of periodontal bacteria *in vitro*. *Oral Microbiol. Immunol.* 17, 296–303. doi: 10.1034/j.1399-302x.2002.170506.x

Rosenbaum, D. M., Rasmussen, S. G., and Kobilka, B. K. (2009). The structure and function of G-protein-coupled receptors. *Nature* 459, 356–363. doi: 10.1038/nature08144

Sandrini, S. M., Shergill, R., Woodward, J., Muralikuttan, R., Haigh, R. D., Lyte, M., et al. (2010). Elucidation of the mechanism by which catecholamine stress hormones liberate iron from the innate immune defense proteins transferrin and lactoferrin. *J. Bacteriol.* 192, 587–594. doi: 10.1128/jb.01028-09

Sandrini, S., Masania, R., Zia, F., Haigh, R., and Freestone, P. (2013). Role of porin proteins in acquisition of transferrin iron by enteropathogens. *Microbiology* 159, 2639–2650. doi: 10.1099/mic.0.071928-0

Scardaci, R., Varese, F., Manfredi, M., Marengo, E., Mazzoli, R., and Pessione, E. (2021). *Enterococcus faecium* NCIMB10415 responds to norepinephrine by altering protein profiles and phenotypic characters. *J. Proteomics* 231:104003. doi: 10.1016/j.jprot.2020.104003

Sharaff, F., and Freestone, P. (2011). Microbial endocrinology. *Cent. Eur. J. Biol.* 6:685. doi: 10.2478/s11535-011-0067-z

Sharma, V. K., and Casey, T. A. (2014a). Determining the relative contribution and hierarchy of *hha* and *qse*BC in the regulation of flagellar motility of *Escherichia coli* O157:H7. *PLoS One* 9:e85866. doi: 10.1371/journal.pone.0085866

Sharma, V. K., and Casey, T. A. (2014b). *Escherichia coli* O157:H7 lacking the qseBC-encoded quorum-sensing system outcompetes the parental strain in colonization of cattle intestines. *Appl. Environ. Microbiol.* 80, 882–892. doi: 10.1128/AEM.03198-13

Sharon, G., Sampson, T. R., Geschwind, D. H., and Mazmanian, S. K. (2016). The central nervous system and the gut microbiome. *Cell* 167, 915–932. doi: 10.1016/j.cell.2016.10.027

Sora, I., Hall, F. S., Andrews, A. M., Itokawa, M., Li, X. F., Wei, H. B., et al. (2001). Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc. Natl. Acad. Sci. U.S.A.* 98, 5300–5305. doi: 10.1073/pnas.091039298

Spencer, H., Karavolos, M. H., Bulmer, D. M., Aldridge, P., Chhabra, S. R., Winzer, K., et al. (2010). Genome-wide transposon mutagenesis identifies a role for host neuroendocrine stress hormones in regulating the expression of virulence genes in *Salmonella*. *J. Bacteriol.* 192, 714–724. doi: 10.1128/jb.01329-09

Sperandio, V., Torres, A. G., Jarvis, B., Nataro, J. P., and Kaper, J. B. (2003). Bacteria-host communication: the language of hormones. *Proc. Natl. Acad. Sci. U.S.A.* 100, 8951–8956. doi: 10.1073/pnas.1537100100

Strosberg, A. D. (1993). Structure, function, and regulation of adrenergic receptors. *Protein Sci.* 2, 1198–1209. doi: 10.1002/pro.5560020802

Torabi Delshad, S., Soltanian, S., Sharifiyazdi, H., and Bossier, P. (2019). Effect of catecholamine stress hormones (dopamine and norepinephrine) on growth, swimming motility, biofilm formation and virulence factors of *Yersinia ruckeri in vitro* and an *in vivo* evaluation in rainbow trout. *J. Fish Dis.* 42, 477–487. doi: 10.1111/jfd.12934

Toulouse, C., Schmucker, S., Metesch, K., Pfannstiel, J., Michel, B., Starke, I., et al. (2019). Mechanism and impact of catecholamine conversion by *Vibrio cholerae*. *Biochim. Biophys. Acta Bioenerg.* 1860, 478–487. doi: 10.1016/j.bbabio.2019.04.003

Vialou, V., Balasse, L., Dumas, S., Giros, B., and Gautron, S. (2007). Neurochemical characterization of pathways expressing plasma membrane monoamine transporter in the rat brain. *Neuroscience* 144, 616–622. doi: 10.1016/j.neuroscience.2006.09.058

Wayment, H. K., Schenk, J. O., and Sorg, B. A. (2001). Characterization of extracellular dopamine clearance in the medial prefrontal cortex: role of monoamine uptake and monoamine oxidase inhibition. *J. Neurosci.* 21, 35–44. doi: 10.1523/jneurosci.21-01-00035.2001

Williams, P. H., Rabsch, W., Methner, U., Voigt, W., Tschape, H., and Reissbrodt, R. (2006). Catecholate receptor proteins in *Salmonella enterica*: role in virulence and implications for vaccine development. *Vaccine* 24, 3840–3844. doi: 10.1016/j.vaccine.2005.07.020

Wilson, V. G., Grohmann, M., and Trendelenburg, U. (1988). The uptake and O-methylation of 3H-(+/-)-isoprenaline in rat cerebral cortex slices. *Naunyn Schmiedebergs Arch. Pharmacol.* 337, 397–405. doi: 10.1007/BF00169530

Wösten, M. M., Kox, L. F., Chamnongpol, S., Soncini, F. C., and Groisman, E. A. (2000). A signal transduction system that responds to extracellular iron. *Cell* 103, 113–125. doi: 10.1016/s0092-8674(00)00092-1

Xu, F., Wu, C., Guo, F., Cui, G., Zeng, X., Yang, B., et al. (2015). Transcriptomic analysis of *Campylobacter jejuni* NCTC 11168 in response to epinephrine and norepinephrine. *Front. Microbiol.* 6:452. doi: 10.3389/fmicb.2015.00452

Yamashita, A., Singh, S. K., Kawate, T., Jin, Y., and Gouaux, E. (2005). Crystal structure of a bacterial homologue of Na^+/Cl^-dependent neurotransmitter transporters. *Nature* 437, 215–223. doi: 10.1038/nature03978

Yang, K., Meng, J., Huang, Y. C., Ye, L. H., Li, G. J., Huang, J., et al. (2014). The role of the QseC quorum-sensing sensor kinase in epinephrine-enhanced motility and biofilm formation by *Escherichia coli*. *Cell Biochem. Biophys.* 70, 391–398. doi: 10.1007/s12013-014-9924-5

Yang, Q., Anh, N. D., Bossier, P., and Defoirdt, T. (2014). Norepinephrine and dopamine increase motility, biofilm formation, and virulence of *Vibrio harveyi*. *Front. Microbiol.* 5:584. doi: 10.3389/fmicb.2014.00584

Yang, Q., Zou, P., Cao, Z., Wang, Q., Fu, S., Xie, G., et al. (2021). QseC Inhibition as a Novel Antivirulence Strategy for the Prevention of Acute Hepatopancreatic Necrosis Disease (AHPND)-Causing *Vibrio parahaemolyticus*. *Front. Cell. Infect. Microbiol.* 10:5946. doi: 10.3389/fcimb.2020.594652

Yernool, D., Boudker, O., Jin, Y., and Gouaux, E. (2004). Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*. *Nature* 431, 811–818. doi: 10.1038/nature03018

Zhou, M., Hank Simms, H., and Wang, P. (2004). Increased gut-derived norepinephrine release in sepsis: up-regulation of intestinal tyrosine hydroxylase. *Biochim. Biophys. Acta* 1689, 212–218. doi: 10.1016/j.bbadis.2004.03.008

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership