DIVERSITY OF THE MICROBIAL WORLD

EDITED BY: Angélica Cibrián-Jaramillo, Edder D. Bustos-Díaz and Francisco Barona-Gómez

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DIVERSITY OF THE MICROBIAL WORLD

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Microbes, or microorganisms, are tiny living beings that cannot be seen by the naked eye. These little guys are one of the oldest living things on Earth, and are extremely diverse in how they live and what they can do. They, for example, can live in many places, from the freezing iciness of glaciers, to the insides of other organisms, like termites or humans. Since they are virtually everywhere, microorganisms are essential for the biological processes that allow plants and animals to breath, eat and thrive. But how were they able to endure, adapt and flourish constantly over millions of years? The secrets of their success are still within them, coded into their genomes, waiting for us to understand them.

Now, genomes, bacterial or otherwise, are the repositories of life. These repositories store almost every bit of information that allows living beings to live in discrete units called genes. Genes are strung together like the sentences in a book, interacting with each other to create meaning, saving the story of that particular book—or that particular living organism's genome—so it can be copied, modified, corrected or enhanced, and then passed on to new generations. After many, many years of studying these "books," we have learned to read and understand them, thanks to the technological innovations of the last decade. Nowadays, it is possible to get the full genomic sequence of practically any organism, and compare it with thousands of genomes from other organisms, letting us peek at the secrets that make each organism who it is. With the current technical abilities, the challenge now is not to obtain the information but to interpret all those chunks of the story.

Finding ways to untangle the riddles of genomic information is the work of Genomics, the science that allows us to obtain, analyze and prioritize information among the many stories that we sequence everyday. To do this, Genomics draws from many sciences, like mathematics and computing sciences, making it a truly interdisciplinary endeavor. Right now , genomics are one of the most important areas of biology, and many, if not most, of current biological studies use at least a little bit of genomics. For example, genomics can be used to identify a microbe and give it a name, to learn about what types of things it can do or places it can live, and to figure out the mechanisms that enable it to survive under particular conditions.

Here, we will dwell on some of the basic questions about microbial adaptation, biodiversity, and their relationships with other living beings using a genomic approach. We will also focus on the environment, trying to understand how such tiny little creatures are capable of solving their daily problems, and how they can alter the places in which they live. Learning about these mechanisms will not only provide us with knowledge about life in general but will also help us to understand these organisms as a fundamental component of our ecosystem, including their harmful and beneficial effects in all aspects of our daily life, which can be translated into useful applications in almost any imaginable way.

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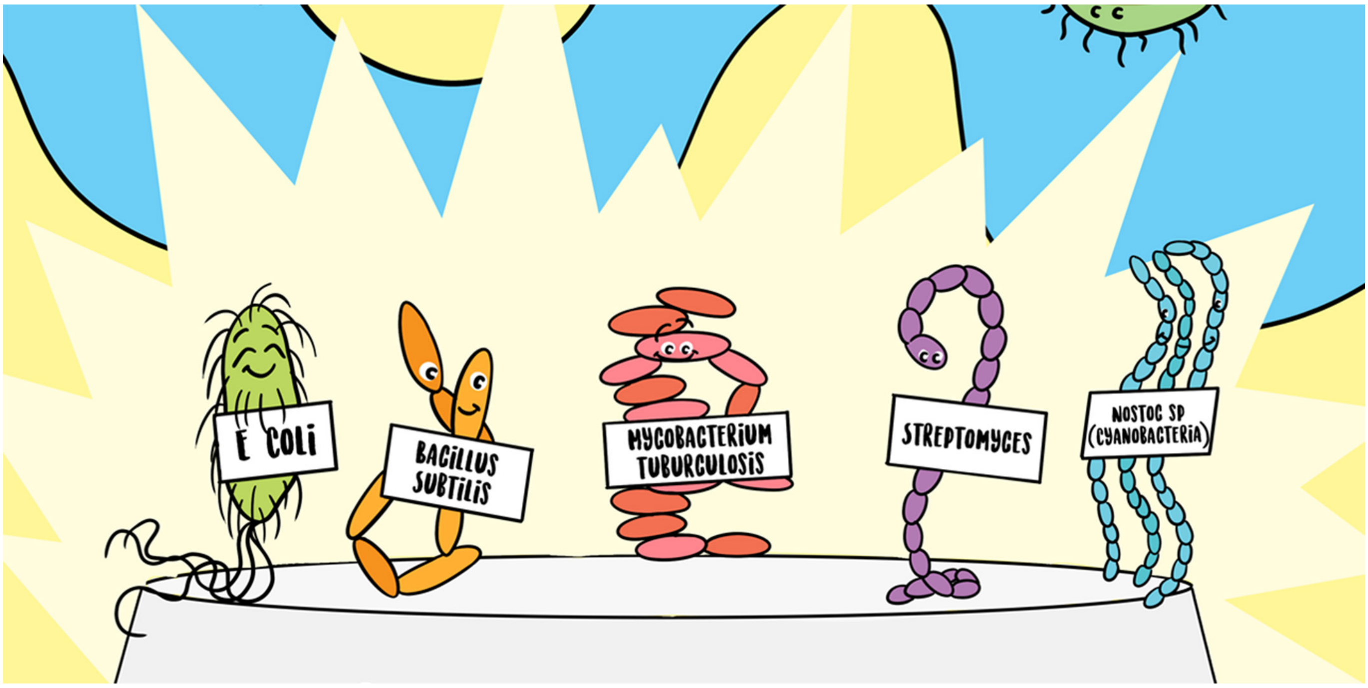

WHAT ARE MODEL MICROORGANISMS?

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YOUNG REVIEWERS:


MARIA
AGE: 15


MARIANA
AGE: 14

When you go to buy a pet fish, you will probably get very detailed instructions on how to take care of it. Even before you go home with your new buddy, you will know a lot of useful stuff, like what it eats and how often you need to clean its tank. Now, if you tried to adopt an octopus, things would not be so easy. I mean, does it even have a mouth? Scientists have a similar problem. When we plan experiments using animals, we need to know a lot about them so that we can tell whether or not our experiments are affecting them. Since scientists cannot hope to learn everything about every animal, they decided to study just a handful of them and use those well-studied examples for their research. These well-studied creatures are called model organisms and, in this article, you will learn about the smallest of them.

INTRODUCTION

There is such an incredible variety of living beings that trying to learn everything about all of them would take us hundreds and hundreds of years. Scientists who want to use living beings for research do not want to wait that long—I mean, who is going to win all those Nobel prizes in the meantime? So, instead of studying every type of fish in the sea, we study just a few of them. This principle is particularly important for **bacteria**, because there are more bacterial species than fish in the sea. In fact, there are so many bacterial species that we can only guess how many there are. And then, to make things even more difficult, bacterial species are so different from each other that sometimes comparing two species feels more like comparing a jellyfish with a horse. Because we know so little about the different kinds of bacteria that exist, the bulk of research done using bacteria has been performed using just a handful of microorganisms called model organisms [1]. Scientists use them when they want to do complicated studies because, since we know a lot about them, it reduces the amount of uncertainty that we could encounter in our experiments. We have learned so much about some model organisms that it is now easy to use them to do the type of experiments we want to do [2].

BACTERIA

Organisms that only have one cell. The singular is bacterium.

BACTERIA AS A MODEL ORGANISM

Bacteria, just like all other living things, are made of a bunch of chemicals. While there are many kinds of chemicals inside them, two of the most important are **DNA** and **proteins**. DNA molecules hold the genetic information of an organism. This genetic information is what gives living organisms their identities—stuff like the color of flowers or the shape of a person's eyes. The DNA regions responsible for these and many other functions are called genes.

DNA

A chain of four chemicals arranged in different orders. It is used to codify and store the necessary information to make proteins.

PROTEINS

The most abundant chemicals in cells. They have various functions and are made of many different chemicals. The instructions to build them are codified in the DNA.

Proteins are also chemicals, but their functions are more diverse. While DNA is like a list of recipes, the proteins are the actual cakes. Proteins come in various forms and sizes and can perform many different functions, but the important thing to know is that they are coded in the DNA, in those genes that I have told you about, and if an organism suffers changes in its DNA sequences, the proteins can be changed in significant ways, sometimes for the better, other times for the worse.

Every organism has DNA and proteins, but what makes bacteria special is that they do not have a lot of these molecules. Well, they do have thousands of genes and proteins, but that is a relatively low number compared with the hundreds of thousands of genes and proteins that animals or plants have. Because of their relative simplicity, it is a little easier to understand the functions and interactions of bacterial genes and proteins. The low numbers of proteins and genes also means that bacteria are generally simpler organisms. While other organisms might have organs dedicated to respiration or food digestion, bacteria do everything at once in their one organ: the **cell**. Each bacterial cell is a whole organism in and of itself.

CELL

The smallest biological sub-unit. It contains DNA, proteins, and many other chemicals.

Another advantage of working with bacteria is that they do not require a lot of food or space. Due to their small size, they are easily fed

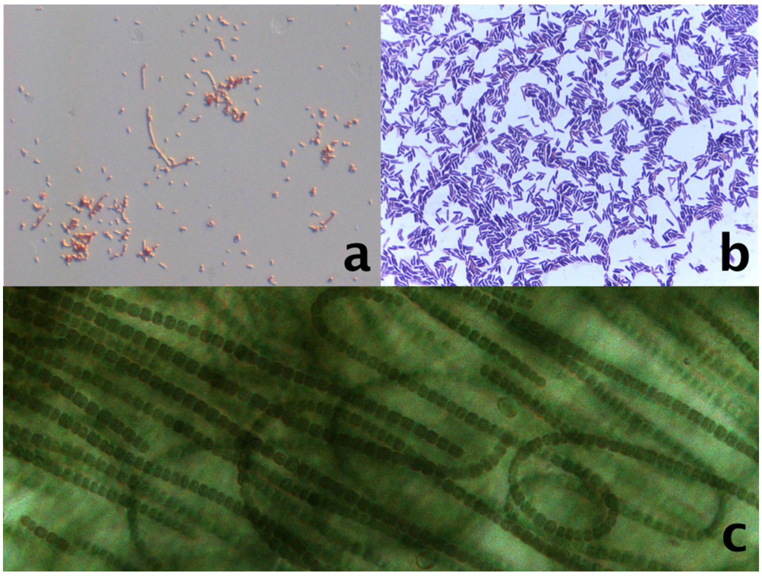

Figure 1

Figure 1

Structures of some of the bacteria that are used as model microorganisms. The images were taken with a microscopy, because these bacteria are very small. **(a)** Stained *Escherichia coli* round cells. **(b)** Stained *Bacillus subtilis* cells. **(c)** The naturally green cyanobacteria *Nostoc* sp., a bacterium that forms filaments of multiple linked cells.

so we can maintain whole bacterial populations for days in very small amounts of what is basically chicken broth. Finally, bacteria grow rapidly by dividing themselves into two new cells, so they grow exponentially, which gives scientists a lot of cells to work with.

Here are a few bacterial model organisms that are used for scientific investigations around the world:

Escherichia coli

E. coli is the undisputed bacterial superstar of the model microorganisms. *E. coli* is used for almost everything, from studies of the bacterial life cycle to experiments on the way bacteria behave in extremely cold temperatures. This small and round bacterium (Figure 1a), discovered by doctor Theodor Escherich in 1885, has been used in many experiments that helped us to understand how bacteria work: how they eat, how they reproduce, questions about their genes and their proteins—about almost everything, really. So, in a way, it can be said that modern microbiology has been built upon *E. coli's* "shoulders." *E. coli* are usually found in our guts, where they live without harming us, although some *E. coli* strains are known to cause diarrhea and other gastrointestinal diseases. But do not worry, the strains used in laboratories are not harmful [1].

Bacillus subtilis

Bacillus is known for the ease with which scientists can manipulate its genes, which allows us to investigate the functions of many of those genes [3]. Another interesting characteristic of this bacterium is that it produces structures called endospores, which are a special cellular form that allows it to survive even when the conditions are not very good for its growth. While *Bacillus* is not the only organism that can create endospores, most of the studies investigating how endospores form were done in *Bacillus* [4]. These bacteria are found in the soil and have a rod-like form (Figure 1b), often with endospores found on one end.

Mycobacterium tuberculosis

These bacteria cause tuberculosis, a disease that used to be very deadly. A lot of the research done using *Mycobacterium* taught us how to use chemicals that can kill dangerous bacteria. Although tuberculosis is not nearly as deadly as it once was, there are now drug-resistant *Mycobacterium* strains. These strains are dangerous, because they can survive in the presence of many antibiotics. Currently, much of the research done using *Mycobaterium* is focused on learning how it infects humans, how the bacteria interact with antibiotics, and how we can defend ourselves from them [5]. Another research field that uses *Mycobacterium* is the study of bacterial communities. Bacterial communities are bunches of individual *Mycobacterium* cells attached tightly to each other by a special chemical compound produced by the bacteria, called mycolic acid. These bundles of *Mycobacterium* cells look like cords, with individual cells wrapping around each other in a disordered way [6].

Streptomyces

You probably know of antibiotics as substances that kill bacteria… but did you know that some bacteria actually produce antibiotics? *Streptomyces* are great antibiotic producers. For about 20 years, these bacteria were intensively studied and used to produce many new antibiotics. Thanks to that work, we now know much more about how antibiotics are made and about the genes and proteins that are involved [7]. *Streptomyces* have also been used to study how bacterial cells develop. These bacteria can produce specialized cells called spores, along with long, branching, filaments that sprout out of the cells, which are called hyphae [8]. Those structures give *Streptomyces* a unique look—the cells are elongated, with branching hyphae that occasionally have round spores around them, making the cells look like colored splashes (Figure 2), due to the array of chemicals that they produce. *Streptomyces* can be found living in many terrestrial habitats.

Cyanobacteria

Cyanobacteria are actually made up of a whole group of related bacteria, which is called a phylum. Within a phylum, all organisms share some characteristics and, in the case of cyanobacteria, the shared characteristic is their lively green color. Their great green look is caused by a protein called chlorophyll, which allows Cyanobacteria to perform photosynthesis. Photosynthesis is a process that has mostly been studied in plants. Like plants, Cyanobacteria can use photosynthesis to change solar energy into chemical energy, which they then use to power themselves [9]. Scientists have studied the proteins and

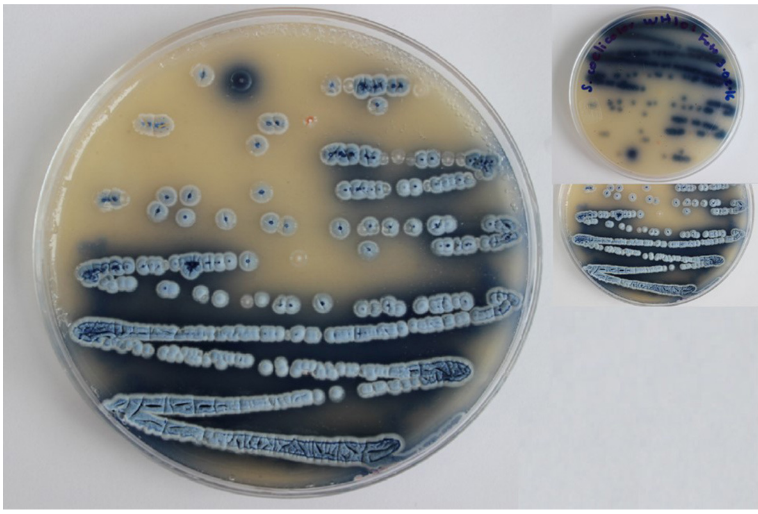

Figure 2

Figure 2

A culture of the blue colored *Streptomyces coelicolor* with white spores. They have a particular smell, like a wheat field after it rains.

genes in Cyanobacteria that allow it to perform photosynthesis and, in recent years, there has been even more interest in Cyanobacteria as people think about renewable energy. There are now a lot of studies attempting to learn how to harness the photosynthetic potential of Cyanobacteria for industrial applications [10]. Cyanobacteria can be found pretty much everywhere, and many Cyanobacteria species have unique structures (Figure 1c), but all of them are green, thanks to their chlorophyll.

CONCLUSIONS

There are a lot of model microorganisms out there, each with unique biological characteristics that can used for different types of scientific studies. We have only shown you the tip of the iceberg—there are many more types of bacteria that are used as model organisms and we could not cover them all here. So, do not get angry if we left your favorite bacteria out!

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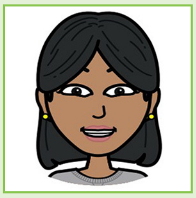
YOUNG REVIEWERS

MARIA, AGE: 15

I am in junior high. I play hockey in Irapuato and I like to read scientific articles about the planet we live on. I Like to learn about living creatures and I love my family and my cat and my sister's dog. I love food and hamburgers and I like to visit my cousins. I like to travel to and learn about different cultures. I also like to see movies and TV series.



MARIANA, AGE: 14

I like basketball, hockey, to read and I play the piano. I also like watching movies with friends and family, and to play with my brother.

AUTHOR

EDDER D. BUSTOS-DIAZ

My name is Edder Daniel Bustos Diaz, although I usually go by Daniel Diaz because it is shorter and easier to remember. I am a Ph.D. student at the National Laboratory of Genomics for Biodiversity in Mexico (usually abbreviated as LANGEBIO). I like to read novels and short stories. I also like to play tabletop and video games. I work with the microorganisms called cyanobacteria. They are green, weird, and do a lot of very interesting stuff. They have cool shapes. I like them a lot. *edder.bustos@cinvestav.mx

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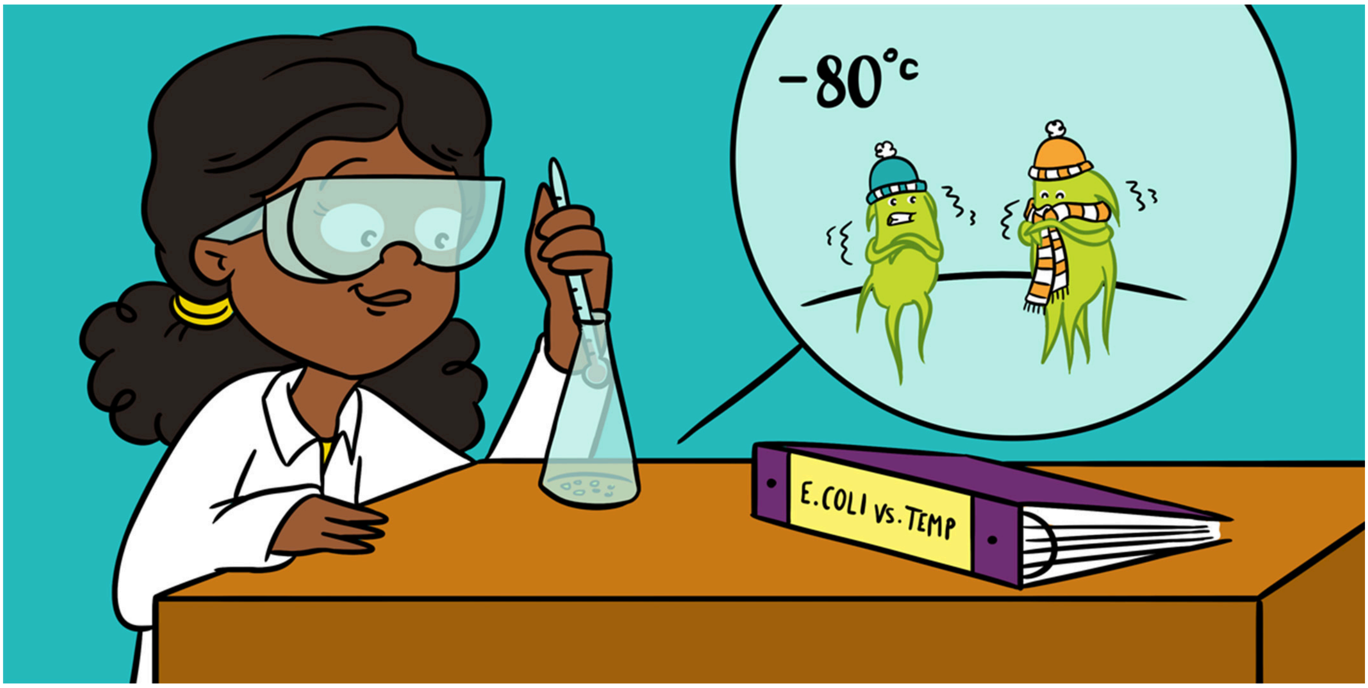

EVOLUTION IN A BOTTLE

César Aguilar*

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CARLOS
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DANIEL
AGE: 14


MARÍA JOSÉ
AGE: 14

Evolution is the process that originates changes in living beings over time, which allows them to adapt to different environments. Evolution has created the big diversity of living things on earth. Using bacteria, scientists are investigating how evolution works, by studying bacterial DNA and its mutations, which are the main cause of the DNA changes that result in evolution. Why did we choose to study bacteria? Well, because they are probably the most well-adapted organisms on earth. They can survive in the most varied and extreme conditions, from underwater volcanoes to Alaskan frozen ground. To conduct this research, a bacterium called *Escherichia coli* was chosen as the test subject some fascinating experiments that we will describe in this article.

WHAT IS EVOLUTION?

On our planet, there are microscopic organisms inhabiting almost every corner: bacteria. There are around one billion species of bacteria on earth. What is the reason for this tremendous diversity of bacteria

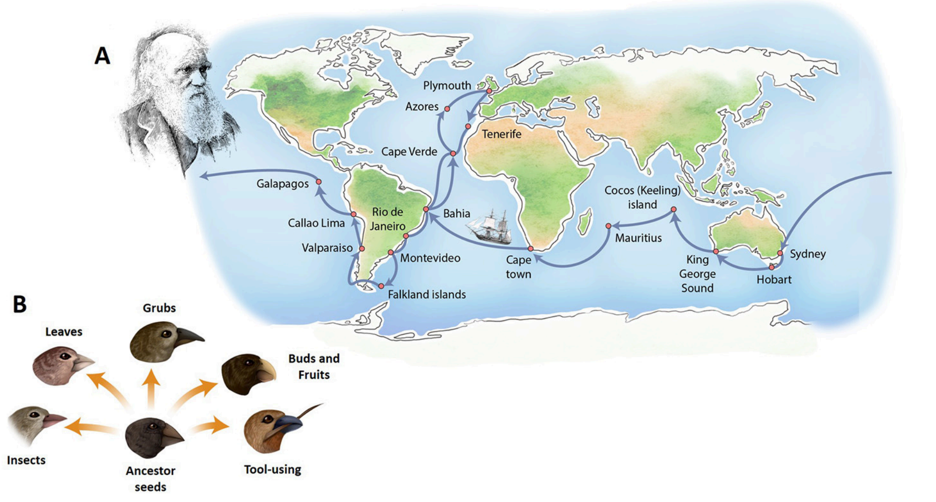

Figure 1

Figure 1

Voyage of the H.M.S. Beagle. **(A)** In 1831, the English naturalist Charles Darwin embarked on a 5-year trip on the British research ship H.M.S. Beagle. The Beagle explored numerous places, shown by the red dots on the map. Darwin's observations of the organisms in these locations showed interesting patterns in the distribution and characteristics of many organisms, such as turtles, finches, mussels, marsupial rats, and platypus, which made Darwin think about adaptation. **(B)** The most famous example of adaptation found by Darwin was the great diversity of finch beaks. The beaks of different finches seemed designed to eat the specific food present in the place where they lived. Using all of his observations, Darwin came up with the theory of evolution by natural selection.

that inhabit our planet? **Evolution** is the key. The word evolution is used to describe changes in organisms that occur over many generations. In 1831, the English naturalist Charles Darwin embarked on a 5-year trip on the British research ship H.M.S. Beagle. During the trip, Darwin observed interesting patterns in the distribution and characteristics of many living creatures. He reflected that these characteristics were changes that helped the organisms to adapt to the environments in which they lived (Figure 1). This trip helped Darwin to come up with a theory of evolution in which he explained how living organisms change every generation, adapting to the conditions of their environment in order to survive [1].

HOW DOES EVOLUTION HAPPEN?

A big question that originated from Darwin's theory was how the changes he observed came to be. Today, we know that these changes are ultimately caused by **mutations**. Mutations are changes in an organism's **genes**. Genes are instructions that all living organisms possess. Every gene is composed of a unique combination of four molecules called **nucleotides**: adenine, cytosine, guanine, and thymine. The order of these nucleotides, which is called the genetic sequence, determines the function of each gene. The total of all the genes in an organism is called the **genome**. The genome contains the information coding for all the characteristics of an organism, so a change in one or more nucleotides of a gene (called a genetic change) can disturb a feature of the organism, like the color of the eyes, its height or the way it processes food. This genetic information is inherited, meaning it is passed on from one generation to another.

EVOLUTION

In biology, is the change in any trait of an organism. Those changes are derived from mutations over the generations.

MUTATION

Are changes in the nucleotide sequence of the genes.

GENE

A defined set of nucleotides. The order and type of these nucleotides determine the functions of a gene.

NUCLEOTIDE

They are four organic molecules called adenine, cytosine, guanine, and thymine that serve as forming units of deoxyribonucleic acid (DNA), the essential biomolecule of all forms of life on Earth.

GENOME

All the genes that an organism possesses.

NATURAL SELECTION

Is the process of slow accumulation of helpful mutations across generations, causing the displacement and ultimately, the extinction of less adapted organisms.

Mutations in genes can happen spontaneously or in response to stress factors in the environment, but regardless of the cause, all mutations are random [2]. This means that the genetic changes can occur anywhere in the genome of the organism. The accumulation of mutations over generations can be helpful, harmful or have no effect on survival. Darwin summarized all these facts in a simple concept: **natural selection** [1]. Natural selection is the process of slow accumulation of helpful mutations over generations, causing organisms to become better adapted to their environments. Organisms that are less well-adapted will have more trouble surviving than will organisms whose mutations have helped them adapt. When mutations produce significant changes in an organism, they can lead to the development of a new species [1].

BACTERIA ARE GREAT FOR STUDYING EVOLUTION

The classical way to demonstrate that species change over time is through the fossil record. Fossils show how primitive life formed and, if we find enough fossils, it is possible to observe how an organism has evolved over time. However, if the fossil record is incomplete, it is impossible to see all of these changes or to figure out how the organism evolved. To solve this problem, it would be easier to study evolution directly, by examining all of the genetic changes that happen in each generation. To do this, researchers must first find an ideal organism to study. Bacteria are an obvious choice, because they are easy to cultivate in the laboratory and they reproduce quickly. *Escherichia coli* is a widely studied bacterium that inhabits the digestive system of humans and other warm-blooded animals (Figure 2). Just to compare, if we wanted to do an evolutionary experiment with humans, we would need to wait, on average, 26 years to have a new generation, while *E. coli* spawns a new generation in 20 min—less time than it takes to get a pizza! Additionally, *E. coli* has a small genome, making it easy and cheaper to study the actual changes in nucleotides that occur in each generation (Figure 2). In the sections that follow, we will describe some of the experiments scientists have performed, using *E. coli*, to study the process of evolution in the laboratory.

HOW DO BACTERIA EVOLVE IN RESPONSE TO EXTREME COLD?

By studying *E. coli* in the lab, one group of scientists hoped to understand how bacteria might adapt to extreme cold. To study this, they devised an experiment in which *E. coli* was subjected to extreme cold for prolonged periods of time. The experiment consisted of 150 cycles of freezing at −80°C for 22.5 h, and then thawing at room temperature for 1.5 h. The result? The survival of *E. coli* increased; they evolved. When scientists studied the genome of these evolved bacteria, they found mutations that disabled a gene that

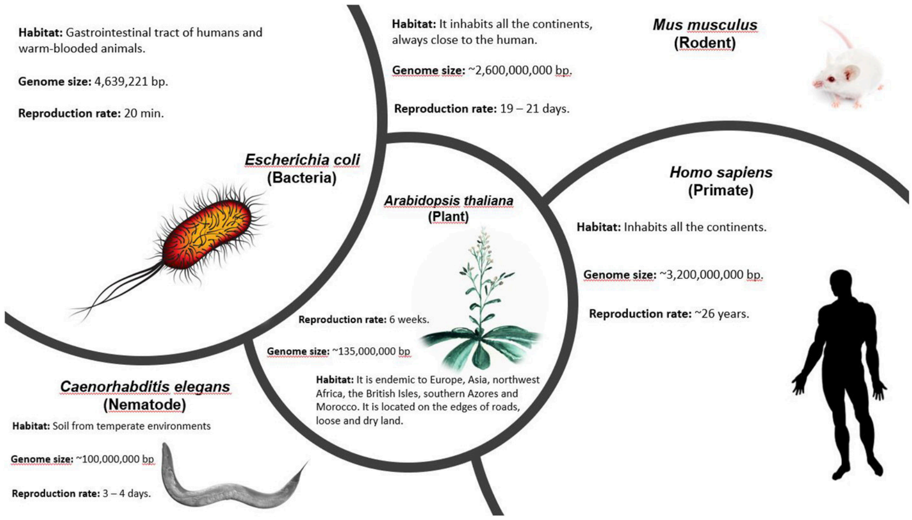

Figure 2

Figure 2

E. coli is a useful organism for evolution experiments. There are several organisms commonly used by scientists to carry out experiments of all kinds. Among the most popular are the plant *Arabidopsis thaliana*, the rodent *Mus musculus*, and the simple worm *Caenorhabditis elegans*. To perform experiments on evolution, however, scientists need an organism with a short reproduction time and a small genome. The *E. coli* bacterium has a reproduction time of only 20 min! This fast division rate and its small genome make it perfect for these experiments, in the laboratories all around the world. In this figure, you can see the reproduction rate and genome size (in bp, which stands for base pairs) of humans and the other organisms commonly used for scientific experiments.

plays a role in the production of cardiolipin, a molecule that makes the outer covering of the bacterial cell sturdy and rigid. Further investigations proved that turning off the production of cardiolipin helped to keep the covering fluid and flexible after freezing, which increased survival [3].

THE MOST AMBITIOUS STUDY OF EVOLUTION

The most ambitious study of evolution was carried out by a scientist named Richard Lenski. Lenski kept *E. coli* in a special bottle with a liquid, called medium, containing limited nutrients (Figure 3). In this culture medium, the bacteria grew rapidly but depleted the nutrients just as quickly. The bacteria then entered a state of "hunger" that lasted 24 h, after which some of those hungry and stressed bacteria were transferred to another bottle with fresh medium containing the same nutrients. He repeated this process again and again. Over a long period of time, the stress experienced by the bacteria during the process led to the generation of mutations and, consequently, to evolution of the bacterial population. Lenski and his collaborators started this long-term evolution experiment (**LTEE**) in 1988 and it is still going on [4]. Now, after more than 74,000 generations, a lot of interesting things have happened.

LTEE

It is the acronym of "long-term evolution experiment," which refers to evolution experiments performed for long periods of time.

AMAZING FINDINGS IN THE LTEE

One of the most interesting observations from the LTEE was the remarkable and sustained increase in **fitness** during the first 5,000 generations (about 2 years). What does that mean? Well, organisms with higher fitness are better adapted to their environment and will tend to produce more offspring compared with their less-fit

FITNESS

How well-adapted an organism is to its environment, which determines how many offspring that organism has.

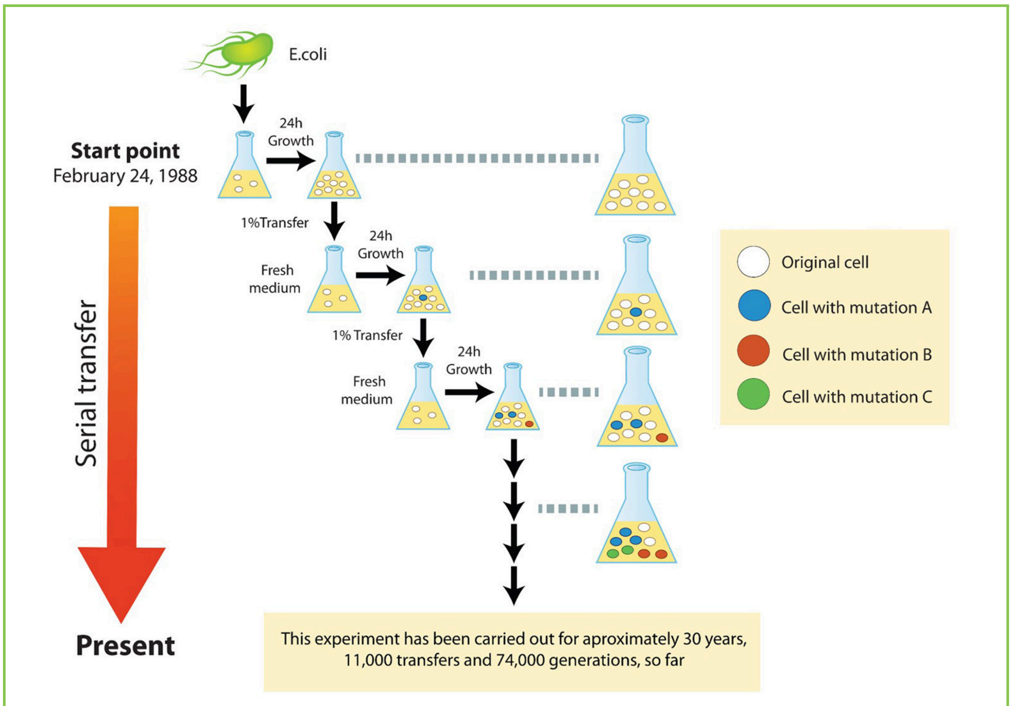

Figure 3

The long-term evolution experiment (LTEE). The LTEE was started in 1988. For this experiment, scientists used a technique called serial transfer, which means that, every day since February 24 of 1988, 1% of an *E. coli* culture bottle is transferred into fresh culture medium. As time passes, harmful and beneficial mutations are generated. The beneficial mutations result in adaptations that help the bacteria to deal with the famine conditions the population faces daily. This project has not ended yet. Now, after more than 74,000 generations, a lot of knowledge has been obtained.

competitors. Lenski observed that, during the first 2 years of the experiment, genetic changes emerged in some bacteria, allowing those bacteria to adapt to the environment, becoming more fit, until they became predominant in the population [4]. Some bacteria adapted to the environment so well that they even lost certain functions that were no longer needed under the experimental conditions. This is similar to what happened to certain animals after hundreds or thousands of years of living in absolute darkness, like in caves. These animals developed an adaptation in which the eyes were eventually lost, since eyes are not useful in those very dark conditions.

Another amazing finding from the LTEE was that, in one experiment, this well-adapted population of *E. coli* was not the only population, but a second minor population co-existed with them because it had evolved the ability to take advantage of acetate, a waste product of the dominant population. This is similar to the relationship between lions and scavenger animals. The lions feed on hunted animals and the remains they leave become food for other animals, like hyenas or vultures.

As previously mentioned, organisms that develop enough genetic and structural changes can become a new species. Could this process have occurred in the LTEE? *E. coli* possess certain characteristics that make them *E. coli* and not another bacterial species. Among these characteristics is the inability to use a substance called citrate as a food source when oxygen is present. Surprisingly, scientists observed that, after 31,500 generations (more than 12 years), one population of *E. coli* began to use citrate as a food source [4]. The evolution of the ability to use citrate is exceptionally rare! How was this possible? Well, *E. coli* possess all the necessary genes to consume citrate, but those genes are turned off and work only in absence of oxygen. When the scientists observed the genome of the evolved organisms, they realized that, due to certain mutations, those genes were turned on, enabling the *E. coli* to use citrate as a food source. Now, is this citrate consumer a new *Escherichia* species? What do you think?

CONCLUSIONS

All the studies of evolution have taught us that life finds a way. For this reason, bacterial genomes can adapt in surprising ways when the bacteria are faced with environmental adversities. This process allows bacteria to colonize new places. *E. coli* has become the model organism of these evolutionary studies because it grows easily, it has a small genome, and we know a lot about it. The data obtained from these experiments contribute to the study of important evolutionary questions and will allow us not only to understand but possibly to even guide evolution in the future. Can you imagine a future in which it is possible to know all the genes that control the physical characteristics and diseases of an organism? Maybe we are not too far from that, and when it happens, we can help organisms to fight against diseases and possibly find other ways to assist organisms, including humans, in becoming more suited to their environments.

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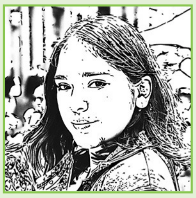
YOUNG REVIEWERS

CARLOS, AGE: 16
My city is located in the middle of Mexico, which is an awesome country to live in because it has such a great culture and its people are just very kind to each other and to other people that are visiting our great country.

DANIEL, AGE: 14
I love sports, specially football and fencing. My favorite meal is tacos and I also like to go watch and learn about cars.



MARÍA JOSÉ, AGE: 14
My name is María José, I am 14 years old and I love ballet, I practice it since I was 3 years old, I also love science and I would like to study medicine.

AUTHOR

CÉSAR AGUILAR
I am a researcher in the National Laboratory of Genomics for Biodiversity in Mexico. I have a Ph.D. in Biochemistry and a background in research on bacterial evolution. I love all life forms, especially microorganisms. I have been studying them for more than 10 years: how they survive, how their metabolism works, how they have evolved, and most importantly, how they can help us make this planet a better place. In my free time, I like to do exercise, play soccer, and read. I love my family, coffee, movies, and comics. My favorite superhero is Captain America.
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THE EVOLUTION OF BACTERIA CAN PRODUCE CHIMERIC CREATURES: THE CASE OF *AZOTOBACTER VINELANDII*

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YOUNG REVIEWER:

DIEGO
AGE: 11

The evolution of bacteria is different from the evolution of other organisms. While we get our genetic information solely from our parents, bacteria can get part of theirs from other sources. In this article, we describe one of these chimeric bacteria, *Azotobacter vinelandii*, and explore how its dormant, seed-like form influenced its evolution.

INTRODUCTION

Earth's first living cell appeared nearly four billion years ago. We call it LUCA, the last universal common ancestor. We do not know much about LUCA, but every plant, every animal, and every one of us humans evolved from LUCA. We know this thanks to studies done with special **proteins** contained in the cells of LUCA's descendants. Proteins are indispensable for every living being, as they are the molecules that do the things that keep everything alive. Every biological process

PROTEINS

Large, complex molecules that play many critical roles in the body. Proteins do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs.

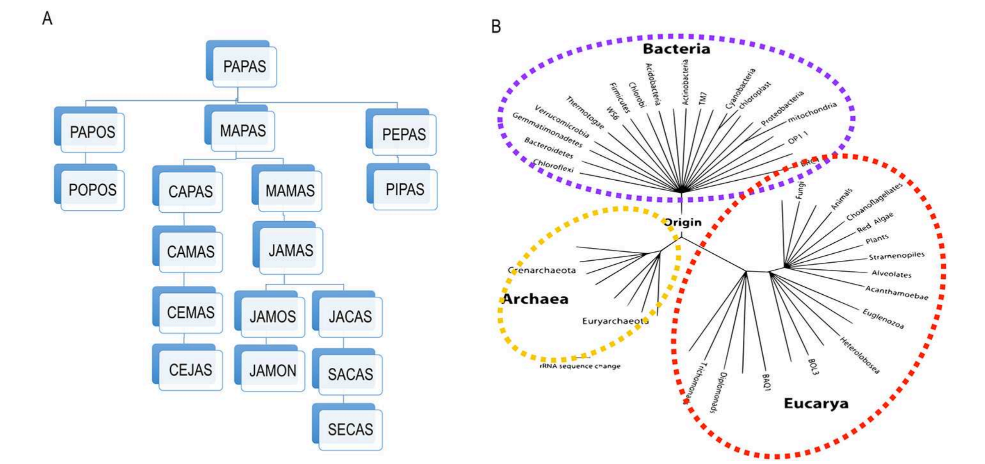

Figure 1

Figure 1

Building phylogenetic trees. **(A)** An example of how phylogenetic trees are constructed, using five-letters words. In this example, each change of a letter represents one unit of distance, so any two words that are just one letter/step apart in the tree are very similar, while those that are separated by more letters/steps (like "cejas" from "secas," which are nine steps apart) are less related. You can see that the most recent common ancestor of any two words is where their paths converge ("mapas" in the case of "cejas" and "secas"). **(B)** A diagram of the tree of life built using the genetic sequence of the ribosome, for species belonging to the three domains of life: Bacteria, Archae (that are also unicellular microscopic organisms), and Eukarya (that include all animals and plants). Since all living organisms are included in this phylogenetic tree it is called the "universal tree of life" (this figure is a modification of a diagram from Pace in 2009).

necessary for life, like respiration, digestion, or reproduction, involves proteins in some way. And while many of these processes and their corresponding proteins vary between organisms—for example, the respiratory proteins of fishes are very different from those of elephants—some proteins are so important that everyone has to produce them. One of these crucial functions for life is the mechanism used to produce proteins, which is carried out by a bunch of proteins coupled with other molecules and collectively called the ribosome. The ribosome is so crucial that it has not really changed much since LUCA was around. Tracking the small changes in the DNA that make the ribosome has allowed us to peek at the evolutionary history of living organisms and follow their relationships.

THE UNIVERSAL TREE OF LIFE

Since all living organisms came from LUCA, and they all have **ribosomes**, it is possible to construct "family trees" that show us the relatedness of different organisms by tracking the small changes that have occurred in their ribosomes. In these family trees, which are called phylogenies by scientists, every species is located at the tips of the leaves, with the size of the branches between two tips indicating how similar the organisms at the tips are to each other. So, for example, the branches that separate cats, lions, and tigers are smaller than the branch that separates these animals from whales. Thus, in a mammals phylogeny all the felines form a tight group of branches that forks at the base from the whales' branch. You can understand how a phylogeny is constructed using words, as describe in Figure 1A. If we grow the size of a phylogenetic tree by adding every known living being to it, we end up with the universal tree of life (UTL), in which we can see the relationships between all of the living beings [1] (Figure 1B).

RIBOSOMES

Ribosomes are complex structures formed of proteins and other molecules. These molecular machines are present in every cell and are the site where new proteins are produced.

In the example shown in Figure 1A we use five letter words, but you can imagine that, if you use larger words, you will be able to measure greater distances. The full length of the gene for the bacterial ribosome is around 1,500 "letters," so the calculation of the relationships between organisms using the ribosome is very sensitive and thus all organisms can be included in the UTL.

The path formed from the bottom of the tree to the leaf tips represents the evolutionary path that led to the species at that tip. The members of that species, having followed that specific path, lost the capacity to mate and have offspring with members of other species that followed different evolutionary paths.

BACTERIA NOT ALWAYS INHERIT THEIR GENES FROM THEIR PARENTS

Every species inherits its DNA, or **genetic information**, directly from its ancestors. Those ancestors got *their* DNA from the generation that came before them, and so on, and so on, tracing the evolutionary path backward until the tree is traced all the way back to LUCA. Because the genetic information of each species is unique to that species, it can only be shared with the members of that same species, flowing from the parents to their offspring. That is, unless we are talking about bacteria. Bacterial species have the ability to share parts of their genetic information with other bacterial species that have not followed the same evolutionary paths; bacteria are able to inherit DNA from other bacteria that are not in any way related to them. This movement of genetic information is called **horizontal gene transfer (HGT)**, since the transfer of genetic information occurs horizontally, between bacteria of the same generation, instead of vertically, between parents and offspring.

Another interesting characteristic of bacteria is that some of them can develop dormant forms that are similar to the seeds of plants. These dormant forms can exist for a long time without reproducing (Figure 2). The dormant forms are like an ancient reservoir of genetic information that, in theory, could be harnessed by other organisms [2]. The genetic information of the dormant forms is preserved for many years without change and can be incorporated into other bacteria by HGT.

ARE BACTERIA ABLE TO FORM CHIMERAS?

To have a clearer picture of how dormant forms of bacteria could affect bacterial evolution, let us imagine that dinosaurs could form eggs that could remain in the environment for tens of millions of years. In that case, once in a while we would bump into a baby dinosaur, which would contain genes that are not present in any other organism that is living today, and would coexist with modern animals. Let us

GENETIC INFORMATION

The DNA in every organism, in which the information required to make proteins and other cellular components is stored. DNA is passed from one generation to the next through inheritance.

HORIZONTAL GENE TRANSFER (HGT)

The process of inheriting genetic information from an unrelated species. This mechanism of inheritance is not common, since organisms usually inherit their genetic information from their parents that are members of the same species as their offspring.

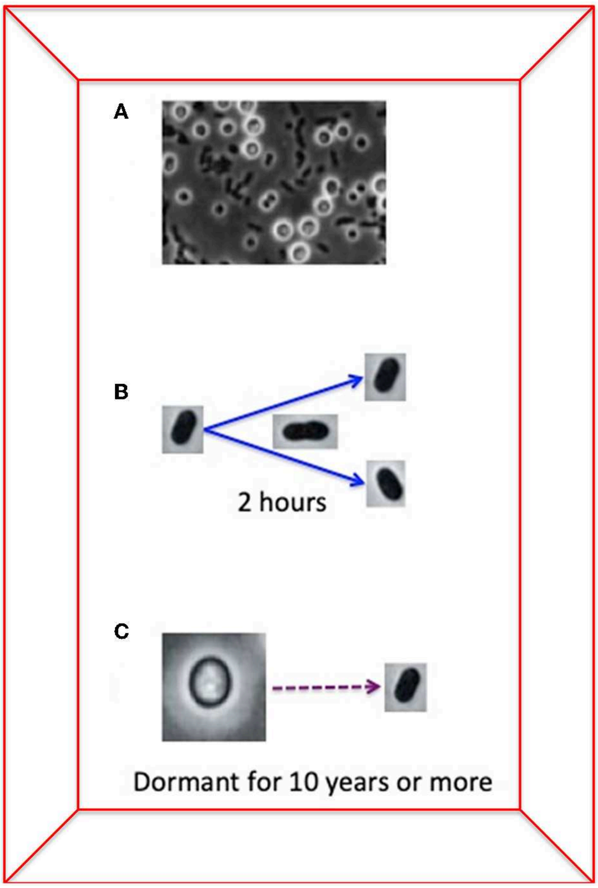

Figure 2

Figure 2

The chimeric bacterium *Azotobacter vinelandii* has two forms of reproduction: its reproduction by cell division takes 2 h, while dormant, seed-like structures can be alive without reproducing for more than 10 years. **(A)** A picture (taken using a light microscope) of bacterial cells and the dormant, seed-like form. You can compare the size and form of the two types of cells: the seed-like form is much larger, while normal bacterial cells are small and dark. **(B)** *Azotobacter vinelandii* takes 2 h to reproduce by cell division. **(C)** The dormant, seed-like form of *Azotobacter* can persist in dry conditions for 10 years or more. So, *Azotobacter vinelandii* "seeds" remain dormant for 44,000 times longer than it takes the cells to reproduce by cell division! It is possible that the genetic information in dormant forms results in a reservoir of genes that can be incorporated from distantly related bacteria by HGT.

CHIMERA

Any mythical animal formed from parts of various animals. This term is used in biology to describe an organism containing a mixture of genetically different tissues. In this article, we use this term to describe bacteria with genetic information inherited from different organisms, some of which are not their ancestors.

imagine further that these newly born dinosaurs could mate with other animals. Then it would be possible to create chimeric animals, which are animals that contain genetic information, and therefore the physical parts, of different species. In this scenario, a chimeric animal with parts of birds, dinosaurs, and mammals could exist!

We imagined that the ability of bacteria to inherit DNA by HGT and their capacity to stay dormant for long periods, will permit that bacterial **chimeras** are formed, just as in the example of the baby dinosaur. This is the question that driven our research [2].

IN THE SEARCH OF A BACTERIAL CHIMERA: THE CASE OF *AZOTOBACTER VINELANDII*

We were interested to find out whether the dormant forms of bacteria could create chimeric bacteria, using their ability to transfer genetic information through HGT. To see if this could happen, we looked at the genome of *Azotobacter vinelandii*, which is a bacterium that is able to form dormant forms called chists (Figure 2), and is very closely related to another bacterium called *Pseudomonas aeruginosa* [3]. It is a little bit of shock that these organisms are related, frankly, because these two species of bacteria are very different from each other! We investigated this interesting fact and found that these organisms are related on the tree of life because nearly half of *Azotobacter vinelandii*'s genetic information, including some of the instructions for making ribosomes, is closely related to that of *Pseudomonas* [4], while the rest of the information is used to make the proteins that give *Azotobacter* its own special characteristics. As if that was not enough, we also found that the genetic information that did not come from *Pseudomonas* was inherited from different unrelated bacterial species [2]. In other words, the core of the genetic information of *Azotobacter vinelandii* came from a shared ancestor of *Azotobacter* and *Pseudomonas*, but during its particular evolutionary path, *Azotobacter* acquired additional genetic information that made it very different from its "cousin" *Pseudomonas*, or to any other organism really, because *Azotobacter* is a chimera.

HOW COMMON ARE BACTERIAL CHIMERAS?

While it is difficult to know if chimeric organisms are common, the existence of *Azotobacter* is strong evidence that chimeras might not be that rare. There are at least two other well-documented cases of bacterial species that seem to have a core derived from a bacterial group called *Firmicutes* and other pieces of genetic information obtained from other organisms. These chimeras are bacteria called *Thematogales*, organisms that acquired genes that allowed them to live at high temperatures, and *Fusobacterium nucleatum*, a bacterium found in dental plaque that acquired genes from neighboring bacteria. Neither *Thermatogales* nor *Fusobacterium nucleatum* form dormant seed-like structures, but the information that they acquired by HGT might have come from an ancient bacteria that did have the capacity to make dormant forms. We still do not know, but maybe someday we will.

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ORIGINAL SOURCE ARTICLE

González-Casanova, A., Aguirre-von-Wobeser, E., Espín, G., Servín-González, L., Kurt, N., Spanò, D., et al. 2014. Strong seed-bank effects in bacterial evolution. *J. Theor. Biol.* 356:62–70. doi: 10.1016/j.jtbi.2014.04.009

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YOUNG REVIEWER

DIEGO, AGE: 11
My favorite thing to do is reading, and I can read a whole book in just a few hours. I also like to play video games. I am good at Math and English, and I always get good grades at school. I practice Football Soccer. When I grow up I would like to be a President of my country because I want to end the injustice and inequality in Mexico.

AUTHOR

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Gloria Soberón-Chávez is a Mexican scientist that studied her undergraduate and Ph.D. degree on Biomedical Research at the Universidad Nacional Autónoma de México (UNAM), where she has worked all her life (39 of her 62 years). Her main research interest is the molecular genetics of bacteria, especially *Pseudomonas aeruginosa*. She enjoys very much working in UNAM and besides doing research she has been involved in administrative jobs, such as Director of the Biomedical Research Institute and of UNAM's Post-graduate Studies. She loves music, she sings in a choir that belongs to UNAM's choral program, and enjoys food from all around the world, and reading novels. *gloria@biomedicas.unam.mx

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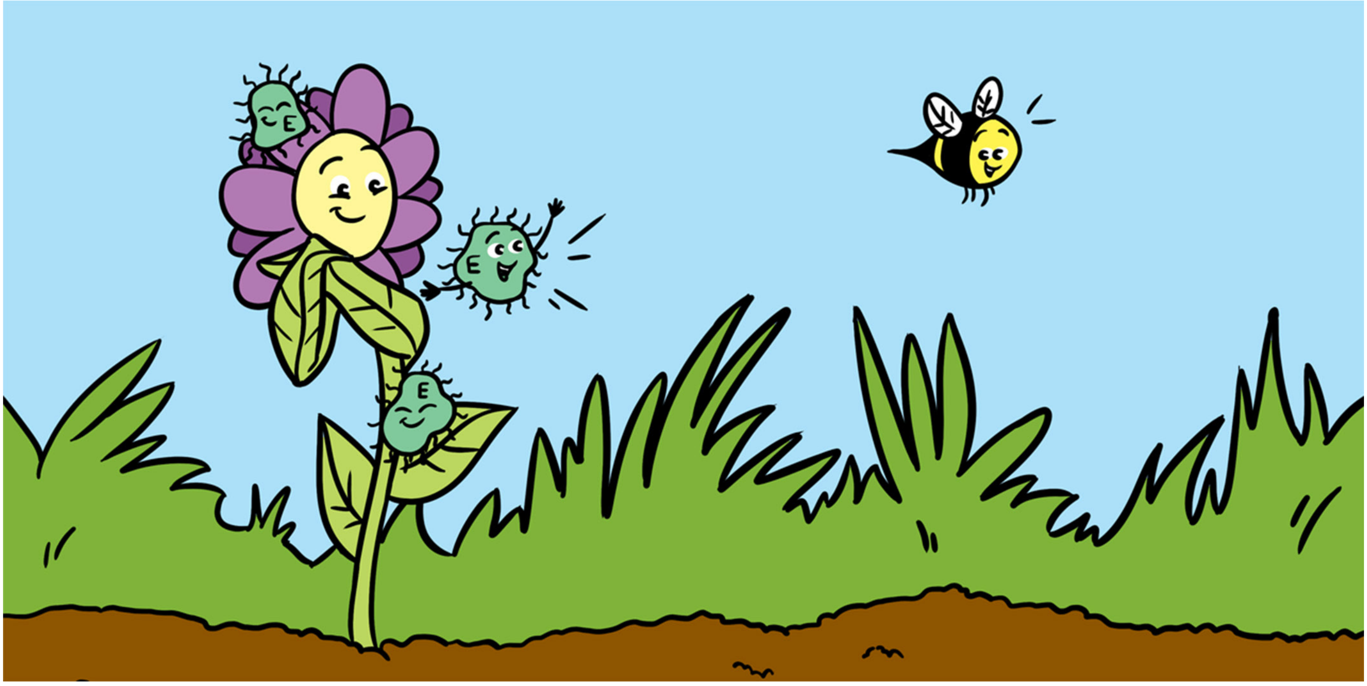

HIDDEN MICROBIAL HELPERS LIVING INSIDE PLANTS: GETTING TO KNOW ENDOPHYTES AND SOME OF THEIR APPLICATIONS IN OUR DAILY LIVES

Rosario del Carmen Flores-Vallejo[1], Jorge Luis Folch-Mallol[2], Ashutosh Sharma[3], Alexandre Toshirrico Cardoso-Taketa[1], María Luisa Villarreal[1]**

[1] *Laboratorio de Investigación de Plantas Medicinales, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico*
[2] *Laboratorio de Biología Molecular de Hongos, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mexico*
[3] *Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Santiago de Querétaro, Mexico*

YOUNG REVIEWERS:

ALAN
AGE: 13

MARIANA
AGE: 14

Microbes are tiny forms of life that can be found everywhere. The microbes that live inside healthy plants are commonly known as endophytes. Plants live together with their endophytes in symbiosis, which means that they work together to help each other out. Plants give shelter and food to their microbial guests, and in exchange, their microbial helpers produce chemicals that support the plants to grow faster, resist droughts, or fight against plant eaters. Scientists are very interested in studying the chemicals produced by endophytes, because new medicines and agricultural products can be developed from them. In this article, we will describe the interactions between plants and their endophytes, and answer some questions, like: How do scientists

select a plant host and extract endophytes from plants? How could the study of endophytes help us in our daily lives? And, which types of endophytes have been isolated from plants growing in Mexico?

PLANTS AND THEIR HIDDEN MICROBIAL HELPERS: ENDOPHYTES

Plants are living organisms that, unlike other forms of life, cannot move to escape from animals or insects that want to eat them (herbivores). So, how did plants compensate for their lack of movement and continue to survive? Well, since very ancient times, plants evolved together with microbes, which are tiny organisms, invisible to the naked eye. This cooperation with microbes is one of the strategies that helped plants to adapt, survive, and thrive [1]. Microbes that live inside the healthy tissues of plants, without causing disease are commonly known as endophytes (from the Latin words "*endo*" = inside, and "*phyton*" = plant).

Endophytes include microbes like fungi, bacteria, and even viruses. Endophytes and their plant hosts work together in **symbiosis**, which means they both obtain benefits from their relationship. When working in symbiosis, plants send signals to their endophytes to warn them when they are being attacked by herbivores. In response, endophytes help to produce chemicals that their plant hosts use to stop herbivores from eating the plant. Endophytes can also help to produce other types of useful chemicals (Figure 1). For example, chemicals that: (1) attract pollinators, like bees and bats; (2) help their plant host to grow faster and absorb the nutrients in the soil more easily; (3) make their plant host resistant to drought and extreme weather conditions; and (4) fight plant infections and diseases. In exchange for those services, plants give shelter and food to their endophytes, which helps the endophytes

SYMBIOSIS

A long-term relationship in which organisms benefit from each other.

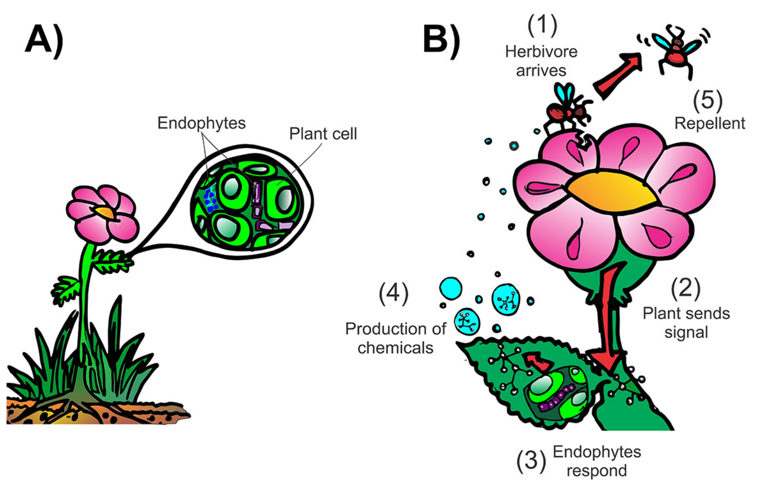

Figure 1

Figure 1

Plants and their endophytes work together in symbiosis. **(A)** Endophytes living inside the tissues of their plant host, where the plant gives them shelter and nutrients. **(B)** An example of how endophytes help to protect their plants. **(1)** A herbivore attacks the plant; **(2)** the plant sends signals that warn its endophytes about the attack; **(3)** endophytes respond to the plant's signals and help to produce chemicals; **(4)** the chemicals are secreted by the plant and reach the herbivore; and **(5)** the herbivore gets repelled by the chemicals produced with the help of the endophytes.

to reproduce and gives them a chance to be passed on to new plants through the seeds of their plant host [1].

At this point, you might be wondering how endophytes get inside plants. Plants have an immune system that normally defends them from anything that tries to invade their cells, including the microbes that are normally found on the plant's outer surface, known as **epiphytes**. Under certain conditions, when the plant's immune system is weakened, epiphytes take advantage and enter the plant through wounds on the roots or the leaves. Inside the plant, some of these microbes will manage to co-exist with the plant's immune system without causing any symptoms of disease—these become endophytes—while some others will provoke sickness to the plant, which are known as **phytopathogens** [1].

EPIPHYTES

Microbes living in the surface of plants.

PHYTOPATHOGENS

Microbes that cause disease to plants.

HOW COULD ENDOPHYTES HELP US IN OUR DAILY LIVES?

Scientists started paying more attention to the study of endophytes when one group demonstrated that endophytes could produce chemicals that were originally thought to be produced by plants. This group discovered an endophyte capable of producing a chemical named Paclitaxel (Taxol®), which is used in the treatment of cancer. This chemical is mainly obtained from bark of the Pacific yew tree (*Taxus brevifolia*). To extract enough of this chemical and treat patients with ovarian and breast cancer for 1 year, it is estimated that an average of 270,000 mature trees (100 years old) must be chopped down, endangering the already reduced populations of this tree [2]. Their discovery that endophytes can produce Paclitaxel means that we might have a feasible way to produce it using microorganisms, without endangering the survival of the Pacific yew tree. This finding also encouraged scientists to explore the microbes that live inside plants, to "hunt" for yet-unknown species capable of producing valuable chemicals that could be useful to humans.

Studying endophytes might bring us a variety of new **biotechnologies** that could help us in our daily lives. For instance: (1) development of new medicines and ways to manufacture them; (2) creation of products that resist extreme temperatures or corrosive conditions; (3) discovery of new ways to decontaminate polluted areas; (4) creation of products that allow crops to be grown with less fertilizer and water; and (5) discovery of environmentally friendly ways of fighting unwanted herbivores or plant diseases in a more sustainable way.

BIOTECHNOLOGIES

Products and services developed from living organisms or their parts.

ON THE LOOKOUT FOR ENDOPHYTES

Endophytes live inside all plant species. They have been found in plants living on the sea, like green algae and plants that live on land, such as the dandelions growing near the sidewalk. Endophytes can live in plants from places with high temperatures and little water, like cacti, or inside tropical palm trees and mangroves from the coastal shores, or in ferns and mosses that live in the cold Antarctic zone [3]. If endophytes live in every plant, how do we choose which plant to study for its endophytes?

While it is true that every plant contains at least one relevant endophyte, the relevance of an endophyte relies on the function that it carries out on the plant and the benefits that the endophyte provides (e.g., help its plant host to resist drought). Some endophytes develop a very close association with their plant host, and contribute to different processes crucial for the survival of the plant. In order to study the biological activity that endophytes carry on inside their plant, first we must select a plant specimen.

There are different ways to select plants to study their endophytes. The selection of a plant is guided by different criteria that go from random selection of a number of different plants in an **ecosystem**, hoping to find something useful. Or the selection might be guided by certain characteristics of the plant to find chemicals with interesting biological activity (**bioprospecting**). This rationale of selection is based on: the plant's particular morphology, age, **ecological niche**, environmental setting, and/or the ethnobotanical history (medicinal uses) [3]. By applying rationales of selection to choose a particular plant and study its endophytes researchers can propose relevant experiments to test their research question (e.g., test if endophytes from desert plants produce chemicals that help their plant host to resist dehydration). The research question is what guides the whole scientific process of discovery, and helps scientists to explain the functions that endophytes carry inside their plant host, and understand their ability to produce chemicals with a particular biological activity.

ECOSYSTEM

A community of organisms and the place where they interact.

BIOPROSPECTING

Study of nature to find chemicals with biotechnological value.

ECOLOGICAL NICHE

The specific place and function of an organism inside an ecosystem.

There are different rationales of selection that guide scientists to choose one particular plant and perform bioprospecting from endophytes [3]. For instance, the scientists that discovered the Paclitaxel-producing endophyte followed an *ethnomedicinal rationale*, and selected a plant that had known medicinal uses. In their case, the plant was known to be used for treating cancer-like symptoms (owed to the presence Paclitaxel in its bark). Other scientists might use an *ecologic rationale*, by selecting plants that remain healthy even when nearby plants have diseases or are being attacked by herbivores. These plants might have endophytes that can produce chemicals that can ward off insects or bacteria. Another way to choose plants to study is by an *adaptability*

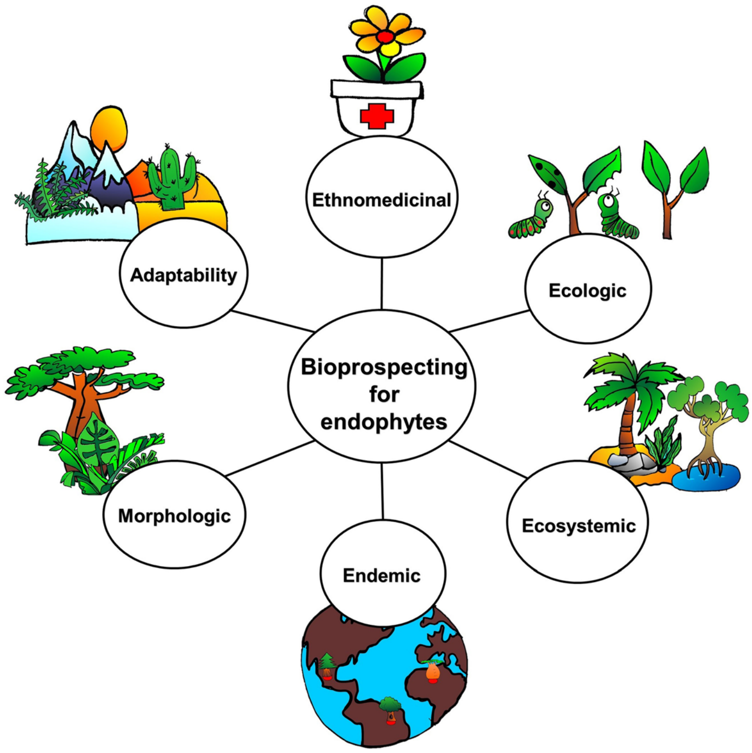

Figure 2

Figure 2

Bioprospecting for endophytes. Rationales followed to select a specific plant to find endophytes with interesting biological activities:
(1) Ethnomedicinal: plants with records of medicinal uses;
(2) Ecologic: plants that resist diseases and herbivore's attack;
(3) Ecosystemic: plants found on habitats with high biodiversity;
(4) Endemic: indigenous and long-lived plants from a particular region;
(5) Morphologic: plants with uncommon physical features;
(6) Adaptability: plants that thrive in extreme conditions.

rationale, selecting plants that live in extreme or unique environments, like in the desert, near volcanoes, or in extremely cold weather. These plants might contain endophytes that help them resist or adapt to those harsh conditions. Using the *ecosystemic rationale*, scientists select plants that live in highly biodiverse ecosystems. Interestingly, it appears that plants growing in tropical or semitropical areas of the world, where there are many types of plants, have a greater diversity of endophytes than those growing in ecosystems with lower plant diversity [3]. The *morphologic rationale* guides scientists to select a plant with uncommon physical features. For example, the form of the leaves, the shape of the trunk, or the presence of unique structures. Finally, using the *endemic rationale*, native plants from a particular region or plants that live a very long time are selected, for example the Mexican "Tule tree" (*Taxodium mucronatum*) from Oaxaca, or the "Yax-ché" tree (*Ceiba petandra*) from Yucatán. These plants could contain endophytes that help to slow down the aging process (Figure 2).

HOW CAN WE EXTRACT THE ENDOPHYTES FROM PLANTS?

There are different steps involved in getting the endophytes out of plants and growing them in the laboratory. In Figure 3, you can see that we selected a plant known in Mexico as "Tulipán moteado" (*Hibiscus rosa-sinensis*). We collected some healthy plants and washed the surface

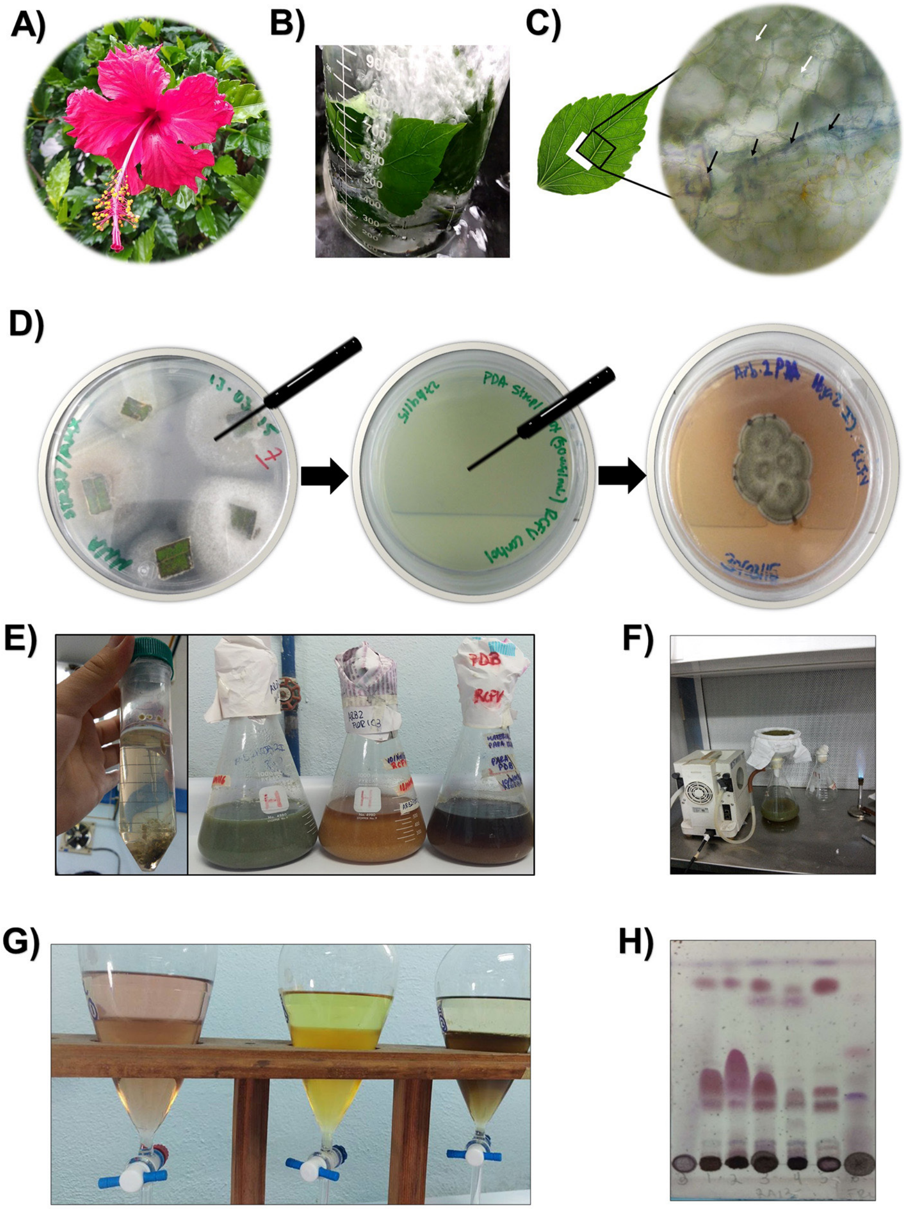

Figure 3

Figure 3

How do we extract the endophytes from a plant? **(A)** Select a plant. **(B)** Disinfect the surfaces of the plant's tissues. **(C)** Cut up the disinfected tissues. In this panel you can appreciate the cells of endophytic fungi stained with Aniline blue (dark arrows) living among the plant cells (white arrows). **(D)** Put the tissues on agar plates so that the endophytes emerge out of the plant's tissues, then isolate the endophytes growing them on other agar plates. **(E)** The endophytes are seeded in bioreactors, where they can grow in a liquid broth and transform nutrients into other chemicals. **(F)** Filter the broth to separate the microbial cells from the broth. **(G)** Extract the chemicals from the broth. **(H)** The chemicals produced by the endophytes are identified using different procedures. Here you cou can observe chemicals known as terpenes (purple bands), which have different biological properties.

of the plant tissues, like the leaves, with different disinfecting agents. Then, the plant tissues get chopped up, and the pieces are placed inside plates that contain a jelly-like substrate known as agar, which includes nutrients and water for the endophytes to grow and come out of the plant tissues. Reliable disinfection procedures include a final step that confirms that the epiphytes living on the surface were removed from the plant's tissues, so that they do not sneak in the study. Next, the endophytes are removed from the agar plates and grown inside bigger containers full of nutrient broth, also known **bioreactors**. While in the bioreactors, the endophytes transform the nutrients into other chemicals. Eventually, the endophytes are separated from the broth, and the

BIOREACTORS

Recipients with nutrients were organisms grow and produce chemicals.

chemicals are extracted from the broth, so that scientists can investigate the identity of the chemicals and their potential uses (Figure 3).

It is important that you know that, up to date, only between 1 and 10% of the microbes living in an ecosystem can be cultured in the laboratory, leaving ~0.9 million fungi and bacteria yet to be identified [3]. If such a great amount of microbes, including the endophytes, cannot be cultured in the laboratory, then how could we study them? For this cases scientists often extract the microbial DNA found inside the plants, then they look for specific pieces of microbial DNA that do not change a lot over time. This conserved pieces of DNA serve as a "barcode" of a particular microbe, this pieces are compared against computational libraries of barcodes that help researchers to identify the majority of unculturable endophytes, and thus have a more integral scope about the biodiversity of microbes that inhabit plants.

ENDOPHYTES FROM PLANTS GROWING IN MEXICO

Different groups of scientists have discovered endophytes from plants that grow in Mexico, and found that the endophytes can be used to make medicines and produce certain food or cosmetic products. Here are some interesting examples of these discoveries.

The medicinal plant known as "Copalchi" (*Hintonia latiflora*) contains an endophyte that produces chemicals that could be used to treat diabetes [4]. From other medicinal plant species, like "Granadilla" (*Callicarpa acuminata*) and the "Copal" tree (*Bursera simaruba*), scientists found endophytes that produce chemicals that can kill weeds and fight against fungi that cause plant diseases [4]. Endophytes were also found from the medicinal tree "Cuachalalate" (*Amphiterygium adstringens*), and these endophytes could be used in the production of chemicals to treat cancer or bacterial infections in humans [5]. From the medicinal plant "Mano de oso" (*Dendropanax arboreous*), scientists discovered endophytes that could produce chemicals to fight against noxious microbes that make humans sick [6].

Endophytes from plants that Mexicans eat in their daily meals were also studied. For example, scientists studied endophytes living in "Tomatillo" (*Physallis ixocarpa*) [7], which is used to prepare delicious sauces. Scientists that studied the coffee plant (*Coffea arabica*) growing in Veracruz were interested in investigating whether environmental conditions played a role in the number or type of endophytes living in a plant. And they found that the diversity of endophytes and their communities inside the coffee plant could be owed mainly to the region in which the coffee is grown [8]. And at last but not least, a group of scientists identified some endophytes from maize plants, which are used to make “tortillas” [9]. These endophytes can help plants to more easily absorb the nutrients that they need, helping the plants to grown even with a lower amount of fertilizer.

In this article, you got to know about endophytes, the hidden microbial helpers living inside plants. And, as you might guess, there are still plenty of endophytes waiting to be discovered. Are you ready to take the challenge and become an amazing Endophyte Hunter?

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CONFLICT OF INTEREST STATEMENT: 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.

YOUNG REVIEWERS

ALAN, AGE: 13

I am currently in the second year of high school. I am very observant, have a very good memory, and am interested in technology and videogames. I am also interested in maritime animals, and in the bacterial world. I would like to study medicine, because I am struck by the functioning of the human body and I want to help others.

MARIANA, AGE: 14

I am a student from Guanajuato, México and I am a squash player. I am in the national junior squash team and I am a volunteer for the Lions Club from Guanajuato. My family consists of my mom, my dad, and my two brothers. I have two lovely pets, one of them is named Han Solo and the other one is called Squash.

AUTHORS

ROSARIO DEL CARMEN FLORES-VALLEJO

From a very young age I was eager to ask a lot of questions, and found in the study of Chemistry and Biology very interesting and breath-taking answers. This later encouraged me to study Biotechnology Engineering. Currently I work as a Research Assistant, and continue in the quest for answers exploring the endomicrobiome of plants. I aim to harness the knowledge of plant-microbe molecular interactions to obtain novel natural products that combat of pro-inflammatory diseases and drug-resistant bacteria. When I am not at the lab some of my favorite activities are painting, photography, and cycling. *rosario.floresv@uaem.edu.mx

JORGE LUIS FOLCH-MALLOL

Since I was a child, for the horror of the women in my family, I started small insect zoo at home. I had praying mantis, dung beetles, stick insects, and other bugs, including a small water snake. Obviously, I studied Biology and became a fan of mushrooms (or fungi). These organisms fascinated me specially because they cannot move, so if a lion comes for them, they cannot escape like zebras or antelopes. So, how do they manage to stay alive with so many predators around? Finally, I got my own lab and devoted myself to study several aspects of fungi.

ALEXANDRE CARDOSO-TAKETA

I am currently a researcher in the Lab of Medicinal Plants at the Autonomous University of the State of Morelos. My research focuses on medicinal plants used in the Mexican traditional medicine, including ferns with neuropharmacological properties. I am also interested in methodologies like NMR, UPLC/GC-MS, and HTPLC for metabolomics analysis of plants. It is fascinating for me to discover new molecules that can improve people's lives. In my free time I like to cook, fusing the flavors of Brazilian and Japanese culture, which I carry in my blood, with the strong flavors of Mexico.

MARIA LUISA VILLARREAL

My interest in Biology started when I was 6 years old, when my dad showed me the beauty of living organisms in a microscope. I studied biology and became a biotechnologist looking for actual tools that can help to improve the quality of life. I focused in plants, searching for the incredible and important secondary metabolites that they produce to be used for human health. For 25 years, I have conducted a research group at the Autonomous University of the State of Morelos that was able to discover interesting molecules with pharmacological value from Mexican medicinal plants. I love to travel in my free times. *luisav@uaem.mx

ASHUTOSH SHARMA

I grew up in Latipur, India. I was awarded with a fellowship and obtained my Ph.D. degree in Mexico, were I studied medicinal plants used in the treatment of nervous disorders. I became a scientist and full-time professor, specialized in the fields of: plant biotechnology, ethnobotany, bioinformatics, plant biotechnology, genomics, and metabolomics. Currently I am the regional leader of the Department of Bioengineering, at Tec de Monterrey, Queretaro. I am a fluent speaker of many languages, and on my free time I enjoy fishing and spending time with my family.

BIODIVERSITY
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CYCADS: ANCIENT PLANTS WITH BACTERIA LIVING IN THEIR ROOTS

Fernando López Restrepo**, Diego Garfias Gallegos and Pablo de Jesús Suarez Moo

National Laboratory of Genomics for Biodiversity, Center for Research and Advanced Studies of the National Polytechnic Institute, Irapuato, Mexico

YOUNG REVIEWERS:

AURA
AGE: 14

MANU
AGE: 14

Have you ever seen a picture of a dinosaur and realized that there is a palm-like plant by its side? Maybe you have walked around your neighborhood and seen the same kind of plant there? Incredibly, those ancient plants, called cycads, have been around for millions of years. A single cycad plant can live up to 2,000 years! We think that the secret to cycads' survival and long life lies inside a very special structure called the coralloid root, which has microbes living inside it. We studied these coralloid roots and found a high diversity of bacterial species living inside of them, more than anyone had ever imagined. When we took a closer look at these bacteria, we found that they can produce many compounds that can help them communicate among themselves and with the plant, transport nutrients, and perform other functions that are still a mystery.

CYCADS: PLANTS THAT ARE OLDER THAN THE DINOSAURS

Cycads are ancient seed-bearing plants that appeared before the age of dinosaurs, during the Permian period, almost 280 million years ago.

CYCAD

Very ancient seed plants that have survived until our days. They can form a unique symbiosis through their coralloid roots.

As great as dinosaurs were, most of them went extinct while others eventually evolved into birds. However, cycads were able to survive until the present day, and still look almost exactly like their ancestors that lived alongside dinosaurs (Figure 1)! If that is not cool enough, cycads can live in extreme environmental conditions with very low nutrients where other plants cannot survive, including sand dunes, slopes, cliffs, and even on rocks [1].

How do cycads manage such amazing feats of survival? Cycads, like superheroes, have different abilities that allow them to resist difficult environments, but maybe their key superpower is inside their roots. Cycads can form unique root structures called **coralloid roots**, which look like tiny marine corals branching off the main root. Coralloid roots get nutrients for the plant, mainly nitrogen. Nitrogen (N_2) is an abundant gas in the atmosphere. N_2 can be taken up by certain organisms and converted into ammonium (NH_3), which is a nutrient that cycads can use to live and grow. This process is called nitrogen fixation. Nitrogen fixation is one function performed by organisms called **endophytes**, which are microbes (bacteria, for example) that live inside of the coralloid roots. The endophytes are in an intimate interaction with the plant, called **symbiosis** [2].

CORALLOID ROOT

Specialized cycad roots where useful bacteria live.

ENDOPHYTE

Any organism that lives inside a plant.

SYMBIOSIS

Any close and long relationship between two or more different organisms.

To understand the cycad-endophyte symbiosis, we should think about our own bodies. We have all heard the saying, "You are what you eat" and you also may have heard that we should include certain bacteria, called probiotics, in our diets. It is true that the diet is an important way to get good bacteria to colonize (live in) some of our tissues and organs, such as the gut (Figure 2). The beneficial bacteria living inside us help us digest our food, generate healthy chemicals, and even protect us against dangerous microbes called pathogens [3]. But what about cycads? Are there bacteria living inside them? If so, what are those bacteria doing and how do they do it? Scientists trying to answer such questions have looked inside the coralloid roots for more than 100 years. However, it was only recently that we uncovered the high diversity of bacteria and the interesting functions of those bacteria inside the coralloid root, including the bacteria that perform nitrogen fixation.

First of all, where do these bacteria even come from? Well, we think that some of these microbes may be inherited from the plant's mother through the seed, similar to the way human babies obtain bacteria from their mothers by breastfeeding. But most of the bacteria inside the coralloid roots seem to come from the soil closest to the roots, called the **rhizosphere**. Cycads produce substances that attract the bacteria to the rhizosphere. Those bacteria then attach to the cycads surface and enter the internal tissues of the coralloid roots, through the numerous tiny holes in the walls of the plant's cells.

RHIZOSPHERE

The soil that is in direct contact with the roots of a plant.

We know little about which soil-living bacteria can enter the cycads' roots, or even how many of them can actually stay in there. How

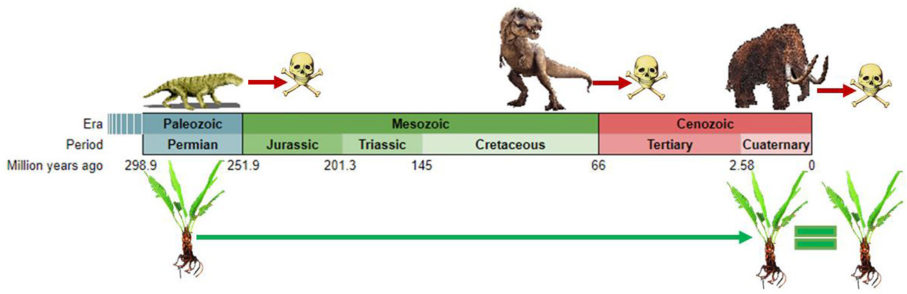

Figure 1

Figure 1

Many living creatures have appeared and disappeared from the planet, while cycads kept on living. Their outside appearance is the same, but we think their roots have changed and are the secret for their survival.

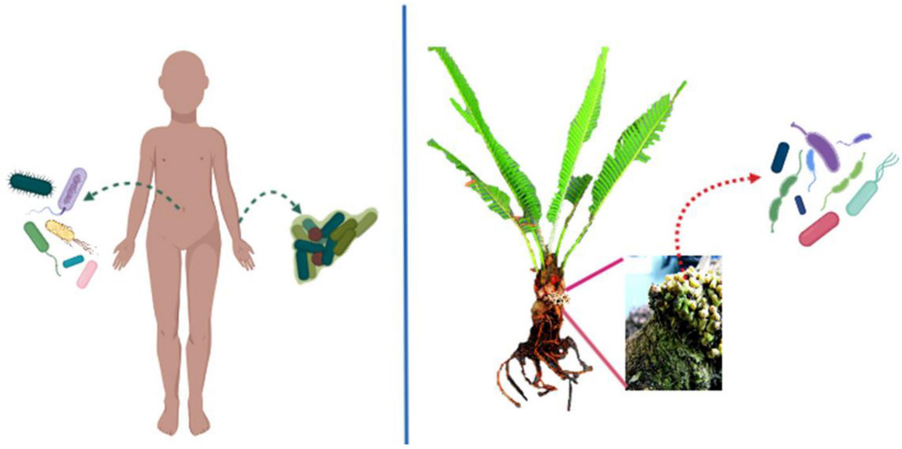

Figure 2

Figure 2

Cycads, just like our bodies, have their own tiny bacterial helpers.

many types of bacteria are there inside the roots? Can all types of bacteria enter, or is this a special bacterial "VIP club"? What effect do these bacteria have on cycads? These key questions have not been answered yet, because most of the earlier studies performed on coralloid roots were focused on a very specific type of bacteria called **cyanobacteria**. Cyanobacteria are experts in nitrogen fixation, making them very important. But we thought that maybe, as in the human gut, there might be multiple types of bacteria, not just cyanobacteria. When we tested this idea, we found that there are many other microbes in the coralloid root, and that the roots might be a kind of "VIP club," in which only certain endophytes are included.

CYANOBACTERIA

Microorganisms with the ability of doing photosynthesis (just like plants!) and fixing nitrogen into useful compounds.

A CLOSER LOOK INTO THE ROOTS OF THE CYCAD

Our hypothesis was that the coralloid roots, and all the different microbes that are inside them, have an important role in the ability of cycads to thrive in difficult environments. To test this idea, we collected coralloid roots and soil samples from the Dioon genus of cycads, both from a botanical garden and from natural areas. A genus is a group comprised of many closely related species. The Dioon cycads are mostly found in Mexico and they are endangered, which makes them very hard to find, so we had to be very careful not to damage them.

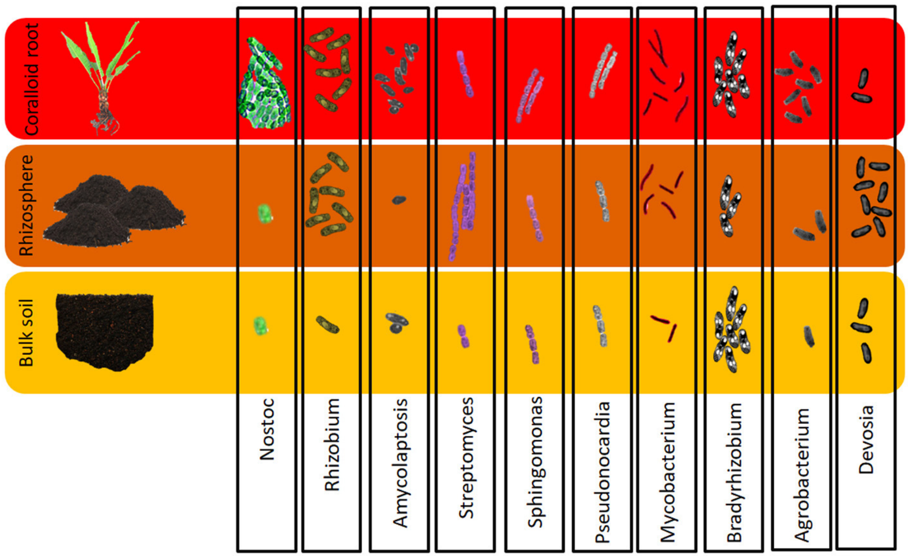

Figure 3

Figure 3

Different amounts and types of bacteria were found in all three compartments: coralloid roots, rhizosphere and bulk soil of all plants studied. The types of bacteria are listed along the bottom, and the number shown next to each sample type shows whether a large or small number of that type was found in that sample. This figure combines the results of plants from both natural populations and botanical gardens. You can see that only some bacteria prosper in the roots, while others barely manage to grow.

Most of the cycads were growing in hard-to-reach places, so we had to have the correct instruments, a lot of determination, and a little bit of luck.

We collected coralloid root samples and stored them in liquid nitrogen (an extremely cold substance with a temperature around −200°C) to preserve the DNA. We also collected the rhizosphere and other soil from the area around the cycads. We separated the coralloid root samples by origin (natural location or botanical garden) and looked at the number of different species of bacteria in each of the soil and root samples.

THE MANY, MANY FRIENDS OF THE CYCAD

We found that there were 246 genera (plural of genus) of bacteria in the root and soil samples, but most of the bacteria in the samples fell into 10 genera (Figure 3). This told us that only a few groups of bacteria have really important roles. Curiously, we found a higher abundance of cyanobacteria species in natural samples than in botanical garden samples. Why did we find these differences? Perhaps because the soil from the natural locations has fewer nutrients than the soil in pots in the botanical garden. This might mean that cyanobacteria help cycads obtain nutrients from the soil, and are less necessary in botanical gardens than in nature. The coralloid-root "VIP club" includes other nitrogen-fixing bacteria in addition to cyanobacteria, such as Rhizobium, Bacillus, and Streptomyces, which are also known to live in the coralloid roots of other cycads besides Dioon.

Why do we call the coralloid root microbiome a VIP club? Because only a small fraction of the microorganisms living outside the cycad are able to get inside of the coralloid root. As a result, the numbers and types of bacteria inside the coralloid root are different than in the bulk soil and rhizosphere, as seen in Figure 3. Does the plant choose which bacteria are members of the VIP club? Or can only some types of bacteria survive inside the roots? Do these VIP bacteria have specific abilities that allow them be members of this club? Scientists are still answering these questions. What do you think?

Since the Dioon cycads that we studied are closely related to each other, we thought that they all might let the same types of bacteria into their coralloid roots. But we found differences in the types of bacteria in the roots of plants growing in natural sites vs. a botanical garden. We think this is due to the soil these cycads are growing in, as well as the small differences between the related plants. Think about humans again: it is known that human genes and diet shape the types of bacteria that are present in our guts. It could be the same with Dioon cycads—they could be sending different signals that attract different kinds of microorganisms to their roots. The exact mechanism for selecting the coralloid endophytes is still unknown, and the factors involved in choosing who is in each plant's VIP club remain to be discovered.

HOW MIGHT CYANOBACTERIA HELP CYCADS SURVIVE?

Are the bacteria living in the coralloid roots the key to the amazing survival of the cycads? To study this, scientists investigated whether cyanobacteria are able to produce special compounds that might help cycads survive. The scientists looked for sections of bacterial DNA that code for proteins called **metabolites**, which are substances that can help organisms survive under difficult environmental conditions. We call these parts of the DNA **biosynthetic gene clusters**, of BGCs for short [4].

METABOLITE

A substance that is formed during the metabolism of an organism.

BIOSYNTHETIC GENE CLUSTER (BGC)

Sections of DNA that produce specific substances.

Scientists searched for 77 known BGCs in the DNA of closely related cyanobacteria species. Of these BGCs, four were shared in all the types of cyanobacteria that we found inside the coralloid roots. These BGCs produced some toxins that can stop other bacteria from growing, possibly protecting the plant from harm. There are also metabolites and molecules that bacteria use to communicate with other endosymbionts and to provide nutrients for the plant, such as metallic-ion-carrier molecules called siderophores. These molecules help in the absorption of certain ions which otherwise would be inaccessible for the plant. These ions can mediate the metabolic response of several bacterial members of the community; thus changing the signals that the microorganisms receive from the environment.

SO, WHAT IS NEXT?

The data gathered in this study gives us a pretty good picture of the bacterial community within the coralloid roots of the cycads. We now think it is possible that cycads have survived since the age of the dinosaurs without having to change on the outside because the cycads endophytes are the ones changing and providing new metabolites and abilities to deal with difficult conditions in the environment. However, there is still a lot we do not know about these plants and their endosymbionts, and scientists are still studying their interactions.

Before we wrap up, we will mention another intriguing observation made during our study. When looking inside the coralloid roots, we saw another type of organism that has barely been studied at all: fungi! So far, all we know about the fungi in the coralloid roots is that different types of fungi live in a single species of cycad. How the fungi affect other organisms and how they interact with the cycad and the bacterial endosymbionts is currently unknown. We think fungi may be acting as a network to move metabolites to the plant, or maybe as protectors of the plant or the bacteria. But the fungi could also be competing for space inside the roots, forcing the bacteria to work together. As you can see, there are still a lot of things to study about these ancient plants and their coralloid roots. What we learn about these plants might help us understand how symbiotic relationships between organisms originally formed in plants, and how these relationships are regulated. Since all known organisms depend upon their relationships with one another, this information could tell us a lot about how living things have survived and developed over the course of evolutionary history, not as isolated beings, but as parts of communities. Only time will tell what future discoveries will show about the beings we share the world with!

ORIGINAL SOURCE ARTICLE

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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.

YOUNG REVIEWERS

AURA, AGE: 14
My name is Aura. I am 14 years old. I live in Guanajuato Mexico and I love dancing. I think all the sciences are very interesting.

MANU, AGE: 14
My name is Manuela but everyone calls me Manu. I am 14 years old and I am in my second year of secondary school. I love horse riding and arts. When I grow up I would like to be a marine biologist or to study gastronomy.

AUTHORS

FERNANDO LÓPEZ RESTREPO
I am a graduate student with a passion for the world around us and all of its inhabitants. I have a background in the study of circadian rhythms, environmental stress, and organism interactions. I first became interested in science and biology

by reading about the animals we share the world with, and my thirst for knowledge never stopped since. I am also passionate about history, music, and creature design.
*fernando.lopez.r@cinvestav.mx

DIEGO GARFIAS GALLEGOS
An art enthusiast since the time when the Bach and Chopin melodies drive me to see that there are questions worth to be asked and answers that are better to attack by different perspectives. Obsessed with fantasy and unseen worlds like the ones Tolkien or Bradbury depicted and what in the end drive me to search answers in science, were there are entire worlds waiting to be discovered.

PABLO DE JESÚS SUAREZ MOO
I am currently an associate professor at the Institute of Ecology (INECOL), Mexico, where I study the role of the microorganisms (microbiome) in the welfare (fitness) of plants and animals. When I started studying biology, my research interest was the biology of sharks (sharks are so awesome), but now I am interested in the microscopic world (mainly fungi and bacteria) and your association (symbiosis) with the plants and animals. With the new technologies, we discover news beneficial properties of the microorganisms to their hosts (is so cool!).

frontiers FOR YOUNG MINDS

THE EARTH AND ITS RESOURCES
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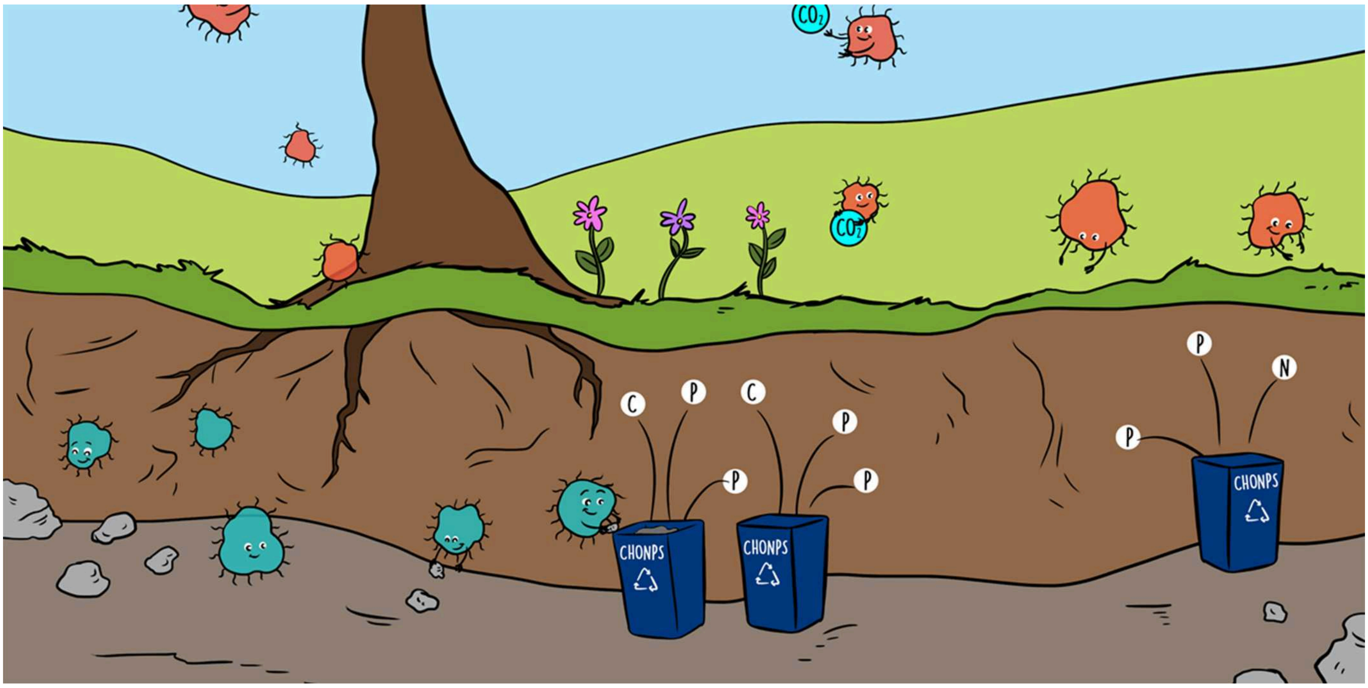

BACTERIA HAVE SUPERPOWERS TO RECYCLE SOIL NUTRIENTS

Yunuen Tapia-Torres* and Alberto Morón-Cruz

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YOUNG REVIEWERS:


ALAN
AGE: 13


DIEGO
AGE: 14


MANU
AGE: 14

Bacteria are everywhere, and they do not only cause diseases! Bacteria are also involved in many processes that are indispensable for our life on Earth. Many of these processes have to do with the recycling (reuse) of chemical elements that have been here since the formation of the planet. Bacteria play an important role in the movement of the chemical elements between air, water, soil, and living things, allowing life as we know it to evolve. How do bacteria recycle chemical elements so that we can use them? Through important superpowers coded for by their genes!

THE CHEMISTRY OF LIFE

All living organisms we know of today are made of **biomolecules** (**proteins**, carbohydrates, nucleic acids, and phospholipids) that are composed of a group of important chemical elements. In the formation of biomolecules, some chemical elements are needed in large quantities; they are known as **macroelements**. The macroelements are carbon (C), hydrogen (H), oxygen (O), nitrogen

BIOMOLECULE

Molecules that make up all living beings, including proteins, carbohydrates, nucleic acids, and phospholipids.

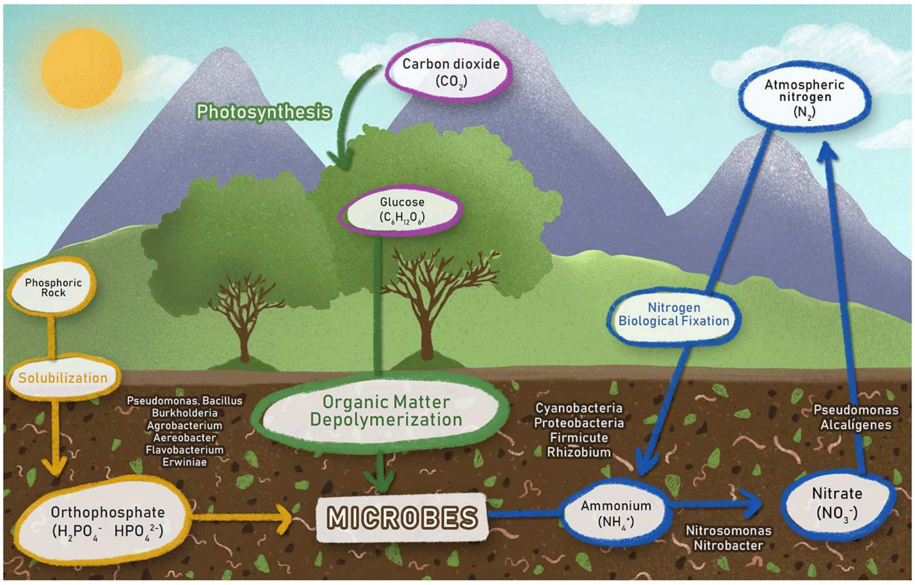

Figure 1

Figure 1

The movement of important molecules containing carbon, nitrogen, and phosphorus through an ecosystem. Boxes represent the nutrient pools (with the names of the compounds and their chemical formulas) and arrows represent processes. The names of some of the bacteria that perform various processes are listed beside the arrows.

PROTEINS

The building blocks of every cell in living beings. Proteins are critical for the chemical reactions of metabolism and they also give cells their structure.

MACROELEMENT

A chemical element required in large quantities for the normal physiological processes of living organisms.

(N), phosphorus (P), and sulfur (S), which we abbreviate as CHONPS. These 6 elements are the main constituents of our cells and represent 95% of the total biomass (all the matter contained in living beings) on the planet. All elements found naturally on Earth are very old; they are approximately the same age as the planet (4.5 million years). Can you imagine that? The same CHONPS atoms have been part of the different molecules of many living beings on the Earth again and again, through a process of biological recycling. This means that all the atoms that form our bodies are the same atoms that could have been part of the dinosaurs millions of years ago! This is amazing!

Atoms of the macroelements can join together to form different molecules. Oxygen can join with hydrogen to make water (H_2O) or it can join with carbon to make carbon dioxide (CO_2). On Earth, large amounts of macroelements are found in the atmosphere as gases. Maybe you have heard of CO_2, molecular oxygen (O_2), and molecular nitrogen (N_2), for example. These are examples of gaseous molecules containing macroelements. In contrast, elements, such as N can be found forming different molecules in the air, water, and soil. However, other macroelements, such as P, do not exist as gases, but we find them in the soil and water (Figure 1).

HOW CAN AN ELEMENT MOVE THROUGHOUT THE ECOSYSTEM?

The movement of the macroelements (CHONPS) within the soil, water, and atmosphere depends mainly on the activity of microscopic organisms: the microbes. "Microbe" refers to a living thing too small to be seen with the naked eye, and this term is used to describe

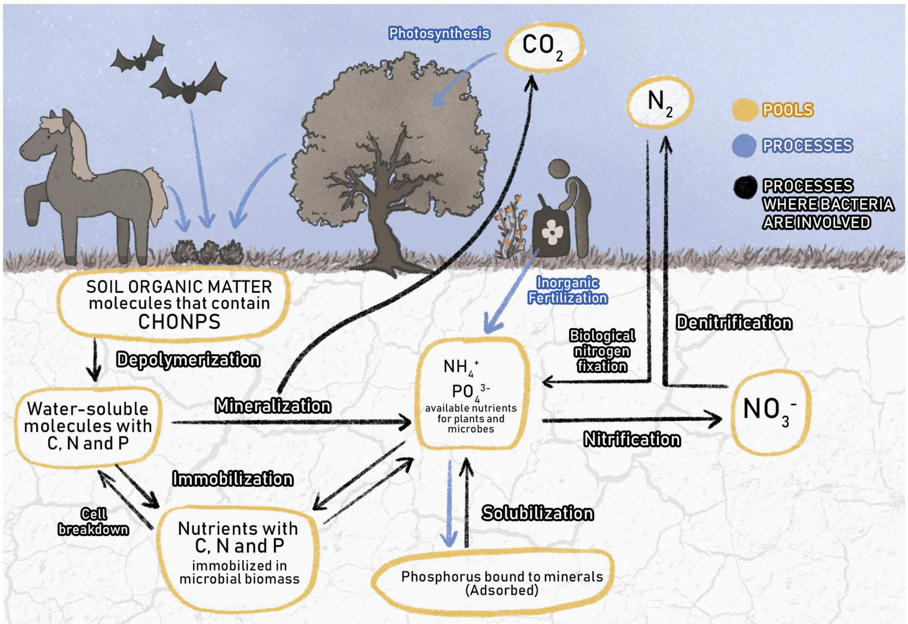

Figure 2

Figure 2

Different ways by which CHONPS move between the air, living things, and soil are shown. Plants and animals incorporate soil organic matter rich in CHONPS; i.e., excrements of animals and leaves that fall from trees. Soil bacteria perform recycling of soil organic matter through different processes, and as a result they produce and release into the soil inorganic molecules (NH_4^+, NO_3^-, PO_4^{3-}, CO_2) that can be consumed by plants and microorganisms to grow and perform their functions. Without the presence of soil microorganisms, CHONPS contained in soil organic matter cannot be reused and ecosystems could no longer function. The boxes represent the nutrient pools, black arrows represent microbial processes, and blue arrows represent processes in which microbes do not participate.

GENE

A piece of DNA that encodes the instructions for making a protein.

BIOLOGICAL NITROGEN FIXATION

The process in which nitrogen gas (N_2) is incorporated into the biomass of living organism and converted to organic nitrogen.

several very different life forms (bacteria, fungi, archaea, viruses, and protists).

Bacteria are responsible for the recycling and transformation of elements on Earth and they perform this task thanks to the superpowers encoded in their **genes**. Genes are the instructions for making the molecules that are needed for many different processes, including the recycling of macroelements. For example, through a process called **biological nitrogen fixation** (Figure 1), bacteria can use N molecules in gaseous form (N_2) to make proteins, which are solid organic molecules rich in C and N. This is extraordinary: bacteria are the only living organisms that can convert, through chemical reactions regulated by genes, gaseous N_2 into proteins that help them to grow and sustain their lives! Humans cannot use N_2 the way bacteria do, but thanks to these bacteria we can also have proteins (and therefore N) in our bodies. Therefore, the chemical elements can go from being in the atmosphere to dissolved in water existing in the soil, or from being in the form of a gas to being inside a living organism (Figures 1, 2). All of these transformations occur thanks to bacterial activity.

Some of the processes used for recycling and transformation of macroelements that form biomolecules are shown in Figure 2. Each arrow indicates a process regulated by different species of soil bacteria, and each box represents a pool of nutrients for soil bacteria and other soil microbes.

To understand Figure 2, think of the leaves that fall from the trees in the forest. These leaves accumulate in the soil and form a layer of organic matter (OM; a mixture of biomolecules). The OM is made up of different kinds of waste from plants and animals and contains very large molecules with CHONPS in them. These large molecules are difficult for plants to use, because they are so big. So, the soil microbes break down the OM molecules into small pieces. The process of breaking down OM molecules is called **depolymerization**, and it results in a set of small molecules that can dissolve in water, such as glucose (Figure 2). These dissolved compounds are very important for the soil microbes that consume them, because they are the microbes' source of C.

DEPOLYMERIZATION

The process of converting a big molecule (polymer) into a small molecule (monomer) or a mixture of small molecules.

HOW DO BACTERIA TRANSFORM THE ELEMENTS THAT ARE ONLY PRESENT IN SOIL AND WATER?

Phosphorus does not have a stable gaseous form. Therefore, we cannot find it in the atmosphere; rather, we can find P in the rocks of the Earth's crust. P is essential for all living beings because it is in many biomolecules, including DNA. P is also widely used as fertilizer in food production. But... how do living organisms acquire P if they do not eat rocks? Bacteria are primarily responsible for P recycling in nature, and they regulate the pool of available P (in the form of phosphate; PO_4^{3-}) through a variety of P-transformation processes (P solubilization, OM depolymerization, P **mineralization**, and P assimilation; Figure 2). Later, plants acquire P from the soil, herbivores acquire it when they eat the plants, and carnivores when they eat the herbivores. In this way, animals can use the P originally found in the rocks to build their bodies.

MINERALIZATION

A biological process in which organic molecules are converted into inorganic molecules.

We know of about 200 different molecules that contain P, and each of these molecules can be the food for a different bacterial group. Can all bacteria use all the different P-containing molecules? To answer this question, we performed an experiment. We isolated 1,163 kinds of bacteria from the soil and sediment of an ecosystem with little P available. We evaluated these bacteria for their ability to use the P contained in six different P-containing molecules (Figure 3). To be able to use and **immobilize** the P contained in each of these molecules, bacteria need different genes to carry out different processes (Figure 2).

IMMOBILIZATION

Processes carried out by microorganisms and plants where they incorporate inorganic molecules into their biomass and convert them into organic molecules.

To understand this experiment, imagine P-hungry soil bacteria in each of the Petri dishes in Figure 3A. All the Petri dishes contained the other nutrients that bacteria need for growth, but P was supplied as a different molecule in each case. This means that, to grow in a certain petri dish, the bacteria needed to have the appropriate genes that helped them to use the specific source of P that was added. If you look at Figure 3B, you can see two Petri dishes with different P sources. The same bacterial were placed in both Petri dishes. Some bacteria could grow in both Petri dishes, but in some cases, bacteria could grow only in one dish, telling us that particular type of bacteria was not able to use both forms of P. We tested each of the 1,163 bacterial isolates with the six different P-containing molecules.

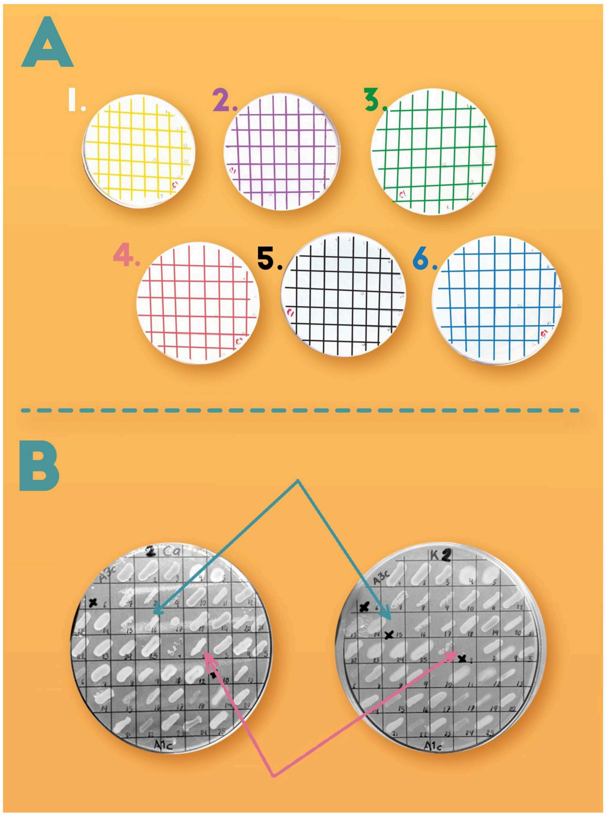

Figure 3

Figure 3

(A) In our experiment, different P sources were present in each Petri dish, to test the ability of different kinds of bacteria to use the P contained in each source. In each Petri dish, bacteria of the same type were located at the same spot in the grid. **(B)** Example of the growth of the same bacteria in two different P sources. The Petri dish on the left contained calcium phosphate and the dish on the right contained potassium phosphate as P source. Arrows indicate the bacteria that could grow using only one of the two P sources. This means that to be able to use the P contained in each of these molecules, bacteria need different genes to carry out different processes.

WHAT DID WE LEARN FROM THESE P-HUNGRY BACTERIA?

Rocks containing P are not equally distributed in ecosystems. Therefore, in extremely P-limited ecosystems, like the ecosystem from which these bacteria were isolated, bacteria can break down and use different forms of P to contribute to P cycling through the ecosystem. In these P-limited ecosystems, soil bacteria have been found to use many different strategies for using P. This is awesome: the less available P that bacteria have, the more powers they acquire for obtaining it.

As we can see, bacteria have many superpowers when it comes to the recycling of chemical elements. These bacteria make it possible for plants and animals to exist. These recycling processes have been at work for billions of years and they have been vital for the evolution of living beings on Earth.

AUTHOR CONTRIBUTIONS

YT-T conceived the study. All authors contributed to all aspects of the preparation and writing of the paper. All authors approved the final version.

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YOUNG REVIEWERS

ALAN, AGE: 13

I am currently in the second year of high school. I am very observant, have a very good memory, and am interested in technology and videogames. I am also interested in maritime animals, and in the bacterial world. I would like to study medicine, because I am struck by the functioning of the human body and I want to help others.

DIEGO, AGE: 14

My name is Diego but they call me "Morrillo," I am 14 years old and I study at the Cumbres de la Independencia school. I like to play videogames and walk. My favorite foods are tacos, chicken wings, and pizza.

MANU, AGE: 14

My name is Manuela but everyone calls me Manu I am in my second year of secondary school. I love horse riding and arts. When I grow up I would like to be a marine biologist or to study gastronomy.

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THE EARTH AND ITS RESOURCES
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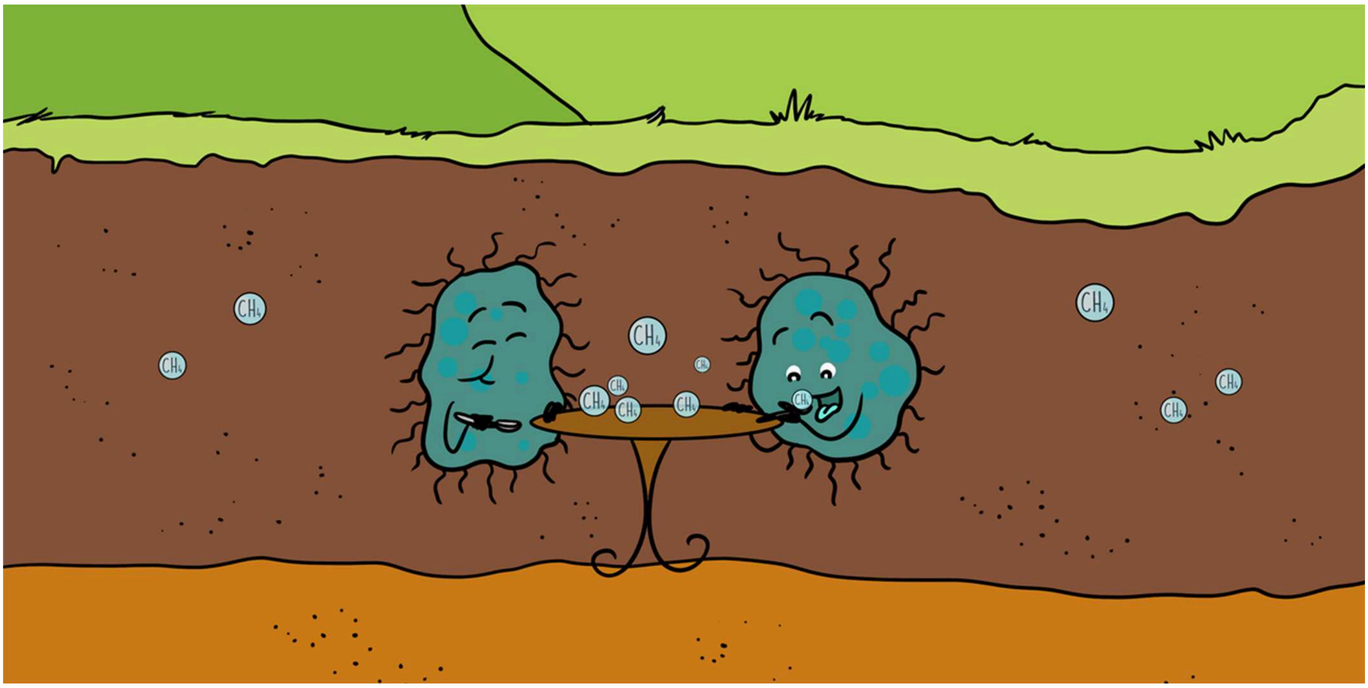

THE ROLE OF MICROORGANISMS IN THE METHANE CYCLE

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YOUNG REVIEWER:

ADAM
AGE: 13

Have you heard about methane gas? Maybe the word methane is not familiar to you, but in fact, this gas is widely found in our daily lives, in our atmosphere, and in the solar system. Methane is a gas that is naturally produced in all kinds of environments, and it comes from the breakdown of organic (formerly living) materials. Methane gas is effective at trapping heat and it also burns very easily. So, methane is one of the most important fuels for humans. Additionally, the methane in the atmosphere helps regulate the climate on Earth. However, the amount of methane in the atmosphere has been steadily increasing for the past 200 years, which concerns the scientific community. Surprisingly, recent studies have indicated that levels of methane are regulated by tiny microbes. In this article, we encourage you to learn about the methane cycle, the microbes

that make and eat methane, and why more research is needed on this gas.

WHAT IS METHANE AND WHY IS IT IMPORTANT TO HUMANS?

Methane is a simple compound, formed by one atom of carbon and four atoms of hydrogen (CH_4). Methane exists as a gas in the environment and is one of the most important fossil fuels for human society. When the methane molecule breaks down, it produces heat. Because of this property, some of our homes are fueled by methane gas, which is used to cook, heat our water, and fuel our furnaces and fireplaces. Methane can also be collected and transformed into electricity, serving as a natural energy source. Methane is also found in animal burps and farts (yes, you read correctly, farts!). Methane is one of the most abundant gases produced in the digestive tract as food is broken down. To summarize, methane is a common atmospheric gas. Remarkably, methane production and breakdown on Earth are processes driven mainly by microorganisms.

MICROBES/ MICROORGANISMS

Very small forms of life including bacteria, fungi, and some diminutive algae.

ORGANIC MATTER

All cells and substances made by living organisms, including living and dead animals and plants.

Microorganisms (microbes) are the smallest life forms known, invisible to unaided eyes. They are found in all habitats and ecosystems on Earth, in our daily surroundings as well as the most hostile and extreme habitats. Although they are extremely small, the diversity and abundance of microorganisms are enormous and remarkable. Recent estimates predict that 90–99% of the microbial species on Earth are still undiscovered [1]. Microbes are the major players in the recycling of **organic matter** and important nutrients on Earth. They also regulate the production and breakdown of some atmospheric gases, including carbon dioxide, the oxygen we breathe, and of course, methane.

Methane has drawn the attention of the scientific community because its concentration in the atmosphere has almost tripled, since the Industrial Revolution began in the eighteenth century. Importantly, some studies indicate that these recent increases in atmospheric methane are happening more quickly as compared to geological time scales. Suggesting the influence of human activities associated to methane emissions. The problem with increased methane in the atmosphere is that, methane gas has the ability to trap the heat energy from the Sun and prevent this heat energy from returning to space, resulting in something known as the green-house effect. This heat-trapping capacity is very important, because it helps the Earth to stay warm enough to sustain life [2]. However, too much methane accumulation impacts the climate and contributes to global warming. Today, the methane cycle is a major research topic, since we need a deeper understanding of where all the methane on earth comes from and how it is transformed.

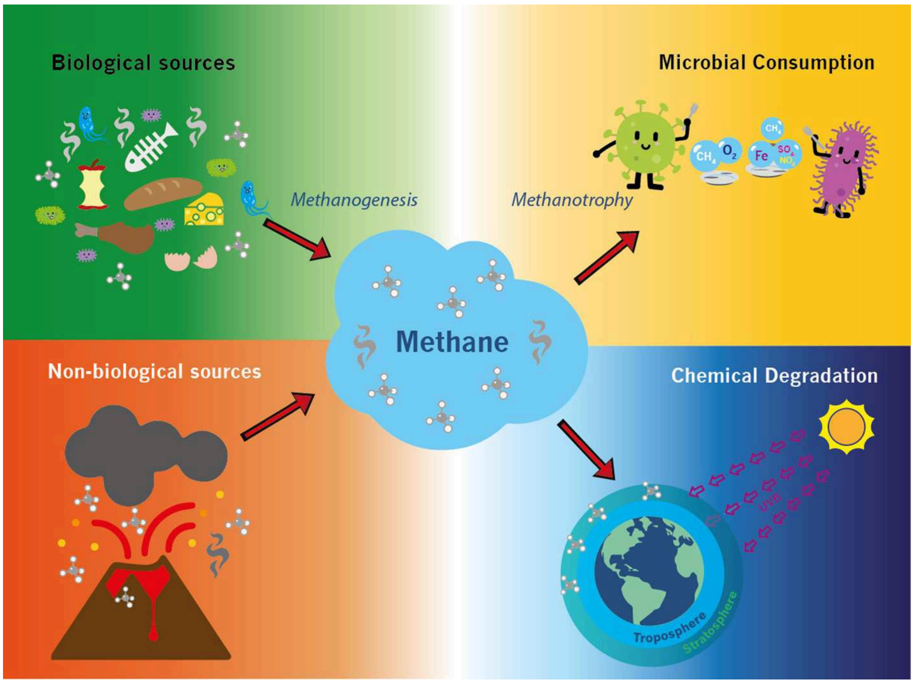

Figure 1

Figure 1

Diagram of the methane cycle showing sources of methane production and methane breakdown on Earth.

METHANE PRODUCTION IN ECOSYSTEMS

There are two known forms of methane production on Earth, called non-biological and biological methane sources. Non-biological methane production occurs without the participation of living organisms. Non-biological methane can be released by volcanoes or formed underground, under high pressures and temperatures. These geological processes normally involve the transformation of rocks that are melted with heat and water (Figure 1). Biological methane production is only done by microorganisms. The current estimates suggest that 90–95% of the methane released into the atmosphere has a biological origin and is produced exclusively as a result of microbial activity!

The process of biological methane production is called *methanogenesis*. The best studied methane-producing microorganisms are named methanogenic archaea or simply *methanogens*. Methanogens have a complex **metabolism** that allows them to create methane as they produce the energy they need to survive. Interestingly, atmospheric oxygen which we need to breath and obtain energy, is toxic to some methanogens, so these microorganisms are generally found in areas where oxygen is limited or absent, such as underground, in the sediments at the bottom of lakes, lagoons, wetlands, and oceans, and even inside the intestines of all types of animals, including worms, termites, cows, and humans.

METABOLISM

All the chemical reactions needed to keep a cell or organism alive. Metabolism refers to how living things make and break down nutrients.

Methanogenesis is the terminal step in the food chain that occurs in the absence of atmospheric oxygen. This gas is produced as a consequence of the total degradation of organic matter, where complex molecules are degraded into their most basic compounds and then are converted to methane by methanogens. This means that in all kinds of environments, the remains of dead organisms, such as plants and animals are slowly decomposed by microbes (Figure 1). This allows the return of the nutrients to the food chain, and the last step involves methane production [3].

ONCE METHANE IS PRODUCED, HOW IS IT REMOVED FROM THE ENVIRONMENT?

Removal of methane from the environment also occurs by both non-biological and biological methods. The main way that atmospheric methane is removed occurs by a non-biological method, which takes place in the zones of the atmosphere known as the troposphere and the stratosphere. These are the lowest layers of Earth's atmosphere, from 0 to 10 km and 10 to 50 km above sea level, respectively. In these zones, methane is broken down by chemical reactions driven by ultraviolet light from the sun. It is calculated that more than 90% of the methane in the atmosphere is broken down through this process (Figure 1).

Biological removal of methane on Earth, as incredible as it seems, is exclusively performed by microbes!

There are some microorganisms that "eat" methane to get energy. This process is named *methano-trophy* and the microbes that carry out this process are called *methanotrophs*. "*Trophos*" means "one who is nourished from." Methanotrophs inhabit ecosystems where methane is produced, mainly under the surface of soil or sediments. Because these methanotrophs live under the soil, atmospheric methane does not come into contact with those organisms. Since the methanotrophs cannot break down the methane in the atmosphere, it accumulates. However, a very interesting phenomenon happens here. Somehow, methane produced in soils gets trapped between the soil particles and is actually there where methanotrophs take the gas for its consumption. This prevents methane from being released from the soil into the atmosphere, significantly impacting the atmospheric methane budget. As an example, it has been estimated that ∼40–60% of the methane produced in wetland habitats is consumed by microbes before it can escape into the atmosphere. This means that methanotrophs are very important in soils, to prevent the release of greenhouse gases into the atmosphere where they can contribute to global warming.

Methanotrophs can eat methane both in the presence and in the absence of atmospheric oxygen. Methanotrophs that can tolerate oxygen, actually use it in the process of breaking down methane. Regularly, these microbes are found in soils where oxygen starts to

Figure 2

Figure 2

If there is methane in other parts of our solar system, could microbes be there, too?

be absent because it cannot penetrate the compressed soil-particles. These oxygen minimum zones contain most of the methanotrophs and are found in all kinds of ecosystems on Earth.

Methanotrophs that do not use oxygen to break down methane, prefer to use other exotic sources of energy, accompanying the methane with some fraction of the organic matter, or with sulfur, nitrogen, and even some metals, such as iron or manganese. Here, methane is the big meal and the other elements are the complements. Interestingly, this process was firstly hypothesized by geo-chemical evidence, but remained elusive until the early 2000s, because it is extremely difficult to grow these microbes in the lab to study them.

METHANE BEYOND EARTH

Here on Earth, microorganisms play a big role in the recycling of methane. So, we could say that methane is related to the presence of life on our planet. Surprisingly, recent evidence obtained by telescopes and remote artifacts has identified methane in other places in our solar system, including on Mars and on Saturn's icy moons Titan and Enceladus [4]. This is very exciting and makes us wonder if there are some kinds of microbes in those places that are producing or consuming that methane!

Methane on Mars was first identified with Earth-based telescopes in the early 2000s, and its presence was proven when the Mars rovers Spirit and Opportunity explored that planet (Figure 2). The scientific community has been wondering if this methane originates from biological processes, but all the scientific evidence collected so far indicates that this methane comes from non-biological sources. Currently, there is no evidence of biological activity on the surface of Mars, but the research continues, because we know that methane also is a source of energy for some microbial life.

Two spacecraft missions, Cassini-Huygens and Voyager, have been studying Saturn. Both spacecrafts have found evidence of organic molecules, including methane on Saturn's moons, Titan and Enceladus (Figure 2). These moons have a lot of water and ice on their surfaces, probably similar to the polar ice caps on Earth. As strange as it seems, data suggest that both Titan and Enceladus have oceans of liquid methane, ethane, and nitrogen that form lakes and rivers, covered with rocks of water-ice.

Figure 2 illustrates methane gas detected on other planetary bodies of our Solar System.

The exploration of extreme environments on Earth, such the Antarctic ice shelves, can help us understand the origin and evolution of extraterrestrial methane. Currently, scientists are studying how microbes can survive in permanently ice-covered ecosystems, because if we can better understand the methane cycle in extreme environments here on Earth, that would help us to also understand how methanogenesis and methanotrophy could potentially exist on the extreme environments of other planets. It is only natural to predict that methanogens and methanotrophs could be amongst the creatures inhabiting other planetary bodies … and that we are not alone in the universe, but share it with a wide range of microbes!

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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.

YOUNG REVIEWER

ADAM, AGE: 13

Hi, my name is Adam. I live with my parents, older brother, dog, fish, and two birds. I am a big fan of Science and History. I like to draw, write, and read. My favorite sport is soccer (or football). I enjoy swimming in the ocean and playing video games.

AUTHORS

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I am a marine biologist dedicated to the study of marine and hypersaline microorganisms. I am very interested in geomicrobiology, astrobiology, and biotechnology. I have experience in the study of the methane and sulfur cycles in marine ecosystems. Currently, I am involved in research on novel oil-degrading microorganisms. Also, we are studying the microbes living in mangrove forests. In brief, I am interested in the study of the role of microorganisms in nature and their potential use for biotechnological purposes. https://www.researchgate.net/profile/Santiago_Cadena

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I am a biotechnology engineer from the Technological Institute of Sonora (ITSON). I obtained a master's degree in Biotechnology from Universidad Autónoma Metropolitana (UAM) Iztapalapa Campus in 1998 and a Ph.D. in Environmental Sciences from Wageningen University (Netherlands) in 2002. I am a professor at the Engineering Institute (Campus Juriquilla) of Universidad Nacional Autónoma de México (UNAM). My research interests include several topics related to environmental biotechnology and microbiology. http://www.iingen.unam.mx/es-mx/SitiosWeb/Laboratorios/LIPATA/Personal/Paginas/default.aspx

LUISA I. FALCÓN

I am a microbial ecologist at UNAM in Mexico, fascinated with the diversity of bacteria and archaea in the environment. I love how microbes interact with each other to complement their metabolic capabilities, allowing for communities and ecosystems to exist. https://sites.google.com/a/ciencias.unam.mx/laboratorio-de-ecologia-bacteriana-instituto-de-ecologia-unam/

JOSÉ Q. GARCÍA-MALDONADO

I am a marine biologist from the Autonomous University of Baja California Sur (UABCS). I obtained my master's and Ph.D. in the Use, Management and Preservation of Natural Resources from the Biological Research Center (CIBNOR). Since September of 2014, I have been a National Council for Science and Technology (CONACYT) researcher for the Marine Resources Department in the Center for research and advanced studies of the National Polytechnic Institute (CINVESTAV), in Mérida, Mexico. My investigations are related to the ecology and biotechnology of complex microbial communities in marine and extreme environments. https://www.mda.cinvestav.mx/Investigaci%C3%B3n/DepartamentodeRecursosdelMar/PersonalAcad%C3%A9mico/Investigadores/JGarcia.aspx *jose.garcia@cinvestav.mx

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MICROBIALITES: WHAT ON EARTH?

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YOUNG REVIEWERS:


DANA
AGE: 14


MARÍA JOSE
AGE: 14


MARIANA
AGE: 15

Microbialites are rock-like underwater structures that look like reefs but are made entirely of millions of microbes. These structures are very ancient and can be found in different environments on every continent on Earth. Mexico has many microbialite reefs, in desert valleys, crater-lakes, and coastal lagoons. Science helps us to understand the microbes that build microbialites, to know whether the same kinds of microbes make up microbialites in different regions of the world, and to figure out how the microbes organize into microbialites. Many things are damaging microbialites in Mexico, including poorly planned development, pollution from lack of sewage treatment, too much water usage, and the fertilizers used for agriculture. Policies that regulate development are urgently needed to help save these diverse and ancient microbial reefs.

WHAT ARE MICROBIALITES?

Do you know what a **microbialite** is? Not many people do. This is because microbialites look like slimy underwater rocks, but they are actually reefs made up of **microbes** (simple, one-celled organisms). Microbialites are fascinating, because these rock-like structures are made by the interaction of millions of microbes that live in certain aquatic environments. The microbes facilitate the precipitation of minerals from the water, to form the microbialite structure [1]. Since microbialites are like rocks, they have remained on earth since extremely ancient times. Fossilized microbialites, known as **stromatolites** (from the Greek *strõma,* meaning bed or layer, and *lithos,* meaning rock), are the oldest evidence of life on Earth, dating back to 3.7 billion years ago [2].

MICROBIALITE

A rock-like structure made by millions of microbes that precipitate carbonate.

MICROBE

An organism that is seen under the microscope.

STROMATOLITE

Fossilized microbialites, and are the oldest fossils dated so far.

Cyanobacteria are one type of microbe found in microbialites, and these bacteria do all kinds of important work. Cyanobacteria build shelters that protect the microbial community from dangerous things in the environment, including protecting them from drying out and from damage by the sun's UV rays. The shelters built by cyanobacteria trap and bind sediments and minerals, which help to grow the microbialites [3, 4]. Cyanobacteria are also involved in producing slimy substances called **exopolymeric substances (EPS)**, which are like mucous. Exopolymeric substances help microbes to stay close to each other and allow the cells to communicate with each other [4]. Cyanobacteria also have pigments that interact with photons from the Sun, allowing them to perform photosynthesis, which leads to the incorporation of carbon from the atmosphere (in the form of CO_2) into their cells. Cyanobacteria take up water and produce oxygen during photosynthesis. Since microbialites are so old, they probably participated in oxygenating the early Earth. Gently and slowly, bubble by bubble, microbialites produced oxygen, and by ~2.4 billion years ago, the chemistry of earth's atmosphere had changed enough to support the evolution of other life forms [5]. Cyanobacteria are one of the food sources for other kinds of microbes, helping many different kinds of microbes work together as a unit to form the microbialite community.

CYANOBACTERIA

A phylum of bacteria that can make photosynthesis.

EXOPOLYMERIC SUBSTANCES (EPS)

Mucous-like molecules released by microorganisms into their environment, which help the microbes remain close together and communicate with each other.

WHERE DO MICROBIALITES LIVE?

There are living microbialites in different aquatic environments all over the globe, including polar, temperate, and tropical locations. In Mexico, there are microbialite communities in different locations, including coastal lagoons, crater-lakes, and desert ponds (Figure 1).

ARE ALL MICROBIALITES IN MEXICO THE SAME?

In our study, we wanted to understand if microbialites from different locations in Mexico are similar or different, in terms of the microbes

Figure 1

Microbialite reef locations in Mexico that were examined in our study: Cuatro Ciénegas basin in Northern México, crater-lake Alchichica in Central México, and Bacalar lagoon in the Yucatán Península.

Figure 1

that make them up. To study this, we explored various sites using catamarans, kayaks, and boats. Once we identified microbialite reefs, we took small samples (∼2 g) of microbialites from the first few millimeters of their surface, using clean, microbe-free tools and containers. We stored these samples in coolers and then froze them when we got back to the laboratory. Later, we extracted the DNA from these microbialite samples, using different enzymes, soaps, and organic molecules. To study this DNA, we first amplified it using a technique known as **polymerase chain reaction (PCR)** and analyzed how similar or different the microbial DNA sequences were to each other.

POLYMERASE CHAIN REACTION (PCR)

A laboratory technique for obtaining millions of copies of pieces of DNA, so that they can be more easily analyzed.

By comparing certain regions of the microbial DNA, thousands of microbes can be identified from a pea-sized rocky sample!

We found that all the microbialite communities we studied had the same groups of microbes, just in slightly different proportions (Figure 2) [6]. The main microbes in microbialites are Cyanobacteria (which we already discussed), Proteobacteria and Bacteroidetes

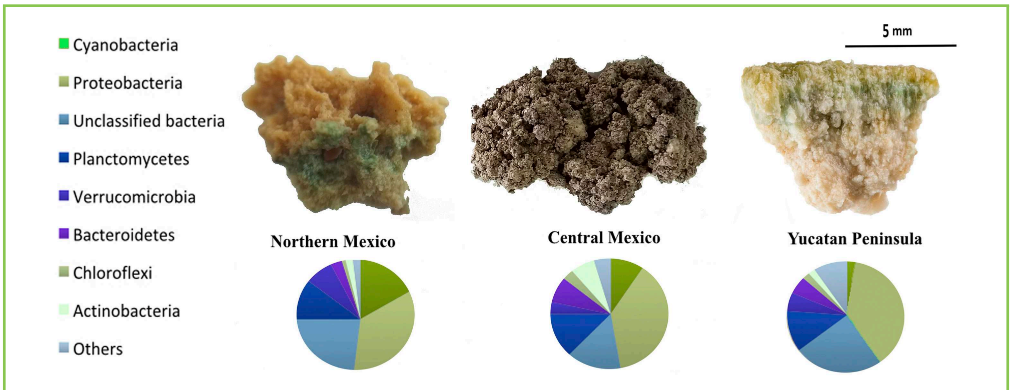

Figure 2

Figure 2

Several different kinds of bacteria make up the microbialites found in various regions of Mexico. The types of bacteria are listed on the left, and the pie charts under the pictures of the microbialites show that microbialites from each location are made up of different proportions of these same types of bacteria.

(which are very diverse and can digest many types of substrates, including carbon, nitrogen, and sulfur), Chloroflexi (which can do a type of photosynthesis that does not produce oxygen, but instead produces a substance called sulfide), and Archaea (which can produce and eat methane) (Figure 3).

Proteobacteria are the most diverse bacteria in microbialites, making up 30–40% of the total diversity in these structures, followed by a group of bacteria that we know very little about and are called unclassified bacteria, which make ∼20% of the bacteria in microbialites. Other bacteria, including Bacteroidetes, Plactomycetes, Verrucomicrobia, Chloroflexi, and Cyanobacteria comprise the remaining diversity (Figure 2). The microbes in microbialites have been interacting amongst themselves and with the environment for many millions of years, and have helped transform the Earth, making our life on this planet possible through the production of oxygen and other important processes. Microbial communities like the ones that form microbialites help the Earth to function as a unit.

ARE MICROBIALITES IN MEXICO DOING WELL?

Since microbialites are self-sufficient, they only need clean water, sunlight, and gases from the atmosphere to grow and develop. All the microbialites we have studied in Mexico live in places with clean and clear water, and we have found massive microbialite formations that have been dated to be ∼10,000 years of age [7]. Some of the beautiful aquatic environments where microbialites have thrived for thousands of years have warm, clear, tropical waters. This is the case for a coastal lagoon called Bacalar in southern Mexico, which has attracted loads of tourists. But a problem arises when there is no infrastructure to treat the sewage from the growing population, leading to pollution of the lagoon. Microbialites in Bacalar lagoon and in other areas of Mexico are being threatened by human activities. In northern Mexico, the microbialites that thrive in the

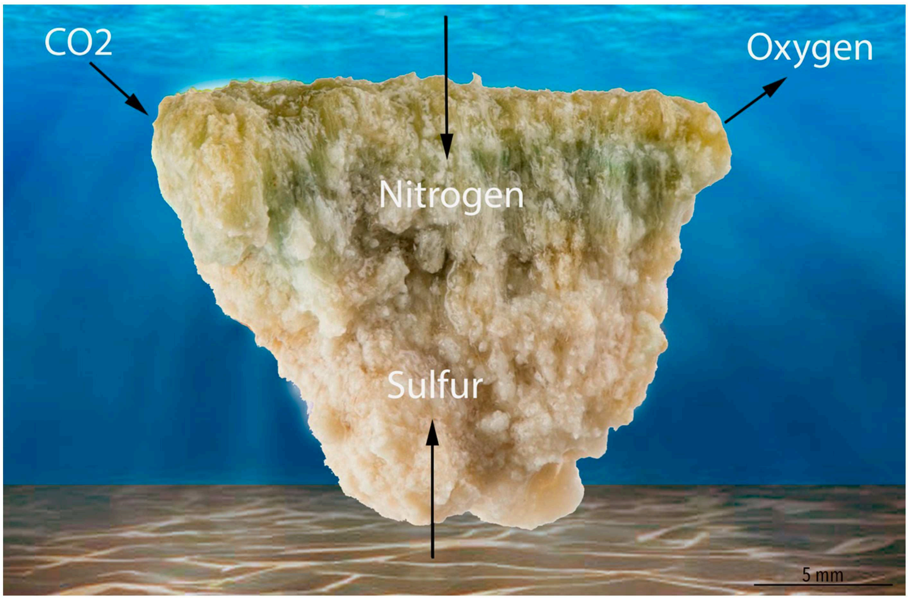

Figure 3

Figure 3

Many microbes build microbialites. They do photosynthesis, and capture CO_2, producing oxygen and are active in the nitrogen, sulfur and methane cycles.

Cuatro Cienegas valley, which has many ponds in the middle of a desert, are at risk because too much water is being removed from the ponds for agriculture. In central Mexico, microbialites that live in crater-lakes are also at risk, due to both pollution and too much water usage.

Humans are the main problem for the world's biodiversity, but we are also the solution. Microbialites are part of Earth's history and it is wonderful that we can observe these ancient communities today! We have the responsibility to be educated about our environment and to make intelligent choices. Let us use efficient water treatment and technology to save microbialites and other aquatic life! Before going on a holiday, read about the places you will visit and learn about the plants, animals, and bacteria that live there, so that you can enjoy their company without harming them or their environment.

AUTHOR CONTRIBUTIONS

The authors contributed equally to writing this paper.

ORIGINAL SOURCE ARTICLE

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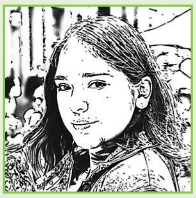
CONFLICT OF INTEREST STATEMENT: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

YOUNG REVIEWERS

DANA, AGE: 14

My name is Dana. I am 14 years old and I live in Guanajuato, Mexico. I have been dancing ballet and jazz since I was 2 years. I love science, specially astronomy. I would like to study it in the future. I also would like to be an engineer or a lawyer.



MARÍA JOSE, AGE: 14

My name is María Jose, my friends call me Majo. I am 14 years old, I live in Guanajuato, Mexico. I like ballet, I have been practice it since I was 3 years old. I like science and I want to be a doctor.

MARIANA, AGE: 15

My Name is Mariana, I am 15 years old and I live in Guanajuato, Mexico. My favorite sport is squash, I have been playing it since I was 6 years old. My favorite subjects are History and Chemistry. In the future I would like to become a Scientist.

AUTHORS

ALFREDO YANEZ-MONTALVO

I am Ph.D. student in Ecology and Sustainable Development. I am passionate about the study of microbial ecology and the understanding of how this microbial diversity is affected by natural and human activities. I love poetry, sports, and Bacalar lagoon. Every day we can learn from people and nature.

BERNARDO ÁGUILA SALGADO

I am Ph.D. student, a curious and geeky person. I am in love with arts, technology and popular science, specially natural science topics, such as biology, geology, astronomy, and environmental sciences. I am very interested in microorganisms that have an important role in the function of ecosystems. I am also aware of the environmental problems we may face in a future and I would like to contribute in some way to the solution.

ELIZABETH S. GÓMEZ-ACATA

I am a microbiologist working as a post-doctoral researcher at Universidad Nacional Autonoma de Mexico. I am fascinated with microbial life. I love studying microorganisms in any environment because they are very important for life on the planet. I also like to investigate biotechnological applications of microorganisms for taking care of the environment. I enjoy looking at them on the microscope and studying them with molecular techniques. In my free time, I enjoy traveling, photography, and cross-stitch embroidery. I love my family and enjoy life.

YISLEM BELTRÁN

I am a post-doctoral fellow at UNAM in Mexico. I am interested in microbial diversity and to understand patterns that characterize the presence of bacterial communities in different environments, as well as their response to environmental changes. What I like about this research is that I have been able to travel and know beautiful places, but also, I can combine field and molecular work and learn about computer sciences.

PATRICIA M. VALDESPINO-CASTILLO

I am a Ph.D. in Marine Science and Limnology. I am fascinated by the microbes of the environment, they are the most ancient and the most powerful mini-machines of life. Science, music and outdoor activities are very important for me; therefore I want to try any possible combination of these. I love being illuminated by curious young minds.

CARLA M. CENTENO

Since I was a little girl I wanted to be a biologist, because I was interested in understanding natural phenomena. When I learned about microorganisms I was fascinated by their shape, their tricky simplicity, the time they spend on our planet (millions of years) and their diversity. I love laboratory work and I really enjoy teaching. I have two small children to whom I teach respect and love toward all life forms.

LUISA I. FALCÓN

I am a microbial ecologist at UNAM in Mexico, fascinated with the diversity of bacteria and archaea in the environment. I love how microbes interact with each other to complement their metabolic capabilities, allowing for communities and ecosystems to exist. *falcon@ecologia.unam.mx

BIODIVERSITY
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WHY WERE THE WATER AND BEACHES IN WEST FLORIDA SO GROSS IN SUMMER 2018? RED TIDES!

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YOUNG REVIEWER:

SEBASTIAN
AGE: 9

The coast of Florida experienced a red tide during summer 2018, an event that turned the sea reddish-brown and caused tons of fish and hundreds of turtles and manatees to die. People also got sick from breathing the sea air. The red tide was caused by the overgrowth of tiny—but toxic—algae, microscopic organisms called *Karenia brevis*. Florida has red tides every year, but this was the largest event in more than 10 years. It is thought that recent hurricanes and wet weather resulted in nutrients, mostly from fertilizer used on land, to be washed into the sea, causing pollution that fed the rapid growth of these unwanted algae. Once the bloom started, there was not much that could be done to stop its growth. We can, however, take actions to reduce nutrient pollution. There is much work to be done to make our waters clean and safe.

WHAT IS A RED TIDE?

Those of you who live in Florida, or whose families planned a trip to western Florida during this past summer (2018), likely saw lots of dead fish on the beach (Figure 1A). The water was gross, a reddish brown color, and maybe you or your family members could not stop coughing if you did walk on the beach. It may have spoiled your vacation. This was the result of **red tide**. What was it and why was it so bad this summer?

RED TIDE

A harmful algal bloom that causes the water to turn a reddish color, due to the accumulation of algae that have a reddish pigment.

Red tide is an event that happens when lots of algae grow in the water. Not just any algae, but a particularly poisonous type of algae. Algae are microscopic plants that live in the water. Every drop of water normally contains hundreds of thousands of these tiny, tiny plants. Algae are natural and important, as they are the vegetables for the food web of the sea. There are many thousands of different types of algae. Algae use sunlight and CO_2 to grow and they, in turn, feed the fish, crabs, oysters, and other organisms. But, some species of algae can be harmful or toxic. Just like some plants on land, such as poison ivy, certain algae can make chemicals that harm both fish and people. When algae increase substantially in number, we say that they bloom (like flowers), and the harmful ones create a **harmful algal bloom** [1]. Some call this

HARMFUL ALGAL BLOOM

Growth of algae that may cause fish kills or seafood contamination through toxins, or that may alter ecosystems in negative ways, or may cause human health problems through toxins that may be carried by the air.

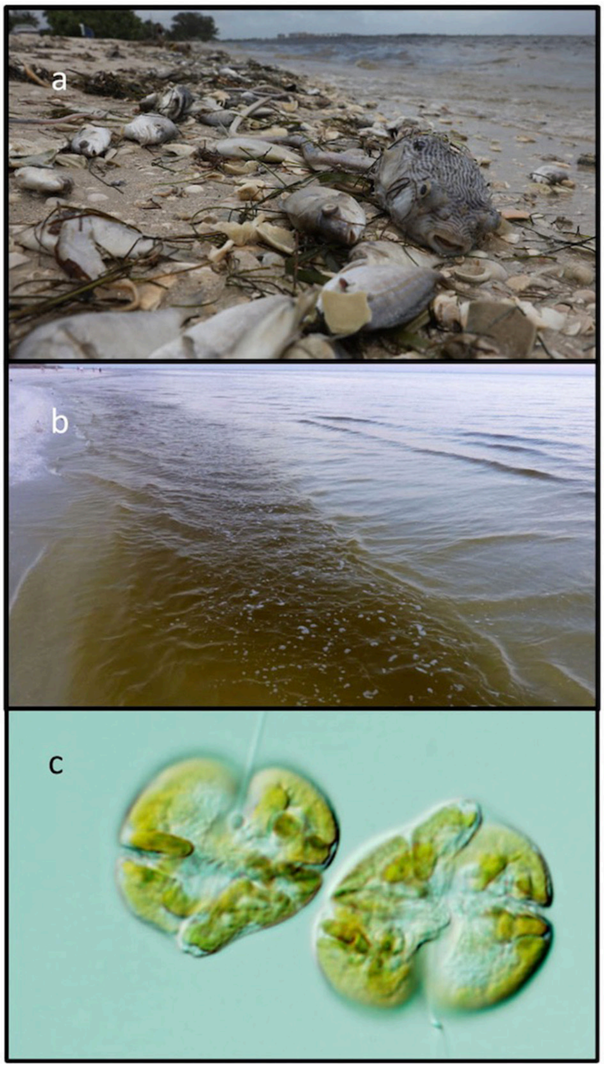

Figure 1

Figure 1

(A) Dead fish that washed ashore in Sanibel, Florida in August 2018. **(B)** This was caused by a red tide that colored the water a reddish brown. **(C)** The red tide is a result of accumulations of the toxic dinoflagellate species *Karenia brevis*, as seen under a microscope. **(A)** is reproduced from Joe Raedle (used with permission of Getty Images), **(B)** is from Rick Bartleson (with permission), and **(C)** from the Florida Fish and Wildlife Research Institute (creative commons license).

type of bloom a red tide, because the algae may be reddish in color, so in large enough numbers they color the sea red (Figure 1B). The most common red tide organism of Florida is named *Karenia brevis* (Figure 1C). It is a type of algae called **dinoflagellates**.

DINOFLAGELLATE

A class of algae, many of which make harmful algal blooms and/or are toxic.

WHY DO RED TIDES OCCUR AND WHY WAS THIS SO BAD DURING THE SUMMER OF 2018?

Karenia brevis are naturally found in the Gulf of Mexico. They have been around since at least the Spanish explorers noticed the red tides, in the sixteenth century [2]. These tiny cells normally grow when waters get warm in spring and early summer, but they may stay offshore in the deeper Gulf of Mexico waters. Just like land plants, algae also need nutrients, nitrogen and phosphorus, to grow and reproduce. Nutrients are the liquid food or natural fertilizers in the water.

There are natural sources of nutrients in the sea, but nutrient pollution can occur when nutrients from the land run off into local waters. Nutrient pollution can be thought of as the rocket fuel that can make algae grow rapidly. It gives them a buffet of nutritious substances to live on [3]. The term for the enrichment of waters with excess nutrients, leading to algal blooms and the problems they cause, is **eutrophication**. There are many sources of nutrients that make their way from the land to the sea. We apply lots of nutrients (fertilizers) on land to make lawns and golf courses look green. The biggest source of nutrients comes from agriculture, and Florida has lots of farmland. Most nutrients used in agriculture stay on land and support the growth of crops. But some can run into rivers and streams when it rains, and can also seep into ground water. Even more nutrients run off into rivers and streams when it rains hard (Figure 2). Nutrients also make their way into waterways when we flush the toilet. Without enough nutrients, red tide blooms do not occur and this is what happens most summers. *Karenia brevis* cells may start to grow, but they die out because there are not a lot of natural nutrients in the Gulf of Mexico.

EUTROPHICATION

Unnatural increase of nutrients in a body of water. The results can be harmful algal blooms, fish kills, or other effects that negatively impact the ecosystem.

During the summer of 2018, we think that there were many more nutrients supporting the growth of *Karenia brevis*. Many scientists think that this bloom actually began in September of 2017, after Hurricane Irma hit Florida. This was a powerful storm, with drenching rains and winds high enough to knock over trees and cause power outages. With all this water washing nutrients from the land, the algae had plenty of nutrients and they started to grow during the fall and winter of 2017. As summer came, the bloom grew even larger. More rains came, as the summer of 2018 was very wet, so the nutrients kept coming too. This was also the case in 2005–2006, when a large red tide followed other hurricanes (Katrina, Rita, Wilma).

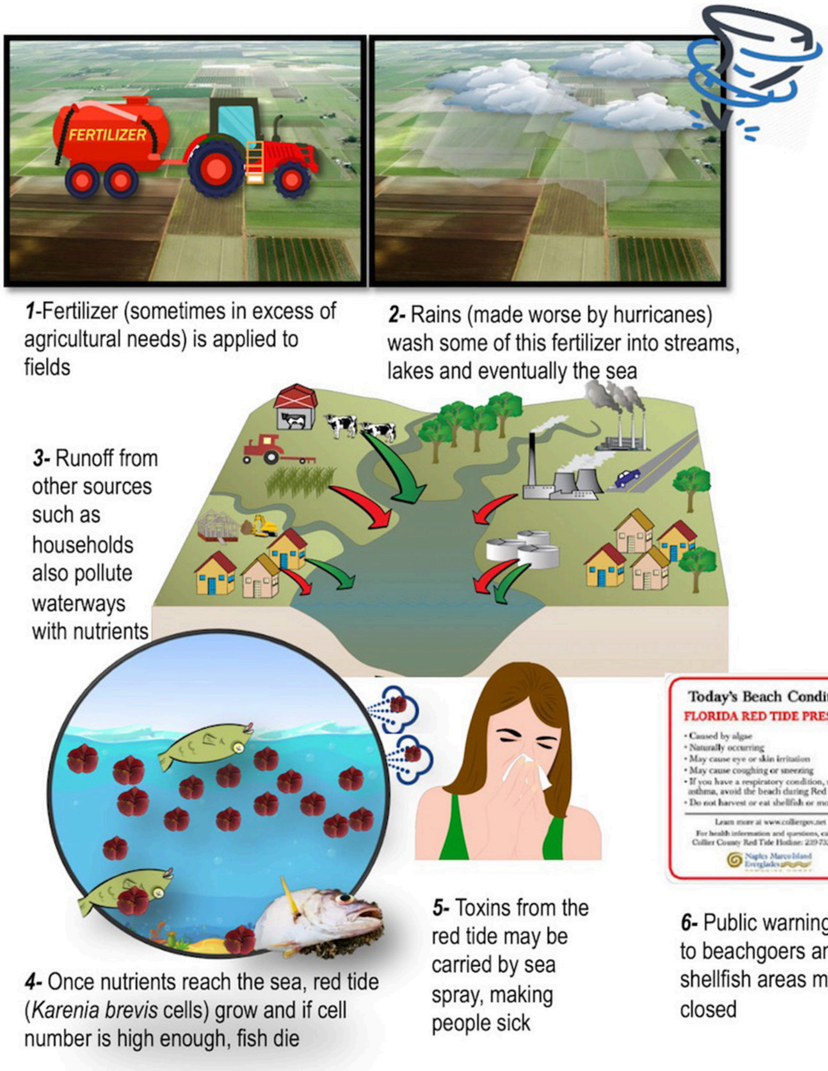

Figure 2

Figure 2

The progression of events that are thought to lead to red tides. Nutrients (fertilizer) from land can get washed into streams, lakes, rivers, and the sea following rain events, and these nutrients provide the fuel to make the red tide algae grow. Once the algae grow in number, its toxins kill fish and can also get carried in sea spray, making people sick. Warnings are issued to beachgoers and shellfishing areas may be closed if the algal cell number is too high. The middle panel (3) is from the University of Maryland Center for Environmental Science Integration and Application Network.

WHY WERE THERE DEAD FISH ON THE BEACH AND WHY WAS EVERYONE COUGHING?

As many as 100 manatees, a 26-foot juvenile shark, 300 turtles (including endangered species) and at least 100 tons of fish died due to this red tide [4]. When these dead animals washed up on beaches, it was smelly and in some places, it was so bad that people said their lungs were burning. Many people started coughing as soon as they came near the beach. Some people had to be taken to the hospital. It was *Karenia brevis* that killed the fish and made people sick.

Karenia brevis makes fish and people sick from its toxin (poison), called brevetoxin. The toxin is tasteless and odorless. When fish come in contact with this toxin, they die. When people come in contact with *Karenia brevis* or its toxins, they cough. People can be exposed to this poison because the algal cells break open in the waves and release the toxin, which is carried in the sea spray that people breathe in when they walk on the beach.

People can also get very sick from these algal blooms when they eat shellfish, like oysters, that have eaten the algae. Oysters are called **filter feeders**, because they get their food by filtering tiny algae from the water. Oysters suck in the water, taking whatever type of algae is present, good or bad. Shellfish do not get sick or die from poisonous algae, but people who eat oysters that have filtered these algae do get sick. The toxin affects the nervous system. Oysters do not have a nervous system, so they do not get sick. People who eat oysters with these toxins may throw up or have diarrhea. Fortunately, they get better in a few days. This disease is known as **neurotoxin shellfish poisoning**, or NSP [5]. People can get sick from oysters when there are about 5,000 *Karenia brevis* cells in each liter of seawater. To protect people from getting sick, the Florida government does not allow oystermen to collect or sell oysters when there are 5,000 or more *Karenia brevis* cells per liter of water. The red tide of summer 2018 was so bad that there were places with 200 times more *Karenia brevis* than this (Figure 3)! The governor had to declare a state of emergency!

FILTER FEEDERS

A group of animals, such as shellfish, that eats by straining food particles from the water without selecting individual food particles.

NEUROTOXIN SHELLFISH POISONING (NSP)

A human health problem resulting from eating shellfish contaminated with the algal toxin called brevetoxin that comes from the harmful algal bloom species *Karenia brevis*. The main symptoms are nausea, diarrhea and headaches.

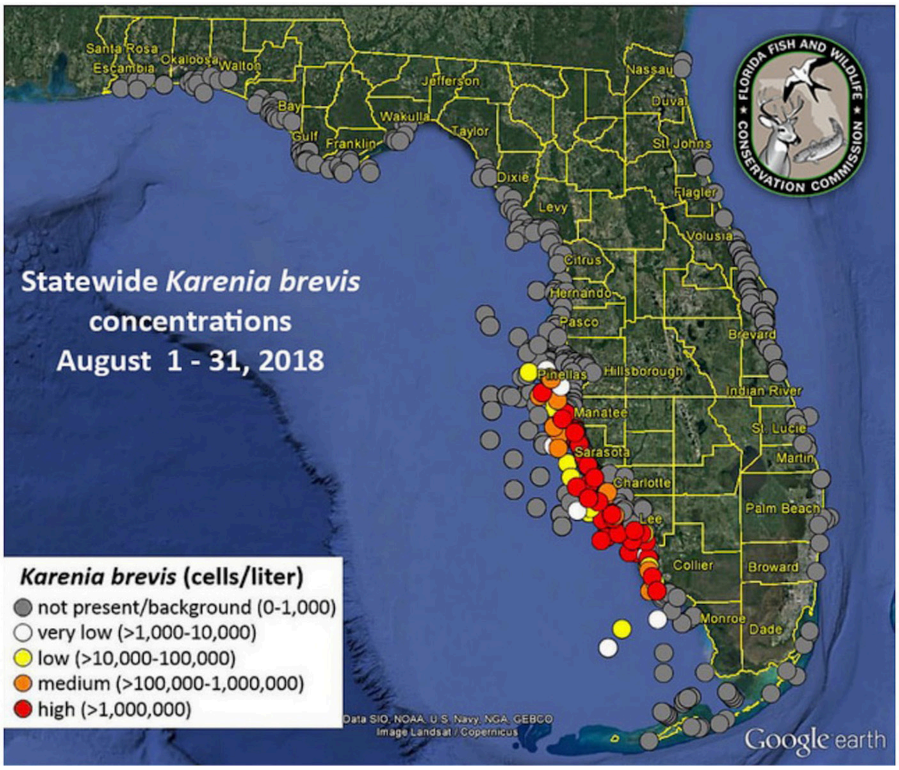

Figure 3

An example of a weekly map of the distribution of the algae that cause red tides, and the number of algal cells found per liter of seawater, in Florida. In this map, the red and orange dots show where the algal cells created the largest problems for beachgoers and for fish.

WHERE ELSE IN THE WORLD DO HARMFUL ALGAL BLOOMS HAPPEN?

There are many harmful algae bloom species found throughout the world, each with a different type of toxin. Due to eutrophication, harmful algal blooms are increasing in frequency, size, duration, and in the human health and ecological problems that they cause. Some harmful algae live only in the water, while others live in the sediment or attached to other surfaces in the water, for some or all of their life cycle. There are also harmful algae that live in freshwater, and many of these are very toxic too. In early summer, Florida experienced a freshwater harmful bloom in the eastern part of the state, turning parts of Lake Okeechobee and the coastal lagoons into a gooey, slimy green mess. Both the eastern Florida bloom and the western red tide are likely related to nutrient pollution and the wet weather that caused nutrients from land to escape into the lake and the sea.

WHAT IS BEING DONE TO STOP HARMFUL ALGAL BLOOMS?

Scientists and politicians are debating what can be done to stop harmful algal blooms. There is no easy way to stop a bloom once it has started. Clearly, we cannot stop hurricanes or rainy weather. What we can do is reduce nutrient pollution. This will take political action to enforce laws, but there is also much we can do ourselves. We should not over-fertilize our lawns. We can talk to our local officials to make sure they take all the actions they can to reduce nutrient pollution. Also, if you are at the beach and you see stranded animals, such as manatees, dolphin or turtles, call the authorities. Pay attention to local warnings about beach closures and seafood closures! Although the science of understanding how and why harmful algal blooms occur has been rapidly advancing, the chances of reducing these events or their impacts are, unfortunately, not very good. When climate change and rising temperatures are also considered, the outlook is even worse. The growth of many of the algal species that form these blooms increases in warmer conditions. There is much work to be done to make our waters clean and safe. *Our waters need your help!*

For More Information on Red Tides and Harmful Algae

https://oceanservice.noaa.gov/facts/redtide.html
https://myfwc.com/redtidestatus
https://www.whoi.edu/redtide/

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YOUNG REVIEWER

SEBASTIAN, AGE: 9

I like playing ice hockey, soccer, tennis, baseball, and reading. I really like animals. I like to camp, ski, play in the snow, swim, climb trees, playing and riding my bike.

AUTHOR

PATRICIA M. GLIBERT

I study algae because I hope what we are doing makes a difference. These are certainly interesting times to be an ecologist studying water quality. Harmful algal blooms are increasingly everywhere, and nutrient pollution and eutrophication are increasing. It used to be difficult to explain what I studied to my non-scientists friends and relatives; now, they read the headlines of water quality issues and algal blooms frequently. I study algae all around the world, from Chesapeake Bay to Florida, from Europe to China! *glibert@umces.edu

BIODIVERSITY
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PLANTS ARE NOT ANIMALS AND ANIMALS ARE NOT PLANTS, RIGHT? WRONG! TINY CREATURES IN THE OCEAN CAN BE BOTH AT ONCE!

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YOUNG REVIEWERS:


FROYLAN
AGE: 11


NICOLAS
AGE: 8

On land, plants make their own food by photosynthesis and animals live by eating. However, in the microscopic world in the oceans, it is not that simple. Many microscopic so-called plants (phytoplankton) can also eat like animals and many microscopic so-called animals (microzooplankton) can also photosynthesize like plants! More amazingly, some of these microzooplankton eat tiny phytoplankton and continue to live off photosynthesis from those ingested phytoplankton. These organisms acting like both plants and animals are called mixotrophs because they mix (combine) different ways of getting nutrition. These fascinating creatures are not rare freaks of nature, but are very common. Some mixotrophs are good food for fish, while others make poisons that can get into our seafood and even

kill fish. Some are increasing in coastal waters due to pollution. We are learning just how important mixotrophs are to ocean ecosystems.

One of the most basic "laws" of science is that plants are plants and animals are animals. Right? Of course! Plants are green. They live using sunlight, carbon dioxide, and nutrients, making their own food through the process of **photosynthesis**. In contrast, animals live by eating other organisms (plants, animals, bacteria, or even bits and pieces of dead organisms). Is this "law" of science correct? Not always! Going against this "law" are oceans full of microscopic organisms that can be both plant-like and animal-like at the same time! They photosynthesize and eat.

PHOTOSYNTHESIS

The process by which green plants and plant-like algae use sunlight, together with carbon dioxide and water, to make their own food.

Have you ever heard of a plant that can eat an animal? There are a few land plants that eat insects. The most commonly known example is the Venus flytrap, which captures insects on its special leaves and then digests them (Figure 1A). Such land plants are considered a bit of a freak of nature. In the ocean, however, these freaks are not freaks at all; they are actually very common. You can find many of these kinds of organisms if you look under the microscope and explore the microbial **plankton**, the tiny organisms that live in the water world. Not only are there plants that eat, there are animals that photosynthesize! These fascinating, mixed ways of getting and making food are called **mixotrophy** and the organisms that perform mixotrophy are called **mixotrophs** (meaning mixed nutrition). A non-science term for these organisms could be "plantimals," since they can be part-plant, part-animal (Figure 1).

PLANKTON/ PHYTOPLANKTON/ MICROZOO-PLANKTON

Plankton are drifting or floating organisms in the sea or in freshwater. Most are microscopic. When plant-like, they are called phytoplankton, and when animal-like, they are called zooplankton. Small-sized zooplankton are termed microzooplankton.

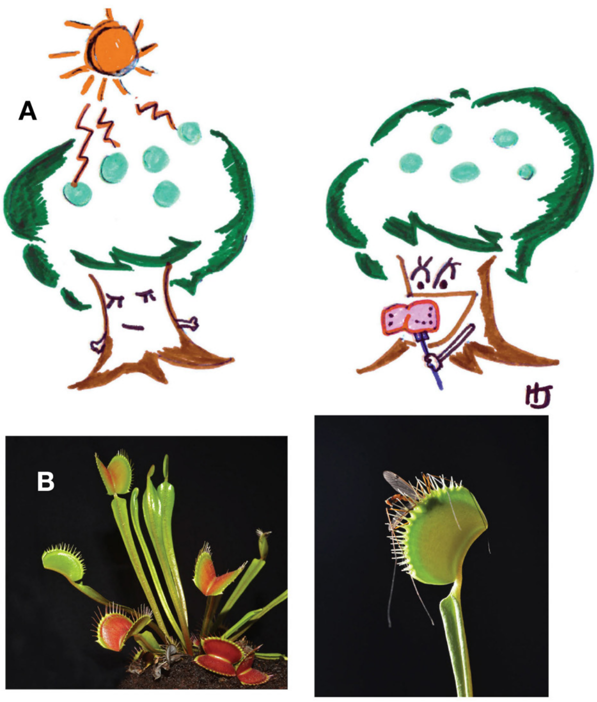

Figure 1

Figure 1

(A) Cartoons of a hungry plant photosynthesizing (left) and eating (right). **(B)** The Venus flytrap plant both photosynthesizes and eats insects. Cartoon by H. J. Jeong, photos from Shutterstock.

PLANKTONIC PLANTS THAT ARE ALSO ANIMALS

Phytoplankton are microscopic plant-like organisms that live in the water. Their name tells us that they live on light (phyto) and drift with the water (plankton). Every drop of water normally contains hundreds of thousands of these tiny, single-celled organisms. Phytoplankton are natural and important; they produce 50% of the oxygen in the air we breathe, and they are also food for fish and other animals in the ocean. There are many hundreds of different types of phytoplankton. For decades, most scientists have thought that phytoplankton lived only by photosynthesis. It turns out that many of these phytoplankton also eat the way animals do [1]. Some eat other phytoplankton, some eat bacteria, and some eat tiny animals (Figure 2). Some of these mixotroph phytoplankton eat only reluctantly or rarely. Some are aggressive and can stuff themselves full of food! These mixotrophs grow much faster when they can eat and photosynthesize at the same time, compared with when they grow by photosynthesis alone.

The ways the mixotrophic phytoplankton eat can be pretty gruesome. Some gobble up entire organisms, while some harpoon their food and suck out the innards using a self-made straw. Some can make their meal explode, leaving a nutritious soup that they can soak up. Some can even eat other organisms that are much bigger than themselves.

MIXOTROPHY/ MIXOTROPH

Mixotrophy is the process of combining photosynthesis (like a plant) and feeding (like an animal) in one organism. A mixotroph is an organism that combines its nutrition in this way.

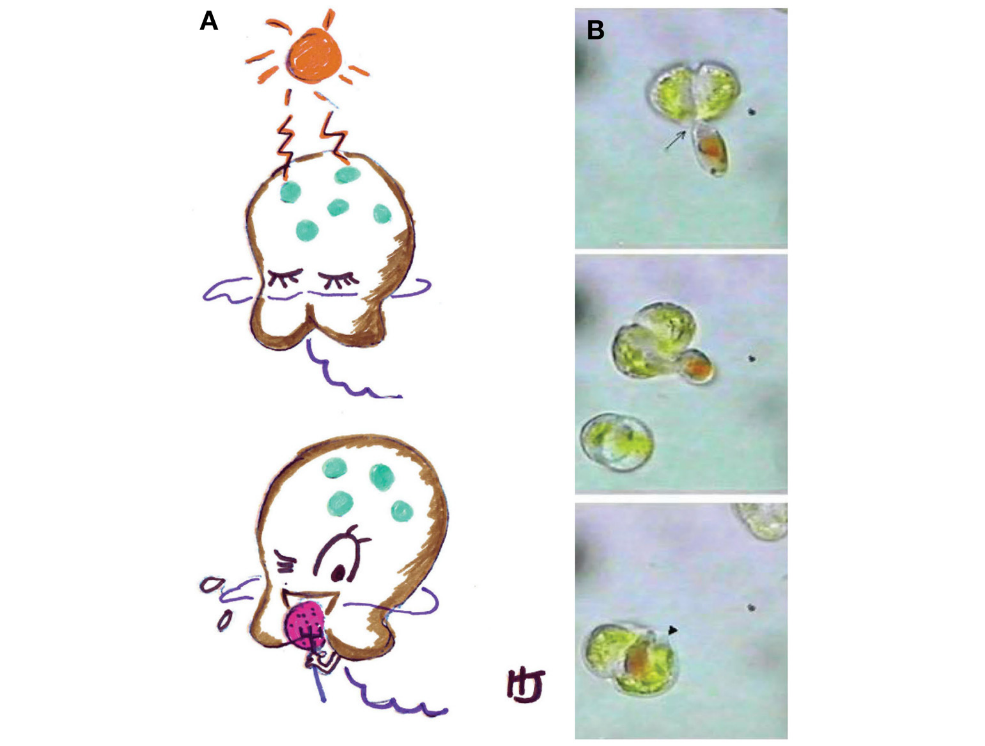

Figure 2

Figure 2

(A) Cartoons of microscopic phytoplankton called mixotrophs. They live off of sunlight and photosynthesis (upper panel) but can also have a meal of another small cell (lower panel). **(B)** The plant-like (phytoplankton-like) mixotroph *Karlodinium* captures (top two panels), then ingests (bottom panel) a small cell. Cartoon by H. J. Jeong and image from Stoecker et al. [2] (reproduced with permission of Springer-Verlag).

Some mixotrophic phytoplankton use poisons to kill what they want to eat. Interestingly, some can make these poisons only when they photosynthesize AND eat at the same time. An example is an organism called *Karlodinium*. *Karlodinium* eats other small algae aggressively, but it seems to only eat during daylight. Why does it not also eat at night? It turns out that *Karlodinium* makes the poisonous compound that it releases to kill its food during daytime, when it is also photosynthesizing.

PLANKTONIC ANIMALS THAT ARE ALSO PLANTS

Along with phytoplankton, there are other, tiny animal-like organisms in the ocean that are called **microzooplankton**, because they are small (micro-), animal (zoo-)-like plankton. Microzooplankton eat lots of different things, but when they eat tiny phytoplankton, they can become part-time plants. How can they do this? One type of microzooplankton eats phytoplankton, but they do not digest the photosynthesizing machinery (the **chloroplasts**; Figure 3). They keep the stolen chloroplasts and use these to photosynthesize! Can you imagine the broccoli

CHLOROPLAST

Photosynthesizing apparatus in plants and marine phytoplankton.

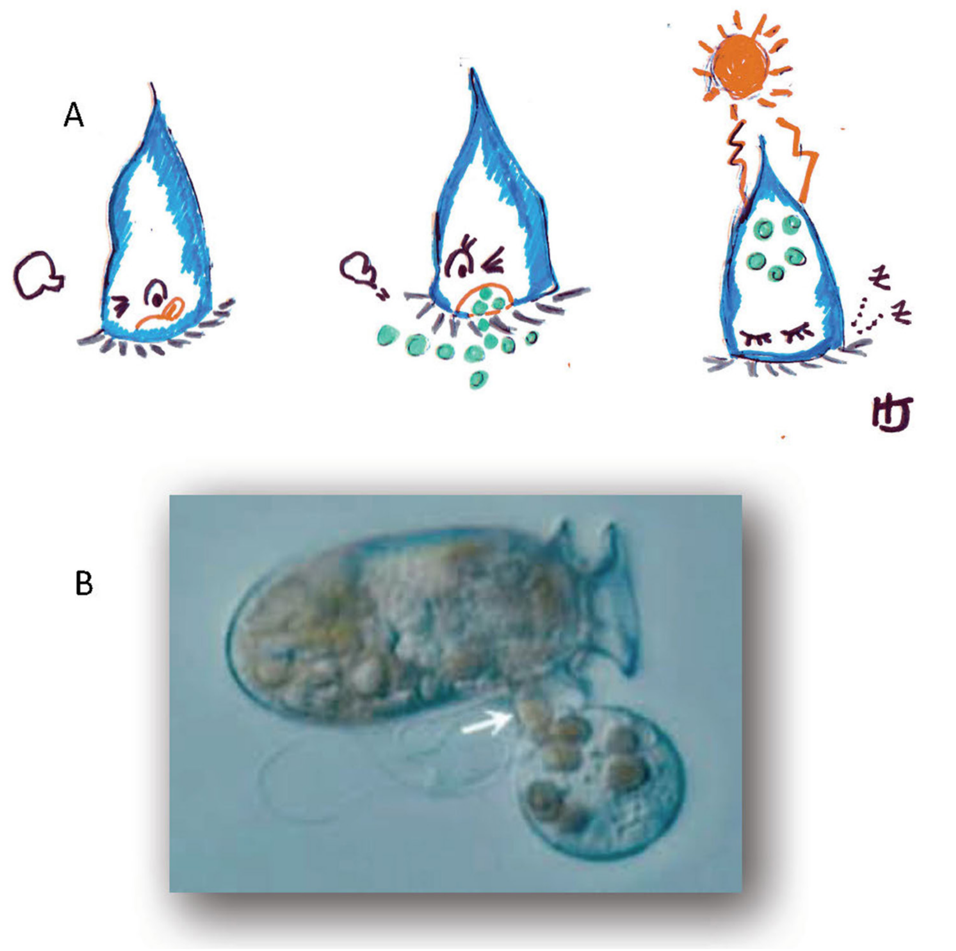

Figure 3

Figure 3

(A) Cartoons of a hungry microzooplankton (left panel), eating small phytoplankton (middle panel), then capturing sunlight for photosynthesis, using the phytoplankton chloroplasts now inside its body (right panel). **(B)** The mixotroph *Dinophysis* beginning to get its meal from *Mesodinium*. Note the small red circles inside the *Mesodinium*-these are the algae the *Mesodinium* ate! The arrow points to the small straw or feeding tube it uses to feed on *Mesodinium*. Cartoon by H. J. Jeong and image from Park et al. [3] (reproduced under Creative commons license).

you eat continuing to photosynthesize in your stomach after you ate it? Other "animal" mixotrophs eat lots of phytoplankton but do not digest them at all—they keep the intact phytoplankton within their bodies and drift in the oceans like microscopic greenhouses; they live off the photosynthesis from the still-growing phytoplankton they ate.

Some mixotrophic microzooplankton are picky eaters, and become plant-like only by eating their favorite foods. One type of these picky mixotrophs is a species called *Dinophysis*, which is found in oceans all over the world. *Dinophysis* wants chloroplasts from one specific type of microscopic phytoplankton but cannot eat those phytoplankton directly. So *Dinophysis* eats another mixotroph named *Mesodinium* that eats the specific phytoplankton with those chloroplasts. The *Dinophysis* then pokes a hole into the *Mesodinium* and sucks all their guts out to finally get the chloroplasts it wants.

Talk about gruesome, picky eaters! It is real microbial warfare in the oceans!

WHERE ARE MIXOTROPHIC PLANKTON FOUND IN THE OCEANS?

All our oceans are home to mixotrophic plankton, but different types live in different parts of the ocean or at different times of year. Some types, such as the *Karlodinium*, are mainly found along coastal areas, while other types are more common in the open waters of the oceans. Other types of mixotrophic plankton are associated with polar waters or tropical waters. Some are more common during certain seasons—especially summer.

EUTROPHICATION

The process of enriching a body of water with nutrients. Eutrophication can result in harmful algal blooms or other negative effects on the ecosystem.

Many mixotrophs grow very well in waters that have become **eutrophic** (enriched with too many nutrients or fertilizers) from all of our human wastes [4]. When we apply fertilizers to lawns or farm land, not all of that fertilizer is used by grass or by crops. Some of the fertilizers are washed out to sea after it rains. These fertilizers then feed the phytoplankton in the ocean water, which then grow, becoming food for other plankton, including the mixotrophs. With more food, mixotrophs can grow more and more. When phytoplankton, including those that are mixotrophs, grow in large numbers it is called a bloom.

WHY SHOULD WE BE INTERESTED IN MIXOTROPHS?

Mixotrophy is now considered so important in the plankton communities that it has been proclaimed as one of the recent revolutions/discoveries in science that could change everything (Scientific American Vol. 27, No. 3, July 2018)! Mixotrophy changes the way we think about all aspects of life under the water [1]. Plankton life does not fall neatly into plant and animal categories, as does life on land. In the world of plankton, there is still much that we do not know or understand. As scientists, it is really cool to try to figure out how mixotrophs work! There are endless numbers of questions that we have and important topics that can be explored with these amazing little creatures [5].

Scientists are also very interested in mixotrophic plankton because they ultimately sustain all the other organisms in the ocean, from oysters and crabs to fish. With climate change, we also want to know how organisms in the oceans, including mixotrophs, are changing and how that may change the populations of fish that humans use for food [1].

Many of the plant-like mixotrophs can harm other types of organisms, including whales, dolphins, or turtles. Figuring out how mixotrophs affect these larger organisms is important if we want to protect those important creatures. The day-time eater *Karlodinium* can release some of its poisons into the water, destroying the gills of fish, which kills the fish almost immediately. *Karlodinium* then eat bits of fish for their dinner. Others, such as *Karenia brevis* off the coast of Florida, produce a poisonous compound that may not only kill fish, but is strong enough to kill even huge manatees! In the summer of 2018, *Karenia brevis* blooms resulted in large fish kills off the Florida coast; many sick and dead animals washed ashore, including over 100 manatees and 300 turtles. This was a terrible loss of marine life and also made the beaches slimy and smelly.

Scientists are especially interested in mixotrophs that make poisonous compounds that can make people sick. If we eat mussels that fed on *Dinophysis*, the picky-eater-mixotroph mentioned above, we can get diarrhetic shellfish poisoning; this means that people get upset stomachs and have diarrhea. The toxic compound made by *Karenia brevis* can get carried in sea spray and makes us cough if we breathe that air at the beach. The types of toxic compounds made by different mixotrophs are very diverse and there is much we still do not know about the chemistry of these compounds. We are very interested in understanding what we can do to stop these tiny, toxic organisms from growing out of control and how we can keep people from getting sick.

These amazing mixotrophs, with their fascinating diversity, are certainly shaping our oceans and the food we get from it. It may seem to be a mixed-up world of microbes in our oceans, but they are major players on our planet. Therefore, they are worthy of our attention.

Scientists, fishermen, seafood lovers, beach goers, environmentalists, and all citizens of the planet should care about what lives and grows in our oceans!

For more information on mixotrophs

www.mixotroph.org

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YOUNG REVIEWERS

FROYLAN, AGE: 11

My favorite hobby is drawing, I can draw almost any character from movies or video games, I also like to create new characters and write new stories. I practice American Football since I was 7 years old, this year I will play my fifth season, and my position is Center.

NICOLAS, AGE: 8

My favorite hobby is the computer I like to research everything; I also like to play Minecraft. I love creating new worlds, I have dozens of them. I would love to travel around the world and meet amazing people and places. I practice American Football since I was 5 years old, this year I will play my fifth season, and my position in the offense is Center and in defense is Nose Tackle.

AUTHORS

PATRICIA M. GLIBERT

I study algae because I hope what we are doing makes a difference in the world. These are certainly interesting times to be an ecologist studying water quality. Phytoplankton blooms are increasingly everywhere, and nutrient pollution and eutrophication are increasing. It used to be difficult to explain what I studied to my non-scientists friends and relatives; now, they read the headlines of water quality issues and algal blooms frequently. I study algae all around the world, from Chesapeake Bay to Florida, and from Europe to China! *glibert@umces.edu

ADITEE MITRA

I have always thrived on challenges—doing something new, finding something different—that is why I love working on mixotrophs! Our research findings revealed that the mixoplankton actually rule our oceans. Mixotrophs are not only cool, but fun to study!

KEVIN J. FLYNN

When I was studying for my Ph.D., I wrote computer games for fun. Now, instead of aliens battling it out against each other, I use that skill to write computer simulations of different plankton "fighting" for life! I found out that you learn quickly when having fun and building and playing with simulations of plankton is a great way to find out how nature works!

PER JUEL HANSEN

I am an experimental biologist that study how plankton organisms interact with each other and how they interact with the environment. I am particularly interested in how harmful algal blooms develop and how bloom-forming toxic algae use their toxins to kill and eat other organisms to get rid of competitors and enemies. I work in the Arctic waters of Greenland, temperate waters in Northern Europe and in tropical waters in Asia.

HAE JIN JEONG

I am very interested in solving harmful algal blooms (HABs), one of the biggest problems at sea. I have developed several methods of eliminating HAB species using mass-cultured predators and some effective chemicals. However, I realized that some HAB species have genomes up to 90 times larger than mine, and I determined that I must become their friend and understand their minds. Now I try to focus on converting "harmful" algae to "useful" algae for humans. For fun I like to draw cartoons and make jokes!

DIANE STOECKER

I study plankton because they are beautiful, live in the sea, and because there are many species that we know little about. I like to go to sea to do research and to work with cultures in the laboratory. Making discoveries about tiny plankton has been fun and has allowed me to work with scientists in many countries.

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