Dimethylsulphoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process

Andrew R. J. Curson[1], Ji Liu[1,2], Ana Bermejo Martínez[1], Robert T. Green[1], Yohan Chan[3], Ornella Carrión[1], Beth T. Williams[1], Sheng-Hui Zhang[4], Gui-Peng Yang[4,5], Philip C. Bulman Page[3], Xiao-Hua Zhang[2,5*], Jonathan D. Todd[1*]

[1]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK. [2]College of Marine Life Sciences, Ocean University of China, 5 Yushan Road, Qingdao 266003, China. [3]School of Chemistry, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK. [4]Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education; College of Chemistry and Chemical Engineering, Ocean University of China, Qingdao 266100, China. [5]Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China.

*corresponding authors

Dimethylsulphoniopropionate (DMSP) is one of the Earth's most abundant organosulphur molecules, a signalling molecule[1], a key nutrient for marine microorganisms[2,3], and the major precursor for gaseous dimethyl sulphide (DMS). DMS, another infochemical in signalling pathways[4], is important in global sulphur cycling[2], and affects the Earth's albedo, and potentially climate, via sulphate aerosol and cloud condensation nuclei production[5,6]. It was thought that only eukaryotes produce significant amounts of DMSP[7-9], but here we demonstrate that many marine heterotrophic bacteria also produce DMSP, likely using the same methionine (Met) transamination pathway as macroalgae and phytoplankton[10]. We identify the first DMSP synthesis gene in any organism, *dsyB*, which encodes the key methyltransferase enzyme of this pathway and is a reliable reporter for bacterial DMSP synthesis in marine alphaproteobacteria.

DMSP production and *dsyB* transcription are upregulated by increased salinity, nitrogen limitation and lower temperatures in our model DMSP-producing bacterium *Labrenzia aggregata* LZB033. With significant numbers of *dsyB* homologues in marine metagenomes, we propose that bacteria likely make a significant contribution to oceanic DMSP production. Furthermore, since DMSP production is not solely associated with obligate phototrophs, the process need not be confined to the photic zones of marine environments, and as such may have been underestimated.

Bacteria isolated from the East China Sea were screened for the ability to produce DMS from DMSP. Surprisingly, one isolate, the alphaproteobacterium *L. aggregata* LZB033 produced DMS (2.4 fmol DMS μg protein^{-1} min^{-1}) when no exogenous DMSP was supplied. We hypothesised that this bacterium might be producing DMSP, and confirmed this by gas chromatography (GC) and liquid chromatography-mass spectrometry (LC-MS) (Fig. 1). LZB033 grown in MBM minimal medium (no added methylated sulphur compounds) produced 99.8 pmol DMSP μg protein^{-1}, and an estimated intracellular concentration of 9.6 mM (Supplementary Table 1). This represents the first report of a heterotrophic bacterium producing DMSP.

Three pathways for DMSP biosynthesis from Met have been identified[10-13] (Fig. 1a), a transamination pathway in marine algae, a methylation pathway in angiosperms and a decarboxylation pathway in one dinoflagellate. Growth in the presence of all transamination pathway intermediates, namely Met, 4-methylthio-2-oxobutyrate (MTOB), 4-methylthio-2-hydroxybutyrate (MTHB) and 4-dimethylsulphonio-2-hydroxybutyrate (DMSHB), significantly enhanced DMSP production (8 to 14-fold) by LZB033 (Fig. 1b). Conversely, methylation pathway intermediates, DMSP-amine and *S*-methylmethionine (SMM), and the decarboxylation pathway intermediate 3-methylthiopropylamine (MTPA) had much less

significant effects on DMSP accumulation in LZB033 (Fig. 1b), suggesting that these molecules are less likely to be bacterial DMSP synthesis intermediates. Cell extracts of LZB033 grown with either 0.5 mM Met (Fig. 1c) or MTOB were shown by LC-MS to accumulate MTHB (30.9 pmol µg protein^{-1} with Met). However, no MTOB was detected when LZB033 was grown with Met, and no DMSHB was detected when LZB033 was grown with Met, MTOB or MTHB, possibly due to the instability and/or low pool sizes of MTOB and DMSHB. Furthermore, LZB033 cell extracts displayed Met aminotransferase (MAT) activity, yielding MTOB from Met, and MTOB reductase (MR) activity, yielding MTHB from MTOB, based on the detection of reaction products by LC-MS. Therefore, we propose that LZB033 most likely uses the transamination pathway to synthesise DMSP, and although addition of pathway intermediates enhances production, this bacterium is able to produce DMSP without addition of methylated sulphur compounds (Supplementary Table 1), presumably through *de novo* production of Met[14].

Previous work on algae predicted that the methylation of MTHB to DMSHB, catalysed by the MTHB methyltransferase (MHM) enzyme, would be the rate-limiting and committing step in the transamination DMSP synthesis pathway[10,15,16]. Summers *et al*.[15] provided further evidence for MHM being the key enzyme of the Met transamination synthesis pathway by showing that MAT and MR enzyme activities exist, although at reduced levels, in non-DMSP-producing algae, whereas MHM activity is specific to DMSP producers[15]. Similarly, we find that the terrestrial alphaproteobacterium *Rhizobium leguminosarum* J391 produced MTHB when provided with 0.5 mM Met or MTOB, but did not convert MTHB to DMSHB or DMSP when grown in the presence of Met, MTOB or MTHB (Supplementary Fig. 1). Furthermore, *R. leguminosarum* was also able to produce DMSP from DMSHB, indicating that it also has DMSHB decarboxylase (DDC) activity (Supplementary Fig. 1). Therefore, although *R. leguminosarum* does not produce DMSP, it has enzymes that can catalyse every step of the

transamination pathway except the MHM reaction. These data, with previous algal work, indicate that MHM likely catalyses the key step of the transamination pathway, and possibly the step that is specific to DMSP synthesis.

We tested the ability of LZB033 to catabolise DMSP (5 mM) and found it cleaved DMSP, generating DMS and acrylate, with a production rate of 5.4 ± 0.2 pmol DMS µg protein^{-1} min^{-1}. The LZB033 genome encodes a DddL DMSP lyase, 66% identical to *Sulfitobacter* sp. EE36 DddL[17]. When expressed in *Escherichia coli*, the LZB033 DddL had DMSP lyase activity (57.9 ± 5.5 pmol DMS µg protein^{-1} min^{-1}) and a *dddL* knockout mutation completely abolished the DMSP lyase activity of LZB033, indicating that DddL is likely the only DMSP lyase in this strain.

To identify the gene encoding MHM activity, a *L. aggregata* genomic library was screened in the heterologous host *R. leguminosarum* J391 for clones conferring the ability to convert MTHB to DMSHB, and ultimately DMSP, since J391 has DDC activity (see above). One such cosmid (pBIO2252) was identified and sequenced. The pBIO2252 cosmid contained a gene, termed *dsyB,* encoding a methyltransferase-like protein (SIAM614_21095) that conferred DMSP production when subcloned and expressed in *R. leguminosarum* (Fig. 1c and Supplementary Table 1). Furthermore, *R. leguminosarum* containing cloned *dsyB* produced DMSP when grown in minimal media alone, but at increased levels when supplemented with Met, MTOB or MTHB (Supplementary Table 1). This shows that the acquisition of a single gene, such as *dsyB,* can enable some bacteria to produce DMSP and indicates the potential for DMSP production to become more widespread, especially in 'stressful' environments where there might be selective pressure for this to occur.

The *dsyB* gene was disrupted in the LZB033 genome to determine its function in DMSP synthesis. The *dsyB*$^{-}$ mutant no longer produced DMSP (Fig. 1c and Supplementary Table 1),

but complementation with cloned *dsyB* restored wild type DMSP production levels. Furthermore, the *dsyB*$^-$ mutant accumulated ~3 fold more MTHB (92.8 pmol µg protein^{-1}) than the wild type strain when grown with 0.5 mM Met (Fig. 1c), consistent with MTHB being the DsyB substrate. Thus, *dsyB* is required for DMSP synthesis in LZB033, and is the first DMSP biosynthesis gene to be reported in any organism.

The LZB033 DsyB protein belongs to a family of *S*-adenosyl methionine-dependent methyltransferases (Pfam family 00891). Close homologues (≥ 39% identity, E value ≤ 1e^{-67}) exist in >50 sequenced alphaproteobacteria, the majority of which were isolated from hypersaline or marine environments. Most of these bacteria are, like *Labrenzia*, in the Rhodobacterales order that are abundant in marine environments[18], but a few homologues are also in the Rhizobiales and Rhodospirillales orders. These DsyB-like proteins in alphaproteobacteria form a distinct group (Fig. 2), with a decline in sequence homology to more distantly related proteins (≤ 33% identity, E value ≥ 2e^{-53}), which are not predicted to be functional DsyB enzymes and are found in more taxonomically distinct bacteria, such as Firmicutes and Actinobacteria.

Representative bacteria with DsyB homologues were all shown to produce DMSP (Supplementary Table 1; Fig. 2), whilst negative control strains that contain no proteins with significant similarity to DsyB or that contain DsyB-like proteins of lower sequence homology (Fig. 2) did not produce DMSP. Furthermore, when cloned and expressed in *R. leguminosarum*, the *dsyB* genes from *Oceanicola batsensis, Pelagibaca bermudensis* and *Amorphus coralli* conferred DMSP production from MTHB (Supplementary Table 1), whereas the less closely related *dsyB*-like gene from *Streptomyces varsoviensis* conferred no detectable activity, as predicted. Thus, the presence of the *dsyB* gene in a bacterial genome is a marker for the ability to synthesise DMSP.

In many bacteria containing *dsyB*, the gene is located adjacent to genes that have either not been characterised or have no known connection to sulphur metabolism or processes related to any reported function of DMSP (Supplementary Fig. 2a). There are exceptions to this, notably in ten Rhodobacterales strains where *dsyB* is closely associated to a predicted *isc*/*suf* gene cluster (Supplementary Fig. 2b). These genes encode proteins involved in Fe-S cluster assembly, which plays a role in oxidative stress protection[19]. *Escherichia coli suf* gene transcription is induced by reactive oxygen species, such as H_2O_2[19]. In another strain, Rhizobiales bacterium HL-109, *dsyB* is downstream of two genes encoding peroxiredoxins (EC 1.11.1.15), which are a family of antioxidant enzymes[20] (Supplementary Fig. 2c). The linkage of *dsyB* to genes potentially involved in oxidative stress protection supports previous hypotheses that DMSP has a role in oxidative stress protection[21].

To identify the potential function/s of DMSP and/or its lysis to DMS and acrylate in *L. aggregata* LZB033, DMSP production and the transcription of the *dsyB* and *dddL* genes were monitored in response to different environmental stimuli or growth conditions that had previously been linked to DMSP synthesis or function in other DMSP-producers[21-23]. Increased DMSP production and *dsyB* transcription levels were observed, to varying degrees, in response to increased salinity, decreased nitrogen, decreased temperature and in stationary phase compared to exponential growth phase (Fig. 3a-b), consistent with findings in previously studied DMSP-producing organisms[22]. Notably, DMSP levels were barely detectable in LZB033 grown under low salinity, where *dsyB* transcription was also downregulated. Although there was a small (~2.5-fold) increase in *dsyB* transcription in response to oxidative stress, there was no significant increase in DMSP production under the same condition. It is possible that DMSP production actually does increase in response to oxidative stress, but that its degradation, possibly by reactions with radicals, increases too. Transcription of *dddL* was significantly reduced under all the conditions that enhanced DMSP accumulation, namely high

146 salinity, nitrogen limitation, decreased temperature and stationary phase growth (Fig. 3c),
147 consistent with DMSP lyase activity being downregulated when DMSP is needed in higher
148 amounts. The transcription of this DMSP lyase gene was also reduced under low salinity (Fig.
149 3c), conditions where DMSP is not produced at detectable levels and thus there is no need for
150 DMSP lyase activity.

151 With nitrogen limitation having the greatest effect on DMSP production in LZB033, we also
152 tested DMSP production by *L. aggregata* IAM12614 in low nitrogen medium. This resulted in
153 a 9-fold increase in *L. aggregata* IAM12614 intracellular DMSP (Supplementary Table 1).
154 Given that marine environments are normally nitrogen-limited, the higher levels of DMSP
155 produced by LZB033 and IAM12614 in low nitrogen medium are probably more similar to the
156 amounts that they would produce in the environment.

157 To further assess the role/s of DMSP and its cleavage to DMS and acrylate in LZB033, we
158 assayed the effects of environmental stimuli and other phenotypic arrays on the growth of the
159 *dsyB*$^{-}$ mutant J571 and *dddL*$^{-}$ mutant J572 in comparison to the LZB033 wild type (see
160 Methods). Surprisingly, we did not observe significant differences in growth phenotype
161 between the wild type, *dsyB*$^{-}$ or *dddL*$^{-}$ mutants under varying conditions of salinity,
162 temperature, oxidative stress, nitrogen supply, in response to freezing, or combinations of these
163 conditions. Thus, contrary to previously identified or predicted functions of DMSP in
164 eukaryotes, our studies show that *de novo* DMSP synthesis does not appear to have a major
165 role in osmoprotection, cryoprotection, protection against thermal stress, oxidative stress, or
166 under conditions where nitrogen is limiting, despite both DMSP production and *dsyB*
167 expression being affected by many of these conditions. It is possible that DMSP has one or
168 more of these predicted functions, in LZB033 or other DMSP-producing bacteria, under

specific environmental conditions that were not replicated here; or that it has only a minor role in these functions and its loss is compensated for by other processes.

The environmental abundance of *dsyB*, and the likely proportion of bacteria capable of DMSP synthesis, was estimated by searching metagenomic datasets (Supplementary Table 2). DsyB protein-encoding genes were predicted to be present in up to 0.5% of bacteria sampled in the *Tara* Oceans and global ocean sampling (GOS) marine metagenomes, but were absent in terrestrial metagenomes (albeit terrestrial datasets being much smaller than marine counterparts; Supplementary Table 2). The *dsyB* abundance in marine and absence in non-marine environments is reminiscent of the bacterial *ddd*, DMSP lyase, genes[24], and was expected since DMSP is thought to be predominantly of marine origin.

Identification of DMSP production in bacteria and the first DMSP biosynthesis gene, likely encoding the key step in the pathway, will greatly improve our ability to model and monitor environmental DMSP production and the climate-active gas DMS. The bacterial contribution to global DMSP production, and the associated DMS production, has not previously been considered. Whilst this contribution is currently unknown, it is possible that prokaryotes make a significant input to the pools of these important environmental sulphur compounds, beyond bacterial DMS production from dissolved DMSP[3]. Furthermore, since *dsyB*-containing bacteria are heterotrophic, there is no requirement for DMSP production to be confined to the photic zone of marine environments, as was thought to be the case with many known obligate phototrophic DMSP-producing organisms. It will be interesting to study the significance of bacterial DMSP production across a broad range of marine environments – from deep sea sediments to pelagic zones.

Methods

Bacterial growth and media

Labrenzia aggregata, *Oceanicola batsensis*, *Sagittula stellata*, *Pelagibaca bermudensis* and *Amorphus coralli* were grown in YTSS[25] complete medium or MBM[26] minimal medium (with 10 mM succinate as carbon source and 10 mM NH_4Cl as nitrogen source) at 30 °C. Where indicated, the salinity of MBM was adjusted by altering the amount of sea salts (Sigma-Aldrich) added and nitrogen levels were altered by adding different amounts of NH_4Cl as nitrogen source. Methylated sulphur compounds, namely DMSP pathway intermediates, were only added to MBM where indicated, in experiments that specifically addressed the effect of adding such compounds. *Thalassobaculum salexigens* and *Nesiotobacter exalbescens* were grown in marine broth 2216 (Becton Dickinson, BD), *Ruegeria pomeroyi* in YTSS and *Sediminimonas qiaohouensis* in marine broth 2216 + 3% NaCl, all at 28 °C. *Streptomyces varsoviensis* and *Bacillus mycoides* were grown in GYM *Streptomyces* medium (4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g calcium carbonate, 12 g agar per litre distilled water) and nutrient broth (5 g peptone, 3 g meat extract, 15 g agar per litre distilled water) respectively at 25 °C. *Escherichia coli* was grown in LB[27] complete medium at 37 °C. *Rhizobium leguminosarum* was grown in TY[28] complete medium or Y[28] minimal medium (with 10 mM succinate as carbon source and 10 mM NH_4Cl as nitrogen source) at 28 °C. Where necessary, antibiotics were added to media at the following concentrations: rifampicin (20 μg ml^{-1}), streptomycin (400 μg ml^{-1}), kanamycin (20 μg ml^{-1}), spectinomycin (200 μg ml^{-1}), gentamicin (20 μg ml^{-1}). Strains used in this study are listed in Supplementary Table 3.

Isolation of *Labrenzia aggregata* LZB033

L. aggregata LZB033 was isolated from seawater of the East China Sea (collected in October 2013) on marine agar 2216 (BD).

General *in vivo* and *in vitro* genetic manipulations

Plasmids (Supplementary Table 4) were transferred to *E. coli* by transformation, or *L. aggregata* LZB033 and *R. leguminosarum* J391 by conjugation using helper plasmid pRK2013[29]. Bacterial genomic DNA was isolated using a Qiagen genomic kit. Routine restriction digestions, ligations, Southern blotting and hybridisations were performed essentially as in Downie *et al*.[30]. The oligonucleotide primers used for molecular cloning were synthesised by Eurofins Genomics and are detailed in Supplementary Table 5. Sequencing of plasmids and PCR products was performed by Eurofins Genomics.

L. aggregata LZB033 *dddL* was amplified from genomic DNA by PCR and cloned into pET21a using *Nde*I and *Bam*HI restriction enzymes. The *dsyB* genes from *L. aggregata* IAM12614 and *O. batsensis* were amplified from genomic DNA and cloned into pET21a using *Nde*I and *Bam*HI or *Eco*RI restriction enzymes. These clones were then digested with *Nde*I and *Sac*I and subcloned into pLMB509[31], a taurine-inducible plasmid for the expression of genes in *Rhizobium*. The *dsyB* genes from *A. coralli*, *P. bermudensis* and *S. varsoviensis* were amplified by PCR and cloned directly into pLMB509 using the primers and restriction enzymes shown in Supplementary Table 6. The *dsyB* gene from *L. aggregata* IAM12614 was subcloned from the pLMB509-based clone pBIO2258 into the wide host-range plasmid vector pRK415[32] using the restriction enzymes *Xba*I and *Eco*RI. All plasmid clones are described in Supplementary Table 4.

Library construction and cosmid screening

A genomic library of *L. aggregata* IAM12614 was constructed essentially as described in Curson *et al.*[17]. *L. aggregata* genomic DNA was partially digested with *Eco*RI, ligated into the wide host-range cosmid vector pLAFR3 and transfected into *E. coli* strain 803, to form a library with an estimated 90,000 clones. The clones were transferred *en masse* to *R. leguminosarum* J391 by conjugation. 200 transconjugants were picked to MBM medium containing 0.5 mM MTHB and screened by gas chromatography (see below) for those containing DMSP (as a result of *Rhizobium* DDC activity).

Sequencing of *Labrenzia aggregata* LZB033 genomic DNA

Genomic DNA of *L. aggregata* LZB033 was extracted using an E.Z.N.A.® Bacterial DNA kit (Omega). Genome sequencing was performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd (China) using the Illumina HiSeq 2000^{TM} sequencer system with a 500 bp pair-end library. The reads were assembled using SOAPdenovo v2.04. Putative genes were identified using Glimmer 3.02[33]. Annotation was performed with BLAST+ 2.2.24[34] searching against databases, including the National Center for Biotechnology Information (NCBI) non-redundant proteins (NR)[35], Clusters of Orthologous Groups of proteins (COG)[36], Kyoto encyclopedia of genes and genomes (KEGG)[37] and Gene ontology (GO)[38].

Mutagenesis of *dsyB* and *dddL*

Primers were designed (Supplementary Table 5) to amplify sequences internal to the *L. aggregata* LZB033 *dsyB* and *dddL* open reading frames and these were cloned into

pBIO1879[39], a derivative of the suicide vector pK19*mob*[40], to form pBIO2253 and pBIO2254 respectively, and then transferred to *L. aggregata* J570 by conjugation. Mutants in which the plasmids had recombined in the target genes were selected for by growth on YTSS agar containing rifampicin (J570), kanamycin (pBIO1879) and spectinomycin (pBIO1879). All mutants were checked by colony PCR and/or Southern blotting.

Phenotyping of *Labrenzia* mutants

Where MBM was used as the minimal medium for the following experiments, this medium lacked any methylated sulphur DMSP pathway intermediates, unless indicated. To identify a phenotype for the mutations in *dsyB* or *dddL*, *L. aggregata* J570 (wild type), J571 (*dsyB*$^-$) and J572 (*dddL*$^-$) strains were grown in MBM with varying levels of salt and nitrogen, or under different environmental conditions. To test the effect of salinity on the mutant, the wild type and mutant strains were grown in MBM minimal medium made with different amounts of sea salts (Sigma-Aldrich) equivalent to salinities of 5, 35 and 50 practical salinity units (PSU), with 35 being the approximate salinity level of seawater, and growth of the strains was monitored spectrophotometrically by optical density at wavelength 600 nm (OD_{600}). To test the effect of nitrogen levels, the strains were grown in modified 35 PSU MBM, made without added nitrogen source, and then supplemented with either 10, 0.5 or 0.05 mM NH_4Cl as nitrogen source, and growth was monitored by OD_{600}. To test the temperature effect, strains were grown in 35 PSU MBM at temperatures of 16, 26, 30 and 37 °C and growth was monitored by OD_{600}. To test tolerance to freezing, cultures of the wild type and mutant strains were grown to stationary phase in 35 PSU MBM (0.5 mM NH_4Cl) then adjusted to the same cell density by measuring the OD_{600} of each culture, spinning down an appropriate volume (~1 ml) of culture and then resuspending the cells in 1 ml of the same medium. 100 µl of each culture was then

284 removed, used to make serial dilutions, and then plated on YTSS agar plates for counting the
285 number of colonies that grew. The remaining 900 µl of culture was placed at -20 °C for 5 days
286 before thawing, serial dilution and plating as above. For testing resistance to oxidative stress
287 in the wild type (J570), *dsyB*$^{-}$ (J571) and *dddL*$^{-}$ (J572) mutant strains, 50 µl of MBM (with 0.1
288 mM Met and with 0.5 mM NH_4Cl as nitrogen source, to increase DMSP production) cultures
289 of each strain, grown overnight, were adjusted to the same cell density and inoculated to 5 ml
290 MBM ± 500 µM H_2O_2. Cultures were incubated at 30 ˚C for 90 minutes then 1.5 µl of a 10%
291 solution of the antioxidant sodium thiosulphate was added to quench the oxidative stress by
292 reducing the H_2O_2. 100 µl of each culture was removed and used to make serial dilutions,
293 dilutions were plated on YTSS agar medium and incubated at 30 ˚C, and colony numbers were
294 determined after 2-3 days growth and used to calculate the percentage of cell survival for the
295 different strains after exposure to H_2O_2. For experiments involving combinations of different
296 conditions (salinity [50 PSU], nitrogen [0.5 mM NH_4Cl] and temperature [16 °C]; salinity [35
297 PSU], nitrogen [0.5 mM NH_4Cl], temperature [30 °C] and oxidative stress [500 µM H_2O_2]),
298 growth under these conditions was monitored by OD_{600} as above. All experiments described
299 here used three biological replicates for each condition.

300 To identify other possible phenotypes of the mutants of *dsyB*$^{-}$ and *dddL*$^{-}$, tests for an array of
301 carbon sources and biochemical properties of the wild type and mutants of *L. aggregata* were
302 investigated by using a GN2 MicroPlate kit (Biolog), and API 20E and API 20NE strips
303 (bioMérieux) according to the manufacturers' instructions, except that strains were first grown
304 on MBM agar, and sterile seawater was used to prepare the inocula. In addition, some
305 independent tests including enzyme activities (protease, DNase, lipase, amylase, chitinase,
306 agarase and cellulase)[41] and quorum quenching activities[42] were performed.

307

Quantification of DMS and DMSP by gas chromatography

All gas chromatography (GC) assays involved measurement of headspace DMS, either directly produced or via alkaline lysis of DMSP, in 2 ml vials containing 0.3 ml liquid samples, using a flame photometric detector (Agilent 7890A GC fitted with a 7693 autosampler) and a HP-INNOWax 30 m x 0.320 mm capillary column (Agilent Technologies J&W Scientific). An eight point calibration curve of DMS standards was used and the detection limit for headspace DMS was 0.015 nmol. For DMS production, *Labrenzia* strains were grown overnight in YTSS medium, adjusted to equal optical density (OD_{600}), washed three times with 35 PSU MBM minimal medium then diluted 1:10 into 35 PSU MBM medium containing 5 mM DMSP (or with no DMSP added) and incubated for 2 hours at 30 °C before assaying by GC. For DddL DMSP lyase expressed in *E. coli*, an overnight culture of cells in LB were diluted 1:100 in LB medium and incubated at 37 °C for two hours then induced with 0.2 mM isopropyl β-D-1-thiogalactopyranoside and incubated for a further 16 hours at 37 °C. Cells were then pelleted, resuspended in Tris-HCl buffer (50 mM, pH 7) and sonicated (3 x 10 s) with a Markson GE50 Ultrasonic Processor. Samples were spun for 5 min at 18,000 x g to remove cell debris, then a sample of the cell-free extract was added to vials with 1 mM DMSP and incubated for an appropriate time (30 min to 2 hours) before assaying by GC. For DMSP measurements in *L. aggregata* LZB033, cultures were grown in complete medium, washed three times in MBM minimal medium then diluted 1:100 in MBM medium, with different additions as indicated and under different temperatures as indicated, and incubated for the required time, dependent on growth conditions (typically 16 hours, but 22 hours for stationary phase samples and 72 hours for 16 °C samples). For H_2O_2 additions, 500 µM H_2O_2 was included in media before inoculation then cultures were spiked with more H_2O_2 (500 µM) and cells were harvested 30 minutes to 3 hours later for DMSP measurement. For all other bacterial strains, cells were cultured in media as detailed in Supplementary Table 1. For all strains where MBM or Y

minimal medium was used, these media lacked any methylated sulphur DMSP pathway intermediates, unless indicated. For *Rhizobium* cultures, 200 µl of culture was added to a vial then 100 µl of 10 M NaOH was added to lyse the DMSP, and vials were immediately sealed and incubated at 30 °C for 6 h (to allow release of DMS into the headspace) before assaying by GC. Following growth of all other bacterial strains in the relevant media and under the specified conditions, 20-40 ml of culture was centrifuged to pellet the cells and cells were resuspended in 1 ml Tris-HCl buffer (50 mM, pH 7) and sonicated and spun down (as above). 200 µl of cell-free extract was added to a vial then 100 µl of 10 M NaOH was added to lyse the DMSP, and vials were immediately sealed and incubated at 30 °C for 6 hours (to allow release of DMS into the headspace) before assaying by GC. All experiments described here used three biological replicates. The protein content in the cells was estimated by the Bradford method (BioRad). DMS production is expressed as pmol µg protein^{-1} min^{-1} and DMSP production typically as pmol µg protein^{-1}. Estimated intracellular concentrations of DMSP (expressed in mM) are based on estimates of protein content per cell[43] and reported cell volumes[44-51].

MTHB methyltransferase (MHM) assays

To measure MHM activity from pLMB509 clones expressing the *dsyB* gene from different bacteria in *R. leguminosarum* J391, cultures were grown overnight in TY complete medium (three biological replicates), 1 ml of culture was spun down, resuspended in the same volume of Y medium and then diluted 1:100 into 5 ml Y with 5 mM taurine (to induce expression), 0.5 mM MTHB and gentamycin, and incubated at 28 °C for 20 hours. 200 µl of culture was then added to a 2 ml vial with 100 µl 10 M NaOH and monitored by GC assay (see above), with MHM activity expressed as pmol DMSP µg protein^{-1}, assuming that all the DMSP is derived from DMSHB through DDC activity.

Met aminotransferase (MAT) and MTOB reductase (MR) assays

LZB033 cultures were grown in 100 ml MBM medium cultures (three biological replicates) to an OD_{600} of ~0.5 then 40 ml of culture was centrifuged and the supernatant removed. Cell pellets were then resuspended in 1 ml of LC-MS grade water and sonicated (3 x 10 s) with a Markson GE50 Ultrasonic Processor. Samples were spun for 5 min at 18,000 x g to remove cell debris and the cell-free extract was retained for the enzyme assays. Assays for these enzymes have been reported previously in algae[15] but were modified here and are described as follows. All chemical solutions were dissolved in LC-MS grade water and reactions were performed in 60 µl volumes. For MAT assays, reactions included 54 µl LZB033 cell-free extract (prepared as described above), 3 µl 0.1 M oxoglutarate (5 mM final concentration) and 3 µl 10 mM Met (0.5 mM). For MR assays, reactions included 51 µl LZB033 cell-free extract, 6 µl 10 mM NADPH (1 mM) and 3 µl 10 mM MTOB (0.5 mM). For no substrate controls, Met or MTOB were replaced with LC-MS grade water. Assays were started by the addition of substrate and incubated at 22 ºC for 1 hour. Reactions were stopped by the addition of 240 µl 100% LC-MS grade acetonitrile to give samples in 80% acetonitrile for use in LC-MS (see below).

Chemical syntheses

DMSP was synthesised from DMS (Sigma-Aldrich) and acrylic acid (Sigma-Aldrich) as described in[52]. DMSHB was synthesised as follows. The calcium salt of 2-hydroxy-4-(methylthio)butyric acid (5.0 g, 14.77 mmol) was dissolved in 6 N HCl (500 ml). The aqueous layer was extracted using ethyl acetate (3 x 75 ml). The organic layers were combined, washed with brine and evaporated under reduced pressure to give the acid as a yellow oil (1.4 g, 63%).

2-Hydroxy-4-(methylthio)butyric acid (0.70 g, 4.66 mmol) was dissolved in methanol (15 ml). Methyl iodide (0.4 ml, 6.99 mmol, 1.5 equiv.) was added and the solution was heated under reflux for 2 hours. The reaction mixture was allowed to reach room temperature and the solvent was removed under reduced pressure to yield DMSHB iodide (0.65 g, 95%). DMSP-amine was synthesised as follows. 3-Methylmercaptopropylamine (1 g, 9.51 mmol) was added to aqueous formic acid (89%, 10 ml) in a 25 ml round-bottomed flask wrapped in foil at 0 ºC. Glacial acetic acid (1 ml) and methyl iodide (0.50 ml, 8 mmol) were added and the resulting mixture stirred for 3 days. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in water (5 ml) and the solution transferred to a separating funnel. The aqueous layer was washed with dichloromethane (2 x 10 ml) and a solution of sodium hydroxide (1 M) was added to bring the pH to neutral. The solvents were removed under reduced pressure and the residue was dissolved in the minimum amount of methanol. Diethyl ether was added to the solution until no further precipitation occurred. The solution was cooled to 0 °C overnight. The precipitate (inorganic salts) was removed by suction filtration and washed with diethyl ether. The filtrate was concentrated under reduced pressure to yield DMSP-amine as a yellow oil (0.8 g, 73%). SMM was synthesised as follows. (L)-Methionine (1.0 g, 6.7 mmol) was dissolved in aqueous formic acid (89%, 10 ml) in a 50 ml round-bottomed flask wrapped in foil. Glacial acetic acid (5 ml) and methyl iodide (1.7 ml, 26.8 mmol) were added and the resulting mixture stirred for 3 days. The reaction mixture was concentrated under reduced pressure. The residue was recrystallised from methanol/diethyl ether. The resulting solid was filtered, washed once with methanol and twice with diethyl ether to yield SMM as the title compound (1.75 g, 90%). Met, MTOB, MTHB and MTPA are commercially available and were obtained from Sigma-Aldrich.

Liquid chromatography-mass spectrometry (LC-MS) analysis

406 The *L. aggregata* LZB033 wild type (J570) and *dsyB*$^-$ mutant (J571) were inoculated 1:100
407 into 5 ml 35 PSU MBM and incubated for 20 hours before the addition of 0.5 mM Met, MTOB
408 or MTHB. After incubation for a further 2 hours, 1.5 ml of culture was aliquoted for LC-MS
409 analysis and 300 µl of culture was used for protein estimation. *Rhizobium* wild type (J391) was
410 inoculated 1:100 into 5 ml Y minimal medium with 0.5 mM Met, MTOB, MTHB, DMSHB or
411 with nothing added. J391 containing cloned *L. aggregata dsyB* (pBIO2258) was inoculated
412 1:100 into 5 ml Y minimal medium with 10 mM taurine (for inducing expression) and
413 gentamicin, and supplemented with 0.5 mM Met or with no addition. *Rhizobium* cultures were
414 incubated with shaking for 48 hours, then for each culture, 3 ml was aliquoted for LC-MS
415 analysis and 300 µl for protein estimation. Culture aliquots (in duplicate) for LC-MS analysis
416 were centrifuged for 4 minutes at 18,000 x g, flash frozen in liquid nitrogen and stored at -80
417 ºC until sample preparation. All cultures described here were grown with three biological
418 replicates. Protein concentrations were estimated by Bradford assays.

419 Samples were extracted as follows. Frozen pellets were resuspended with 300 µl of 80% LC-
420 MS grade acetonitrile (extraction solvent), centrifuged at 18,000 x g for 3 minutes and 200 µl
421 of the supernatant was collected. For a second round of extraction, another 200 µl of the
422 extraction solvent was then added to the pellet and remaining supernatant, and the pellet was
423 resuspended before centrifugation at 18,000 x g for 3 minutes and another 200 µl of supernatant
424 was collected. A third round of extraction was then performed in the same way to give a total
425 volume of 600 µl of the collected supernatant for LC-MS analysis.

426 LC-MS was carried out using a Shimadzu Ultra High Performance Liquid Chromatography
427 (UHPLC) system formed by a Nexera X2 LC-30AD Pump, a Nexera X2 SIL-30AC
428 Autosampler, a Prominence CTO-20AC Column oven, and a Prominence SPD-M20A Diode
429 array detector; and a Shimadzu LCMS-2020 Single Quadrupole Liquid Chromatograph Mass

Spectrometer. Samples were analysed in hydrophilic interaction chromatography (HILIC) mode using a Phenomenex Luna NH2 column (100 x 2 mm with a particle size of 3 µm) at pH 3.75. Mass spectrometry spray chamber conditions were capillary voltage 1.25 kV, oven temperature 30 ºC, desolvation temperature 250 ºC and nebulising gas flow 1.50 L min^{-1}. Solvent A is 5% acetonitrile + 95% 5 mM ammonium formate in water. Solvent B is 95% acetonitrile + 5% 100 mM ammonium formate in water. Flow rate was 0.6 ml min^{-1} and gradient (% solvent A/B) was t = 1 min, 100% B; t = 3.5 min, 70% B; t = 4.1 min, 58% B; t = 4.6 min, 50% B; t = 6.5 min, 100% B; t = 10 min, 100% B. The injection volume was 15 µl. All samples were analysed immediately after being extracted. Samples were run in positive and negative mode in a single run. The targeted mass transitions corresponded to $[M+H]^+$ of methionine (m/z 150), DMSHB (m/z 165) and DMSP (m/z 135) in positive mode; and $[M-H]^-$ of MTOB (m/z 147) and MTHB (m/z 149) in negative mode. A calibration curve was performed for quantification of MTHB. The compounds present in the biological samples were ratified by the addition of the corresponding standards (20 µM) to the sample, and correct peaks remained as single peaks, with the same shape but increased ion intensity.

qRT-PCR

L. aggregata LZB033 wild type (J570) was inoculated to 100 ml MBM cultures (lacking methylated sulphur DMSP pathway intermediates) and incubated with shaking, with different cultures having different additions or under different growth conditions depending on the stress condition being tested. Standard conditions were salinity of 35 PSU, nitrogen source concentration of 10 mM NH_4Cl, incubation temperature of 30 °C and sampling in exponential phase growth (OD_{600} of ~0.5). Cultures were exposed to different stress conditions: low salt (5 PSU), high salt (50 PSU), stationary phase (OD_{600} of ~1.0); nitrogen limitation (0.5 mM

454 NH_4Cl), oxidative stress (grown for 16 hours and spiked with 500 uM H_2O_2 30 minutes before
455 sampling), and low temperature (16 °C). For each condition, there were three biological
456 replicates.

457 For each culture, RNA for qRT-PCR was extracted as follows: 40 ml of phenol-ethanol
458 (10/90% v/v) was added to the 100 ml culture and then incubated on ice for 1 hour. 50 ml of
459 cultures were then spun down at 5,000 x g for 15 min at 4°C. The supernatant was decanted,
460 the cell pellet resuspended in the remaining liquid (~0.5 ml) and transferred to an RNase-free
461 1.5 ml centrifuge tube and the suspension was then centrifuged at 11,000 x g for 2 min at 4 °C.
462 All supernatant was removed then left for 2 hours in dry ice. The frozen cell pellet was
463 resuspended in 200 µl of 100 mg ml^{-1} lysozyme (prepared using RNase-free TE buffer) then
464 incubated at 37°C for 10 minutes. Samples were then subjected to five cycles of freezing on
465 dry ice and thawing at 37 ºC before a final freezing step. RNA extraction was performed using
466 a Promega SV total RNA isolation kit according to the manufacturer's protocol (including the
467 optional on-column DNase treatment), with the exception of the following changes. 300 µl of
468 lysis reagent and 630 µl of RNA dilution buffer were added to the frozen sample and inverted
469 to mix thaw. 300 mg of glass beads were then added to the samples and cells were disrupted
470 using an MP FastPrep®-24 instrument set at maximum speed for 40 seconds. Samples were
471 then heated at 70 ºC for 3 minutes and centrifuged at 18,000 x g at 4 ºC for 10 minutes. The
472 supernatant was transferred to an RNase-free 1.5 ml centrifuge tube before adding the 95%
473 Ethanol. Samples were purified and concentrated using an RNA Clean & Concentrator™ Kit
474 (Zymo Research). Samples grown under nitrogen limitation were treated differently as they
475 had a lower yield of RNA when using the RNA extraction method as described above. For
476 these samples, RNA was stabilised using RNA Later (Ambion®, Life Technologies) according
477 to the manufacturer's protocol. RNA samples were then purified using RNeasy® spin columns
478 (Qiagen) according to the manufacturer's protocol.

To eliminate traces of genomic DNA, all samples were treated with TURBO DNA-free™ DNAse (Ambion®, Life Technologies) according to the manufacturer's protocol. The quantity and quality of the samples was determined using Qubit™ 3.0 Fluorometer, following the protocol of the Qubit RNA HS Assay Kit (Thermo Fisher Scientific).

Reverse transcription of 1 µg of DNA-free RNA per sample was done using the QuantiTect® Reverse Transcription Kit (Qiagen). No reverse transcriptase and no template controls were performed to confirm that samples were DNA-free and that the reactions were free of contaminants.

Primers for qRT-PCR for *L. aggregata* LZB033 *dsyB* and *dddL*, as well as the housekeeping genes *recA* and *rpoD*, were designed using Primer3[53,54] (http://bioinfo.ut.ee/primer3-0.4.0/) and are listed in Supplementary Table 5. The optimum primer melting temperature was 60 °C, but melting temperatures from 57 °C to 63 °C were accepted. The maximum melting temperature difference between primers in a primer pair was 2 °C and primer GC content was kept between 40-60 %. The primer pairs were checked to avoid stable homo- and heterodimers as well as hairpin structures using IDT (Integrated DNA Technologies) Oligoanalyzer 3.1 tool (https://www.idtdna.com/calc/analyzer). Primer efficiencies were all 90-110 % and within recommended limits.

Quantitative PCR was performed using a StepOnePlus instrument (Applied Biosystems). Quantification was performed using a standard SensiFAST™ SYBR® Hi-ROX Kit (Bioline) following manufacturer's instructions. 20 µl reactions contained 10 ng of cDNA, primers at concentrations of 400 nM (*dsyB*, *recA*) or 300 nM (*rpoD*, *dddL*), and with an annealing/elongation temperature of 60 °C. A single gene was quantified per run, with three biological replicates and three technical replicates. Manually detected outliers were excluded from further analysis. Controls run in each plate were the no reverse transcriptase and no

503 template controls from the reverse transcriptase step. Standard curves were included in each
504 run to calculate the reaction efficiency (five points in 1:2 dilutions starting from 50 ng of cDNA
505 and water only as negative control). Analysis of post-run melting curve was also performed.

506 For each condition and gene, the cycle threshold (Ct) values of the technical and biological
507 replicates were averaged. The *rpoD* and *recA* Ct values and efficiencies were then averaged
508 and the relative expression ratio was calculated[55] and expressed as normalised fold change
509 relative to the standard conditions.

510

511 **Statistics**

512 Statistical methods for qRT-PCR are described in the relevant section above. All measurements
513 for DMSP and DMS production (in bacterial strains or enzyme assays) are based on the mean
514 of three biological replicates per strain/condition tested and error bars are shown from
515 calculations of standard deviations, with all experiments performed at least twice. To identify
516 statistically significant differences between standard and experimental conditions in Fig. 1b
517 and Fig. 3a-c, a single-tailed independent Student's *t*-test ($P<0.05$) was applied to the data,
518 using Microsoft Excel.

519

520 **Bioinformatic analysis**

521 BLAST searches[34] to identify homologues of the *Labrenzia* DsyB and DddL proteins were
522 performed using BLASTP at NCBI or JGI. DsyB homologues, along with selected other more
523 distantly related methyltransferases in Pfam family PF00891 below the predicted cut-off for
524 DsyB functionality (E values $\leq 1e^{-67}$), were aligned by ClustalW in MEGA v6[56]. Evolutionary

analyses were conducted using MEGA and different amino acid substitution models were tested to identify the most suitable. The evolutionary history was inferred by using the Maximum Likelihood method based on the Le Gascuel 2008 model[57]. The tree with the highest log likelihood (-8097.3139) was used. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.8781)). The analysis involved 47 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 203 positions in the final dataset.

Protein sequences used in the phylogenetic tree were obtained from either NCBI or JGI. Accession numbers of protein sequences from NCBI are: *B. mycoides* DSM2048, EEL96501; *L. aggregata* CECT4801, CTQ43687; *L. aggregata* LZB033, KT989543; *Labrenzia* sp. C1B10, ERP98606; *Labrenzia* sp. C1B70, ERR00112; *Labrenzia* sp. DG1229, WP_051644456; *Ponticoccus* sp. UMTAT08, WP_043143384; *Pseudooceanicola atlanticus* 22II-S11g, WP_043748339; Rhodobacteraceae bacterium PD-2, WP_023852424; *Roseibacterium elongatum* DSM19469, WP_025312975; *S. varsoviensis* DSM40346, WP_030879264. Gene IDs of protein sequences from JGI are: *A. coralli* DSM19760, 2517908241; *Antarctobacter heliothermus* DSM11445, 2620496472; *Caenispirillum salinarum* AK4, 2520173307; *Citreicella aestuarii* DSM22011, 2616590712; *Citreicella* sp. 357, 2514595470; *Defluviimonas* sp. 20V17, 2579689465; *Donghicola* sp. S598, 2553022978; *L. aggregata* IAM 12614, 639943763; *Labrenzia alexandrii* DFL-11, 2517312627; *Litorimicrobium taeanense* DSM22007, 2616591799; *Maritimibacter alkaliphilus* HTCC2654, 648280724; *Nisaea denitrificans* DSM18348, 2525377636; *Nisaea* sp. BAL199, 641429164; *O. batsensis* HTCC2597, 638883374; *Pseudooceanicola nanhaiensis* DSM18065,

2558678304; *Oceanicola* sp. HL-35, 2541035415; *Oceanicola* sp. S124, 2527024186; *P. bermudensis* HTCC2601, 648285806; *Pseudodonghicola xiamenensis* DSM18339, 2524485630; Rhizobiales bacterium HL-109, 2609135787; Rhodobacteraceae bacterium bin09, 2609105254; Rhodospirillales bacterium URHD0017, 2593183274; *Roseibium hamelinense* ATCC BAA-252, 2597124009; *Roseivivax halodurans* JCM 10272, 2565720611; *Roseivivax sediminis* DSM26472, 2635168442; *Roseovarius indicus* DSM26383, 2620334468; *S. stellata* E-37, 640641694; *Salipiger mucosus* DSM16094, 2523510257; *S. qiaohouensis* DSM21189, 2523943366; *Stappia stellulata* DSM5886, 2525928126; *Thalassobaculum litoreum* DSM18839, 2599852567; *T. salexigens* DSM19539, 2523405058; *Thalassobius gelatinovorus* DSM5887, 2622865483; *Tropicibacter naphthalenivorans* DSM19561, 2623181840; *Yangia pacifica* CGMCC 1.3455, 2617877652; *Yangia pacifica* DSM26894, 2620103054.

BLAST searches to identify DsyB homologues in metagenomes from different environments (Supplementary Table 2) were performed using standalone BLASTP algorithm and custom peptide databases using the *L. aggregata* LZB033, *L. aggregata* IAM12614, *S. stellata* E-37, *O. batsensis* HTCC2597 and *A. coralli* DSM19760 DsyB proteins as probes (see above for sequences) and with a cutoff of E value $\leq 1e^{-67}$. To estimate the number of bacteria represented in each metagenome, a selection of RecA proteins (as in [24]) were used as probes to BLAST the same metagenome databases, with a cutoff of E value $\leq 1e^{-50}$.

Data availability

Sequence data that support the findings of this study have been deposited in GenBank with the accession codes KT989543 (*L. aggregata* LZB033 *dsyB* gene sequence) and JXMT00000000 (*L. aggregata* LZB033 whole genome shotgun project).

574

575 **References**

576 1 Seymour, J. R., Simo, R., Ahmed, T. & Stocker, R. Chemoattraction to
577 dimethylsulfoniopropionate throughout the marine microbial food web. *Science* **329**, 342-345,
578 doi:10.1126/science.1188418 (2010).
579 2 Sievert, S. M., Kiene, R. P. & Schulz-Vogt, H. N. The Sulfur Cycle. *Oceanography* **20**,
580 117-123 (2007).
581 3 Curson, A. R., Todd, J. D., Sullivan, M. J. & Johnston, A. W. Catabolism of
582 dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nat Rev Microbiol* **9**, 849-
583 859, doi:10.1038/nrmicro2653 (2011).
584 4 Nevitt, G. A. The neuroecology of dimethyl sulfide: a global-climate regulator turned
585 marine infochemical. *Integr Comp Biol* **51**, 819-825, doi:10.1093/icb/icr093 (2011).
586 5 Stefels, J., Steinke, M., Turner, S., Malin, G. & Belviso, S. Environmental constraints
587 on the production and removal of the climatically active gas dimethylsulphide (DMS) and
588 implications for ecosystem modelling. *Biogeochemistry* **83**, 245-275 (2007).
589 6 Vallina, S. M. & Simo, R. Strong relationship between DMS and the solar radiation
590 dose over the global surface ocean. *Science* **315**, 506-508, doi:10.1126/science.1133680
591 (2007).
592 7 Yoch, D. C. Dimethylsulfoniopropionate: its sources, role in the marine food web, and
593 biological degradation to dimethylsulfide. *Appl Environ Microbiol* **68**, 5804-5815 (2002).
594 8 Van Alstyne, K. L. & Puglisi, M. P. DMSP in marine macroalgae and
595 macroinvertebrates: Distribution, function, and ecological impacts. *Aquat Sci* **69**, 394-402
596 (2007).

9 Raina, J. B. *et al.* DMSP biosynthesis by an animal and its role in coral thermal stress response. *Nature* **502**, 677-680, doi:10.1038/nature12677 (2013).

10 Gage, D. A. *et al.* A new route for synthesis of dimethylsulphoniopropionate in marine algae. *Nature* **387**, 891-894 (1997).

11 Uchida, A., Ooguri, T., Ishida, T., Kitaguchi, H. & Ishida, Y. in *Biological and Environmental Chemistry of DMSP and Related Sulfonium Compounds* (eds R. P. Kiene, P.T. Visscher, M.D. Keller, & G.O. Kirst) Ch. 9, 97-107 (Plenum Press, 1996).

12 Rhodes, D., Gage, D. A., Cooper, A. J. L. & Hanson, A. D. S-methylmethionine conversion to dimethylsulfoniopropionate: Evidence for an unusual transamination reaction. *Plant Physiol* **115**, 1541-1548 (1997).

13 Kocsis, M. G. & Hanson, A. D. Biochemical evidence for two novel enzymes in the biosynthesis of 3-dimethylsulfoniopropionate in Spartina alterniflora. *Plant Physiol* **123**, 1153-1161 (2000).

14 Ferla, M. P. & Patrick, W. M. Bacterial methionine biosynthesis. *Microbiology* **160**, 1571-1584, doi:10.1099/mic.0.077826-0 (2014).

15 Summers, P. S. *et al.* Identification and stereospecificity of the first three enzymes of 3-dimethylsulfoniopropionate biosynthesis in a chlorophyte alga. *Plant Physiol* **116**, 369-378 (1998).

16 Ito, T., Asano, Y., Tanaka, Y. & Takabe, T. Regulation of biosynthesis of dimethylsulfoniopropionate and its uptake in sterile mutant of Ulva pertusa (Chlorophyta). *J Phycol* **47**, 517-523 (2011).

17 Curson, A. R., Rogers, R., Todd, J. D., Brearley, C. A. & Johnston, A. W. Molecular genetic analysis of a dimethylsulfoniopropionate lyase that liberates the climate-changing gas dimethylsulfide in several marine alpha-proteobacteria and Rhodobacter sphaeroides. *Environ Microbiol* **10**, 757-767, doi:10.1111/j.1462-2920.2007.01499.x (2008).

18 Dang, H., Li, T., Chen, M. & Huang, G. Cross-ocean distribution of Rhodobacterales bacteria as primary surface colonizers in temperate coastal marine waters. *Appl Environ Microbiol* **74**, 52-60, doi:10.1128/AEM.01400-07 (2008).

19 Ayala-Castro, C., Saini, A. & Outten, F. W. Fe-S cluster assembly pathways in bacteria. *Microbiol Mol Biol Rev* **72**, 110-125, table of contents, doi:10.1128/MMBR.00034-07 (2008).

20 Dubbs, J. M. & Mongkolsuk, S. Peroxiredoxins in bacterial antioxidant defense. *Subcell Biochem* **44**, 143-193 (2007).

21 Sunda, W., Kieber, D. J., Kiene, R. P. & Huntsman, S. An antioxidant function for DMSP and DMS in marine algae. *Nature* **418**, 317-320 (2002).

22 Stefels, J. Physiological aspects of the production and conversion of DMSP in marine algae and higher plants. *J Sea Res* **43**, 183-197 (2000).

23 Otte, M. L., Wilson, G., Morris, J. T. & Moran, B. M. Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants. *J Exp Bot* **55**, 1919-1925 (2004).

24 Carrion, O. *et al.* A novel pathway producing dimethylsulphide in bacteria is widespread in soil environments. *Nat Commun* **6**, 6579, doi:10.1038/ncomms7579 (2015).

25 Gonzalez, J. M., Whitman, W. B., Hodson, R. E. & Moran, M. A. Identifying numerically abundant culturable bacteria from complex communities: an example from a lignin enrichment culture. *Appl Environ Microbiol* **62**, 4433-4440 (1996).

26 Baumann, P. & Baumann, L. *The marine Gram-negative eubacteria: genera Photobacterium, Benecekea, Alteromonas, Pseudomonas and Alcaligenes.*, 1302-1331 (Springer-Verlag, 1981).

27 Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning, A Laboratory Manual*. 2nd edn, Vol. 3 (Cold Spring Harbor Laboratory Press, 1989).

28 Beringer, J. E. R factor transfer in Rhizobium leguminosarum. *J Gen Microbiol* **84**, 188-198, doi:10.1099/00221287-84-1-188 (1974).

29 Figurski, D. H. & Helinski, D. R. Replication of an origin-containing derivative of plasmid Rk2 dependent on a plasmid function provided in trans. *P Natl Acad Sci USA* **76**, 1648-1652, doi:10.1073/pnas.76.4.1648 (1979).

30 Downie, J. A. *et al.* Cloned nodulation genes of Rhizobium-leguminosarum determine host range specificity. *Mol Gen Genet* **190**, 359-365, doi:10.1007/Bf00331059 (1983).

31 Tett, A. J., Rudder, S. J., Bourdes, A., Karunakaran, R. & Poole, P. S. Regulatable vectors for environmental gene expression in alphaproteobacteria. *Appl Environ Microb* **78**, 7137-7140, doi:10.1128/Aem.01188-12 (2012).

32 Keen, N. T., Tamaki, S., Kobayashi, D. & Trollinger, D. Improved broad-host-range plasmids for DNA cloning in Gram-negative bacteria. *Gene* **70**, 191-197, doi:10.1016/0378-1119(88)90117-5 (1988).

33 Delcher, A. L., Bratke, K. A., Powers, E. C. & Salzberg, S. L. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* **23**, 673-679, doi:10.1093/bioinformatics/btm009 (2007).

34 Altschul, S. F. *et al.* Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389-3402, doi:10.1093/nar/25.17.3389 (1997).

35 Pruitt, K. D., Tatusova, T. & Maglott, D. R. NCBI reference sequences (RefSeq): a curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res* **35**, D61-D65, doi:10.1093/nar/gkl842 (2007).

36 Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* **28**, 33-36, doi:10.1093/nar/28.1.33 (2000).

37 Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y. & Hattori, M. The KEGG resource for deciphering the genome. *Nucleic Acids Res* **32**, D277-D280, doi:10.1093/nar/gkh063 (2004).

38 Ashburner, M. *et al.* Gene Ontology: tool for the unification of biology. *Nat Genet* **25**, 25-29 (2000).

39 Todd, J. D. *et al.* DddQ, a novel, cupin-containing, dimethylsulfoniopropionate lyase in marine roseobacters and in uncultured marine bacteria. *Environmental Microbiology* **13**, 427-438, doi:10.1111/j.1462-2920.2010.02348.x (2011).

40 Schafer, A. *et al.* Small mobilizable multipurpose cloning vectors derived from the Escherichia-coli Plasmids pK18 and pK19 - selection of defined deletions in the chromosome of Corynebacterium-glutamicum. *Gene* **145**, 69-73, doi:10.1016/0378-1119(94)90324-7 (1994).

41 Zhang, Y. H., Tang, K. H., Shi, X. C. & Zhang, X. H. Flaviramulus ichthyoenteri sp nov., an N-acylhomoserine lactone-degrading bacterium isolated from the intestine of a flounder (Paralichthys olivaceus), and emended descriptions of the genus Flaviramulus and Flaviramulus basaltis. *Int J Syst Evol Micr* **63**, 4477-4483, doi:10.1099/ijs.0.053744-0 (2013).

42 Tang, K. H. *et al.* Evaluation of a new high-throughput method for identifying quorum quenching bacteria. *Sci Rep-Uk* **3**, doi:10.1038/srep02935 (2013).

43 Simon, M. & Azam, F. Protein-Content and Protein-Synthesis Rates of Planktonic Marine-Bacteria. *Mar Ecol Prog Ser* **51**, 201-213, doi:10.3354/meps051201(1989).

44 Uchino, Y., Hirata, A., Yokota, A. & Sugiyama, J. Reclassification of marine Agrobacterium species: Proposals of Stappia stellulata gen. nov., comb. nov., Stappia aggregata sp. nov., nom. rev., Ruegeria atlantica gen. nov., comb. nov., Ruegeria gelatinovora comb. nov., Ruegeria algicola comb. nov., and Ahrensia kieliense gen. nov., sp. nov., nom. rev. *J Gen Appl Microbiol* **44**, 201-210 (1998).

45 Cho, J. C. & Giovannoni, S. J. Oceanicola granulosus gen. nov., sp. nov. and Oceanicola batsensis sp. nov., poly-beta-hydroxybutyrate-producing marine bacteria in the order 'Rhodobacterales'. *Int J Syst Evol Microbiol* **54**, 1129-1136, doi:10.1099/ijs.0.03015-0 (2004).

46 Gonzalez, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. Sagittula stellata gen. nov., sp. nov., a lignin-transforming bacterium from a coastal environment. *Int J Syst Bacteriol* **47**, 773-780 (1997).

47 Zeevi Ben Yosef, D., Ben-Dov, E. & Kushmaro, A. Amorphus coralli gen. nov., sp. nov., a marine bacterium isolated from coral mucus, belonging to the order Rhizobiales. *Int J Syst Evol Microbiol* **58**, 2704-2709, doi:10.1099/ijs.0.65462-0 (2008).

48 Cho, J. C. & Giovannoni, S. J. Pelagibaca bermudensis gen. nov., sp. nov., a novel marine bacterium within the Roseobacter clade in the order Rhodobacterales. *Int J Syst Evol Microbiol* **56**, 855-859, doi:10.1099/ijs.0.64063-0 (2006).

49 Urios, L., Michotey, V., Intertaglia, L., Lesongeur, F. & Lebaron, P. Thalassobaculum salexigens sp. nov., a new member of the family Rhodospirillaceae from the NW Mediterranean Sea, and emended description of the genus Thalassobaculum (vol 60, pg 209, 2010). *Int J Syst Evol Micr* **60**, 2507-2507 (2010).

50 Wang, Y. X. *et al.* Sediminimonas qiaohouensis gen. nov., sp nov., a member of the Roseobacter clade in the order Rhodobacterales. *Int J Syst Evol Micr* **59**, 1561-1567, doi:10.1099/ijs.0.006965-0 (2009).

51 Dong, J., Signo, K. S. L., Vanderlinde, E. M., Yost, C. K. & Dahms, T. E. S. Atomic force microscopy of a ctpA mutant in Rhizobium leguminosarum reveals surface defects linking CtpA function to biofilm formation. *Microbiol-Sgm* **157**, 3049-3058, doi:10.1099/mic.0.051045-0 (2011).

52 Todd, J. D. *et al.* Molecular dissection of bacterial acrylate catabolism--unexpected links with dimethylsulfoniopropionate catabolism and dimethyl sulfide production. *Environ Microbiol* **12**, 327-343, doi:10.1111/j.1462-2920.2009.02071.x (2010).

53 Koressaar, T. & Remm, M. Enhancements and modifications of primer design program Primer3. *Bioinformatics* **23**, 1289-1291, doi:10.1093/bioinformatics/btm091 (2007).

54 Untergasser, A. *et al.* Primer3-new capabilities and interfaces. *Nucleic Acids Res* **40**, doi:10.1093/nar/gks596 (2012).

55 Pfaffl, M. W. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, doi:10.1093/nar/29.9.e45 (2001).

56 Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol Biol Evol* **30**, 2725-2729, doi:10.1093/molbev/mst197 (2013).

57 Le, S. Q. & Gascuel, O. An improved general amino acid replacement matrix. *Mol Biol Evol* **25**, 1307-1320, doi:10.1093/molbev/msn067 (2008).

Corresponding Author

Correspondence and requests for materials should be addressed to J.D.T. (jonathan.todd@uea.ac.uk) or X.-H.Z. (xhzhang@ouc.edu.cn).

Acknowledgements

Funding from the Natural Environmental Research Council (NE/J01138X, NE/M004449 and NE/N002385) supported work in J.D.T.'s laboratory; funding from the National Natural Science Foundation of China (41521064, 41476112 and 41276141) supported the research in X.-H.Z.'s laboratory. We acknowledge Lionel Hill of the John Innes Centre Metabolite

Services for technical support with LC-MS experiments and Pamela Wells for general technical support. We thank Charles Brearley, Elena Mercadé, Colin Murrell and Leanne Sims for advice and discussion of results. We also acknowledge the *Tara* Oceans Consortium for providing metagenomic sequence data.

Author contributions

J.D.T. wrote the paper, designed all experiments and performed experiments (genomic library screening, gene cloning, enzyme assays) and analysed data; A.R.J.C. wrote the paper, performed experiments (genomic library construction, gene cloning, enzyme assays, mutant construction, GC experiments to quantify DMSP and DMS production, growth experiments to measure DMSP production under different environmental conditions, mutant phenotyping, bioinformatics analysis to identify homologues in bacterial genomes, phylogenetic trees), analysed data and prepared figures/tables; A.B.M. performed experiments (sample preparation, method development and operation of LC-MS, RNA isolation, and qRT-PCR experiments) and analysed data; X.-H.Z. and J.L. performed experiments (isolation of LZB033 and generation of a draft genome sequence, identification of DMS production in LZB033, mutant phenotyping) and analysed data; R.T.G. performed bioinformatic analysis of metagenomes and prepared figures/tables; Y.C. and P.C.B.P. performed chemical syntheses; O.C. and B.W. performed GC experiments and discussed results; S.-H.Z. and G.-P.Y. performed preliminary GC experiments on DMS production in LZB033. All authors reviewed the manuscript before submission.

Additional information

Supplementary information is available for paper. Reprints and permissions information is available at www.nature.com/reprints.

Competing interests

The authors declare no competing financial interests.

Figure legends

Figure 1. DMSP biosynthesis pathways and bacterial DMSP production

a, Predicted pathways for DMSP biosynthesis in higher plants (*Spartina*,*SMM converted to DMSP-aldehyde directly in *Wollastonia*) (left); macroalgae (*Ulva*, *Enteromorpha*), diatoms (*Thalassiosira*, *Melosira*), prymnesiophytes (*Emiliania*) and prasinophytes (*Tetraselmis*) (centre); and dinoflagellates (*Crypthecodinium*) (right). **b**, *L. aggregata* LZB033 DMSP production with or without pathway intermediates (0.5 mM) in MBM minimal medium (10 mM succinate as carbon source, 10 mM NH_4Cl as nitrogen source). Three biological replicates for each condition. Error bars of standard deviations are shown (n=3). Student's *t*-test ($P<0.05$): Met, MTOB, MTHB and DMSHB were all significantly different to no addition. **c**, LC-MS chromatograms for MTHB (m/z 149) and DMSP (m/z 135) in LZB033 wild type and *dsyB*$^-$ strains, and for DMSP in *R. leguminosarum* J391 with or without cloned *dsyB* (pBIO2258). Three biological replicates for each strain. Abbreviations: DMSP-ald, DMSP-aldehyde; MMPA, methylmercaptopropionate.

Figure 2. Maximum likelihood phylogenetic tree of DsyB proteins.

The tree is drawn to scale, with branch lengths measured in the number of substitutions per site, as indicated on scale bar. All DsyB proteins came from alphaproteobacteria in the order Rhodobacterales, except where other orders are shown in brackets. Methyltransferase enzymes whose corresponding genes were cloned and shown to be functional are marked with a circle and those non-functional are marked with a square. Strains experimentally confirmed to produce DMSP are marked with a star and those not producing DMSP with a cross.

Figure 3. DMSP production, *dsyB* transcription and *dddL* transcription in *L. aggregata* LZB033 under different conditions.

a, DMSP production, **b**, *dsyB* transcription and **c**, *dddL* transcription in *L. aggregata* under varying conditions of salinity, temperature, nitrogen concentration, oxidative stress and growth phase. Standard conditions were growth in MBM medium (with succinate as carbon source and no added methylated sulphur compounds) at a salinity of 35 PSU, nitrogen source concentration of 10 mM NH_4Cl, incubation temperature of 30 °C and sampling in exponential phase growth. For DMSP production, three biological replicates were used for each condition and for qRT-PCR, three biological replicates and three technical replicates were used for each gene/condition. Error bars of standard deviations are shown (n=3). Significance was determined using a Student's *t*-test ($P<0.05$), with all experimental conditions, except oxidative stress in **a** and **c**, shown to be significantly different from the standard conditions.