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The abundant marine bacterium *Pelagibacter* simultaneously catabolizes dimethylsulfoniopropionate to the gases dimethyl sulfide and methanethiol

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Marine phytoplankton produce ~10^9 tonnes of dimethylsulfoniopropionate (DMSP) per year[1,2], an estimated 10% of which is catabolized by bacteria through the DMSP cleavage pathway to the climatically active gas dimethyl sulfide[3,4]. SAR11 Alphaproteobacteria (order Pelagibacterales), the most abundant chemo-organotrophic bacteria in the oceans, have been shown to assimilate DMSP into biomass, thereby supplying this cell's unusual requirement for reduced sulfur[5,6]. Here, we report that *Pelagibacter* HTCC1062 produces the gas methanethiol, and that a second DMSP catabolic pathway, mediated by a cupin-like DMSP lyase, DddK, simultaneously shunts as much as 59% of DMSP uptake to dimethyl sulfide production. We propose a model in which the allocation of DMSP between these pathways is kinetically controlled to release increasing amounts of dimethyl sulfide as the supply of DMSP exceeds cellular sulfur demands for biosynthesis.

In an experiment designed to measure the stoichiometry of dimethylsulfoniopropionate consumption, we observed that Pelagibacterales strain HTCC1062 produced methanethiol (MeSH), the gaseous end product of a catabolic pathway in which the first step involves DMSP demethylation. This was consistent with the presence in the genome of *dmdA*, which encodes DMSP demethylase[3,7]. However, we were surprised to observe that axenic cultures of this strain also produced large amounts of dimethyl sulfide (DMS, Fig. 1a). This observation indicated that, despite widespread attention to Pelagibacterales genomics and metagenomics, a *Pelagibacter* DMSP cleavage metabolic pathway leading to DMS formation had gone undetected. The amounts of DMS and MeSH increased linearly over 18 h of incubation in the presence of live cells, but DMS production by killed cell controls was either low or undetectable. Over 80% of the DMSP sulfur decrease could be accounted for, with 59% converted to DMS, 21% to MeSH and ~1% for biosynthesis (Table 1). These observations were confirmed by real-time measurements of DMS and MeSH production by cultured cells, using a proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF/MS, Fig. 2a).

The discovery that *Pelagibacter* expresses two DMSP degradation pathways simultaneously is particularly striking given its small genome size (1.28–1.46 Mb) and simple metabolism[8]. Enzymes for the DMSP demethylation pathway (DmdABC) have been described in *Pelagibacter*, but not DmdD, which catalyses the release of MeSH from methylthioacryloyl–coenzyme A (MTA-CoA)[9]. Nor has a gene for any DMSP lyase, which catalyses the alternative catabolic pathway leading to DMS production, been annotated or reported in *Pelagibacter*. Thus, the data shown in Fig. 1a confirm a complete demethylation pathway leading to MeSH production in *Pelagibacter*[3,7] and are the first evidence of a DMSP cleavage pathway in this organism.

Assimilation of DMSP sulfur into biomass is potentially a strong evolutionary driver for retention of DMSP metabolism in Pelagibacterales, which lack genes for assimilatory sulfate reduction[10]. To identify intermediates of DMSP metabolism that could support the demand for reduced sulfur for biosynthesis, HTCC1062 cultures were inoculated into artificial seawater medium (ASW) in the presence of MeSH, DMS or methionine (Fig. 1b). Only MeSH and methionine supported growth above the negative control. This is the first data showing that free MeSH can serve as a sulfur source for Pelagibacterales cells and it is consistent with the observation that, under DMSP-replete conditions, more sulfur is released as MeSH than is used for growth. The lower molar yield observed with MeSH, relative to methionine, is probably a consequence of the susceptibility of MeSH to spontaneous oxidation. DMS is apparently a metabolic waste product and cannot serve as a source of reduced sulfur in Pelagibacterales, in accord with the observations that DMS monooxygenase and DMS dehydrogenase are missing from Pelagibacterales genomes (Supplementary Fig. 1).

The unexpected observation of DMS production by HTCC1062 cultures (Fig. 1a) suggested that a DMSP lyase gene had gone undetected in the genome[7,9]. Reviewing the genome annotation, we noticed that hypothetical gene SAR11_0394 was predicted to have a C-terminal cupin, a very widely distributed protein fold that resembles a small barrel[11]. Of the DMSP lyases identified to date, three (DddL, DddQ and DddW) have C-terminal cupin domains and are members of the cupin superfamily[12–14] (Supplementary Fig. 2).

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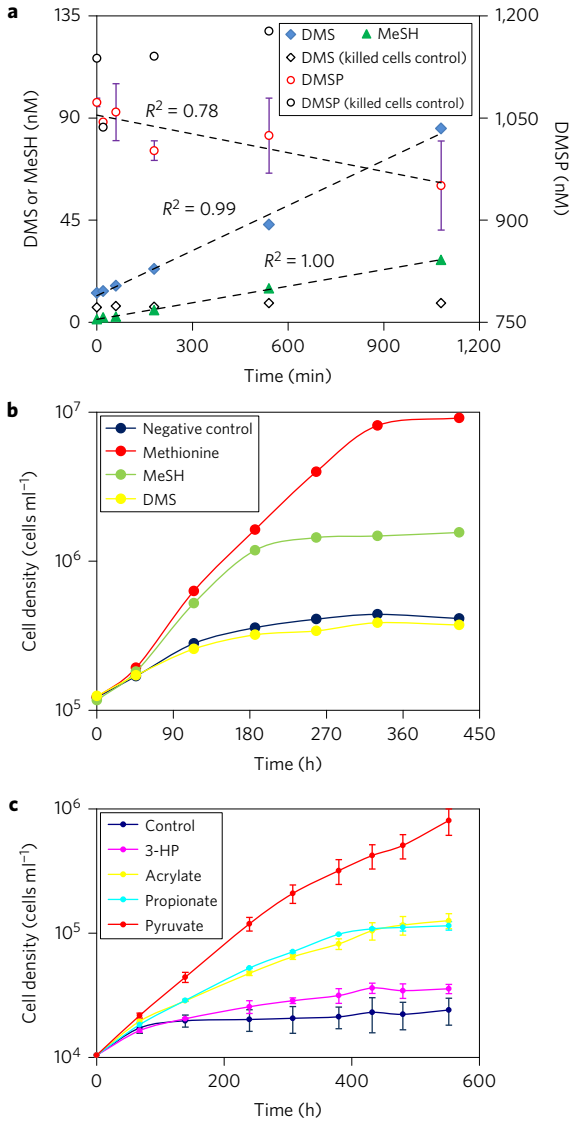

Figure 1 | DMSP metabolism in HTCC1062. a, Accumulated sulfur gases in headspaces as a function of time. Left axis: production of DMS (blue diamonds; control, black diamonds) or MeSH (green triangles) from DMSP in HTCC1062 culture. Right axis: DMSP decline (red circles; control, black circles). Results are the average of two biological replicate samples. Error bars indicate the range of the duplicates. Where no error bars are visible, they are smaller than the size of the symbols. Killed cell controls were performed in single vials, and the last data point for the killed cell (DMSP) was absent. No MeSH was detected in killed-cell controls. The Pearson's correlation *P* value for the DMSP regression is 0.007, with a correlation of −0.726. **b**, Growth of HTCC1062 on MeSH and DMS. Cultures were incubated in ASW amended with methionine (positive control), MeSH or DMS. A culture without any sulfur source was treated as negative control. Each point represents a single vial and the experiments were repeated three times, with similar results. **c**, Growth of HTCC1062 on C3 compounds. Cultures were incubated in ASW amended with 10 μM pyruvate (positive control), 3-HP, acrylate and propionate. The culture without pyruvate was treated as negative control. Points are the average of triplicate cultures and error bars indicate standard deviation ($n = 3$). Student's *t*-test was used to assess significance.

We confirmed that the SAR11_0394 gene encoded a product with DMSP lyase activity, by cloning and expressing it in *Escherichia coli* strain BL21. When grown in M9 medium containing 1 mM DMSP, the transformed *E. coli* strain converted ~15.4% of this substrate to corresponding molar amounts of DMS plus acrylate, as determined by gas chromatography (GC) and NMR, respectively. Genes homologous to SAR11_0394 in two additional Pelagibacterales strains, HTCC9022 and HIMB5, were cloned and also confirmed to encode DMSP lyases (Supplementary Table 1). The genes were named *dddK*. The DddK of HTCC1062 was purified and values of $V_{max} = 3.61 \pm 0.27$ μmol DMS min^{-1} (mg protein)$^{-1}$ and $K_m = 81.9 \pm 17.2$ mM were determined (Supplementary Figs 3 and 4).

Table 1 | Mass balance calculated from Fig. 1a.

	Concentration (nM)	Percentage
ΔDMSP	−122	100
ΔDMS	+73	59
ΔMeSH	+26	21
ΔCellular sulfur*	+1	1
Missing sulfur†		19

*Estimated cellular sulfur demands according to ref. 10.
†The DMSP loss not accounted for could be due to measurement errors, oxidation of MeSH, or DMSP accumulation within cells.

DMSP catabolism also benefits cells by providing a source of organic carbon that can be oxidized for energy production or assimilated into biomass[15]. The data suggest that when cells are supplied with an excess of DMSP, 99% of DMSP oxidation is probably supporting carbon metabolism (Fig. 1a and Table 1). DMSP lyase enzymes are distributed among multiple protein families, but all lead to the production of DMS and either acrylate (DddL, P, Q, W, Y) or 3-hydroxypropionate (3-HP; DddD)[9]. The HTCC1062 genome encodes annotated genes for all steps in the degradation of acrylate to propionyl–CoA or acetyl–CoA (Fig. 3). To test the capacity of strain HTCC1062 to assimilate acrylate, propionate or 3-HP, we relied on the unusual requirement of *Pelagibacter* strains for growth substrates that can be metabolized to pyruvate, which these cells require for alanine synthesis[16]. As predicted, acrylate and propionate each could substitute for pyruvate in defined media. Enhancement of growth by 3-HP was slight, but statistically significant (Student's *t*-test, $n = 3$, $P < 0.05$) (Fig. 1c).

Comparisons of Pelagibacterales genomes across the Group Ia subclade revealed that *dddK* homologues were found in eight of twelve Pelagibacterales Ia genomes (Supplementary Fig. 5). In addition to *dddK*, strain HIMB5 has a homologue of *dddQ*, also a member of the cupin superfamily[13]. As predicted, *E. coli* transformants containing cloned HIMB5_00000220 (*dddQ*) displayed DMSP lyase activity ($K_m = 56$ mM, $V_{max} = 0.78$ μmol min^{-1} (mg protein)$^{-1}$). Strain HTCC7211 and the more distantly related subclade V strain HIMB59 lacked *dddK* homologues, but encoded gene products (respectively PB7211_1082 and HIMB59_00005110) that are ~30% identical to DddP, a DMSP lyase in the M24 family of metallo-peptidases[17,18]. However, *E. coli* transformants containing cloned PB7211_1082 (*dddP*-like) showed very low DMSP lyase activity (0.5 ± 0.1 nmol min^{-1} mg^{-1}) and therefore this protein may not be a bona fide DMSP lyase.

We compared the abundance of the Pelagibacterales genes for DMSP cleavage with those for demethylation (*dmdABC*) in the Global Ocean Survey (GOS) metagenomic data set (Supplementary Fig. 6). The DMSP lyases *dddK* and *dddQ*, and *dddP*, the putative lyase with low activity, were much less abundant than *dmdABC* or the single-copy marker *recA*. This supports the interpretation that either the cleavage pathway is less important than the demethylation pathway, or undiscovered DMSP lyase analogues are present in other Pelagibacterales strains. Interestingly, Pelagibacterales genes for metabolism of acrylate are more abundant than DMSP lyases and similar in abundance to demethylation genes and *recA*, which supports either the interpretation that DMSP lyases are underestimated because of their diversity, or that *Pelagibacter* cells lacking DMSP lyase use acrylate from other sources, perhaps dissolved acrylate.

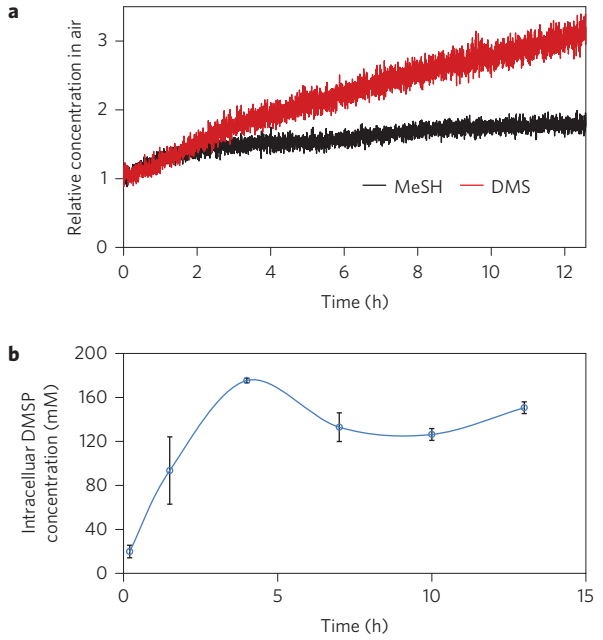

Figure 2 | Production of gaseous DMSP products as cells accumulate intracellular DMSP. a, Real-time gas-phase MeSH and DMS production measurements by PTR-TOF/MS. HTCC1062 cell suspensions that were not previously exposed to DMSP were incubated in ASW and subjected to a flow of fine bubbles. DMSP (1 μM) was added at $T = 0$ to cells that had been grown in the absence of DMSP. Measurements are presented in relative concentration units and were normalized to the gas-phase concentrations of MeSH and DMS (m/z 49 and 63, respectively) at $T = 0$. This experiment was repeated three times, with similar results, but with variation that we attribute to differences between batch cultures (Supplementary Fig. 9). **b**, Intracellular DMSP concentrations. HTCC1062 cell suspensions were incubated under the same condition as in **a**. Negative controls were killed cell cultures. DMSP (1 μM) was added at $T = 0$ to cells that had been grown in the absence of DMSP and 10 ml aliquots were filtered through 0.1 μm membranes at different time points to retain the cells. The intracellular DMSP concentrations were quantified by measuring DMS release after hydrolysis in NaOH. Results are the average of triplicate samples. Error bars indicate standard deviation ($n = 3$).

Most of the Pelagibacterales strains with DddK genes belong to the temperate surface ocean ecotypes (Ia.1)[19], whereas most of the strains that possess DddP are subtropical ocean surface ecotypes (Ia.3) (Supplementary Fig. 5). This may indicate that the lyase system is more common in Pelagibacterales strains, such as HTCC1062, that originate from higher-productivity ocean regions, a distribution that is consistent with its inferred role as an auxiliary system that metabolizes excess DMSP. However, the presence of an alternative gene, *DddP*, that has weak DMSP lyase activity in most SAR11 Ia.3 strains, suggests that further investigations of the phenotypes of live strains will be needed before the distribution of DMSP metabolism across the clade is fully understood.

Metabolic reconstruction with eight Pelagibacterales genomes revealed that, consistent with the observation of MeSH production in HTCC1062, this and other examined Pelagibacterales strains (except those in the distantly related subclade IIIa) contain homologues of the *dmdABC* genes found in *Ruegeria pomeroyi*[20] (Supplementary Fig. 1). Also reported in nearly all Pelagibacterales are genes encoding methyl group oxidation pathways (Supplementary Fig. 1), which produce energy from DMSP demethylation and are essential to the demethylation pathway because they perform the function of regenerating the methyl-group-accepting cofactor tetrahydrofolate (THF)[15,20]. Pelagibacterales strains also contain homologues of cystathionine-gamma-synthetase (cys-γ-synth), predicted to catalyse the conversion of MeSH to methionine[21] and thus necessary for growth when using MeSH as sole sulfur source. However, none of these examined Pelagibacterales strains had homologues of *dmdD* (methylthioacryloyl-CoA hydratase), which converts MTA–CoA to MeSH. The absence of this gene from Pelagibacterales is also reflected in its low abundance in ocean metagenomic databases[9]. As in HTCC1062, *dmdD* is not required for complete demethylation of DMSP to MeSH in *Ruegeria lacuscaerulensis*[7], suggesting that an undescribed analogous enzyme fills this pathway gap.

One of the unexpected findings reported above is that both the cleavage and demethylation pathways operate simultaneously. We investigated transcription changes using Affymetrix microarrays and observed no significant changes in the expression of DMSP catabolic pathway genes between HTCC1062 cells grown in the presence and absence of DMSP (Supplementary Section II). Because no changes in transcription were observed, we used isobaric tags for relative and absolute quantitation (iTRAQ) to compare the proteomes of HTCC1062 cultures grown in the presence and absence of DMSP, confirming that proteins for both pathways of DMSP catabolism are expressed constitutively (Supplementary Fig. 7 and Supplementary Table 2). Further support for this conclusion came from real-time measurements of DMS and MeSH production by cells, which showed that DMS and MeSH were immediately released when DMSP was added to cells that had been grown in the absence of DMSP (Fig. 2a).

We propose that constitutive, simultaneous expression of the cleavage and demethylation pathways in *Pelagibacter* is an adaptation that provides these cells with a kinetically regulated system that favours the pathway to DMS formation when intracellular DMSP concentrations are high. We modelled this process (Supplementary Fig. 8) using the measured properties of cloned enzymes and intracellular DMSP concentration (Fig. 2b). In *Pelagibacter*, DMSP active transport is thought to be mediated by the ABC transporter (OpuAC), which was the sixth most highly detected Pelagibacterales protein in a previous study of the Sargasso Sea metaproteome[22]. The properties of ABC transport functions are consistent with the model in that they predict that cells can achieve high intracellular DMSP concentrations from naturally measured DMSP abundances (Supplementary Table 3), provided that DMSP remains within the range of transporter affinity for a period of hours (Supplementary Section V). The K_m we measured for DddK, 81.9 ± 17.2 mM (Supplementary Fig. 4), is high compared to the K_m of DmdA (13.2 ± 2.0 mM)[23]. Intracellular DMSP concentrations increased following DMSP addition, reaching a maximum of 180 mM after 4 h (Fig. 2b). When DMSP flux into cells is low, the model predicts that most is channelled to MeSH production, producing energy via oxidation of the products CH_3–THF and acetaldehyde, sulfur for biosynthesis and MeSH losses caused by oxidation and diffusion (Fig. 1a). As intracellular DMSP concentrations rise, the model predicts that DMSP cleavage to DMS increases (Supplementary Fig. 8). There is a precedent for simple, kinetically driven switches controlling the flow of vital metabolites in HTCC1062, where intracellular glycine concentrations control the flow of carbon from exometabolites, such as glycolic acid, via glycine-mediated riboswitches[16,24]. Kinetic regulation of metabolic processes is well known, but here we see evidence that it plays an unexpected role in large-scale biogeochemical processes mediated by metabolically streamlined cells.

The model presented in Supplementary Fig. 8 captures the observations we report, and provides an explanation for why cells might simultaneously express two pathways that compete for a single substrate. Although the model in Supplementary Fig. 8 is based on *in vitro* enzyme kinetics, which can deviate from the kinetic properties of enzymes in the intracellular environment, the model successfully approximates the behaviour of whole cells

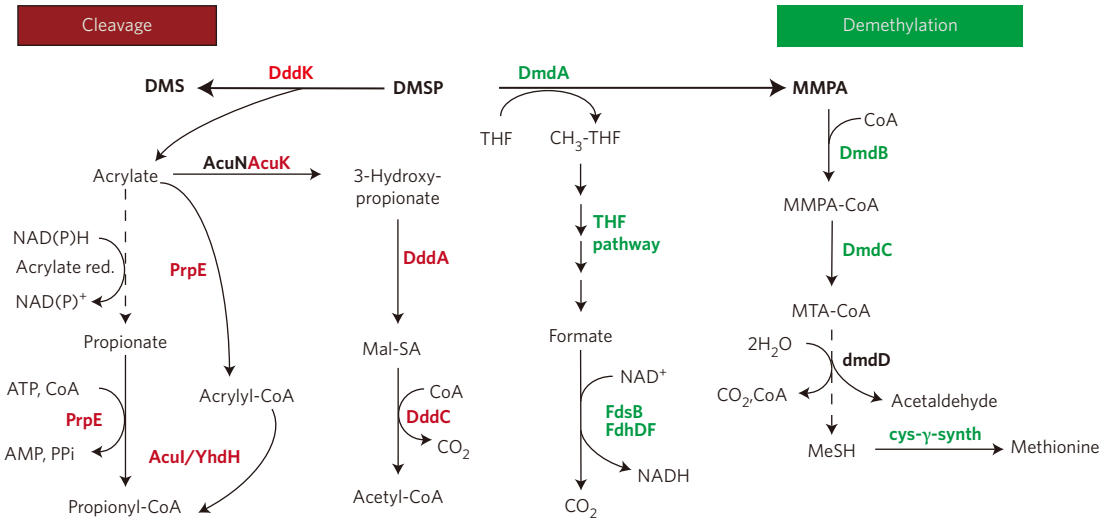

Figure 3 | DMSP catabolic pathways and homologues identified in HTCC1062. Predicted enzymes in the cleavage pathway are in red, enzymes in the demethylation pathway are in green. Proteins in black indicate that no homologues were identified in the HTCC1062. The distributions of these pathways across the Pelagibacterales are described in more detail in Supplementary Figs 1 and 5. ATP, adenosine triphosphate; AMP, adenosine monophosphate; PPi, pyrophosphate.

(Fig. 2a). However, a number of aspects of this model will need to be tested and refined before it can be validly implemented for geochemical predictions. In particular, we observed cells accumulating DMSP to high intracellular concentrations over a period of a few hours when supplied with excess DMSP. It remains to be determined how frequently such sustained supplies of DMSP occur in nature.

Recognition that the relative expression of the demethylation and cleavage pathways by bacteria in nature controls the fate of DMSP sulfur led to a concept that is referred to as the 'bacterial switch' in discussions of DMSP biogeochemistry[2]. In principle, the 'bacterial switch' could involve different bacterial taxa, each potentially having a different organization of DMSP metabolic pathways. Although the bacterial switch is largely hypothetical[4,25,26], insight has emerged from studies of cells in culture. Like *Pelagibacter*, the marine bacterium *R. pomeroyi* strain DSS-3 has both the DMSP demethylation and cleavage pathways, which are transcriptionally regulated, although the changes in expression that were reported were not very large[18,27]. Further work is needed to determine whether kinetic switching plays a role in the *R. pomeroyi* response to DMSP. Recent field observations indicate that *Roseobacter* species HTCC2255 regulates transcriptional expression of both the lyase and demethylase pathways for DMSP catabolism in response to changing environmental conditions[28]. The findings we report here provide important details about the mechanisms of the bacterial switch that will be vital to the design of future research and to modelling transformations of DMSP in ocean ecosystems[29,30]. Many factors, including DMSP leakage from phytoplankton, the action of free (dissolved) DMSP lyases and the activity of many different microbial taxa, contribute to natural fluxes of DMSP and its volatile derivatives[28,29]. The findings we present here describe an unexpected and simple mechanism that is probably an important part of this complex process.

Methods

Measurements of DMSP and its metabolic products. HTCC1062 was grown in autoclaved, filtered ASW[16] amended with 1 mM NH_4Cl, 100 μM KH_2PO_4, 1 μM $FeCl_3$, 100 μM pyruvate, 50 μM glycine, 1 μM DMSP and excess vitamins[16]. Cultures were collected by centrifugation, washed once, and resuspended in ASW. Cells (final concentration of ~1.5×10^6 cells ml^{-1}) were distributed into 20 ml sealed vials (10 ml per vial). DMSP (1 μM) was injected into vials and incubated in the dark at 16 °C. Biological activity was stopped by the addition of 0.1 M sodium azide (100 μl per vial) at 0, 20 min and 1, 3, 9 and 18 h. Biological duplicate samples were refrigerated before chemical analysis.

DMS and MeSH were analysed using the solid-phase microextraction-gas chromatography-pulsed flame-photometric detection (SPME-GC-PFPD) method[31,32]. DMSP was quantified by measuring released DMS after hydrolysis in NaOH (0.1 M final concentration), at room temperature for 12 h.

DMS and MeSH utilization in HTCC1062. HTCC1062 was cultured in 40 ml clear sealed vials with autoclaved, filtered ASW amended with 1 mM NH_4Cl, 100 μM KH_2PO_4, 1 μM $FeCl_3$, 100 μM pyruvate, 50 μM glycine, excess vitamins[16] and 100 nM DMSP, methionine, DMS or MeSH. Each vial contained a 10 ml aliquot, which was incubated on a shaker at 16 °C. Cell densities were monitored with a Guava flow cytometer[33].

C3 compounds utilization in HTCC1062. Cells (biological triplicates) were grown in autoclaved, filtered ASW amended with 100 μM NH_4Cl, 10 μM KH_2PO_4, 100 nM $FeCl_3$, 50 μM glycine, 50 μM methionine and excess vitamins[16]. Each compound (3-HP, acrylate or propionate) was tested at a concentration of 10 μM. The positive control was amended with 10 μM pyruvate. The negative control contained no pyruvate.

Real-time measurements of DMS and MeSH by PTR-TOF/MS. HTCC1062 was grown in autoclaved, filtered ASW amended with 1 mM NH_4Cl, 100 μM KH_2PO_4, 1 μM $FeCl_3$, 100 μM pyruvate, 25 μM glycine, 25 μM methionine and excess vitamins[16]. Cultures were collected by centrifugation, washed once and re-suspended in ASW. Cells (~$3–5 \times 10^6$ cells ml^{-1} final concentration) were distributed into 100 ml ASW and placed in a 200 ml polycarbonate dynamic stripping chamber. DMSP (1 μM) was spiked into the chamber and the suspensions were incubated at 16 °C under a continuous flow of fine air bubbles. A PTR-TOF mass spectrometer 1000 (IONICON Analytik) was used to quantify the production of MeSH and DMS from HTCC1062 cultures. The fundamentals of PTR-MS are described elsewhere[34]. Primary ions (protonated water, H_3O^+) were produced from pure water vapour in the hollow cathode ion source at a flow rate of 5 s.c.c.m., from which they entered the drift tube. The sample air stream produced from the dynamic stripping chamber was introduced to the drift tube via a separate orifice, where proton transfer reactions occurred between H_3O^+ and volatile organic compounds (VOCs) that had proton affinities greater than that of water (691 kJ mol^{-1}):

$$VOC + H_3O^+ \rightarrow VOC \cdot H^+ + H_2O$$

Within the drift tube, the pressure, temperature and voltage conditions were kept constant at 2.0 mbar, 80 °C and 600 V, respectively, which equated to a field strength (E/N) of 153 Td (where Td = 10^{-17} cm^2 V $molecule^{-1}$). One advantage of PTR-MS is that reactions occurring in the drift tube are non-dissociative and compounds are not usually fragmented during ionization and exhibit a protonated mass of M+1. Thus, for DMS and MeSH, we monitored m/z 63 and 49, respectively. Although interference at these masses is likely to be low, we cannot rule out the possibility that more than one compound was contributing to the signal. Mass spectra were recorded up to 250 a.m.u. at 10 s integration intervals. Quantification of gas-phase DMS and MeSH concentrations was achieved using the relative transmission (kinetic) approach and also accounted for the influence of the hydrated water cluster at m/z 37 (due to the high sample humidity introduced by bubbling air through

seawater). For MeSH, a default collision rate constant of 2.00×10^{-9} cm^{-2} was assumed, whereas a literature value of 2.53×10^{-9} cm^{-2} was used for DMS[35].

Intracellular DMSP concentration. HTCC1062 was grown under the same conditions as described above (see 'Real-time measurements of DMS and MeSH by PTR-TOF/MS'). Cultures were collected by centrifugation, washed once and resuspended in ASW. Cells (final concentration of $\sim 4 \times 10^6$ cells ml^{-1}) were distributed into five 200 ml chambers (100 ml per chamber) and treated using the same air bubbling method as described for the dynamic stripping chambers above. DMSP (1 μM) was spiked into the chambers, which were subsequently incubated at 16 °C. Duplicate negative (killed cells) controls were performed by the addition of 0.1 M sodium azide (100 μl per vial). Cultures (10 ml, biological triplicates) were filtered through 0.1 μm polytetrafluoroethylene membranes at 10 min and 1.5, 4, 7, 10 and 13 h. The cells on the membranes were washed once with ASW, then transferred into 20 ml sealed vials, and finally resuspended in 10 ml ASW. DMSP was quantified by measuring DMS release after hydrolysis in NaOH (0.1 M final concentration), at room temperature for 12 h. DMS was analysed using the SPME-GC-PFPD method.

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Author contributions

J.S. and S.J.G. conceived and designed the experiments. J.S., Y.Q. and M.C.Q. measured DMSP products and intracellular DMSP concentration. J.S. and J.G. performed the physiological growth experiments for HTCC1062. J.S. and J.C.T. analysed and proposed DMSP metabolic pathways. J.D.T., E.K.F. and A.W.B.J. designed and implemented the cloning, expression and characterization of DddK. B.T. performed metagenomics analyses. B.T., J.T.A., C.D.N., M.S.L., R.D.S. and S.H.P. performed iTRAQ and data analyses. P.D.L. and S.J.G. proposed the model. C.L.D.-M. and K.H.H. measured real-time DMS/MeSH production by PTR-TOF/MS. S.J.G. contributed reagents, materials and analysis tools.

Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.J.G.

Competing interests

The authors declare no competing financial interests.