



DMSP Production by Coral-Associated Bacteria

Felicity W.I. Kuek^{1,2,3,4,5}, Cherie A. Motti^{1,5†}, Jia Zhang^{3,4}, Ira R. Cooke^{3,4}, Jonathan D. Todd⁶, David J. Miller^{2,3,4†}, David G. Bourne^{1,5,7†} and Jean-Baptiste Raina^{8*†}

¹ AIMS@JCU, Division of Research and Innovation, James Cook University, Townsville, QLD, Australia, ² Australian Research Council (ARC) Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD, Australia, ³ Centre for Tropical Bioinformatics and Molecular Biology, James Cook University, Townsville, QLD, Australia, ⁴ College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, QLD, Australia, ⁵ Tropical Marine Water Quality and Impacts, Australian Institute of Marine Science, Townsville, QLD, Australia, ⁶ School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, United Kingdom, ⁷ College of Science and Engineering, James Cook University, Townsville, QLD, Australia, ⁸ Climate Change Cluster (C3), University of Technology Sydney, Ultimo, NSW, Australia

OPEN ACCESS

Edited by:

Gabrielle Nevitt,
University of California, Davis, CA,
United States

Reviewed by:

Chuya Shinzato,
The University of Tokyo, Japan
Kshitij Tandon,
The University of Melbourne, Australia

*Correspondence:

Jean-Baptiste Raina
Jean-Baptiste.Raina@uts.edu.au

†These authors have contributed
equally to this work

Specialty section:

This article was submitted to
Coral Reef Research,
a section of the journal
Frontiers in Marine Science

Received: 04 February 2022

Accepted: 06 April 2022

Published: 04 May 2022

Citation:

Kuek FWI, Motti CA, Zhang J,
Cooke IR, Todd JD, Miller DJ,
Bourne DG and Raina J-B (2022)
DMSP Production by Coral-
Associated Bacteria.
Front. Mar. Sci. 9:869574.
doi: 10.3389/fmars.2022.869574

Dimethylsulfoniopropionate (DMSP) is an important molecule in the marine sulfur cycle, produced in large amounts by corals and their dinoflagellate endosymbionts, Symbiodiniaceae. Although corals are known to harbour bacteria that can catabolise DMSP, the recent discovery of bacteria capable of producing DMSP in coastal and deep-sea environments raises the possibility of a bacterial contribution to the DMSP output of corals. Here, 157 bacteria associated with four common coral species were isolated and screened for their ability to produce DMSP by targeting *dsyB*, a key gene involved in DMSP biosynthesis. Approximately 9% (14 out of 157) of the bacterial isolates harboured *dsyB*, all being members of the Alphaproteobacteria. The ability of these isolates to produce DMSP was confirmed by liquid chromatography-mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) measurements. A *dsyB*-harbouring strain, *Shimia aestuarii* AMM-P-2, was selected for genome sequencing. This strain harbours the complete genetic machinery to (i) assimilate sulfate and synthesise the DMSP precursors, cysteine and methionine; (ii) demethylate DMSP and generate methanethiol; (iii) cleave DMSP, generating dimethyl sulfide (DMS) and acrylate; and (iv) utilise or detoxify acrylate. The impacts of varied environmental factors (temperature, salinity, light and UV radiation) on *S. aestuarii* AMM-P-2 DMSP biosynthesis were characterised. DMSP levels in *S. aestuarii* AMM-P-2 increased almost two-fold under both hypersaline conditions (40 PSU) and high UV exposure. DMSP catabolism through the cleavage pathway also increased under these conditions, producing the antioxidants DMS and acrylate, a potential response to the oxidative stress generated. Overall, our results reveal that coral-associated bacteria can synthesise DMSP and may therefore contribute to DMSP production by the coral holobiont.

Keywords: DMSP, sulfur cycle, coral-associated bacteria, holobiont, acrylate

INTRODUCTION

Dimethylsulfoniopropionate (DMSP) is one of the most abundant organic sulfur compounds in the ocean (Sievert et al., 2007; Johnston et al., 2012), with global production estimates ranging between 12 and 103 Tmol of sulfur per year (Howard et al., 2006). Functionally speaking, this molecule is the chemical equivalent of a Swiss Army knife, being a key source of carbon and reduced-sulfur for marine microbes (Kiene et al., 2000), a potent chemoattractant affecting the behaviour of organisms ranging from bacteria to fish (DeBose et al., 2008; Seymour et al., 2010), an antioxidant (Sunda et al., 2002), a cryoprotectant (Karsten et al., 1996), a protectant against hydrostatic pressure (Zheng et al., 2020), and an osmolyte (Kirst, 1996; Stefels, 2000). In addition, its role as the main precursor of dimethyl sulfide (DMS) has received considerable attention because this highly abundant gaseous compound is released into the atmosphere where it ultimately plays a role in cloud formation, bridging marine and atmospheric sulfur cycles (Andreae et al., 1983; Bates et al., 1987; Ayers and Gras, 1991).

DMSP biosynthesis was long thought to be restricted to marine photosynthetic eukaryotes (Kiene et al., 1996). However, recent studies have demonstrated that photosynthesis is not a prerequisite for DMSP production with marine heterotrophic bacteria found in saltmarshes, the photic zone and even deep seafloor sediments producing this compound (Curson et al., 2017; Williams et al., 2019). Many marine Alphaproteobacteria belonging to the orders Rhodobacterales, Rhizobiales, and Rhodospirillales produce DMSP *via* the methionine transamination pathway (Curson et al., 2017). The *dysB* gene in these bacteria encodes the key S-adenosyl methionine-dependent methylthiohydroxybutyrate (MTHB) methyltransferase enzyme in this pathway. Furthermore, some Alphaproteobacteria and Actinobacteria have been shown to produce DMSP *via* a different methionine methylation pathway in which a methionine S-methyltransferase, termed MmtN, is an important enzyme (Williams et al., 2019). Approximately 0.3–0.6% of marine bacteria are predicted to produce DMSP, with the majority containing *dysB* (Curson et al., 2017; Curson et al., 2018; Williams et al., 2019). Importantly, Alphaproteobacteria orders that are known to harbour *dysB* can represent up to 50% of the bacterial communities associated with some reef-building coral species (Luo et al., 2021).

Coral reefs are recognised as DMSP hotspots (Jones and Trevena, 2005), thought to largely result from the photosynthetic endosymbionts of the family Symbiodiniaceae within the coral tissue that produce large amounts of the compound (Hill et al., 1995). However, the coral host itself can also produce DMSP and contain a DsyB-like MTHB S-methyltransferase, termed DSYB (Raina et al., 2013; Aguilar et al., 2017; Curson et al., 2018). Bacteria are also abundant in and around corals (Bourne et al., 2016; Pogoreutz et al., 2020) and, together with protists, fungi, archaea, and viruses, they form a metaorganism referred to as the coral holobiont (Rohwer et al., 2002). These microorganisms likely support central metabolic processes of the coral host and their microalgal partners through

the fixation of carbon, cycling of nitrogen, synthesis of essential B-vitamins, and antimicrobial production (Rädecker et al., 2015; Robbins et al., 2019; Matthews et al., 2020; Ngugi et al., 2020). A large proportion of coral-associated bacteria can also catabolise DMSP, and this compound constitutes an important source of carbon and reduced sulfur for microorganisms in the holobiont (Raina et al., 2009; Raina et al., 2010; Frade et al., 2016; Tandon et al., 2020). Yet, the potential contribution of coral-associated bacteria to the production of the high DMSP concentrations measured in some reef-building corals has never been considered.

Here, bacteria associated with four common species of reef-building corals from the Great Barrier Reef (GBR) were investigated. We hypothesized that these corals harbour DMSP-producing bacteria that may contribute to DMSP concentrations measured in the holobiont. Bacterial isolates were screened for the presence of the *dysB* gene, and their capacity to produce DMSP was investigated through chemical analyses. A representative DMSP-producing coral-associated bacterial isolate was exposed to abiotic stressors relevant for coral reefs and the role environmental factors may play in driving DMSP production characterised.

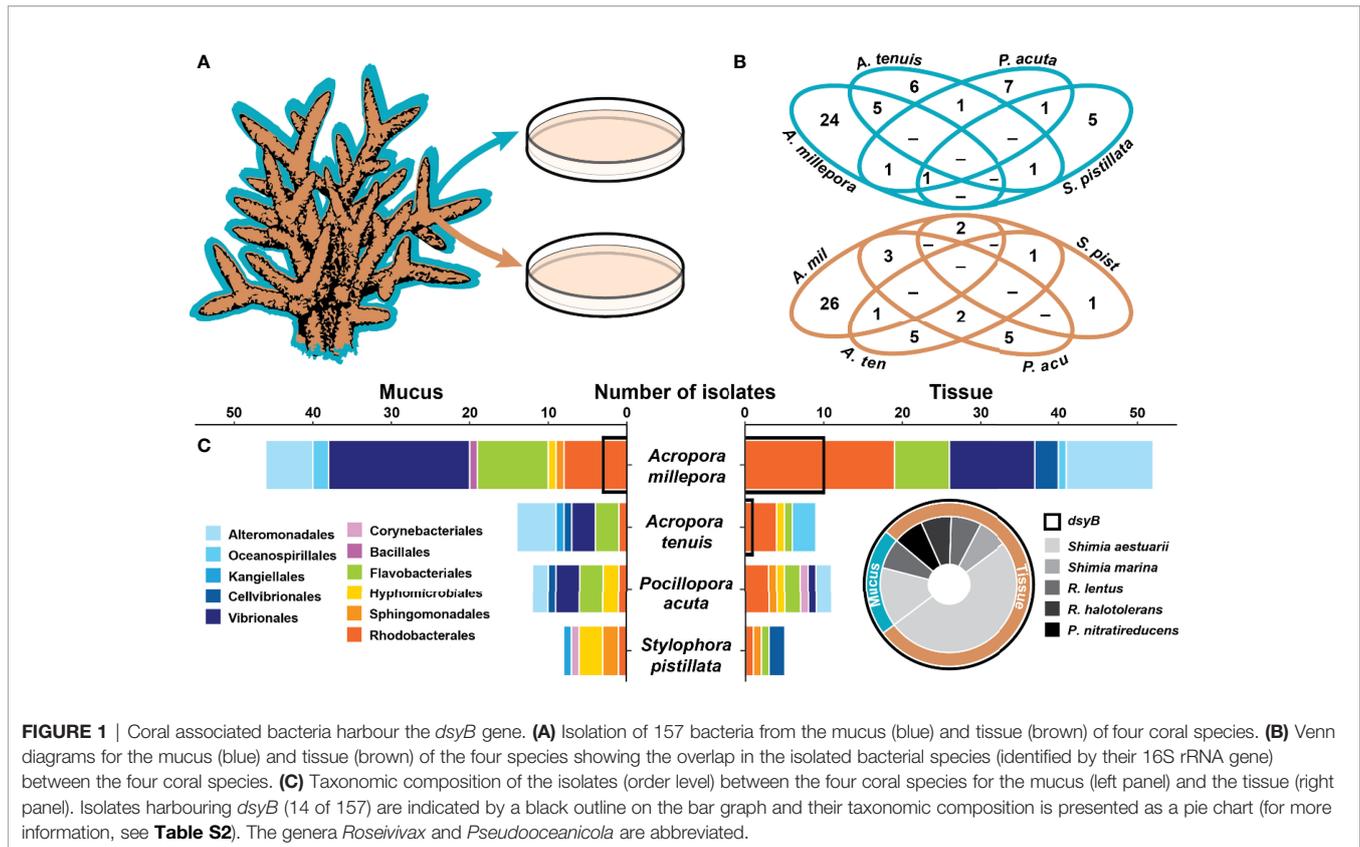
MATERIALS AND METHODS

Sample Collection

Coral-associated bacteria were isolated from four scleractinian coral species, *Acropora millepora*, *Acropora tenuis*, *Pocillopora acuta*, and *Stylophora pistillata*, all collected from Davies Reef (18°49'03.7"S, 147°38'39.6"E). Corals were maintained at the Australian Institute of Marine Science (AIMS) National Sea Simulator (SeaSim) and were healthy when the samples were collected, with no visual signs of bleaching or disease. Five coral fragments per colony were rinsed in sterile artificial sea water (ASW) prior to mucus collection using sterile 50 mL syringes fitted with 20-gauge hypodermic needles. Mucus samples were kept on ice and processed within an hour. In addition, two coral fragments per colony were placed in separate Whirl-Pak sterile sample bags (Nasco, United States) and immediately air-brushed with 5 mL of sterile ASW to remove coral tissue and their associated microorganisms from the coral skeleton. The tissue slurry was homogenised and transferred into sterile 50 mL centrifuge tubes, placed on ice, and processed within an hour.

Bacterial Isolation

To isolate coral-associated bacteria, each sample type (mucus and tissue slurry) was serially diluted in ASW (2-, 10-, 100-, and 1,000-fold; **Figure 1A**). Aliquots (50 μ L) of each dilution were then spread onto Difco Marine Agar 2216 (MA; Becton Dickinson, United States) or modified minimal basal medium (MBM) agar enriched with mixed carbon sources [300 mM; details in **Table S1**; (Curson et al., 2017)], methionine (C₅H₁₁NO₂S; 0.5 mM), and ammonium chloride, (NH₄Cl; 20 mM) as nitrogen source. All agar plates were incubated at 28°C in the dark for one week and inspected daily for growth and the



formation of morphologically distinct individual colonies. Colonies were picked using sterile 20 μL pipette tips and resuspended in 5 mL of Difco Marine Broth 2216 (MB; Becton Dickinson, United States). The isolates were incubated at 28°C and 180 RPM until growth was visible. These liquid cultures were replated on MA and this procedure repeated until pure isolates were obtained. Isolates were then cultured in MB and aliquots of each isolate stored in 20% v/v glycerol at -80°C.

Isolate Identification

A 5 mL liquid culture of each isolate was grown overnight in MB at 28°C with agitation (orbital shaker at 180 RPM) before being centrifuged for 5 min at 10,000 g and the supernatant decanted. DNA extraction was performed using the DNeasy UltraClean Microbial Kit (Qiagen, Germany) according to the manufacturer's instructions. Extracted DNA was resuspended in 20 μL of UltraPure DNase/RNase-Free Distilled Water (Invitrogen, United States) and quantified by spectrophotometry (NanoDrop ND-1000, ThermoFisher, United States). Aliquots of extracted DNA were diluted with sterile Milli-Q water to 10 ng μL^{-1} and stored at -20°C until required.

PCR Amplification of Bacterial 16S rRNA and *dsyB* Genes

Extracted DNA was used as template in PCR with the universal 16S rRNA genes primers 27F and 1492R (Lane, 1991), as well as the *dsyB* specific primers *dsyB_deg1F* and *dsyB_deg2R* that amplify a 246 bp region of the gene (Williams et al., 2019).

Each PCR reaction mixture contained 1 \times reaction buffer, 2 mM of MgCl_2 solution, 1 mM of deoxyribonucleotide triphosphate (dNTP) mix, 0.4 μM of each primer, 0.5 μL of BIOTAQ DNA Polymerase (Bioline, United Kingdom), 1 ng μL^{-1} of template DNA, and adjusted to a final volume of 25 μL with UltraPure DNase/RNase-free distilled water (Thermo Fisher Scientific, United States). PCR amplifications were as follows: (i) *dsyB_deg1F/dsyB_deg2R*: initial step at 95°C for 5 min; 30 cycles at 95°C for 30 s, 61°C for 1 min and 72°C for 15 s; and a final extension step at 72°C for 5 min; (ii) 27F/1492R: as described by Bourne and Munn (2005). PCR products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, United States) and visualised *via* electrophoresis on a 1% agarose gel stained with ethidium bromide.

Sanger Sequencing and Phylogenetic Analysis

PCR products were sequenced at Macrogen Inc. (Seoul, South Korea). The forward and reverse 16S rRNA and *dsyB* amplicon sequences were paired and the overlapping fragments were merged using Geneious Prime 2019.2.3 (Biomatters, New Zealand). Some *dsyB* amplicon sequences were too short (or of poor quality) to be merged and only one sequence (forward or reverse) was used.

To identify the closest taxonomic-relative of each isolate, BLAST searches (Altschul et al., 1990) were conducted through the National Center for Biotechnology Information (NCBI). 16S rRNA gene sequences were then aligned using MAFFT (Multiple

Alignment using Fast Fourier Transform) v7 (Katoh et al., 2002; Katoh and Standley, 2013) with default settings, then trimmed using trimAl v1.4 (Capella-Gutiérrez et al., 2009) to remove sites with more than 50% missing or degraded data. Following the Bayesian Information Criterion (Schwarz, 1978), the maximum likelihood phylogeny for each sample niche was calculated. Phylogenetic trees were constructed using IQ-TREE v1.6.12, with 1,000 ultrafast bootstrap replicates (Minh et al., 2013), and formatted using the ggtree package (Yu et al., 2017) in R (R Core Team, 2020).

The *dsyB* amplicons were translated into protein sequences and searches to find regions of local similarity were performed using BLASTP. These translated sequences were also aligned with DsyB from *Labrenzia aggregata* (AOR83342) (Curson et al., 2017) to assess their similarity using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970). A multiple sequence alignment (MSA) of the prokaryotic DsyB protein sequences was visualised to show conserved residues, conservative mutations, and divergence between the different homologues. The MSA was conducted using T-Coffee v11.00 (Notredame et al., 2000; Di Tommaso et al., 2011) with default settings and formatted using Boxshade v3.21. Conserved domains within the predicted DsyB sequence were detected using CD-Search (Marchler-Bauer and Bryant, 2004) against the Conserved Domain Database (CDD) v3.18 (Lu et al., 2019).

Culture of *dsyB*-Positive Bacterial Strains

To confirm the DMSP biosynthesis capability of bacteria harbouring *dsyB*, each strain was cultured in 500 mL of either MB, yeast tryptone sea salts [YTSS; (González et al., 1996)], MBM broth or methionine-enriched MBM broth (final concentration 0.5 mM). The cultures were incubated at 28°C with agitation (orbital shaker at 180 RPM) for 24 hours before being harvested by centrifugation (3,000 g for 15 min at 4°C). The clarified medium was discarded and the remaining cell pellets snap-frozen with liquid nitrogen, lyophilised overnight (Dynavac freeze dryer, Massachusetts, United States; model FD12) and stored at -20°C until required.

Chemical Extraction

The freeze-dried cell pellets were resuspended in 1 mL of deuterated methanol (CD₃OD; Cambridge Isotope Laboratories, Massachusetts, United States), vortexed at maximum speed for 5 min, and sonicated for 5 min at room temperature. A further 1 mL of CD₃OD and 666 μL of deuterium oxide (D₂O; Cambridge Isotope Laboratories, Massachusetts, United States) were added into the mixture (for a final CD₃OD to D₂O ratio of 3:1), which were then vortexed at maximum speed for 5 min and sonicated for 10 min at room temperature. Bacterial extracts were subsequently centrifuged at 3,000 g for 5 min. The particulate-free extracts (final volume of ~2.6 mL) were then used for subsequent analyses on the LC-MS and NMR.

Liquid Chromatography-Mass Spectrometry

LC-MS was used to assess the presence of intracellular DMSP in *dsyB*-positive bacterial isolates. Particulate-free bacterial extracts

were analysed on an Agilent 1100 series high performance liquid chromatograph coupled to a Bruker Esquire 3000 quadrupole ion trap mass spectrometer (LC-MS; Bruker Daltonics, Massachusetts, United States) equipped with an electrospray ionisation interface (ESI). Extracts (5 μL) were separated on a reverse-phase Luna 3 μm HILIC column (Phenomenex, California, United States; 150 × 3 mm, with a particle size of 3 μm) maintained at 25°C. Separation was achieved using a programmed step gradient consisting of solvent A: 0.1% formic acid (HCOOH) in Milli-Q water and solvent B: methanol (CH₃OH, HPLC grade OmniSolv), at a flow rate of 0.5 mL min⁻¹. The column was pre-equilibrated at 60% B for 10 min prior to injection. The programmed step gradient was t = 0 min, 60% B; t = 12 min, 10% B; t = 14 min, 10% B; t = 15 min, 60% B; t = 20 min, 60% B; t = 22 min, 60% B. The ESI was operated in positive mode and the target mass of *m/z* 135, corresponding to the [M+H]⁺ of DMSP, monitored (established from a DMSP standard).

Nuclear Magnetic Resonance

The presence of intracellular DMSP in *dsyB*-positive bacterial isolates was also assessed using NMR. A 700 μL aliquot of each bacterial particulate-free extract was transferred into a 5 mm Norell 509-UP NMR tube (North Carolina, United States) and analysed immediately using quantitative NMR (qNMR) *via* the ERETIC method (Electronic Reference To access *In vivo* Concentrations) (Akoka and Trierweiler, 2002) to measure the concentration of DMSP, as described in Tapiolas et al. (2013).

NMR spectra of the bacterial extracts were recorded on a Bruker Avance 600 MHz NMR spectrometer (Bruker BioSpin, United States) with a triple resonance cryoprobe (TXI), referenced using CD₃OD (δ_H 3.31). ¹H NMR spectra were acquired as outlined in Tapiolas et al., (2013) using a standard Bruker solvent suppression pulse sequence. 2D NMR spectra were also acquired to confirm the assignment of DMSP. All spectra were referenced to residual ¹H and ¹³C resonances in CD₃OD. In addition, one extract was spiked with 14 μL of 50 mM DMSP to confirm the position of the methyl singlet, as NMR signals can shift as the sample matrix changes.

Genome Sequencing of Isolate AMM-P-2

A phenol:chloroform extraction method, outlined in detail in Raina et al. (2016), was used to extract high-molecular weight DNA from a representative bacterial isolate producing DMSP (called AMM-P-2 hereafter). Extracted DNA was sent to the Ramaciotti Centre for Genomics (Sydney, Australia) for library preparation using the Nextera XT DNA Library Preparation Kit (Illumina, United States) and sequenced on the Illumina MiSeq system using V2 with 2×250 bp paired-end reads.

Genome Assembly and Annotation

The MiSeq read set was trimmed, assembled, and error-corrected using Trimmomatic 0.38 (Bolger et al., 2014), SPAdes v3.13.0 (Bankevich et al., 2012), and Pilon v1.23 (Walker et al., 2014), respectively. All were implemented through Shovill v1.0.4 using default settings. Prediction of coding regions and annotation were performed with Prokka v1.14.6 (Seemann, 2014) using standard databases (i.e., ISfinder, NCBI Bacterial Antimicrobial

Resistance Reference Gene Database, and UniProtKB (SwissProt)) and default settings.

Bioinformatic Analyses

To confirm the presence of *dsyB* within the genome of AMM-P-2, we used a reciprocal BLAST approach between the protein sequence reported by Curson et al. (2017) and the AMM-P-2 genome (E -value $\geq 1 \times 10^{-50}$). Similarly, to explore the genomic potential of the bacterium to utilise and metabolise other sulfur compounds (e.g., sulfate, cysteine), target genes were obtained from KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa et al., 2004) and compared against the AMM-P-2 genome. The orthology of the highest scoring match (E -value $\geq 1 \times 10^{-50}$) was confirmed by conducting BLASTP analyses against the NCBI NR (non-redundant) database, followed by BLASTP analyses of retrieved best match against the AMM-P-2 predicted proteins.

Stress Experiment

Isolate AMM-P-2 was grown in MBM broth with methionine (final concentration 0.5 mM) (Curson et al., 2017), and the culture incubated at 27°C, 180 RPM, ambient lighting, and 35 practical salinity units (PSU). After three days, a 1:10 dilution of this starter culture was inoculated into 60 mL replicate cultures and incubated under different environmental conditions simulating stress experienced by tropical corals: (i) high temperature (32°C; T₃₂), (ii) low temperature (22°C; T₂₂), (iii) high UV (through a combination of Deluxlite Black Light Blue (18W) and Reptile One UVB 5.0 (18W) with an average total radiation in the incubator of 1.328 mW cm⁻² measured using a Solarmeter Model 5.0 UVA + UVB meter (Solar Technology, Pennsylvania, United States)), (iv) complete darkness, (v) high salinity (40 PSU), and (vi) low salinity (25 PSU). Three biological replicates were grown for each of the described conditions, including control conditions maintained at ambient lighting, and 35 PSU.

Culture density was monitored through time using spectrophotometry (2 μ L was measured at 600 nm (OD₆₀₀) on a NanoDrop 1000 spectrophotometer; Thermo Fisher Scientific, United States). Cultures were sampled over four time points, corresponding to the mid-exponential (24 h), late exponential (28 h), early stationary (32 h), and late stationary (36 h) growth stages of the bacterium, as previously established by a standard growth curve. At each time point, one culture per treatment was removed and samples were taken for quantitative nuclear magnetic resonance (qNMR) analysis (50 mL; centrifuged for 5 min at 3,000 g, pellet snap frozen and stored at -20°C until analysis).

Bacterial Cell Counts

To convert the optical density data recorded with spectrophotometry into bacterial cell numbers, OD₆₀₀ and flow cytometry counts were carried out simultaneously on isolate AMM-P-2 grown under standard conditions in triplicate (27°C at 180 RPM, ambient lighting, 35 PSU, in modified MBM). After each OD₆₀₀ measurement, 100 μ L of cells were fixed for 15 min in 2% glutaraldehyde for subsequent flow cytometry analysis.

Samples were then stained with SYBR Green (1:10,000 final dilution; ThermoFisher, Massachusetts, United States), incubated for 15 min in the dark and analysed on a CytoFLEX S flow cytometer (Beckman Coulter, California, United States) with filtered MilliQ water as the sheath fluid. For each sample, forward scatter (FSC), side scatter (SSC), and green (SYBR) fluorescence were recorded. The samples were analysed at a flow rate of 25 μ L min⁻¹. Microbial populations were characterized according to SSC and SYBR Green fluorescence (Marie et al., 1997) and cell abundances were calculated by running a standardized volume (50 μ L) per sample.

Statistical Analyses

Statistics were performed using IBM SPSS Statistics v27.0.1. qNMR signals associated with DMSP and acrylate were normalised to cell density and tested for significance using repeated-measures ANOVA, with Greenhouse-Geisser correction applied (a correction for sphericity). A simple main effect test, applied following significant interactions between treatments, was used to determine the difference between treatments at each time point for both DMSP and acrylate. A Pearson product-moment correlation was used to determine the relationship between DMSP and acrylate concentrations in AMM-P-2.

RESULTS

Corals Harbour DMSP Producing Bacteria

A total of 157 isolates were recovered from coral mucus (51%) and tissue (49%) (**Figure 1B** and **Table S2**). The highest proportion of these were members of the Gammaproteobacteria (49%), followed by Alphaproteobacteria (32%) and Flavobacteriia (17%) (**Table S2**). The taxonomic composition of the isolates in each compartment was different (**Figure 1** and **Table S2**), and few isolates were shared between coral species (**Figure 1B**).

From the 157 bacterial isolates screened, 14 harboured the *dsyB* gene (9% of total isolates). These strains all belong to the Alphaproteobacteria class, specifically the family Rhodobacteraceae, and include members of the *Shimia* (n=10), *Roseivivax* (n=3), and *Pseudooceanicola* (n=1) genera (**Figure 1C** and **Table S2**). All of these *dsyB*-harbouring strains were isolated from *Acropora millepora* and *Acropora tenuis*, with 3 strains derived from the mucus and 11 from the tissue. More specifically, bacteria harbouring *dsyB* represented 19% (10 of 52) of the isolates from *A. millepora* tissue. The average sequence identity of the predicted (partial) DsyB amino acid sequences derived from the isolates to the protein from *Labrenzia aggregata* was ~65% (**Figure S1**) (Curson et al., 2017; Curson et al., 2018). Interestingly, three different *Labrenzia* strains were isolated from corals, including two strains with more than 98% 16S rRNA gene sequence identity to *L. aggregata*, but *dsyB* could not be amplified from these isolates.

The 14 *dsyB*-harbouring strains were assessed for their ability to produce DMSP. The isolates were initially grown in MB and YTSS, but DMSP could not be detected in any of the cultures using either LC-MS (**Figure S2**) or NMR (**Figure S3**). However,

LC-MS did detect a peak at m/z 135 and retention time of 5.9 min, consistent with DMSP, in all isolates grown in methionine-enriched MBM (Figure S4). ^1H NMR analysis established the presence of a well-resolved diagnostic singlet at δ_{H} 2.95 ppm ($2 \times \text{CH}_3$) (Tapiolas et al., 2013) (Figure 2) and ^1H - ^1H correlation spectroscopy (COSY) revealed two coupled methylene groups, S-CH_2 - (δ_{H} 3.45, t) and $-\text{CH}_2\text{-CO}_2\text{H}$ (δ_{H} 2.70, t) (Figure S5). In addition, a ^1H - ^{13}C heteronuclear multiple bond correlation (HMBC) revealed long-range chemical shift correlations between: (i) the protons of the two methyl groups (S-CH_3) and the carbon of the S -methylene group (δ_{H} 2.94 - δ_{C} 43.21); (ii) the carboxyl-methylene protons of the carboxyl carbon (δ_{H} 2.69 - δ_{C} 172.03) and the S -methylene carbon (δ_{H} 2.69 - δ_{C} 43.21) (Figure S6). Together, these COSY and HMBC correlations confirmed the structure of DMSP, thus verifying the presence of DMSP in the bacterial extracts.

Sulfur Transformation Potential of the DMSP Producer *Shimia aestuarii* AMM-P-2

To further characterise the genomic underpinnings of DMSP production in coral-associated bacteria, isolate AMM-P-2 was selected for whole-genome sequencing (Table 1). This isolate belongs to the *Shimia* genus, which accounted for 71% (or 10 of 14) of *dsyB*-positive bacteria isolated. This strain exhibited >97% similarity with *Shimia aestuarii* based on its full 16S rRNA gene sequence.

Comparison of the predicted amino acid sequence of DsyB from *S. aestuarii* AMM-P-2 with previously characterised homologues representing the diversity of this protein family ($n=14$) revealed the presence of two conserved domains which are common to all *dsyB* orthologues (Figure S7): (i) an S -adenosylmethionine-dependent methyltransferase (AdoMet-MTase) class I superfamily domain (pfam00891; E-value 4.02×10^{-16}) and (ii) a dimerization2

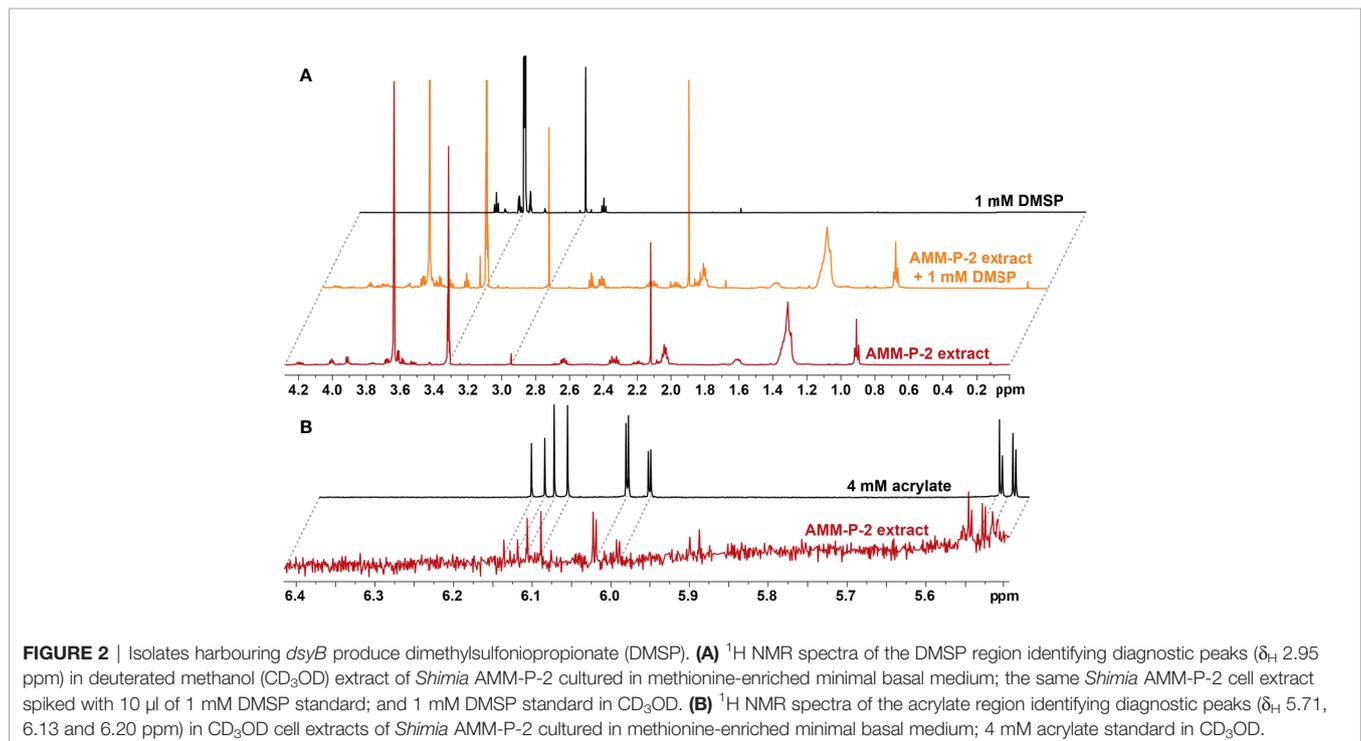


TABLE 1 | Genome statistics for *Shimia aestuarii* AMM-P-2.

Attribute	Value
Estimated genome size (bp)	4,151,190
Assembly size (bp)	3,972,669
No. of sequences	75
GC content (%)	60.6
N50	181,193
Gap ratio (%)	0
Number of CDSs	3,906
Number of rRNA	3
Number of tRNA	46
Number of CRISPRs	0
Coding ratio (%)	89.7

superfamily domain (pfam16864; E-value 4.13×10^{-7}). As in several other Rhodobacterales strains (Curson et al., 2017), *isc* [iron sulfur cluster] or *suf* [sulfur formation] gene clusters were present 5' of *dsyB* in *S. aestuarii* AMM-P-2 (Figure S8). However, in the region 3' of *dsyB*, only limited synteny was observed between *S. aestuarii* AMM-P-2 and other Rhodobacterales.

To determine the source of sulfur used by *S. aestuarii* AMM-P-2 for DMSP biosynthesis, its sulfur metabolic potential was assessed. Distinct orthologues of all the enzymes in the sulfate reduction pathway were identified (Figure 3 and Table S3), confirming *S. aestuarii* AMM-P-2 has the genetic machinery required to uptake and convert extracellular inorganic sulfate to sulfide (Table S3). Following sulfide production, *S. aestuarii*

AMM-P-2 can produce cysteine (through serine or homocysteine) and ultimately the DMSP precursor methionine (via aspartate; **Figure 3**).

Turning the attention to DMSP catabolism, *S. aestuarii* AMM-P-2 has the genetic potential to both demethylate and cleave this sulfur compound (**Figure 3** and **Table S3**). *S. aestuarii* AMM-P-2 has the entire *dmdABCD* suite of genes enabling conversion of DMSP to MeSH (Zhang et al., 2019). This bacterium also contains the DMSP lyase genes *dddP* and *dddW*, whose products liberate equimolar amounts of DMS and acrylate from DMSP (Zhang et al., 2019). It is also able to metabolise acrylate to propionyl-CoA through *acuI*, *acuH* and *prpE* genes (Wang et al., 2017). Significant matches to *dddA* and *dddC* (E-values $\geq 1.39 \times 10^{-93}$) which catabolise 3-hydroxypropionyl-CoA (3HP) to acetyl-CoA (Wang et al., 2017) (**Table S3**) were also identified.

DMSP Production by *Shimia aestuarii* AMM-P-2 Under Different Environmental Conditions

To assess the influence of environmental factors on DMSP production by *S. aestuarii* AMM-P-2, cultures were grown in methionine-enriched MBM under six different conditions and sampled four times over a 48-hour incubation period. The conditions tested are known to elicit stress responses in corals (high and low temperature, high and low salinity, high UV, and darkness), and were compared to controls. Intracellular DMSP levels per cell within each treatment were not statistically different through time (repeated-measure ANOVA, $p = 0.078$; see **Table S4**) and no significant interaction was identified between time and treatments (repeated-measure ANOVA, $p = 0.204$; **Table S4**).

Changes in salinity affected DMSP levels per cell (**Figure 4A**). Levels of DMSP in cultures grown under hyposaline conditions (25 PSU) were 63% lower than the controls (35 PSU) over all four time points. In contrast, the highest DMSP levels were recorded under hypersaline conditions (40 PSU), which were 80% higher than the controls. DMSP levels also increased when cells were grown under

high UV (65% higher) or in complete darkness (43% higher) compared to control ambient light conditions. Finally, at higher temperature (32°C), DMSP levels were 42% lower than the controls, while the low temperature treatment (22°C) had no effect throughout the experiment.

Although it was not possible to quantify DMS with qNMR due to the volatility of this compound, DMSP lysis releases equimolar concentrations of acrylate, which was detected in every sample. Overall, the level of acrylate per cell was approximately one order of magnitude lower than that of DMSP (**Figure 4B**). These measurements confirm that a fraction of the synthesized DMSP is channelled through the cleavage pathway. A significant interaction between time and treatments was identified for acrylate levels per cell (ANOVA, $p = 0.047$; **Table S5**). However, the differences between control conditions and the different treatments were not as pronounced for acrylate compared to DMSP. The 32°C treatment exhibited the lowest levels of acrylate across all timepoints (with 68% less acrylate on average than the control), while the high salinity treatment gave rise to the highest concentrations (with 36% more acrylate).

The six treatments were divided into two categories: (i) those likely to elicit a stress response in bacteria (i.e., high UV, low and high salinity), and (ii) those unlikely to be stressors (i.e., darkness, 22 and 32°C), and the level of correlation of the intracellular levels of DMSP and acrylate between these assessed. No significant correlation was identified between DMSP and acrylate levels for the mild conditions (Pearson's $R = 0.23$, $p = 0.39$; **Figure 4D**). However, a strong positive correlation was identified for the stress treatments (Pearson's $R = 0.87$, $p < 0.001$; **Figure 4C**).

DISCUSSION

Coral-Associated Bacteria Can Produce DMSP

Of the 157 bacteria isolated from the mucus and tissues of four coral species, approximately 9% harboured the *dsyB* gene (14 out

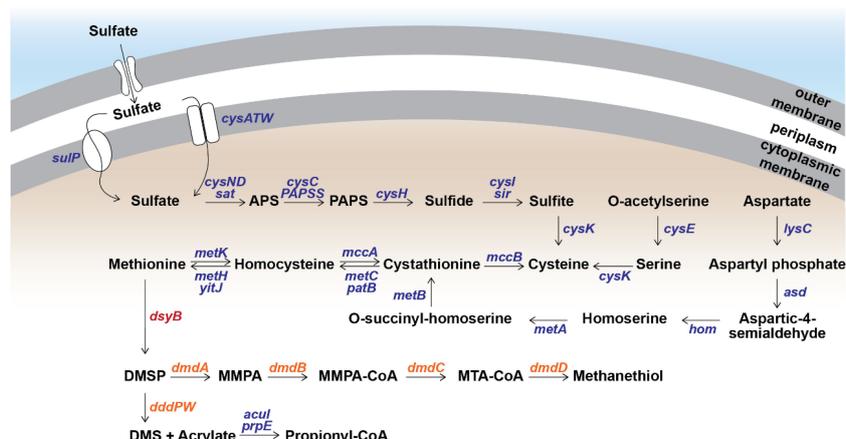


FIGURE 3 | Metabolism of methylated sulfur compounds in *Shimia aestuarii* AMM-P-2. Orthologous genes for each step are presented in blue. The DMSP production gene *dsyB* is presented in red, while the catabolism genes are in orange. E-values and additional information can be found in **Table S3**.

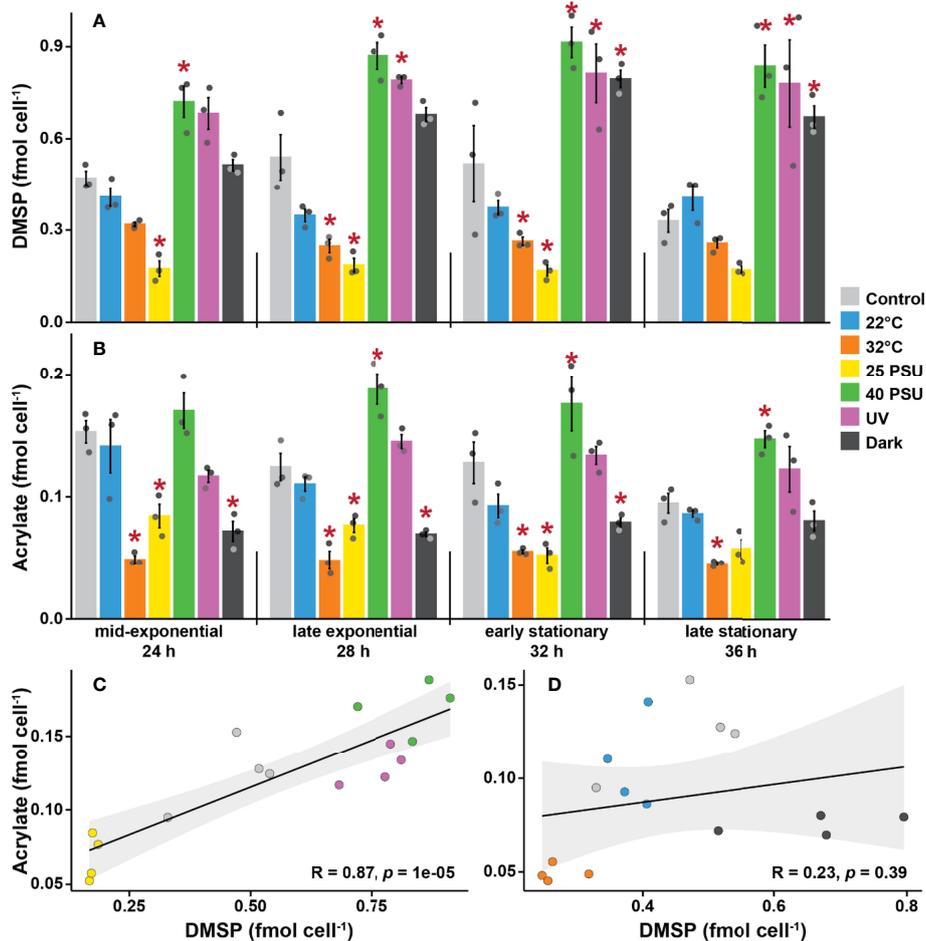


FIGURE 4 | Dimethylsulfoniopropionate (DMSP) and acrylate levels are strongly influenced by environmental stressors in *Shimia aestuarii* AMM-P-2. Levels of (A) DMSP and (B) acrylate in bacterial cells exposed to high and low temperatures (32 and 22°C), high and low salinity (40 and 25 PSU), constant darkness, and high UV. Controls were grown at 27°C under ambient light in a modified MBM media adjusted to 35 PSU and supplemented with 0.5 mM methionine. Error bars indicate standard error ($n = 3$). Experimental conditions marked with an asterisk are significantly different ($p < 0.05$) from the controls (Repeated-measure ANOVA). Correlation between DMSP and acrylate levels in *Shimia aestuarii* AMM-P-2 under conditions (C) likely to stress the cells (high-low salinity and high UV), (D) unlikely to stress the cells (22–32°C and darkness). Pearson correlation coefficient and associated p values are displayed on each graph.

of 157). Although three genera (four species) of corals were screened, *dsyB*-positive bacteria were only isolated from the two *Acropora* species, which correlates with the high DMSP production by members of this genus (Tapiolas et al., 2013). Note, it is possible that other DMSP-producing bacteria were present in the corals sampled, potentially containing *mmtN* or other unknown DMSP synthesis genes (Williams et al., 2019). All bacteria with *dsyB* belonged to the Rhodobacterales order (Curson et al., 2017), with *Shimia* species representing 71% of these isolates (10 out of 14). *Shimia* are metabolically versatile members of the Roseobacter clade (Choi and Cho, 2006), and are abundant in the water column, marine sediments, and commonly associated with eukaryotic hosts (Lenk et al., 2012; Luo and Moran, 2014), particularly phytoplankton (Ajani et al., 2018; Behringer et al., 2018) and reef-building corals (Chen et al., 2011; Zhang et al., 2021). The other *dsyB*-harbouring bacteria isolated in our screen belonged to the genera *Pseudoceanicola* –

which are common DMSP producers in the water column (Zheng et al., 2020) – and *Roseivivax* – which have been isolated from corals (Chen et al., 2012) and can induce coral larval settlement (Sharp et al., 2015).

DMSP was unambiguously identified in extracts of *dsyB*-harbouring bacteria when the cells were grown in methionine-supplemented MBM. In MBM lacking methionine, DMSP signal intensity was close to or below the detection limits of the LC-MS and NMR. Addition of pathway intermediates, including methionine, have been shown to enhance DMSP production in bacteria, but most *dsyB*-harbouring strains can also produce methionine *de novo* (through sulfate assimilation) (Curson et al., 2017). This study therefore reveals the presence of bacteria capable of DMSP production in corals and shows that these microorganisms are likely to contribute to the DMSP production by coral holobionts.

Three strains of *Labrenzia*, the bacterial genus from which the *dsyB* gene was initially identified (Curson et al., 2017), were

isolated from *Pocillopora acuta* and *Stylophora pistillata*. The 16S rRNA genes of one of the strains was 98.6% similar to *Labrenzia alba*, while of the other two were more than 98% similar to *L. aggregata*. However, the *dsyB* gene was not detected in any of the three strains. This is consistent with the previously-reported lack of *de novo* DMS production from a Symbiodiniaceae-associated *Labrenzia* strain (Lawson et al., 2020), suggesting that the ability to produce DMSP is not conserved across the *Labrenzia* genus.

Shimia aestuarii* AMM-P-2 Can Produce DMSP *de novo

To investigate the potential of a representative DMSP-producing bacterium from corals to metabolise sulfur compounds, the genome of *S. aestuarii* AMM-P-2 was fully sequenced. *S. aestuarii* is capable of assimilating sulfate from seawater to produce sulfide and sulfur-containing amino acids. Although all the genes required for methionine synthesis were present, its growth on methionine-enriched media indicates that it can also use exogenous methylated sulfur compounds, enhancing the pool of reduced sulfur intermediates available for the synthesis of DMSP. The *S. aestuarii* AMM-P-2 genome also encodes both the demethylation and the cleavage pathways, allowing it to produce the sulfurous gases methanethiol and DMS. Given that metabolic interdependencies between different partners are common in symbiotic systems, it is important to note that *S. aestuarii* AMM-P-2 has all the required genes to produce DMSP *de novo*.

As in other Rhodobacterales (Curson et al., 2017), *dsyB* in *S. aestuarii* AMM-P-2 is located downstream of multiple genes (*iscRS*, *sufBCDS*) encoding iron-sulfur cluster (Isc) proteins involved in the formation of Fe-S clusters and cellular defence against oxidative stress. Specifically, the cysteine desulfurase IscS provides the sulfur that is then incorporated into Fe-S clusters and its deletion renders the cells hypersensitive to oxidative stress (Ayala-Castro et al., 2008; Rybniker et al., 2014). In addition, the IscR protein is a transcriptional regulator of the *suf* operon (Yeo et al., 2006) and both the *suf* and *isc* operons are highly induced by oxidative stress (Yeo et al., 2006). Therefore, the tight linkage of *dsyB* to the *suf* and *isc* operons suggests that DMSP transcription may be directly affected by oxidants (Sunda et al., 2002; Curson et al., 2017).

DMSP Production by *Shimia aestuarii* AMM-P-2 Is Enhanced by Environmental Stress

DMSP levels in *S. aestuarii* cells were affected by changes in salinity. Fluctuations in salinity are known to affect DMSP levels in corals (Gardner et al., 2016; Aguilar et al., 2017), while also impacting DMSP degradation in bacteria (Salgado et al., 2014; Liu et al., 2018). The nearly 5-fold increase in intracellular DMSP under hypersaline compared to hyposaline conditions reported here is consistent with previous reports in algae (Vairavamurthy et al., 1985; Kirst et al., 1991; Trossat et al., 1998), and bacteria (Curson et al., 2017), and supports the proposed function of this molecule as an osmolyte (Kirst et al., 1991).

UV exposure and complete darkness both caused significant increases in DMSP levels in *S. aestuarii* cells. The level of UV radiation applied here was approximately 50% higher than values previously measured at 1 m depth on the Great Barrier Reef (Nordborg et al., 2018), and is therefore likely to cause oxidative stress in the *S. aestuarii* cells. Indeed, because of their lack of pigmentation and low internal self-shading due to small cell volume, heterotrophic bacteria are amongst the most UV-sensitive organisms (Ruiz-Gonzalez et al., 2013). Similar increases in DMSP levels triggered by UV exposure have been reported in phytoplankton (Sunda et al., 2002; Slezak and Herndl, 2003) and have been attributed to the antioxidant capacity of DMSP (Sunda et al., 2002). More surprising was the increase in DMSP levels in cells grown in darkness. Recent studies have revealed that bacteria in aphotic environments, such as the deep ocean as well as coastal and deep sediments, produce substantial amounts of DMSP (Williams et al., 2019; Zheng et al., 2020). Although the effect of sunlight on bacterioplankton has been extensively studied at the community level (Ruiz-Gonzalez et al., 2013), the impact of prolonged darkness on the physiology of heterotrophic bacteria has received little attention to date. Our results suggest that the absence of light influences sulfur metabolism in *S. aestuarii*, and further investigation should aim to identify the mechanism driving the production of DMSP in aphotic conditions.

Intracellular acrylate levels in *S. aestuarii* AMM-P-2 were almost one order of magnitude lower than those of DMSP and decreased significantly over time in all growth conditions. Acrylate and DMSP levels were strongly and positively correlated under conditions likely to stress the bacteria (e.g., salinity, UV), but were decoupled under conditions that are stressful for the coral host but not necessarily for the bacteria (e.g., small temperature variations, darkness). This suggests that under conditions stressful for the bacteria, more DMSP is channelled towards the DMSP cleavage pathway, which generates equal amounts of DMS and acrylate, rather than the demethylation pathway (Gao et al., 2020). DMS and acrylate are efficient scavengers of hydroxyl radicals produced by the cells during stress (Sunda et al., 2002), which may explain why the cleavage pathway is preferred during stressful conditions.

CONCLUSION

This study demonstrates that some coral-associated bacteria can produce the important organosulfur compound DMSP, implying that, along with the animal host and Symbiodiniaceae, bacterial communities may contribute to DMSP production by the coral holobiont. It has recently been shown that bacteria can also use a second (*dsyB*-independent) pathway to produce DMSP, involving the *mmtN* gene (Williams et al., 2019). Since this gene is also present in Gammaproteobacteria and Actinobacteria, it is likely that some DMSP-producing bacteria were overlooked by the *dsyB*-centric screening approach and thus that the proportion of DMSP-producing bacteria in corals might be greater than estimated here. Analysis of the genome of a *Shimia* strain, representing the most abundant *dsyB*-harbouring genus of those isolated from corals, revealed that this bacterium has the genetic machinery to

assimilate sulfate, to synthesise the sulfur-based amino acids cysteine and methionine, to catabolise DMSP to methanethiol, DMS and acrylate, and to utilise or detoxify acrylate. Furthermore, DMSP production in *S. aestuarii* AMM-P-2 is regulated by specific environmental conditions, some of which are not necessarily tied to coral stress. The capacity of coral-associated bacteria to produce DMSP not only adds this trait to the functional repertoire of prokaryotes associated with corals, but also indicates that bacteria may contribute to the large DMSP pool produced by this metaorganism.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The 16S rRNA gene amplicon sequences of the isolated bacteria have been deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) under the accession numbers MW828351 to MW828429 and MW828507 to MW828582. The assembled genome of isolate AMM-P-2 has been deposited in GenBank under the accession number PRJNA810763.

AUTHOR CONTRIBUTIONS

CM, DM, DB, FK and J-BR designed the experiments. FK collected the samples and performed all laboratory work and data analysis with the assistance of CM, DM, DB, JT and J-BR. FK, JZ, IC and DM analysed the genomic data. FK, CM, DM, DB and J-BR wrote the manuscript, with input from all authors before submission. All authors contributed to the article and approved the submitted version.

REFERENCES

- Aguilar, C., Raina, J.-B., Motti, C. A., Fôret, S., Hayward, D. C., Lapeyre, B., et al. (2017). Transcriptomic Analysis of the Response of *Acropora Millepora* to Hypo-Osmotic Stress Provides Insights Into DMSP Biosynthesis by Corals. *BMC Genomics* 18, 612. doi: 10.1186/s12864-017-3959-0
- Ajani, P. A., Kahlke, T., Siboni, N., Carney, R., Murray, S. A., and Seymour, J. R. (2018). The Microbiome of the Cosmopolitan Diatom *Leptocyclus* Reveals Significant Spatial and Temporal Variability. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.02758
- Akoka, S., and Trierweiler, M. (2002). Improvement of the ERETIC Method by Digital Synthesis of the Signal and Addition of a Broadband Antenna Inside the NMR Probe. *Instrum. Sci. Technol.* 30, 21–29. doi: 10.1081/CI-100108768
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic Local Alignment Search Tool. *J. Mol. Biol.* 215, 403–410. doi: 10.1016/S0022-2836(05)80360-2
- Andreae, M. O., Barnard, W. R., and Ammons, J. M. (1983). The Biological Production of Dimethylsulfide in the Ocean and Its Role in the Global Atmospheric Sulfur Budget. *Ecol. Bull.* 35, 167–177. doi: jstor.org/stable/20112852
- Ayala-Castro, C., Saini, A., and Outten, F. W. (2008). Fe-S Cluster Assembly Pathways in Bacteria. *Microbiol. Mol. Biol. Rev.* 72, 110–125. doi: 10.1128/MMBR.00034-07
- Ayers, G. P., and Gras, J. L. (1991). Seasonal Relationship Between Cloud Condensation Nuclei and Aerosol Methanesulphonate in Marine Air. *Nature* 353, 834–835. doi: 10.1038/353834a0
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: A New Genome Assembly Algorithm and Its

FUNDING

FWIK was supported through scholarships and funding from the AIMS@JCU Division of Research & Innovation and the College of Public Health, Medical and Veterinary Sciences at James Cook University. This research was funded in part by the Pilot Research Awards from AIMS@JCU.

ACKNOWLEDGMENTS

The coral colonies were collected under permit G14/36802 issued by the Great Barrier Reef Marine Park Authority. The authors thank the staff of AIMS SeaSim for providing coral samples as needed, and Aurélie Moya, Sara Bell, Peter Thomas-Hall, and Nahshon Siboni for discussions and assistance in the lab. The authors acknowledge the Traditional Owners of the land on which the research was conducted, the Wulgurukaba, Bindal and Gadigal people; we recognise their connection to land, sea, and community, and pay our respects to Elders past, present and emerging. Finally, a special thanks goes to Libby Evans-Illidge (AIMS@JCU) for her unwavering support throughout the project.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.869574/full#supplementary-material>

- Applications to Single-Cell Sequencing. *J. Comput. Biol.* 19, 455–477. doi: 10.1089/cmb.2012.0021
- Bates, T. S., Charlson, R. J., and Gammon, R. H. (1987). Evidence for the Climatic Role of Marine Biogenic Sulphur. *Nature* 329, 319–321. doi: 10.1038/329319a0
- Behringer, G., Ochsenkühn, M. A., Fei, C., Fanning, J., Koester, J. A., and Amin, S. A. (2018). Bacterial Communities of Diatoms Display Strong Conservation Across Strains and Time. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.00659
- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170
- Bourne, D. G., Morrow, K. M., and Webster, N. S. (2016). Insights Into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annu. Rev. Microbiol.* 70, 317–340. doi: 10.1146/annurev-micro-102215-095440
- Bourne, D. G., and Munn, C. B. (2005). Diversity of Bacteria Associated With the Coral *Pocillopora Damicornis* From the Great Barrier Reef. *Environ. Microbiol.* 7, 1162–1174. doi: 10.1111/j.1462-2920.2005.00793.x
- Capella-Gutiérrez, S., Silla-Martínez, J. M., and Gabaldón, T. (2009). trimAl: A Tool for Automated Alignment Trimming in Large-Scale Phylogenetic Analyses. *Bioinformatics* 25, 1972–1973. doi: 10.1093/bioinformatics/btp348
- Chen, M.-H., Sheu, S.-Y., Chen, C. A., Wang, J.-T., and Chen, W.-M. (2011). *Shimia Isopora* Sp. Nov., Isolated From the Reef-Building Coral *Isopora Palifera*. *Int. J. Syst. Evol. Microbiol.* 61, 823–827. doi: 10.1099/ijs.0.022848-0
- Chen, M.-H., Sheu, S.-Y., Chen, C. A., Wang, J.-T., and Chen, W.-M. (2012). *Rosevivax Isopora* Sp. Nov., Isolated From a Reef-Building Coral, and Emended Description of the Genus *Rosevivax*. *Int. J. Syst. Evol. Microbiol.* 62, 1259–1264. doi: 10.1099/ijs.0.032961-0

- Choi, D. H., and Cho, B. C. (2006). *Shimia Marina* Gen. Nov., Sp. Nov., a Novel Bacterium of the *Roseobacter* Clade Isolated From Biofilm in a Coastal Fish Farm. *Int. J. Syst. Evol. Microbiol.* 56, 1869–1873. doi: 10.1099/ijls.0.64235-0
- Curson, A. R. J., Liu, J., Bermejo Martínez, A., Green, R. T., Chan, Y., Carrión, O., et al. (2017). Dimethylsulfoniopropionate Biosynthesis in Marine Bacteria and Identification of the Key Gene in This Process. *Nat. Microbiol.* 2, 17009. doi: 10.1038/nmicrobiol.2017.9
- Curson, A. R. J., Williams, B. T., Pinchbeck, B. J., Sims, L. P., Martínez, A. B., Rivera, P. P. L., et al. (2018). DSYB Catalyses the Key Step of Dimethylsulfoniopropionate Biosynthesis in Many Phytoplankton. *Nat. Microbiol.* 3, 430–439. doi: 10.1038/s41564-018-0119-5
- DeBose, J. L., Lema, S. C., and Nevitt, G. A. (2008). Dimethylsulfoniopropionate as a Foraging Cue for Reef Fishes. *Science* 319, 1356–1356. doi: 10.1126/science.1151109
- Di Tommaso, P., Moretti, S., Xenarios, I., Orobitg, M., Montanyola, A., Chang, J.-M., et al. (2011). T-Coffee: A Web Server for the Multiple Sequence Alignment of Protein and RNA Sequences Using Structural Information and Homology Extension. *Nucleic Acids Res.* 39, W13–W17. doi: 10.1093/nar/gkr245
- Frade, P. R., Schwanning, V., Glas, B., Sintes, E., Hill, R. W., Simó, R., et al. (2016). Dimethylsulfoniopropionate in Corals and its Interrelations With Bacterial Assemblages in Coral Surface Mucus. *Environ. Chem.* 13, 252–265. doi: 10.1071/EN15023
- Gao, C., Fernandez, V. I., Lee, K. S., Fenizia, S., Pohnert, G., Seymour, J. R., et al. (2020). Single-Cell Bacterial Transcription Measurements Reveal the Importance of Dimethylsulfoniopropionate (DMSP) Hotspots in Ocean Sulfur Cycling. *Nat. Commun.* 11, 1942. doi: 10.1038/s41467-020-15693-z
- Gardner, S. G., Nielsen, D. A., Laczka, O., Shimmon, R., Beltran, V. H., Ralph, P. J., et al. (2016). Dimethylsulfoniopropionate, Superoxide Dismutase and Glutathione as Stress Response Indicators in Three Corals Under Short-Term Hyposalinity Stress. *Proc. R. Soc. B: Biol. Sci.* 283, 20152418. doi: 10.1098/rspb.2015.2418
- González, J. M., Whitman, W. B., Hodson, R. E., and Moran, M. A. (1996). Identifying Numerically Abundant Culturable Bacteria From Complex Communities: An Example From a Lignin Enrichment Culture. *Appl. Environ. Microbiol.* 62, 4433–4440. doi: 10.1128/aem.62.12.4433-4440.1996
- Hill, R. W., Dacey, J. W. H., and Krupp, D. A. (1995). Dimethylsulfoniopropionate in Reef Corals. *Bull. Marine Sci.* 57, 489–494.
- Howard, E. C., Henriksen, J. R., Buchan, A., Reisch, C. R., Bürgmann, H., Welsh, R., et al. (2006). Bacterial Taxa That Limit Sulfur Flux From the Ocean. *Science* 314, 649–652. doi: 10.1126/science.1130657
- Johnston, A., Todd, J., and Curson, A. (2012). Microbial Origins and Consequences of Dimethyl Sulfide. *Microbe* 7, 181–185. doi: 10.1128/microbe.7.181.1
- Jones, G. B., and Trevena, A. J. (2005). The Influence of Coral Reefs on Atmospheric Dimethylsulfide Over the Great Barrier Reef, Coral Sea, Gulf of Papua and Solomon and Bismarck Seas. *Marine. Freshwater. Res.* 56, 85–93. doi: 10.1071/MF04097
- Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., and Hattori, M. (2004). The KEGG Resource for Deciphering the Genome. *Nucleic Acids Res.* 32, D277–D280. doi: 10.1093/nar/gkh063
- Karsten, U., Kück, K., Vogt, C., and Kirst, G. O. (1996). “Dimethylsulfoniopropionate Production in Phototrophic Organisms and its Physiological Functions as a Cryoprotectant,” in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*. Eds. R. P. Kiene, P. T. Visscher, M. D. Keller and G. O. Kirst (Boston, MA: Springer US), 143–153.
- Katoh, K., Misawa, K., i. Kuma, K., and Miyata, T. (2002). MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform. *Nucleic Acids Res.* 30, 3059–3066. doi: 10.1093/nar/gkf436
- Katoh, K., and Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010
- Kiene, R. P., Linn, L. J., and Bruton, J. A. (2000). New and Important Roles for DMSP in Marine Microbial Communities. *J. Sea. Res.* 43, 209–224. doi: 10.1016/S1385-1101(00)00023-X
- Kiene, R. P., Visscher, P. T., Keller, M. D., and Kirst, G. O. (1996). *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds* (Boston, MA: Springer).
- Kirst, G. O. (1996). “Osmotic Adjustment in Phytoplankton and Macroalgae,” in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds*. Eds. R. P. Kiene, P. T. Visscher, M. D. Keller and G. O. Kirst (Boston, MA: Springer US), 121–129.
- Kirst, G. O., Thiel, C., Wolff, H., Nothnagel, J., Wanzek, M., and Ulmke, R. (1991). Dimethylsulfoniopropionate (DMSP) in Icealgae and its Possible Biological Role. *Marine. Chem.* 35, 381–388. doi: 10.1016/S0304-4203(09)90030-5
- Lane, D. (1991). “16s/23s rRNA Sequencing,” in *Nucleic Acid Techniques in Bacterial Systematics*. Eds. E. Stackebrandt and M. Goodfellow (New York, NY, USA: John Wiley & Sons), 115–175.
- Lawson, C. A., Seymour, J. R., Possell, M., Suggett, D. J., and Raina, J.-B. (2020). The Volatilomes of Symbiodiniaceae-Associated Bacteria Are Influenced by Chemicals Derived From Their Algal Partner. *Front. Marine. Sci.* 7. doi: 10.3389/fmars.2020.00106
- Lenk, S., Moraru, C., Hahnke, S., Arnds, J., Richter, M., Kube, M., et al. (2012). *Roseobacter* Clade Bacteria are Abundant in Coastal Sediments and Encode a Novel Combination of Sulfur Oxidation Genes. *ISME. J.* 6, 2178–2187. doi: 10.1038/ismej.2012.66
- Liu, J., Liu, J., Zhang, S.-H., Liang, J., Lin, H., Song, D., et al. (2018). Novel Insights Into Bacterial Dimethylsulfoniopropionate Catabolism in the East China Sea. *Front. Microbiol.* 9. doi: 10.3389/fmicb.2018.03206
- Luo, H., and Moran, M. A. (2014). Evolutionary Ecology of the Marine *Roseobacter* Clade. *Microbiol. Mol. Biol. Rev.* 78, 573–587. doi: 10.1128/MMBR.00020-14
- Luo, D., Wang, X., Feng, X., Tian, M., Wang, S., Tang, S.-L., et al. (2021). Population Differentiation of Rhodobacteraceae Along With Coral Compartments. *ISME. J.* 15, 3286–3302. doi: 10.1038/s41396-021-01009-6
- Lu, S., Wang, J., Chitsaz, F., Derbyshire, M. K., Geer, R. C., Gonzales, N. R., et al. (2019). CDD/SPARCLE: The Conserved Domain Database in 2020. *Nucleic Acids Res.* 48, D265–D268. doi: 10.1093/nar/gkz991
- Marchler-Bauer, A., and Bryant, S. H. (2004). CD-Search: Protein Domain Annotations on the Fly. *Nucleic Acids Res.* 32, W327–W331. doi: 10.1093/nar/gkh454
- Marie, D., Partensky, F., Jacquet, S., and Vault, D. (1997). Enumeration and Cell Cycle Analysis of Natural Populations of Marine Picoplankton by Flow Cytometry Using the Nucleic Acid Stain SYBR Green I. *Appl. Environ. Microbiol.* 63, 186–193. doi: 10.1128/aem.63.1.186-193.1997
- Matthews, J. L., Raina, J.-B., Kahlke, T., Seymour, J. R., van Oppen, M. J. H., and Suggett, D. J. (2020). Symbiodiniaceae-Bacteria Interactions: Rethinking Metabolite Exchange in Reef-Building Corals as Multi-Partner Metabolic Networks. *Environ. Microbiol.* 22, 1675–1687. doi: 10.1111/1462-2920.14918
- Minh, B. Q., Nguyen, M. A. T., and von Haeseler, A. (2013). Ultrafast Approximation for Phylogenetic Bootstrap. *Mol. Biol. Evol.* 30, 1188–1195. doi: 10.1093/molbev/mst024
- Needleman, S. B., and Wunsch, C. D. (1970). A General Method Applicable to the Search for Similarities in the Amino Acid Sequence of Two Proteins. *J. Mol. Biol.* 48, 443–453. doi: 10.1016/0022-2836(70)90057-4
- Ngugi, D. K., Ziegler, M., Duarte, C. M., and Voolstra, C. R. (2020). Genomic Blueprint of Glycine Betaine Metabolism in Coral Metaorganisms and Their Contribution to Reef Nitrogen Budgets. *iScience* 23, 101120. doi: 10.1016/j.isci.2020.101120
- Nordborg, F. M., Flores, F., Brinkman, D. L., Agustí, S., and Negri, A. P. (2018). Phototoxic Effects of Two Common Marine Fuels on the Settlement Success of the Coral *Acropora Tenuis*. *Sci. Rep.* 8, 8635. doi: 10.1038/s41598-018-26972-7
- Notredame, C., Higgins, D. G., and Heringa, J. (2000). T-Coffee: A Novel Method for Fast and Accurate Multiple Sequence Alignment. *J. Mol. Biol.* 302, 205–217. doi: 10.1006/jmbi.2000.4042
- Pogoreutz, C., Voolstra, C. R., Rådecker, N., Weis, V., Cardenas, A., and Raina, J.-B. (2020). “The Coral Holobiont Highlights the Dependence of Cnidarian Animal Hosts on Their Associated Microbes,” in *Cellular Dialogues in the Holobiont*. Eds. T. C. G. Bosch and M. G. Hadfield (CRC Press: Taylor & Francis Group), 91–118.
- Rådecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., and Wild, C. (2015). Nitrogen Cycling in Corals: The Key to Understanding Holobiont Functioning? *Trends Microbiol.* 23, 490–497. doi: 10.1016/j.tim.2015.03.008
- Raina, J.-B., Dinsdale, E. A., Willis, B. L., and Bourne, D. G. (2010). Do the Organic Sulfur Compounds DMSP and DMS Drive Coral Microbial Associations? *Trends Microbiol.* 18, 101–108. doi: 10.1016/j.tim.2009.12.002
- Raina, J.-B., Tapiolas, D. M., Foret, S., Lutz, A., Abrego, D., Ceh, J., et al. (2013). DMSP Biosynthesis by an Animal and its Role in Coral Thermal Stress Response. *Nature* 502, 677–680. doi: 10.1038/nature12677

- Raina, J.-B., Tapiolas, D., Motti, C. A., Foret, S., Seemann, T., Tebben, J., et al. (2016). Isolation of an Antimicrobial Compound Produced by Bacteria Associated With Reef-Building Corals. *PeerJ* 4, e2275. doi: 10.7717/peerj.2275
- Raina, J.-B., Tapiolas, D., Willis, B. L., and Bourne, D. G. (2009). Coral-Associated Bacteria and Their Role in the Biogeochemical Cycling of Sulfur. *Appl. Environ. Microbiol.* 75, 3492–3501. doi: 10.1128/AEM.02567-08
- R Core Team (2020). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing).
- Robbins, S. J., Singleton, C. M., Chan, C. X., Messer, L. F., Geers, A. U., Ying, H., et al. (2019). A Genomic View of the Reef-Building Coral *Porites Lutea* and its Microbial Symbionts. *Nat. Microbiol.* 4, 2090–2100. doi: 10.1038/s41564-019-0532-4
- Rohwer, F., Seguritan, V., Azam, F., and Knowlton, N. (2002). Diversity and Distribution of Coral-Associated Bacteria. *Marine. Ecol. Prog. Ser.* 243, 1–10. doi: 10.3354/meps243001
- Ruiz-Gonzalez, C., Simo, R., Sommaruga, R., and Gasol, J. M. (2013). Away From Darkness: A Review on the Effects of Solar Radiation on Heterotrophic Bacterioplankton Activity. *Front. Microbiol.* 4. doi: 10.3389/fmicb.2013.00131
- Rybniker, J., Pojer, F., Marienhagen, J., Kolly, G. S., Chen, J. M., van Gumpel, E., et al. (2014). The Cysteine Desulfurase IscS of Mycobacterium Tuberculosis is Involved in Iron–Sulfur Cluster Biogenesis and Oxidative Stress Defence. *Biochem. J.* 459, 467–478. doi: 10.1042/BJ20130732
- Salgado, P., Kiene, R., Wiebe, W., and Magalhães, C. (2014). Salinity as a Regulator of DMSP Degradation in *Ruegeria Pomeroyi* DSS-3. *J. Microbiol.* 52, 948–954. doi: 10.1007/s12275-014-4409-1
- Schwarz, G. (1978). Estimating the Dimension of a Model. *Ann. Stat* 6, 461–464. doi: 10.1214/aos/1176344136
- Seemann, T. (2014). Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* 30, 2068–2069. doi: 10.1093/bioinformatics/btu153
- Seymour, J. R., Simó, R., Ahmed, T., and Stocker, R. (2010). Chemoattraction to Dimethylsulfoniopropionate Throughout the Marine Microbial Food Web. *Science* 329, 342–345. doi: 10.1126/science.1188418
- Sharp, K. H., Sneed, J. M., Ritchie, K. B., McDaniel, L., and Paul, V. J. (2015). Induction of Larval Settlement in the Reef Coral *Porites Astreoides* by a Cultivated Marine *Roseobacter* Strain. *Biol. Bull.* 228, 98–107. doi: 10.1086/BBLv228n2p98
- Sievert, S. M., Kiene, R. P., and Schulz-Vogt, H. N. (2007). The Sulfur Cycle. *Oceanography* 20, 117–123. doi: 10.5670/oceanog.2007.55
- Slezak, D., and Herndl, G. J. (2003). Effects of Ultraviolet and Visible Radiation on the Cellular Concentrations of Dimethylsulfoniopropionate (DMSP) in *Emiliania Huxleyi* (Strain L). *Marine. Ecol. Prog. Ser.* 246, 61–71. doi: 10.3354/meps246061
- Stefels, J. (2000). Physiological Aspects of the Production and Conversion of DMSP in Marine Algae and Higher Plants. *J. Sea. Res.* 43, 183–197. doi: 10.1016/S1385-1101(00)00030-7
- Sunda, W., Kieber, D. J., Kiene, R. P., and Huntsman, S. (2002). An Antioxidant Function for DMSP and DMS in Marine Algae. *Nature* 418, 317–320. doi: 10.1038/nature00851
- Tandon, K., Lu, C.-Y., Chiang, P.-W., Wada, N., Yang, S.-H., Chan, Y.-F., et al. (2020). Comparative Genomics: Dominant Coral-Bacterium *Endozoicomonas* Acroporae Metabolizes Dimethylsulfoniopropionate (DMSP). *ISME. J.* 14, 1290–1303. doi: 10.1038/s41396-020-0610-x
- Tapiolas, D. M., Raina, J.-B., Lutz, A., Willis, B. L., and Motti, C. A. (2013). Direct Measurement of Dimethylsulfoniopropionate (DMSP) in Reef-Building Corals Using Quantitative Nuclear Magnetic Resonance (qNMR) Spectroscopy. *J. Exp. Marine. Biol. Ecol.* 443, 85–89. doi: 10.1016/j.jembe.2013.02.037
- Trossat, C., Rathinasabapathi, B., Weretilnyk, E. A., Shen, T.-L., Huang, Z.-H., Gage, D. A., et al. (1998). Salinity Promotes Accumulation of 3-Dimethylsulfoniopropionate and Its Precursor S-Methylmethionine in Chloroplasts. *Plant Physiol.* 116, 165–171. doi: 10.1104/pp.116.1.165
- Vairavamurthy, A., Andreae, M. O., and Iverson, R. L. (1985). Biosynthesis of Dimethylsulfide and Dimethylpropiothetin by *Hymenomonas Carterae* in Relation to Sulfur Source and Salinity Variations. *Limnol. Oceanogr.* 30, 59–70. doi: 10.4319/lo.1985.30.1.0059
- Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., et al. (2014). Pilon: An Integrated Tool for Comprehensive Microbial Variant Detection and Genome Assembly Improvement. *PLoS One* 9, e112963. doi: 10.1371/journal.pone.0112963
- Wang, P., Cao, H.-Y., Chen, X.-L., Li, C.-Y., Li, P.-Y., Zhang, X.-Y., et al. (2017). Mechanistic Insight Into Acrylate Metabolism and Detoxification in Marine Dimethylsulfoniopropionate-Catabolizing Bacteria. *Mol. Microbiol.* 105, 674–688. doi: 10.1111/mmi.13727
- Williams, B. T., Cowles, K., Bermejo Martinez, A., Curson, A. R. J., Zheng, Y., Liu, J., et al. (2019). Bacteria are Important Dimethylsulfoniopropionate Producers in Coastal Sediments. *Nat. Microbiol.* 4, 1815–1825. doi: 10.1038/s41564-019-0527-1
- Yeo, W.-S., Lee, J.-H., Lee, K.-C., and Roe, J.-H. (2006). IscR Acts as an Activator in Response to Oxidative Stress for the Suf Operon Encoding Fe-S Assembly Proteins. *Mol. Microbiol.* 61, 206–218. doi: 10.1111/j.1365-2958.2006.05220.x
- Yu, G., Smith, D. K., Zhu, H., Guan, Y., and Lam, T. T.-Y. (2017). Ggtree: An R Package for Visualization and Annotation of Phylogenetic Trees With Their Covariates and Other Associated Data. *Methods Ecol. Evol.* 8, 28–36. doi: 10.1111/2041-210X.12628
- Zhang, X.-H., Liu, J., Liu, J., Yang, G., Xue, C.-X., Curson, A. R. J., et al. (2019). Biogenic Production of DMSP and its Degradation to DMS—their Roles in the Global Sulfur Cycle. *Sci. China Life Sci.* 62, 1296–1319. doi: 10.1007/s11427-018-9524-y
- Zhang, Y., Yang, Q., Zhang, Y., Ahmad, M., Ling, J., Tang, X., et al. (2021). Shifts in Abundance and Network Complexity of Coral Bacteria in Response to Elevated Ammonium Stress. *Sci. Total. Environ.* 768, 144631. doi: 10.1016/j.scitotenv.2020.144631
- Zheng, Y., Wang, J., Zhou, S., Zhang, Y., Liu, J., Xue, C.-X., et al. (2020). Bacteria are Important Dimethylsulfoniopropionate Producers in Marine Aphotic and High-Pressure Environments. *Nat. Commun.* 11, 4658. doi: 10.1038/s41467-020-18434-4

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

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

Copyright © 2022 Kuek, Motti, Zhang, Cooke, Todd, Miller, Bourne and Raina. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.