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## **DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton**

Curson, A. R. J., Williams, B. T., Pinchbeck, B. J., Sims, L. P., Martínez, A. B., Rivera, P. P. L., Kumaresan, D., Mercadé, E., Spurgin, L. G., Carrión, O., Moxon, S., Cattolico, R. A., Kuzhiumparambil, U., Guagliardo, P., Clode, P. L., Raina, J. B., & Todd, J. D. (2018). DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nature Microbiology*, 3, 430–439 . <https://doi.org/10.1038/s41564-018-0119-5>

**Published in:**  
Nature Microbiology

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
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1 **DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many**  
2 **phytoplankton**

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18 **Dimethylsulfoniopropionate (DMSP) is a globally important organosulfur molecule, and**  
19 **the major precursor for dimethyl sulfide (DMS). These compounds are important info-**  
20 **chemicals, key nutrients for marine microorganisms, and are involved in global sulfur**  
21 **cycling, atmospheric chemistry and cloud formation<sup>1-3</sup>. DMSP production was thought**  
22 **to be confined to eukaryotes, but heterotrophic bacteria can also produce DMSP, via**  
23 **the pathway used by most phytoplankton<sup>4</sup>, and the DsyB enzyme catalysing the key step**

24 of this pathway in bacteria was recently identified<sup>5</sup>. However, eukaryotic phytoplankton  
25 likely produce most of Earth's DMSP, yet no DMSP biosynthesis genes have been  
26 identified in any such organisms. Here we identify functional *dsyB* homologues, termed  
27 *DSYB*, in many phytoplankton and corals. *DSYB* is a methylthiohydroxybutyrate  
28 (MTHB) methyltransferase enzyme localised in the chloroplasts and mitochondria of  
29 the haptophyte *Prymnesium parvum*, and stable isotope tracking experiments support  
30 these organelles as sites of DMSP synthesis. *DSYB* transcription levels increased with  
31 DMSP concentrations in different phytoplankton and were indicative of intracellular  
32 DMSP. The identification of the eukaryotic *DSYB* sequences, along with bacterial *dsyB*,  
33 provide the first molecular tools to predict the relative contributions of eukaryotes and  
34 prokaryotes to global DMSP production. Furthermore, evolutionary analysis suggests  
35 that eukaryotic *DSYB* originated in bacteria and was passed to eukaryotes early in their  
36 evolution.

37 Not all phytoplankton produce DMSP, and in those that do, intracellular DMSP  
38 concentrations vary considerably across groups and within genera<sup>6</sup>. Previous studies  
39 identified candidate genes<sup>7,8</sup> involved in DMSP synthesis via the transamination pathway  
40 (Fig. 1a), which is common to DMSP-producing bacteria<sup>5</sup> and algae<sup>4</sup>. A proteomic study of  
41 the diatom *Fragilariopsis cylindrus* identified putative DMSP synthesis enzymes<sup>7</sup>, including  
42 the MTHB methyltransferase reaction catalysed by DsyB in bacteria. Another study on corals  
43 identified homologues of two of the *F. cylindrus* enzymes in *Acropora millepora*, one being a  
44 candidate MTHB methyltransferase<sup>8</sup>. None of these enzymes have been functionally ratified,  
45 and the putative MTHB methyltransferases share no significant sequence similarity to DsyB.  
46 When we cloned and expressed the *F. cylindrus* and *A. millepora* putative MTHB  
47 methyltransferase genes they had no such enzyme activity (Supplementary Table 1),  
48 suggesting that the identity of an algal MTHB methyltransferase was still unknown.

49 We identified homologues to the bacterial MTHB methyltransferase gene *dsyB*<sup>5</sup> in available  
 50 genomes and/or transcriptomes of all marine prymnesiophytes; most dinoflagellates, some  
 51 corals, and ~20% of diatoms and Ochrophyta (Fig. 1b, Supplementary Table 2,  
 52 Supplementary Table 3 and Supplementary Data 1). The only dinoflagellate transcriptomes  
 53 lacking *dsyB* were from *Oxyrrhis marina*, a heterotroph which produces no detectable  
 54 DMSP<sup>6,9</sup>. Furthermore, many dinoflagellates, and some haptophytes, diatoms and corals,  
 55 have multiple *dsyB* homologs. The grouping of these multiple homologues across the  
 56 phylogeny was consistent with multiple gene duplication and gene loss events over the  
 57 evolutionary history of eukaryotes<sup>10</sup> (Fig. 1b, Supplementary Table 2, and Supplementary  
 58 Table 3). These *dsyB*-like genes, termed *DSYB*, from representatives of the corals (*Acropora*  
 59 *cervicornis*), diatoms (*F. cylindrus*), dinoflagellates (*Alexandrium tamarense*, *Lingulodinium*  
 60 *polyedrum*, *Symbiodinium microadriaticum*) and prymnesiophytes (*Chrysochromulina tobin*,  
 61 *Prymnesium parvum*) were cloned and shown to have MTHB methyltransferase activity, at  
 62 similar levels to bacterial DsyB from *Labrenzia* (Supplementary Table 1). These algal *DSYB*  
 63 enzymes fully complement bacterial *dsyB*<sup>-</sup> mutants, defective in DMSP production.  
 64 Furthermore, enzyme assays with purified *DSYB* and MTHB substrate alone showed no  
 65 activity, but *in vitro* S-adenosyl methionine (SAM)-dependent MTHB methyltransferase  
 66 activity was observed when the same assays were incubated with heat-denatured *P. parvum*  
 67 cell lysates (Supplementary Table 4). This suggests that a co-factor(s) present in *P. parvum*  
 68 lysates might be required for activity. The K<sub>M</sub> values of *DSYB* for MTHB and SAM were  
 69 88.2 μM and 60.1 μM respectively (Supplementary Table 4, Supplementary Fig. 1). *DSYB*  
 70 showed no detectable methyltransferase activity with other potential substrates (including  
 71 methionine (Met), 4-methylthio-2-oxobutyrate (MTOB) and methylmercaptopropionate  
 72 (MMPA); Supplementary Table 4). Thus, *DSYB* encodes the first DMSP synthesis enzyme to  
 73 be identified and functionally ratified from any eukaryotic algae.

DSYB is found across many, but by no means all, major groups of eukaryotes, and eukaryotes are monophyletic in the DsyB/DSYB phylogeny, suggesting either i) that *DSYB* was present in the last eukaryotic common ancestor (LECA) and has been lost across many eukaryotic groups, or ii) that *dsyB* has been transferred to eukaryotes multiple times. Homology and phylogenetic analyses place Alphaproteobacteria as the sister clade to the eukaryotes for this gene (Fig. 1b); we note that Alphaproteobacterial genes make up a significant proportion of eukaryotic genomes, due to endosymbiotic events with the ancestor of mitochondria<sup>11</sup>. We suggest that DMSP production originated in prokaryotes, and was transferred to the eukaryotes, either via endosymbiosis at the time of mitochondrial origin, or more recently via horizontal gene transfer (HGT). Interestingly, coral DSYB paralogs grouped with dinoflagellate sequences from coral symbionts of the genus *Symbiodinium* (Fig. 1b). This is consistent with HGT between corals and their symbionts, as documented for other genes<sup>12</sup>, and suggests that DMSP production in corals may be a result of recent HGT of *DSYB* from dinoflagellates. However, we cannot discount the possibility that coral DSYB sequences might be contaminant sequences unintentionally extracted from their symbionts.

No *DSYB* homologs were identified in available transcriptomes from marine ascomycota, cercozoa, chlorophyta, ciliophoran, cryptophyta, euglenozoa, glaucophyta, labyrinthista, perkinsozoa, or rhodophyta (Supplementary Table 3), although some members of these taxa, such as chlorophyta and rhodophyta<sup>9,13</sup>, are known to produce DMSP. DSYB homologs were also absent in the genomes of the DMSP-producing diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*<sup>14</sup>. Some marine eukaryotes lack *DSYB* simply because they do not produce DMSP<sup>6,9</sup>. Others may (i) have *DSYB* but not express it under the tested transcriptome conditions, (ii) contain a MTHB methyltransferase isoform, or (iii) produce DMSP via a different synthesis pathway<sup>15</sup>.

Intracellular DMSP concentrations are generally high in dinoflagellates (reported up to 3.4 M, but unlikely to be this high given seawater osmolarity is  $\sim 1 \text{ Osm l}^{-1}$ ) and haptophytes (up to 413 mM), but significant intra-group variance exists, with some representatives not producing DMSP at detectable levels<sup>6,9</sup>. Since eukaryotic DSYB enzymes had MTHB methyltransferase rates similar to bacterial DsyB enzymes (Supplementary Table 1), it is unlikely that variation in DsyB and DSYB amino acid sequences is responsible for the differing intracellular DMSP concentrations in these organisms (Supplementary Table 1). To understand this variance, we studied model DMSP-producing phytoplankton, starting with *Chrysochromulina tobin* CCMP291 and *Chrysochromulina* sp. PCC307, two haptophytes adapted to different salinity levels (fresh-brackish and marine waters<sup>16,17</sup>, respectively). Both *Chrysochromulina* strains produced very low intracellular DMSP concentrations (Supplementary Table 1, Supplementary Fig. 2), which were unaffected by variation in salinity and nitrogen availability, conditions that have been shown previously to affect DMSP production in bacteria<sup>5</sup> and phytoplankton<sup>7,18</sup>. Consistent with these findings, *C. tobin* CCMP291 *DSYB* was transcribed at very low levels (Supplementary Fig. 2), perhaps indicating a DMSP function in these haptophytes that only requires low concentrations. Many haptophytes produce high DMSP concentrations, consistent with an osmoregulatory function, but this contrasts the low *C. tobin* DMSP concentrations and highlights the variability in the process and requirement for a methodology to predict which phytoplankton are high and low DMSP producers. Perhaps other compatible solutes, possibly sugars or amino acids, are the major osmolytes in CCMP291 and PCC307. Consistent with this, the osmolyte glycine betaine ( $551 \pm 6 \text{ nmol}$ ) was present in  $\sim 10$ -fold higher amounts than DMSP ( $52 \pm 6 \text{ nmol}$ ) in CCMP291.

Next, we investigated DMSP production in six *Prymnesium* strains, from brackish/marine sources, and found they had similar intracellular DMSP concentrations, which were much

higher than those for *C. tobin* (Supplementary Fig 2). *P. parvum* CCAP946/6 *DSYB* transcription was also higher than that for *C. tobin* *DSYB* under standard conditions (Supplementary Fig 2). Interestingly, *DSYB* transcription, *DSYB* protein levels and DMSP concentration in *P. parvum* were all enhanced by increased salinity but unaffected by other environmental conditions, including nitrogen availability or temperature (Supplementary Fig. 2; Supplementary Fig. 3). Increased salinity enhances DMSP production in many phytoplankton, notably *P. parvum*, where DMSP is thought to be a significant osmolyte<sup>19</sup>. Our findings, and those of Dickson and Kirst<sup>19</sup>, are consistent with DMSP playing an osmoregulatory role in this haptophyte. However, *dsyB* transcription and DMSP production is regulated by salinity in bacteria, yet no detrimental effect on growth was observed in a bacterial *dsyB*<sup>-</sup> mutant when grown in saline conditions<sup>5</sup>. Thus, increased *DSYB* expression and DMSP production with raised salinity does not necessarily indicate a major role for DMSP in osmoprotection.

*P. parvum* *DSYB* protein was concentrated to the chloroplasts and mitochondria (Fig. 2; Supplementary Fig. 4). We propose these organelles as sites of DMSP synthesis in *P. parvum* and perhaps other eukaryotic phytoplankton. Although DMSP production in mitochondria has not been reported, DMSP is produced in the chloroplasts of the higher plant *Wollastonia*, albeit using a different pathway<sup>20</sup>. Based on *in silico* sequence analysis (see Methods), *DSYB* from *P. parvum* and some other phytoplankton are predicted to be targeted to the mitochondria and/or chloroplasts (Supplementary Table 5). However, chromophyte algae, such as haptophytes and diatoms, have complex plastids<sup>21</sup>, which may render such *in silico* predictions less reliable.

Nanoscale secondary-ion mass spectrometry (NanoSIMS), with a cryopreservation method previously shown to preserve cytosolic DMSP<sup>22</sup>, was used to identify potential sub-cellular

sites of DMSP production and storage in *P. parvum* through tracking  $^{34}\text{SO}_4$  uptake. After 48 h incubation,  $\sim 23.2\% \pm 0.2$  of the *P. parvum* intracellular DMSP pool was labelled with  $^{34}\text{S}$  ( $^{34}\text{S}$ -DMSP; Supplementary Fig. 5). Within the cells, the  $^{34}\text{S}$  appeared to be localized in sub-cellular compartments, with increasing levels appearing over time in the chloroplasts ( $^{34}\text{S}/^{32}\text{S}$ :  $3.4 \pm 0.17$  after 48 h) and in submicrometer hotspots ( $^{34}\text{S}/^{32}\text{S}$ :  $3.1 \pm 0.15$  after 48 h) (Fig. 3). Given the size and location of these hotspots, they are likely to be mitochondria or small lipid vesicles (Fig. 3). Although many sulfur compounds are present in algal cells, DMSP represents more than 50% of the total organosulfur compounds in marine phytoplankton<sup>23</sup> and it is expected to account for a significant fraction of the  $^{34}\text{S}$  signal detected by NanoSIMS. However, it cannot be discounted that the increased  $^{34}\text{S}$  content in the chloroplast could be due to transport of sulfur and subsequent assimilation via plastid-located enzymes, such as ATP sulfurylase, APS reductase and sulfite reductase. Nonetheless, the simultaneous increase in  $^{34}\text{S}$  in the chloroplasts and potentially mitochondria supports our hypothesis that these organelles are indeed sites of DMSP synthesis and storage in *P. parvum* and likely other phytoplankton. Given the role of these organelles in energy production, it is perhaps not surprising that DMSP production, an energy-demanding process<sup>24</sup>, may occur at these sites. With DMSP being far less concentrated in the cytosol, it is less likely that its primary function in *P. parvum* is as a typical cytosolic osmolyte, but it may be a key osmolyte in the chloroplasts and/or mitochondria, as proposed in *Wollastonia* chloroplasts<sup>20</sup>. Also, considering reactive oxygen species (ROS) are generated in the mitochondria and chloroplasts, and that DMSP is an effective scavenger of ROS<sup>25</sup>, the production of DMSP in these organelles is in line with its putative role in oxidative stress protection<sup>24,25</sup>.

Diatoms are thought to produce the lowest intracellular DMSP levels (typically  $< 50\text{ mM}$ )<sup>9</sup>. We studied DMSP production in the polar ice diatom *Fragilariopsis cylindrus*, one of the few diatoms with a functional *DSYB* (Supplementary Table 2), finding that, under standard



conditions, intracellular DMSP levels and *DSYB* transcription were relatively low, when compared to (e.g.) *P. parvum* (Supplementary Fig. 2). However, consistent with work in other diatoms<sup>18</sup>, both *F. cylindrus* DMSP production and *DSYB* transcription increased with nitrogen limitation and increased salinity (Supplementary Fig. 2). The latter supports a role for DMSP in osmoregulation and salinity-induced oxidative stress protection in *F. cylindrus*, as suggested by Lyon et al.<sup>7</sup>. *DSYB* was not detected as one of the salinity-induced proteins in Lyon et al.<sup>7</sup>, despite using the same salinity conditions for our experiments, reflecting the nature of 2D gel electrophoresis studies, whereby not all proteins are identified.

Given the trend of intracellular DMSP concentration increasing with *DSYB* transcription, we studied *Symbiodinium microadriaticum* CCMP2467, a dinoflagellate from a genus producing high DMSP concentrations<sup>6</sup>. *S. microadriaticum* gave the highest intracellular DMSP (282 mM) and cumulative *DSYB* transcription of the tested phytoplankton (Supplementary Fig. 2). Similarly, available transcriptomic data showed that high DMSP-producing dinoflagellate and haptophyte phytoplankton (see above) had the highest average *DSYB* transcription, which was ~3 and 8-fold higher, respectively, than that in diatoms (Supplementary Table 2). Transcriptomic data was also congruent with high variability in intracellular DMSP levels within dinoflagellates and haptophytes<sup>6,9</sup>. While additional factors, such as *DSYB* protein levels, DMSP excretion, DMSP catabolism and cell volume, will affect an organism's intracellular DMSP concentration, the data presented here on a small number of phytoplankton supports the hypothesis that *DSYB* transcription is a reasonably good indicator of DMSP concentration. Some *DSYB*-containing phytoplankton may also contain MTHB methyltransferase isoform enzymes or utilise other DMSP synthesis pathways, in which case such predictions may be inaccurate. Further work is required to substantiate this hypothesis.

195 The prominence of environmental DMSP-producing bacteria and eukaryotes was examined  
 196 in the ocean microbial reference gene catalogue (OM-RGC) metagenomic dataset, generated  
 197 from samples fractionated to  $< 3 \mu\text{m}^{26}$  (Supplementary Table 6 and Supplementary Fig. 6).  
 198 The *dsyB* gene was predicted to be present in 0.35% of total bacteria in these samples. For  
 199 comparison, DMSP lyase genes (*dddD*, *dddL*, *dddK*, *dddP*, *dddQ*, *dddW*, *dddY* and *AlmaI*)<sup>27</sup>,  
 200 were also used. The *dsyB* gene was more abundant than *dddL*, *dddW*, *dddY*, and the algal  
 201 DMSP lyase gene *AlmaI*, but was less abundant than *dddD*, *dddK*, *dddP* and *dddQ* in the  
 202 OM-RGC dataset. Despite only 3% of the OM-RGC microorganisms likely being  
 203 eukaryotes<sup>26</sup>, *DSYB* genes were detected and were ~25-fold less abundant than bacterial *dsyB*.  
 204 Since no *DSYB* sequences have been identified in bacteria, we conclude that picoeukaryotes  
 205 in these samples contain *DSYB* and thus, the genetic potential to make DMSP. The  
 206 production of DMSP by *DSYB*-containing picoeukaryotes could contribute, along with  
 207 DMSP-producing bacteria, to the DMSP measured from particles  $< 2 \mu\text{m}$  in size in seawater  
 208 samples<sup>28</sup>.

209 We also investigated the occurrence of *dsyB* and *DSYB* in marine metatranscriptomes  
 210 (Supplementary Table 7). *dsyB* transcripts were detected in all tested *Tara* oceans  
 211 metatranscriptomic datasets apportioned to marine bacteria (Supplementary Table 8 and  
 212 Supplementary Fig. 6). *dsyB* transcript abundance (normalised to total sequence numbers)  
 213 was similar to *dddD* and greater than *dddL*, *dddW*, *dddY* and *AlmaI*, but was far less than  
 214 *dddK*, *dddP* and *dddQ*. Although these datasets do not consider phytoplankton  $> 3 \mu\text{m}$ , *DSYB*  
 215 transcripts, likely from picoeukaryotes, were detected at levels only 3-fold lower than the  
 216 bacterial *dsyB* gene, again suggesting that these smaller eukaryotes, like bacteria, should be  
 217 considered as potentially significant DMSP producers (Supplementary Table 8).

We also analysed the North Pacific Ocean metatranscriptomes (GeoMICS) which used appropriate fractionation methods for bacteria and larger phytoplankton<sup>29</sup>. As expected, eukaryotic *DSYB* transcript numbers were higher than those of bacterial *dsyB* in all of the 2-53  $\mu\text{m}$  fractions, which should contain relatively more phytoplankton than bacteria, and the opposite was true in most of the 0.2-2  $\mu\text{m}$  fractions, which should have relatively more bacteria but not contain the larger phytoplankton (Supplementary Table 9). Analysing data from both the large and small size fractions at different sites allowed us to gauge the relative total transcript numbers of *DSYB* and *dsyB* in these samples, as well as those of the DMSP lyase genes. Prokaryotic *dsyB* transcripts (normalised to the recovery of an internal standard) were more abundant than those for the bacterial DMSP lyase genes *dddK*, *dddL*, *dddQ*, *dddY* and *dddW*, 3-fold less than *dddP* and *AlmaI* and 27-fold less than *dddD* (Supplementary Table 9). Eukaryotic *DSYB* transcripts were slightly less abundant than those for the eukaryotic DMSP lyase (*AlmaI*), but, were ~2-fold more abundant than those for bacterial *dsyB*. With similar DsyB and DSYB enzyme rates (Supplementary Table 1), this metatranscriptomic data suggests that eukaryotic phytoplankton may be the major contributors to DMSP production via the DsyB/DSYB pathway in these samples. However, direct extrapolation from these data to predict eukaryotic versus bacterial DMSP production (via DsyB/DSYB) is not likely accurate since other factors, such as DsyB/DSYB protein stability or the differing expression and activities of other enzymes in the pathway, may also affect DMSP production. Nonetheless, *dsyB* and *DSYB* sequences provide invaluable tools for future, in-depth studies to investigate the relative contribution of bacterial and algal DMSP production in varied marine environments. Molecular studies are also required to identify DMSP synthesis genes in DMSP-producing organisms which lack *dsyB* or *DSYB*.

## 242    **Methods**

### 243    **Media and general growth of algae and bacteria**

244    *Prymnesium parvum* CCAP941/1A, *Prymnesium parvum* CCAP941/6, *Prymnesium parvum*  
245    CCAP946/1B, *Prymnesium parvum* CCAP946/1D, *Prymnesium parvum* CCAP946/6,  
246    *Prymnesium patelliferum* CCAP946/4, *Chrysochromulina* sp. PCC307 and *Symbiodinium*  
247    *microadriaticum* CCMP2467 were grown in F/2<sup>30</sup> medium made with ESAW artificial  
248    seawater<sup>31</sup> and without any added Na<sub>2</sub>SiO<sub>3</sub>. Axenic *Fragilariopsis cylindrus* CCMP1102 was  
249    supplied by Mock et al.<sup>32</sup> and grown in F/2 medium made with ESAW artificial seawater at 4  
250    °C with a light intensity of 120  $\mu\text{E m}^{-2} \text{s}^{-1}$  and constant illumination. *Chrysochromulina tobin*  
251    CCMP291 was grown in the proprietary medium RAC-5<sup>33</sup>. All algal cultures (except *F.*  
252    *cylindrus*) were grown at 22 °C with a light intensity of 120  $\mu\text{E m}^{-2} \text{s}^{-1}$  and a light dark cycle  
253    of 16 h light/8 h dark, unless otherwise stated. Where necessary, media for algal growth were  
254    modified according to the requirements of the experimental conditions being tested. Where  
255    strains were not already known to be axenic, cultures were treated with multiple rounds of  
256    antibiotic treatment prior to experiments. Test cultures with and without antibiotic treatments  
257    showed no significant difference in total DMSP in samples. For *P. parvum* CCAP946/6, and  
258    *Chrysochromulina* sp. PCC307 cultures, streptomycin (400  $\mu\text{g ml}^{-1}$ ), chloramphenicol (50  $\mu\text{g}$   
259     $\text{ml}^{-1}$ ), gentamicin (20  $\mu\text{g ml}^{-1}$ ) and ampicillin (100  $\mu\text{g ml}^{-1}$ ) were added, and for *S.*  
260    *microadriaticum* cultures, streptomycin (100  $\mu\text{g ml}^{-1}$ ) and neomycin (100  $\mu\text{g ml}^{-1}$ ) were  
261    added. *E. coli* was grown in LB<sup>34</sup> complete medium at 37 °C. *R. leguminosarum* was grown  
262    in TY<sup>35</sup> complete medium or Y<sup>35</sup> minimal medium (with 10 mM succinate as carbon source  
263    and 10 mM NH<sub>4</sub>Cl as nitrogen source) at 28 °C. *L. aggregata* J571 was grown in YTSS<sup>36</sup>  
264    complete medium or MBM<sup>37</sup> minimal medium (with 10 mM succinate as carbon source and  
265    10 mM NH<sub>4</sub>Cl as nitrogen source) at 30 °C. Where necessary, antibiotics were added to

bacterial cultures at the following concentrations: streptomycin (400  $\mu\text{g ml}^{-1}$ ), kanamycin (20  $\mu\text{g ml}^{-1}$ ), spectinomycin (200  $\mu\text{g ml}^{-1}$ ), gentamicin (20  $\mu\text{g ml}^{-1}$ ), ampicillin (100  $\mu\text{g ml}^{-1}$ ). Strains used in this study are listed in Supplementary Table 10.

#### **Staining with 4',6-diamidino-2-phenylindole (DAPI)**

The absence of bacterial contamination was confirmed by epifluorescence microscopy of culture samples stained with DAPI<sup>38</sup>. Briefly, 13 ml of culture was removed and fixed with 765  $\mu\text{l}$  paraformaldehyde, then 130  $\mu\text{l}$  of DAPI stain (1  $\text{mg ml}^{-1}$  in  $\text{H}_2\text{O}$ ) was added and samples were stored in the dark at 4 °C for 16 h. After staining, 3 ml of the stained cells were removed and filtered onto a Whatman Nuclepore track-etched membrane (25 mm, 0.2  $\mu\text{m}$ , polycarbonate). To prepare slides, one drop of immersion oil was added onto the slide then the sample filter was placed on the oil and another drop of immersion oil was added onto the filter. A cover slip was then placed on top of the filter and pressed down with forceps to remove air bubbles. The slide was then tilted and left on absorbent paper towel to allow any excess oil to drain/wick away. Slides were examined using an Olympus BX40 microscope equipped with an Olympus Camedia C-7070 digital camera.

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

Plasmids (Supplementary Table 10) were transferred to *E. coli* by transformation, and *Rhizobium leguminosarum* J391 or *Labrenzia aggregata* J571 by conjugation in a triparental mating using the helper plasmid pRK2013<sup>39</sup>. Routine restriction digestions and ligations for cloning were performed essentially as in Downie et al.<sup>40</sup>. The oligonucleotide primers used

for molecular cloning were synthesised by Eurofins Genomics and are detailed in Supplementary Table 11. Sequencing of plasmids and PCR products was performed by Eurofins Genomics.

The *DSYB* gene from *P. parvum* CCAP946/6 was PCR-amplified from cDNA and cloned into the IPTG-inducible wide host range expression plasmid pRK415<sup>41</sup>. All other *DSYB* genes were synthesised by Eurofins Genomics, from sequences codon-optimised (using Invitrogen GeneArt) for expression in *E. coli*, in the vector pEX-K4 (Eurofins Genomics). The synthesised genes were then subcloned into pLMB509<sup>42</sup>, a taurine-inducible plasmid for the expression of genes in *Rhizobium* and *Labrenzia*, using *NdeI* and *BamHI* or *EcoRI* restriction enzymes. All plasmid clones are described in Supplementary Table 10.

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#### 299 **MTHB methyltransferase (MMT) assays**

To measure MMT activity from pLMB509 clones expressing the *dsyB* or *DSYB* gene in *R. leguminosarum* J391, cultures were grown (in triplicate) overnight in TY complete medium, 1 ml of culture was centrifuged at 20,000g for 2 min, resuspended in the same volume of Y medium and then diluted 1:100 into 5 ml Y with 10 mM taurine (to induce expression, Sigma-Aldrich, T0625), 0.5 mM DL-MTHB (Sigma-Aldrich, 55875), 0.1 mM L-methionine and gentamycin, and incubated at 28 °C for 60 h before sampling for gas chromatography (GC) analysis (see ‘Quantification of DMS and DMSP by gas chromatography’) to determine the amount of DMSP product.

To measure MMT activity from pLMB509 clones expressing the *DSYB* gene in the *L. aggregata dsyB* mutant strain J571, cultures were grown (in triplicate) overnight in YTSS complete medium. Following incubation, 1 ml of culture was then centrifuged at 20,000g for 2 min, resuspended in the same volume of MBM medium and then diluted 1:50 into 5 ml

MBM with 10 mM taurine (to induce expression, Sigma-Aldrich), rifampicin and gentamycin, and incubated at 30 °C for 24 h. Samples were taken for GC analysis and determining protein concentration (t = 0 h timepoint). DL-MTHB (0.5 mM) and L-methionine (0.1 mM) were then added as substrates to the remaining cultures and these were incubated for 4 h at 30 °C before sampling for GC and protein again (t = 4 h timepoint), with activity calculated based on the difference in measured DMSP product between t=0 h and t=4 h.

To measure DMSP in *Rhizobium* or *Labrenzia* assay mixtures, 200 µl of culture was added to a 2 ml glass serum vial then 100 µl 10 M NaOH was added and vials were crimped immediately, incubated at 22 °C for 24 h and monitored by GC assay (see ‘Quantification of DMS and DMSP by gas chromatography’). DsyB/DSYB activity is expressed as pmol DMSP mg protein<sup>-1</sup> min<sup>-1</sup>, assuming that all the DMSP is derived from DMSHB through DDC activity. LC-MS analysis shows no detectable DMSHB in *Rhizobium* or *Labrenzia* expressing DsyB/DSYB, presumably due its conversion to DMSP by DDC activity, so DMSP production is used as a proxy for DsyB activity. Protein concentrations were determined using the Bradford method (BioRad). Control assays of *Rhizobium* or *Labrenzia* J571 containing pLMB509 were carried out, as above, and gave no detectable DsyB/DSYB activity.

### **Growth of algae under non-standard conditions**

For all *P. parvum*, *F. cylindrus* and *C. tobin* cultures described here, all samples were taken in mid-exponential phase growth before growth rates started to decline (checked by continuing to monitor growth following sampling). To measure DMSP production or DSYB/DSYB expression in *P. parvum* CCAP946/6 under different conditions, the growth

conditions or F/2 medium were modified as follows. Standard growth conditions were a temperature of 22 °C, light intensity of 120  $\mu\text{E m}^{-2} \text{s}^{-1}$ , salinity of 35 practical salinity units (PSU) and nitrogen concentration of 882  $\mu\text{M}$ . For increased or decreased salinity, the amount of salts added to the artificial seawater were adjusted to give a salinity of 50 or 10 PSU respectively. For reduced nitrogen concentration cultures, the F/2 medium contained 88.2  $\mu\text{M}$  (10% of standard F/2). For changes in temperature, cultures were grown at 15 °C or 28 °C. To measure the effect of increased salinity and nitrogen limitation in *F. cylindrus* CCMP1102, this strain was grown in F/2 medium with increased salts in the artificial seawater (to 70 PSU) or reduced nitrogen (88.2  $\mu\text{M}$ , 10% of standard F/2). To measure the effect of increased salinity and nitrogen limitation in *C. tobin* CCMP291, this strain was grown in F/2 medium with sea salts added to the RAC-5 medium (to 5 PSU) or reduced nitrogen (85  $\mu\text{M}$ , 10% of standard RAC-5).

## **Sampling methods**

To measure growth of algal cultures, samples were removed, diluted (dependent on level of growth) in artificial seawater and cell counting was done using a Multisizer 3 Coulter counter (Beckman Coulter). The effect of stress on photosystem II was determined by measuring Fv/Fm values using a Phyto-Pam phytoplankton analyzer (Heinz Walz, Germany). To obtain samples for DMSP quantification by GC or liquid chromatography-mass spectrometry (LC-MS), 25 ml of culture was filtered onto 47 mm GF/F glass microfiber filters (Fisher Scientific, UK) using a Welch WOB-L 2534 vacuum pump, and filters were then blotted on paper towel to remove excess liquid and stored at -80 °C in 2 ml centrifuge tubes for particulate DMSP (DMSPp) measurement. To obtain samples for RNA, 50 ml of culture was filtered onto 47 mm 1.2  $\mu\text{m}$  RTTP polycarbonate filters (Fisher Scientific, UK) and filters



were stored in 2 ml centrifuge tubes at -80 °C. To obtain samples for protein for Western blotting, 50 ml of culture was centrifuged at 600g for 10 min in a 50 ml centrifuge tube, the supernatant was decanted and cells were transferred in the residual liquid to a 2 ml centrifuge tube and centrifuged at 600g for 5 mins. All residual liquid was then aspirated and the pelleted cells were stored at -80 °C.

### **Quantification of DMS and DMSP by GC**

All GC assays involved measurement of headspace DMS, either directly produced or via alkaline lysis of DMSP or DMSHB, 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). All GC measurements were performed using 2 ml glass serum vials containing 0.3 ml liquid samples and sealed with PTFE/rubber crimp caps. Quantification of DMSP from algal samples filtered on GF/F glass microfiber filters (see ‘Sampling methods’) was performed following methanol extraction. Filters were folded, placed in a 2 ml centrifuge tube and 1 ml 100% methanol was added. Samples were stored for 24 h at -20 °C to allow the extraction of cellular metabolites, then 200 µl of the methanol extract was added to a 2 ml vial, 100 µl 10 M NaOH was added, vials were crimped immediately, incubated at 22 °C for 24 h in the dark and monitored by GC. Control samples in which DMSP standards were added to algal sample filters prior to methanol extraction showed that all standard was recovered following our extraction and measurement procedure. Calibration curves were produced by alkaline lysis of DMSP standards in water (for *Rhizobium/Labrenzia* MMT assays) or 100% methanol (for algal methanol extracts), or DL-DMSHB (chemically synthesised as in Curson et al.<sup>5</sup>) standards in water with heating at 80 °C for 10 mins (to release DMS from DMSHB) (for assays with purified DSYB protein). The

384 detection limit for headspace DMS from DMSP was 0.015 nmol in water and 0.15 nmol in  
385 methanol, and from DMSHB was 0.3 nmol in water.

386

### 387 **Quantification of DMSP by LC-MS**

388 LC-MS was used to confirm that phytoplankton were producing DMSP and at similar levels  
389 to that shown by GC, ruling out the possibility that DMS detected by GC was due to some  
390 other compound and not DMSP. Samples were extracted as follows: GF/F filters of  
391 phytoplankton (see 'Sampling methods') were resuspended in 1 ml of 80% LC-MS grade  
392 acetonitrile (extraction solvent), and mixed by pipetting and vortexing for 2 min. The  
393 resulting mixture was transferred into a fresh 2 ml Eppendorf tube. For a second round of  
394 extraction, another 1 ml of the extraction solvent was then added and mixed as previously  
395 described. Then the filters were centrifuged at 18,000g for 10 min and the supernatant was  
396 collected, giving a total volume of 2 ml of the collected supernatant. The collected  
397 supernatant was then centrifuged at 18,000g for 10 min and 1.5 ml of the supernatant was  
398 collected for LC-MS analysis. To extract the metabolites from *Chrysochromulina* sp.  
399 CCMP291, 20 ml of sample was centrifuged at 600g for 10 min and the cell pellet was  
400 resuspended in a total volume of 0.7 ml of the extraction solvent and mixed by pipetting and  
401 vortexing for 2 min. Samples were then centrifuged at 18,000g for 10 min and 0.5 ml of the  
402 supernatant was collected for LC-MS analysis.

403 LC-MS was carried out using a Shimadzu Ultra High Performance Liquid Chromatography  
404 (UHPLC) system formed by a Nexera X2 LC-30AD Pump, a Nexera X2 SIL-30AC  
405 Autosampler, a Prominence CTO-20AC Column oven, and a Prominence SPD-M20A Diode  
406 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 NH<sub>2</sub> 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 °C, desolvation temperature 250 °C and nebulising gas flow 1.50 L min<sup>-1</sup>. 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<sup>-1</sup> 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. The targeted mass transition corresponded to [M+H]<sup>+</sup> of DMSP (m/z 135) and of glycine betaine (m/z 118) in positive mode. A calibration curve was performed for quantification of DMSP and glycine betaine using a mixture of DMSP and glycine betaine standards in the extraction solvent.

#### **Reverse transcription quantitative PCR (RT-qPCR)**

For each culture, RNA was extracted as follows: 1 ml Trizol reagent (Sigma-Aldrich), prewarmed at 65 °C, was added directly to the frozen phytoplankton filter (see ‘Sampling methods’), followed by 600 mg of < 106 µm glass beads (Sigma-Aldrich). Cells were disrupted using an MP FastPrep®-24 instrument set at maximum speed for 3 x 30 seconds. Following a 5 min recovery time at 22 °C, samples were centrifuged at 13,000g, 4 °C, for 2 min. The supernatant was transferred to a 2 ml screwcap tube containing 1 ml 95% ethanol and RNA was extracted using a Direct-zol<sup>TM</sup> RNA MiniPrep kit (Zymo Research, R2050), according to the manufacturer’s specifications.

Genomic DNA was removed by treating samples with TURBO DNA-free<sup>TM</sup> DNase (Ambion®) according to the manufacturer’s protocol. The quantity and quality of the RNA

was determined by a NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Scientific) using 1  $\mu$ l of sample.

Reverse transcription of 1  $\mu$ g DNA-free RNA was achieved using the QuantiTect® Reverse Transcription Kit (Qiagen). Primers (Supplementary Table 11) were designed, using Primer3Plus<sup>43</sup>, to amplify ~130 bp region, with an optimum melting temperature of 60 °C. Melting temperature difference between primers in a pair was 2 °C and GC content was kept between 40-60%.

Quantitative PCR was performed with a C1000 Thermal cycler equipped with a CFX96 Real-time PCR detection system (BioRad), using a SensiFAST™ SYBR® Hi-ROX Kit (Bioline) as per the manufacturer's instructions for a 3-step cycling programme. Reactions (20  $\mu$ l) contained 50 ng cDNA and a final concentration of 400 nM of each primer, with a 60 °C annealing temperature. Gene expression for each condition was performed upon three biological replicates, each with three technical replicates. Control DNA consisted of pGEMT-Easy (Promega) containing the fragment created by the RT-qPCR primer pair for each gene tested (made through PCR on synthesised cDNA, cloning in *E. coli* 803 and purifying via a Miniprep Kit [Qiagen]).

For each condition and gene, the cycle threshold (Ct) values of the technical and biological replicates were averaged and manually detected outliers were excluded from further analysis. Standard curves of control DNA were calculated from 3 points of 1:10 serial dilutions, starting with 0.01 ng, to absolutely quantify the *DSYB* transcripts for comparison between organisms<sup>44</sup>. For an individual organism, relative *DSYB* expression was normalised to the  $\beta$ -actin housekeeping gene, and calculated using the  $2^{-\Delta\Delta CT}$  method<sup>45</sup> to observe changes in response to various conditions.

## Analysis of DSYB expression by Western blotting

A polyclonal rabbit IgG was designed against *P. parvum* DSYB using the OptimumAntigen<sup>TM</sup> software (GenScript Ltd.). The purified IgG was used as a primary antibody in Western blotting and immunogold labelling (see 'DSYB immunogold labelling'). The specificity of this antibody was ensured by Western blot analysis of DSYB expressed in the heterologous host *R. leguminosarum* J391. J391 strains containing pBIO2275 (positive control) and pRK415 with no cloned insert (negative control) were grown overnight in TY medium with 0.5 mM IPTG. Proteins were extracted by harvesting 1 ml culture, resuspending cell pellet in 200 µl 20 mM HEPES, 150 mM NaCl, pH 7.5 and disrupting with an ultrasonic processor (Cole Palmer) for 2 x 10 s cycles on ice. Cell debris was separated by centrifugation at 18,000g for 10 mins, following which the supernatant was mixed with SDS sample buffer and incubated at 95 °C for 5 min, before resolution on a 15 % (v/v) acrylamide gel.

The specificity of the anti-DSYB antibody was additionally tested on *P. parvum* 946/6, where protein samples were prepared from cell pellets (see 'Sampling methods') as for *R. leguminosarum*, without the removal of cell debris. Cell lysate containing 5.5 µg protein was mixed with SDS sample buffer and heat-treated at 95 °C for 20 min, before resolution on a 15 % (v/v) acrylamide gel.

Following SDS-PAGE, proteins were transferred to a PVDF membrane (Amersham Hybond<sup>TM</sup>-P, GE Healthcare) by semi-dry Western blot as outlined by Mahmood and Yang<sup>46</sup>. After 1 hour blocking with 5 % (w/v) skimmed milk powder in TBS (20 mM Tris, 150 mM NaCl, pH 7.5), the anti-DSYB antibody was added at a final concentration of 0.386 µg ml<sup>-1</sup>. Specific interactions were left to form overnight at 4 °C, before the membrane was washed 4 x 10 min with TBST (TBS + 0.1 % (v/v) Tween 20). TBST (20 ml) was added with 3 µl anti-

rabbit IgG-alkaline phosphatase at 1 mg ml<sup>-1</sup> (Sigma). Following 1 h incubation, the membrane was washed as before with two 10 min TBS washes. Colorimetric detection with NBT/BCIP (Thermo Fisher) was used to detect the target protein as per the manufacturer's instructions. All SDS-PAGE gels were run with Bio-Rad Precision Plus Dual Colour protein size standards and stained with Coomassie using InstantBlue Protein stain (Expedeon).

#### **Purification of DSYB and *in vitro* catalytic assays**

A 1.1 kb fragment of DNA containing the synthesised coding region of *Chrysochromulina tobin* DSYB was subcloned (from pBIO2272) into pET16b as an *NdeI/EcoRI* restriction fragment, downstream of a 10-histidine coding sequence, and transformed into *E. coli* BL21 DE3 (New England BioLabs), for protein purification. Batch cultures were grown aerobically in LB medium at 37 °C until reaching an OD<sub>600</sub> value of ~0.6 and were then supplemented with 0.2 mM IPTG and incubated at 28 °C overnight to induce recombinant protein expression. Cells were harvested at 5,000g for 20 min and resuspended in buffer A (20 mM HEPES, 150 mM NaCl, 25 mM imidazole, pH 7.5). The mixture was supplemented with protease inhibitor (Roche cOmplete Tablets, Mini EDTA-free, EASYpack (cat. no. 04 693 159 001)), lysed via sonication and separated at 15,000g, 4 °C for 30 min.

DSYB was purified via an immobilized metal affinity chromatography (IMAC, HiTrap Chelating HP, GE Healthcare) column charged with NiSO<sub>4</sub> and equilibrated with buffer A. All steps were performed at 24 °C with a flow rate of 1 ml min<sup>-1</sup>. Soluble cell lysate was loaded and washed through with 4 column volumes of buffer A. Bound protein was eluted into 1 ml fractions using a stepped gradient of 25 to 150 mM imidazole, applied for 2 column volumes each. Fractions were visualised via SDS-PAGE analysis (Supplementary Fig. 7) and

502 those containing DSYB were pooled and dialysed at 4 °C overnight against 20 mM HEPES,  
503 150 mM NaCl, pH 7.5.

504 *P. parvum* lysate was prepared by centrifuging 100 ml of culture at late exponential phase for  
505 10 min at 2,500g. The pellet was washed with 20 mM HEPES, 150 mM NaCl, pH 7.5 and  
506 resuspended in 2 ml buffer supplemented with EDTA-free protease inhibitor (Roche  
507 cOmplete Tablets, Mini EDTA-free, *EASY*pack (cat. no. 04 693 159 001)). Cells were  
508 sonicated 3 x 10 s to lyse, with a 50 s recovery time at 4 °C. Resulting lysate was heat-treated  
509 at 80 °C for 10 min to denature proteins (ensuring no activity from native DSYB protein) and  
510 centrifuged for 2 min 14,000g. Supernatant was removed to a fresh Eppendorf tube and used  
511 for downstream catalytic assays.

512 DSYB MTHB methyltransferase activity was monitored by performing *in vitro* enzyme  
513 assays in 400 µl reactions with 50 µl *P. parvum* lysate and 350 µl purified DSYB (~0.1 mg  
514 ml<sup>-1</sup>) or buffer. All enzyme substrates were added to a final concentration of 1 mM and  
515 reactions were incubated at 28 °C for 30 mins. Following this, 800 µl of finely ground  
516 charcoal (38 mg ml<sup>-1</sup> in 0.1 M acetic acid) was added to the samples and mixed to remove  
517 SAM. Samples were centrifuged for 10 mins, 14,000g and the supernatant was retained. For  
518 GC analysis, 200 µl of the supernatant was added to a 2 ml vial, 100 µl 10 M NaOH was  
519 added, vials were crimped immediately, then heated at 80 °C for 10 minutes (to release DMS  
520 from DMSHB) and finally incubated at 22 °C for 24 h in the dark. These samples were  
521 subsequently used for quantification of DMSHB by GC analysis as described earlier and  
522 activities are reported as nmol DMSHB mg protein<sup>-1</sup> min<sup>-1</sup>. DMS produced from background  
523 DMSHB/DMSP present in the *P. parvum* lysate was subtracted from the reported activities.

524

525 **DSYB immunogold labelling**

Cells from *P. parvum* 946/6 were cryoimmobilized using a Leica EMPACT High-Pressure Freezer (Leica Microsystems), freeze-substituted in an EM AFS (Leica Microsystems) and embedded in Lowicryl HM20 resin (EMS, Hatfield, USA) as in Perez-Cruz et al.<sup>47</sup>. Gold grids containing Lowicryl HM20 ultrathin sections were immunolabeled with a specific primary antibody to *P. parvum* DSYB (polyclonal rabbit IgG, GenScript), whose stock concentration was 0.550 mg ml<sup>-1</sup> and this was diluted 1:15,000. Secondary antibody was an IgM anti-rabbit coupled to 12 nm diameter colloidal gold particles (Jackson) diluted 1:30. As controls, pre-immune rabbit serum was used as primary antibody, or the gold-conjugated secondary antibody was used without the primary antibody. Sections were observed in a Tecnai Spirit microscope (FEI, Eindhoven, The Netherlands) at 120 kV.

#### ***Prymnesium* growth and experimental conditions for NanoSIMS**

*P. parvum* were grown as previously described in F/2 medium (35 PSU)<sup>30</sup>. Sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>, 25 mM) was used as the sole sulfur source, with either <sup>34</sup>S (90% <sup>34</sup>S (Sigma-Aldrich, USA; hereafter called <sup>34</sup>S-F/2) or natural abundance of <sup>32</sup>S (95% <sup>32</sup>S, 0.7% <sup>33</sup>S, 4.2% <sup>34</sup>S; hereafter called <sup>nat</sup>S-F/2). Consequently, the composition of the both the trace metals and vitamin complement had to be slightly modified (with Riboflavin replacing the sulfur-containing Biotin and Thiamine)<sup>22</sup>. *P. parvum* cells in late exponential phase (grown in <sup>nat</sup>S-F/2) were centrifuged at low speed (1,000g) for 5 mins, rinsed with <sup>34</sup>S-F/2 (to remove potential leftover <sup>nat</sup>S) and transferred in <sup>34</sup>S-F/2, whereas a batch incubated only in <sup>nat</sup>S-F/2 acted as a control. Culture were sampled at four time-points: directly after the medium exchange, and after 6 hrs, 24 hrs and 48 hrs. At each timepoint, cultures were sampled for NanoSIMS, mass-spectrometry and cell counts (see below).



**Flow cytometry for NanoSIMS samples**

Cells were enumerated in triplicate *via* flow cytometry (BD Accuri C6, Becton Dickinson, USA). For each sample, forward scatter (FSC), side scatter (SSC), and red (chlorophyll) fluorescence were recorded. The samples were analysed at a flow rate of 35  $\mu\text{l min}^{-1}$ . *Prymnesium* populations were characterized according to SSC and chlorophyll fluorescence and cell abundances were calculated by running a standardized volume of sample (50  $\mu\text{l}$ ).

**Sample collection for mass spectrometry (NanoSIMS)**

At each time point, 1 ml of culture was centrifuged at low speed (1,000g) for 5 mins, the supernatant was discarded and the cell pellet was extracted with 80% methanol, sonicated on ice for 30 mins and dried.

Dried extracts were reconstituted in methanol to perform LC-MRM-MS analysis. The LC-MS system consisted of an Agilent 1290 series LC interfaced to an Agilent G6490A QQQ mass spectrometer (Agilent, Santa Clara, CA, USA). The MS was equipped with an electrospray ionization source and was controlled by Mass Hunter workstation (version B07) software. A HILIC column (Luna Phenomenex, 150 $\times$ 3 mm, 5  $\mu\text{m}$ , 300 Å) was used for the on-line separations, at a flow rate of 1  $\text{ml min}^{-1}$ . The gradient used consisted of a 95 % solvent B (Acetonitrile, 0.1% formic acid), followed by a 2 min linear gradient to 40% solvent A (Milli Q, 0.1 % formic acid), then a 10 min linear gradient to 90% A, and returning to initial conditions at 12.25 min. The injection volume was 2  $\mu\text{l}$ . The MS acquisition parameters were: positive ion mode; capillary voltage, 3,000 V; gas flow 12  $\text{l min}^{-1}$ ; nebulizer gas, 20 p.s.i.; sheath gas flow rate 7  $\text{l/min}^{-1}$  at a temperature of 250 °C. Acquisition was done in MRM mode with transitions  $m/z$  135- > 63 and  $m/z$  137- > 65 for quantifying  $^{32}\text{DMSP}$  and

<sup>34</sup>DMSP respectively. The collision energy was optimised as 10 eV to detect the highest possible intensity.

## **Sample collection and preparation for NanoSIMS**

Samples for NanoSIMS were collected and processed following the method described by Raina et al.<sup>22</sup>. Briefly, samples were snap-frozen, and embedded following by a water-free embedding procedure to effectively prevent the loss of highly soluble compounds such as DMSP from the samples. This method does retain elements in solution by effectively replacing the ‘solution’ with resin, without displacing the ions and osmolytes. *Prymnesium* cultures (20 µl) were dropped onto Thermanox strips (Thermo Fisher Scientific, Waltham, USA, 4×18 mm) and placed in humidified chambers. After 20 min, the cells settled onto the strips and the excess medium was carefully removed with filter paper. The strips were then immediately snap-frozen by immersion into liquid nitrogen slush<sup>22</sup>. Samples were stored in liquid nitrogen until required. Frozen samples for NanoSIMS were freeze-substituted in anhydrous 10% acrolein in diethyl ether, and warmed progressively to room temperature over three weeks in an EM AFS2 automatic freeze-substitution unit (Leica Microsystems, Wetzlar, Germany) as described recently in step-by-step detail by Kilburn and Clode<sup>48</sup>. The samples were subsequently infiltrated and embedded in anhydrous Araldite 502 resin, after which the Thermanox strip was removed and the sample re-embedded and stored in a desiccator. No sulfur was present in processing or resin components. Resin sections (1 mm thick) of embedded *Prymnesium* cells were cut dry using a Diatome-Histo diamond knife on an EM UC6 Ultramicrotome (Leica Microsystems, Wetzlar, Germany), mounted on a silicon wafer and coated with 10 nm of gold.

## NanoSIMS analysis

The NanoSIMS-50L (Cameca, Gennevilliers, France) at the Centre for Microscopy, Characterisation and Analysis (CMCA) at the University of Western Australia was used for all subsequent analyses. The NanoSIMS-50L allows simultaneous collection and counting of seven isotopic species, which enables the determination of  $^{34}\text{S}/^{32}\text{S}$  ratio. Enrichments of the rare isotope  $^{34}\text{S}$  was confirmed by an increase in the sulfur ( $^{34}\text{S}/^{32}\text{S}$ ) ratio above natural abundance values recorded in controls (0.0438). NanoSIMS analysis was undertaken by rastering a 2.5 pA  $\text{Cs}^+$  beam (~100 nm diameter) across defined  $20\text{ }\mu\text{m}^2$  sample areas ( $256\times 256$  pixels), with a dwell time of 30 ms per pixel. The isotope ratio values are represented hereafter using a colour-coded transform (hue saturation intensity (HSI)) showing natural abundance levels in blue, and grading to high enrichment in pink. Images were processed and analysed using Fiji (<http://fiji.sc/Fiji>)<sup>49</sup> with the Open-MIMS plug-in (<http://nrims.harvard.edu/software>). All images were dead-time corrected<sup>50</sup>. Ratio data were tested for QSA (quasi-simultaneous arrivals) by applying different beta values from 0.5 to 162. No differences in the data were observed, indicating that the secondary ion count rates were too low to be affected by QSA. Quantitative data were extracted from the mass images through manually drawn regions of interest, at T0 (whole cells n = 7, hotspot n = 10, chloroplasts n = 3), at T6 (whole cells n = 14, hotspot n = 10, chloroplasts n = 6), at T24 (whole cells n = 12, hotspot n = 10, chloroplasts n = 9), and at T48 (whole cells n = 6, hotspot n = 10, chloroplasts n = 4).

## Statistics

Statistical methods for RT-qPCR are described in the relevant section above. All measurements for DMSP production or DSYB/DsyB enzyme activity (in algal strains or

enzyme assays) are based on the mean of at least three biological replicates per strain/condition tested, with all experiments performed at least twice. To identify statistically significant differences between standard and experimental conditions in Supplementary Fig. 2, a two-tailed independent Student's *t*-test ( $P < 0.05$ ) was applied to the data, using R<sup>51</sup>.

## Identification of DSYB proteins in eukaryotes

BLASTP and TBLASTN searches<sup>52</sup> were used to identify homologues of the *Labrenzia* DsyB protein in available eukaryotic genomes and/or transcriptome assemblies at NCBI or JGI. Any eukaryotic DsyB-like proteins ( $E$  values  $\leq 1e^{-30}$ ), were aligned to ratified bacterial DsyB sequences and to non-functional DsyB-like proteins, e.g., in *Streptomyces varsoviensis*, see below. Representative DsyB-like proteins, more similar to DsyB than to non-functional *S. varsoviensis* DsyB-like proteins, were cloned and assayed for MMT activity (as above).

Ratified eukaryotic DSYB peptide sequences were used in BLASTP searches of 119 eukaryotic transcriptomes (with replicates) downloaded from the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP)<sup>53</sup> via the sequencing repositories iMicrobe (<http://imicrobe.us/project/view/104>) and ENA (European Nucleotide Archive)<sup>54</sup>. Of these, 45 contained at least one hit to DSYB ( $E$  values  $\geq 1e^{-30}$ ) (Supplementary Table 3). Each potential DsyB/DSYB sequence was manually curated by BLASTP analysis against the RefSeq database and discounted as a true DSYB sequence if the top hits were not to ratified DSYB sequences detailed in Fig. 1b. DSYB sequences identified from iMicrobe transcriptomes were aligned to ratified DsyB and DSYB sequences and included in the evolutionary analysis (Fig. 1b). All DsyB and DSYB protein sequences identified from genomes or transcriptomes are listed in Supplementary Data 1. Kallisto<sup>55</sup> was used to

quantify transcript abundances. Firstly, Kallisto indexes were created for the combined nucleotide assemblies of each organism. Next, Kallisto quant was used to obtain Transcripts Per kilobase Million (TPM) expression values for all datasets using the relevant reference transcriptome index for that organism. Nucleotide sequences corresponding to the DSYB hits were obtained using TBLASTN, and the CAMNT ID number was used to identify the TPM values for each *DSYB* read, giving an estimate of gene expression for organisms grown in standard conditions.

### **Phylogenetic analysis of DSYB and DsyB proteins**

All prokaryotic DsyB and eukaryotic DSYB amino acid sequences were aligned in MAFFT<sup>56,57</sup> version 7 using default settings, then visually checked. Prior to phylogeny construction, model selection was carried out and the best supported model of sequence evolution based on the Bayesian Information Criterion (BIC)<sup>58</sup> was selected for phylogeny construction (the LG+I+G4 model<sup>59</sup>). A maximum likelihood phylogeny was then constructed using IQ-TREE<sup>60</sup> version 1.5.3, implemented in the W-IQ-TREE web interface<sup>61</sup>, with 1,000 ultrafast bootstrap replicates<sup>62</sup> used to assess node support. The resulting tree was rooted using a non-DsyB methyltransferase sequence from *Streptomyces varsoviensis*<sup>5</sup>, and was formatted for publication using the ggtree package<sup>63</sup> in R<sup>51</sup>.

### **Analysis of DSYB sequences for localisation signals**

Searches for localisation signals in the DSYB protein sequences used the prediction software packages SignalP 4.1 (<http://www.cbs.dtu.dk/services/SignalP/>), TargetP 1.1

(<http://www.cbs.dtu.dk/services/TargetP/>) and ChloroP 1.1

(<http://www.cbs.dtu.dk/services/ChloroP/>).

## **Analysis of marine metagenomes and metatranscriptomes**

Hidden Markov Model (HMM)-based searches for *dsyB* and *DSYB* homologs in metagenome and metatranscriptome datasets were performed as described in<sup>64</sup> using HMMER tools (version 3.1, <http://hmmer.janelia.org/>). The *DsyB*/*DSYB* protein sequences, shown in Fig. 1b, and ratified DddD<sup>65–68</sup>, DddK<sup>69</sup>, DddL<sup>70</sup>, DddP<sup>71</sup>, DddQ<sup>72</sup>, DddY<sup>73</sup>, DddW<sup>74</sup> and Alma1<sup>75</sup> sequences were used as training sequences to create the HMM profiles. Profile HMM-based searches eliminate the bias associated with single sequence BLAST queries<sup>76</sup>. HMM profiles for the *recA* gene were downloaded from the functional gene pipeline and repository (FunGene<sup>77</sup>). The *Ruegeria pomeroyi* DddW<sup>74</sup> sequence was used to search metagenome and metatranscriptome datasets via BLASTP<sup>52</sup> since it is the only ratified DddW. HMM and BLASTP searches were performed against peptide sequences predicted from OM-RGC database assemblies (Supplementary Table 6) and all hits with an E value cut-off of  $1e^{-30}$  were retrieved. In the case of metatranscriptome datasets (*Tara* Oceans and GeoMICS metatranscriptomes), homologs with an E value cutoff of  $1e^{-5}$  were retrieved. Each potential *DsyB*/*DSYB* sequence retrieved from the analysis of metagenomes and metatranscriptomes was manually curated by BLASTP analysis against the RefSeq database and discounted as a true *DsyB* sequence if the top hits were not to *DsyB* or *DSYB* sequences detailed in Fig. 1b. If the top hits were to eukaryotic *DSYB* then the sequence was counted as a true *DSYB* sequence, and vice versa for bacterial *DsyB*. Each of the DddD, DddK, DddL, DddP, DddQ, DddW, DddY and Alma1 peptide sequences retrieved were aligned to curated reference sequences using hmmlalign and an approximate maximum likelihood tree was constructed

690 using FastTree<sup>78</sup> v2.1. Putative Ddd or Alma1 peptide sequences not aligning most closely to  
691 functional Ddd or Alma1 enzymes were removed. To estimate the percentage of bacteria  
692 containing *dsyB*, the number of unique hits to DdsB in metagenomes was normalised to the  
693 number of RecA sequences. Retrieved DsyB/DSYB homolog sequences were aligned to the  
694 training sequences using the *dsyB* HMM alignment and this was used to construct an  
695 approximately maximum likelihood phylogenetic tree inferred using FastTree<sup>78</sup> v2.1. The  
696 resulting tree (Supplementary Fig. 6) was visualised and annotated using the Interactive Tree  
697 Of Life (iTOL)<sup>79</sup> version 3.2.4.

698 The GeoMICS metatranscriptome database<sup>29</sup> generated from North Pacific Ocean samples  
699 offered an opportunity to compare prokaryotic and eukaryotic gene expression. Sequences  
700 from both the 0.2 µm – 2 µm and 2 µm – 53 µm filtrate fractions for sites P1 and P6 (those  
701 samples that had duplicates) were obtained from NCBI (Accession: PRJNA272345)  
702 (Supplementary Table 7). Sequences were trimmed using TrimGalore (default parameters,  
703 paired-end mode, [https://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) and  
704 overlapping paired-end reads were joined using PandaSeq<sup>80</sup>. To create peptide databases, the  
705 joined reads were translated using the translate function in Sean Eddy's squid package  
706 (<http://selab.janelia.org/software.html>) to generate all ORFs above 20 amino acids in length.  
707 The resulting peptide sequences were used to retrieve *dsyB* and *DSYB* sequences using HMM  
708 searches and BLASTP (as above). Read numbers for *dsyB/DSYB* were normalised to the read  
709 numbers of internal standard<sup>29</sup> recovered in each sample by dividing the number of reads by  
710 the internal standard number and multiplying by 100. Normalised reads from the same site  
711 and fraction were averaged (Supplementary Table 9) and ratios of *dsyB/DSYB* calculated.

712

**Data availability statement** The datasets analysed during the current study are available in the iMicrobe (<https://www.imicrobe.us/#/projects/104>), European Nucleotide Archive (<https://www.ebi.ac.uk/ena>), NCBI (<https://www.ncbi.nlm.nih.gov/>) and Ocean Microbiome (<http://ocean-microbiome.embl.de/companion.html>) repositories or are available within the paper in Methods section ‘Analysis of marine metagenomes and metatranscriptomes’ and in Supplementary Tables 7, 8 and 9. All data that support the findings of this study are available from the corresponding author upon reasonable request.

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**Acknowledgements** Funding from the Natural Environment Research Council  
(NE/J01138X/1, NE/M004449/1, NE/N002385/1 and NE/P012671/1) supported work in  
J.D.T.'s laboratory. B.T.W. was supported by a NERC EnvEast grant (NE/L002582/1) and  
A.B.M was supported by a BBSRC Norwich Research Park Biosciences Doctoral Training  
Partnership grant (BB/M011216/1). The NanoSIMS work was supported by an Australian  
Research Council Grant (DE160100636) to J-B.R. We thank Pamela Wells and Marco  
Giardina for general technical support, Thomas Mock for supplying *Fragilariopsis cylindrus*,  
and Robert Green, Ji Liu and Colin Murrell for advice and discussion of results. We also  
acknowledge the *Tara* Oceans Consortium for providing metagenomic sequence data, and the  
facilities at the Australian Microscopy & Microanalysis Research Facility at the Centre for  
Microscopy, Characterisation & Analysis, University of Western Australia, a facility funded  
by the University, State and Commonwealth Governments.

**Author contributions** J.D.T. wrote the paper, designed experiments and performed  
experiments (gene cloning, enzyme assays, bioinformatics) and analysed data; A.R.J.C. wrote  
the paper, designed experiments, performed experiments (gene cloning, enzyme assays, gas  
chromatography to quantify DMSP/DMSHB, phytoplankton growth experiments), analysed  
data and prepared figures/tables; B.T.W. performed experiments (bioinformatics analysis of  
DsyB/DSYB in transcriptomes, metagenomes and metatranscriptomes, phylogenetic tree  
construction), analysed data and prepared figures/tables; B.J.P. performed experiments (gene

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## **Competing interests**

The authors declare no competing financial interests.

## **Additional Information**

**Supplementary Information** is linked to the online version of the paper.

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## Figure legends

### Figure 1. Transamination pathway for DMSP biosynthesis pathway in bacteria and marine algae, and phylogenetic tree of DsyB/DSYB proteins

**a**, Predicted pathway for DMSP biosynthesis in bacteria (*Labrenzia*), macroalgae (*Ulva*, *Enteromorpha*), diatoms (*Thalassiosira*, *Melosira*), prymnesiophytes (*Emiliana*) and prasinophytes (*Tetraselmis*). Abbreviations: Met, methionine; MTOB, 4-methylthio-2-oxobutyrate; MTHB, 4-methylthio-2-hydroxybutyrate; DMSHB, 4-dimethylsulphonio-2-hydroxybutyrate. **b**, Maximum likelihood phylogenetic tree of DsyB/DSYB proteins. Species are colour-coded according to taxonomic class as shown in the key, with proteins shown to be functional marked with an asterisk. Bootstrap support for nodes is marked. Based on 145 protein sequences.

### Figure 2. Immunogold localisation of DSYB in *Prymnesium parvum* CCAP946/6

Representative electron micrographs of *P. parvum* cells showing location of DSYB by immunogold labelling. **a**, **b**, Immunostaining of cell with DSYB antibody and secondary antibody with gold. **c**, **d**, Control immunostaining with pre-immune serum. **e**, **f**, Control immunostaining with only secondary antibody. Boxes in **a**, **c**, and **e**, correspond to area magnified in **b**, **d**, and **f** respectively. Scale bars are all 500 nm. Abbreviations: ch, chloroplast; g, golgi apparatus; ig, immunogold; m, mitochondrion; nu, nucleus; py, pyrenoid; ri, ribosome; v, vacuole. Experiments were repeated twice and two samples (n=2) were used for each experiment.

**Figure 3. Sub-cellular distribution of  $^{34}\text{S}$  in *Prymnesium parvum* CCAP946/6 following sulfur uptake for 48 h. a-d**, Representative  $^{12}\text{C}^{14}\text{N}/^{12}\text{C}_2$  mass images showing cellular structures of *P. parvum* cells. The cells were imaged straight after the start of the incubation (a), and after 6 h (b), 24 h (c) and 48 h (d). **e-h**,  $^{34}\text{S}/^{32}\text{S}$  ratio of the same cells, shown as Hue Saturation Intensity (HSI) images where the colour scale indicates the value of the  $^{34}\text{S}/^{32}\text{S}$  ratio, with natural abundance in blue, changing to pink with increasing  $^{34}\text{S}$  levels. Each image was only acquired once. **i**, Isotope ratio of  $^{34}\text{S}/^{32}\text{S}$  in different cellular regions (biological replicates, number of cells analysed: T0: whole cells n = 7, chloroplasts n = 3, hotspot n = 10; T6: whole cells n = 14, chloroplasts n = 6, hotspot n = 10; T24: whole cells n = 12, chloroplasts n = 9, hotspot n = 10; and T48: whole cells n = 6, chloroplasts n = 4, hotspot n = 10; error bars are shown for standard error). Abbreviations, ch: chloroplast; h: hotspot; py: pyrenoid; v: vacuole. Scale bars: 1  $\mu\text{m}$ .









