LETTER

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Novel pathway for assimilation of dimethylsulphoniopropionate widespread in marine bacteria

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Dimethylsulphoniopropionate (DMSP) accounts for up to 10% of carbon fixed by marine phytoplankton in ocean surface waters[1,2], producing an estimated 11.7–103 Tmol S per year[3], most of which is processed by marine bacteria through the demethylation/demethiolation pathway[4]. This pathway releases methanethiol (MeSH) instead of the climatically active gas dimethylsulphide (DMS) and enables marine microorganisms to assimilate the reduced sulphur[5–7]. Despite recognition of this critical microbial transformation for over two decades, the biochemical pathway and enzymes responsible have remained unidentified. Here we show that three new enzymes related to fatty acid β-oxidation constitute the pathway that assimilates methylmercaptopropionate (MMPA), the first product of DMSP demethylation/demethiolation, and that two previously unknown coenzyme A (CoA) derivatives, 3-methylmercaptopropionyl-CoA (MMPA-CoA) and methylthioacryloyl-CoA (MTA-CoA), are formed as novel intermediates. A member of the marine roseobacters, *Ruegeria pomeroyi* DSS-3, requires the MMPA-CoA pathway for MMPA assimilation and MeSH production. This pathway and the ability to produce MeSH from MMPA are present in diverse bacteria, and the ubiquitous SAR11 clade bacterium *Pelagibacter ubique* possesses enzymes for at least the first two steps. Analysis of marine metagenomic data indicates that the pathway is widespread among bacterioplankton in the ocean surface waters, making it one of the most important known routes for acquisition of reduced carbon and sulphur by surface ocean heterotrophs.

The global importance of DMSP lies in its availability as a carbon and sulphur source for marine microorganisms and as a precursor of the gas dimethylsulphide (DMS)[8], the oceanic emission of which leads to the formation of cloud condensation nuclei and promotion of solar radiation backscatter[9]. Two competing pathways exist for the bacterial catabolism of DMSP, one releasing DMS and the other releasing methanethiol (MeSH). Only recently has there been progress on identifying the specific biochemical pathways and genes responsible for these transformations. To date, four genes have been identified that encode proteins which catalyse the cleavage reaction that releases DMS[10–13], and one gene (*dmdA*) has been identified that encodes the initial demethylase in the pathway to MeSH[14]. Following demethylation, the intermediate MMPA is further catabolized to the highly reactive volatile sulphur gas MeSH[15]. This demethiolation pathway has long been proposed to be either an elimination or reductive cleavage, producing either acrylate or propionate, respectively[16,17]. An alternative proposal suggested that MMPA is catabolized similarly to β-oxidation of fatty acids[18–20]. In either case, MeSH has not been shown to be a major product of DMSP metabolism as its rapid turnover results in very low concentrations in both culture-based (Supplementary Table 1) and environmental experiments[15]. Thus, this report is the first direct evidence that MeSH is indeed a major degradation product of DMSP in at least some organisms.

To elucidate the pathway of DMSP demethiolation in the marine roseobacter *Ruegeria pomeroyi* DSS-3, CoA-containing intermediates were examined. A *dmdA*$^-$ strain, which is incapable of DMSP demethylation, was supplied with DMSP, and the wild type was given MMPA. Upon CoA extraction and HPLC separation, an unknown CoA-containing intermediate was highly abundant in cells fed MMPA, compared to the mutant strain, which could only use the cleavage pathway (Supplementary Fig. 1). Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry indicated that the mass of this unknown product was 870.137 (Supplementary Fig. 2a), consistent with the theoretical monoisotopic mass of a CoA-MMPA thioester (MMPA-CoA) of 870.137 $[M+H]^+$. MMPA-CoA was synthesized chemically and shown to have the same molecular weight and chromatographic retention time as the compound isolated from cell extracts. When [1,2,3-^{13}C] DMSP was used as the carbon source for a mutant in this pathway (see below), the mass of MMPA-CoA purified from cell extracts increased as expected to 873.147 $[M+H]^+$ (Supplementary Fig. 2b), indicating that this compound was in fact a product of DMSP metabolism.

To identify the enzyme catalysing the production of MMPA-CoA, the native enzyme was purified from *R. pomeroyi* DSS-3 cell extracts. One of four proteins remaining after purification, as judged by silver-stained SDS–polyacrylamide gel electrophoresis (SDS–PAGE), was identified as a medium chain fatty-acid CoA ligase by matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) analysis (Supplementary Table 2). This protein, now designated as 3-methylmercaptopropionyl-CoA ligase (DmdB), was encoded by gene SPO2045. Confirmation that the gene encoded the correct protein was obtained by cloning SPO2045 into the pET101 expression vector and expressing it in *Escherichia coli*. The recombinant *E. coli* strain possessed MMPA-CoA ligase activity, whereas the host strain alone did not. The enzymatic reaction consumed ATP and produced AMP (Supplementary Fig. 3a) and is presumed to have produced pyrophosphate as well.

To elucidate the next step of the pathway, MMPA-CoA-consuming activity in *R. pomeroyi* DSS-3 was examined by incubating crude cell extracts with MMPA-CoA and various redox cofactors. Upon the addition of the artificial electron acceptors phenazine methosulphate or ferrocenium hexafluorophosphate, MMPA-CoA was consumed and an unknown intermediate was produced, the molecular mass of which was 868.121 $[M+H]^+$ (Supplementary Fig. 2c), exactly two hydrogen atoms less than MMPA-CoA. This mass was consistent with the theoretical monoisotopic molecular mass of methylthioacryloyl-CoA (MTA-CoA; Fig. 1).

Upon the addition of MTA-CoA to *R. pomeroyi* DSS-3 crude cell extract, MeSH and free CoA were released. The enzyme catalysing the release of MeSH was purified to electrophoretic homogeneity from cell extracts of MMPA-grown *R. pomeroyi* DSS-3 (Supplementary Fig. 4) and identified as SPO3805, an enoyl-CoA hydratase and a member of the crotonase superfamily (cd06558) (Supplementary Table 3). Immediately upstream in the *R. pomeroyi* genome was a gene annotated as acyl-CoA dehydrogenase. We proposed that this gene, SPO3804, encoded the enzyme catalysing the production of MTA-CoA. SPO3804 and SPO3805 were each cloned into the pET101 expression vector and

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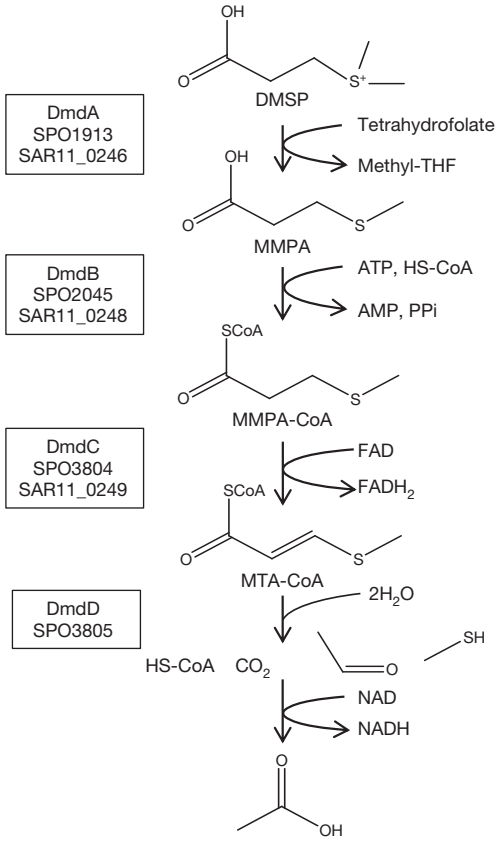

Figure 1 | Pathway of DMSP demethylation as identified in *R. pomeroyi* DSS-3. The genes identified thus far in both *R. pomeroyi* DSS-3 and *P. ubique* are indicated at each step. DMSP is first demethylated to MMPA in a tetrahydrofolate-dependent reaction catalysed by DmdA as described previously[25]. A MMPA-CoA thioester is then formed in a reaction that consumes ATP and produces AMP. MMPA-CoA is then dehydrogenated forming an enoyl-CoA intermediate, MTA-CoA. In *R. pomeroyi* DSS-3, MTA-CoA is then hydrated by MTA-CoA hydratase in a reaction that releases the volatile sulphur product MeSH as well as free CoA, CO_2 and acetaldehyde. Acetaldehyde is then oxidized to acetic acid by acetaldehyde dehydrogenase.

expressed in *E. coli*. The recombinant *E. coli* strains had activity for MMPA-CoA dehydrogenase (SPO3804) and MTA-CoA hydratase (SPO3805), whereas the host strain alone did not (Supplementary Fig. 3b and 3c). Therefore, we designate these genes as *dmdC* and *dmdD*, respectively.

The DmdD-catalysed reaction produced stoichiometric amounts of free CoA and MeSH. Using ^{1}H and ^{13}C NMR spectroscopy it was found that the three-carbon moiety was transformed into acetaldehyde (Supplementary Figs 5 and 6). The thioester bonded carbon of MTA-CoA was lost completely, presumably as CO_2, in the reaction (Supplementary Fig. 7). Quantification of acetaldehyde produced from MTA-CoA found 90% of the theoretical yield. *R. pomeroyi* DSS-3 is capable of further oxidizing acetaldehyde to acetate, based on the levels of acetaldehyde dehydrogenase activity (Table 1).

There are multiple lines of evidence that confirm the physiological significance of these activities. In extracts of chemostat-grown cells the levels of DmdB, DmdC and DmdD activities exceeded the minimum level, 57 nmol min^{-1} per mg of protein, necessary to support growth (Table 1 and Methods). In addition, the amount of transcripts for *dmdB*, *dmdC* and *dmdD* increased during growth on MMPA or DMSP, as expected if the pathway was required for MMPA metabolism (Supplementary Fig. 8).

Mutations in each of the MMPA-CoA pathway genes also yielded phenotypes consistent with their participation in the pathway. A *dmdC*$^{-}$ mutant (SPO3804::Tn*5*) could not grow on MMPA as the sole source of carbon (Supplementary Fig. 9), indicating that this pathway was essential for growth on MMPA. In contrast, the mutant grew similarly to wild-type with DMSP, indicating that the cleavage pathway, initiated by DddQ or DddP[13], remained capable of supporting growth. Following growth with DMSP, the levels of DmdC and DmdD activity in the mutant were greatly reduced (Table 1). The low level of DmdC activity was consistent with the presence of additional *dmdC* orthologues in *R. pomeroyi* DSS-3 (see below). The low level of DmdD activity indicated that *dmdC* and *dmdD* were cotranscribed. This hypothesis was consistent with the absence of a recognizable promoter preceding *dmdD*, the coregulation of both genes, and the reduced levels of *dmdD* transcript in the *dmdC*$^{-}$ mutant (Supplementary Fig. 8). Presumably, read-through of the transcriptional terminator on the kanamycin resistance marker was responsible for the low level of DmdD activity. The growth defect of the *dmdC*$^{-}$ mutant was complemented with SPO3804 but not SPO3805 expressed on a plasmid, indicating that the failure of the *dmdC*$^{-}$ mutant to grow on MMPA was not due to a polar affect on *dmdD*.

A *dmdD* (SPO3805::*tet*) mutant also failed to grow with MMPA, and growth on DMSP was severely inhibited (Supplementary Fig. 9d). Following growth on acetate, DmdD activity of the mutant was <0.5 nmol min^{-1} mg^{-1}, whereas wild-type had activity of 12.7 nmol min^{-1} mg^{-1}. Lastly, growth of a *dmdB* mutant (SPO2045::*tet*) was somewhat delayed during growth on MMPA (Supplementary Fig. 9e). Following growth with DMSP, the DmdB activity was reduced by only 40% compared to the wild-type (data not shown), which was consistent with the presence of an additional *dmdB* orthologue in *R. pomeroyi* DSS-3 (see below).

Homologues to the *R. pomeroyi* DSS-3 genes are abundant in the genomic database. The genomic database contained 36 bacteria that possessed *dmdA*, and all 36 also possessed *dmdB* and *dmdC*. However, the distribution of *dmdB* and *dmdC* was not limited to bacteria possessing *dmdA*, and many β and γ-proteobacteria as well as other bacteria not typically associated with marine systems possessed homologues with high sequence similarity to *dmdB* and *dmdC*. To confirm that *dmdA*-negative bacteria were capable of producing of MeSH from MMPA, pure cultures of representative bacteria were grown in the presence of MMPA. *Burkholderia thailandensis*, *Pseudomonas aeruginosa*, *Pseudoalteromonas atlantica*, *Myxococcus xanthus* and *Deinococcus radiodurans* all produced MeSH from MMPA. In contrast, *Escherichia coli*, which does not possess highly similar homologues, did not produce MeSH under the same conditions (Supplementary Table 4). Although a strain of *Burkholderia* was previously shown to possess a gene product capable of catalysing the DMSP cleavage reaction[10], it is unlikely that many of the organisms possessing

Table 1 | MMPA-CoA pathway enzyme activities in cell extracts

Strain	Growth conditions	Enzyme assay*			
		MMPA-CoA ligase (DmdB)	MMPA-CoA dehydrogenase (DmdC)	MTA-CoA hydratase (DmdD)	Acetaldehyde dehydrogenase
R. pomeroyi DSS-3	2 mM MMPA (chemostat)	118 ± 17	1,226 ± 213	759 ± 36	132 ± 11
R. pomeroyi DSS-3	2 mM DMSP (batch)†	11 ± 1	281 ± 15	186 ± 18	34 ± 6
R. pomeroyi DmdC^{-} (SPO_3804::Tn*5*)	2 mM DMSP (batch)†	27 ± 2	43 ± 5	12 ± 1	26 ± 3
R. lacuscaerulensis	1 mM MMPA (chemostat)	198 ± 30	39 ± 5	44 ± 13	n.d.

* Activities are reported as nmol min^{-1} per mg of protein and are the result of triplicate experiments, ± s.d.
† The mutant and control wild-type strains were grown in batch culture to avoid selection for revertants in the chemostat.
n.d., not detected.

the MMPA-CoA pathway naturally encounter DMSP or MMPA derived from DMSP, and an alternative source of MMPA is likely. One possibility is that MMPA is derived from methionine, via the "off-pathway" reaction in the salvage pathway[21]. Regardless of the source of MMPA, the presence of the MMPA-CoA pathway in diverse bacteria further emphasizes its importance.

Both *dmdB* and *dmdC* are members of large gene families that encode enzymes with many different functions. To distinguish *dmdC* from closely related homologues with different functions, a selection of *dmdC* homologues from MeSH-producing bacteria were cloned and expressed in *E. coli*. Genes from *B. thailandensis*, *P. aeruginosa* and *Ruegeria lacuscaerulensis* encoded proteins with DmdC activity. Similarly, all three copies of *dmdC* from *R. pomeroyi* DSS-3 yielded functional proteins when expressed in *E. coli*. Lastly, SAR11_0249, the *dmdC* homologue from the SAR11 clade bacterium *Pelagibacter ubique* HTCC1062, was synthesized, expressed in *E. coli*, and shown to possess DmdC activity. These genes encompassed a well-defined clade within the acyl-CoA dehydrogenases (Fig. 2). Likewise, both copies of *dmdB* from *R. pomeroyi* DSS-3 and SAR11_0248, the *Pelagibacter ubique* HTCC1062 *dmdB* homologue, yielded functional proteins when expressed in *E. coli*. These genes defined a similar DmdB clade of acyl-CoA ligases (Supplementary Fig. 10). Of the 49 sequenced genomes currently available in the JGI genomic database from the family *Rhodobacteraceae*, which contains the marine roseobacters, 47 and 49 possess *dmdB* and *dmdC* genes, respectively. The metabolism of reduced sulphur compounds in the marine roseobacters is complex, with some organisms producing MeSH from MMPA while lacking the ability to demethylate DMSP[22]. Thus, some roseobacters possess *dmdB* and *dmdC* even though they lack *dmdA* and are unable to demethylate DMSP. In addition to demonstrating that the MMPA-CoA pathway was widespread among marine bacteria, these experiments demonstrated that homologues are widespread in bacteria from a variety of habitats.

Given the abundance of *Pelagibacter* and the roseobacters in the ocean, both *dmdB* and *dmdC* are likely to be abundant in ocean surface waters. Analysis of the GOS metagenomic database[23] confirmed this hypothesis, as over 6200 homologues to DmdB and DmdC were found, indicating that these genes may be present in up to 61% of surface ocean bacterioplankton (Supplementary Table 5).

In contrast, the *P. ubique* homologue to the *R. pomeroyi* DSS-3 DmdD possessed only low protein identity of 24%. When synthesized

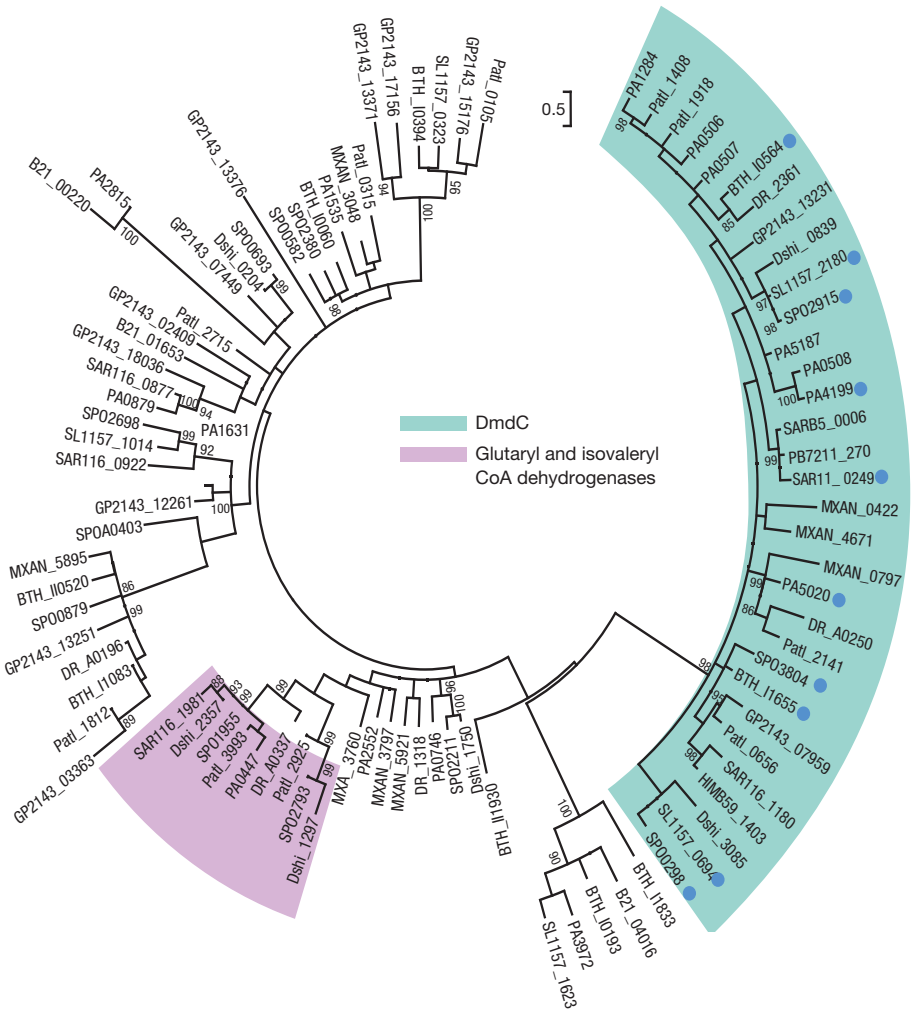

Figure 2 | Phylogenetic tree of DmdC from representative bacteria. Proteins from representative bacteria from marine surface waters as well as bacteria shown to produce MeSH from MMPA were included in the phylogenetic analysis. Proteins whose function was verified by recombinant expression in *E. coli* are indicated with blue dots. Methods for selecting homologous sequences are described in the supplementary methods. The original alignment contained 194 sequences, but for clarity, 101 out-group sequences were removed. Clusters with an identified or annotated function are labelled, whereas the remaining sequences possess conserved domains in the acyl-CoA dehydrogenase superfamily (cl09966). Locus tags correspond to organisms as follows: *Escherichia coli* BL21(DE3) (B21), *Burkholderia thailandensis* (BTH), *Deinococcus radiodurans* (DR), *Dinoroseobacter shibae* (Dshi), marine γ-proteobacteria HTCC2143 (GP2143), SAR11 HIMB59 (HIMB59), *Myxococcus xanthus* (MXAN), *Pseudomonas aeruginosa* (PA), *Pseudoalteromonas atlantica* (Patl), *P. ubique* HTCC7211 (PB7211), *P. ubique* HTCC1062 (SAR11), *Puniceispirillum marinum* IMCC1322 (SAR116), SAR11 HIMB5 (SARB5), *R. lacuscaerulensis* (SL1157), *R. pomeroyi* (SPO).

and expressed in *E. coli*, it did not possess activity with MTA-CoA as the substrate. The gene SAR11_0247, annotated as an α-β fold hydratase, was also cloned and expressed because it was located in between *dmdA* and *dmdB*. However, this gene product also did not have activity with MTA-CoA as the substrate. These results suggested that *dmdD* orthologues were not widely distributed and may have been replaced by non-orthologous isofunctional enzymes in some organisms. To investigate the pathway being used by *dmdD*-negative bacteria, the activity of the enzymes of the MMPA-CoA pathway were assayed in the *dmdD*-negative strain *Ruegeria lacuscaerulensis*. Cell extracts had activity for both DmdB and DmdC, as expected, but also for DmdD (Table 1). Therefore, a non-orthologous isofunctional enzyme may have replaced *dmdD* in this bacterium. Whether an isofunctional enzyme is also catalysing this step in *P. ubique* is unknown, but it is clear that orthologous proteins are not abundant in ocean surface waters as a BLASTp search against the GOS metagenomic database[23] yielded only 16 homologues with scores corresponding to an *e*-value of less than e^{-30}.

In conclusion, the novel MMPA-CoA pathway is widespread in marine and other bacteria. In oceans, this pathway leads to the formation of MeSH and acetate from the common osmolyte DMSP and prevents formation of the anti-greenhouse gas DMS. Acetate as a final product for the three-carbon moiety of DMSP is significant in that possible fates of acetate are numerous in cells. The DMSP cleavage pathway in a strain of *Halomonas* also resulted in the production of acetate as an end product, although the distribution and abundance of this pathway are unclear[24]. The ecological function of the MMPA-CoA pathway outside the ocean is less understood. However, its ubiquity is strong evidence for an important role. Further investigations on the abundance, enzymology and expression of this pathway will be critical to our understanding of biosequestration and flux of reduced sulphur and carbon in marine and other ecosystems.

METHODS SUMMARY

R. pomeroyi DSS-3 and *R. lacuscaerulensis* were grown in an artificial seawater medium as described previously[25]. Continuous cultures were grown in a carbon-limited chemostat with a dilution rate of $0.0416\ h^{-1}$ and a doubling time of 24 h. To determine the minimum enzyme activity required to sustain growth in the chemostat, the dry weight of the chemostat culture was estimated using the attenuance at 660 nm (D_{660}) and the equation ($\mu g\ ml^{-1}$) = $364.74D_{660} + 6.7D_{660}$ (ref. 37). Using a substrate concentration of 1 mM for *R. lacuscaerulensis* or 2 mM for *R. pomeroyi* DSS-3 and a flow rate of $0.1\ ml\ min^{-1}$, there was a total of 100 or 200 $nmol\ min^{-1}$ of substrate entering the chemostat. Assuming that 55% of dry weight was protein[38], the minimum enzyme activity required to sustain the observed MMPA consumption in the chemostat was estimated at 57 and 46 $nmol\ min^{-1}\ mg^{-1}$ for *R. pomeroyi* DSS-3 and *R. lacuscaerulensis*, respectively. For full details of growth conditions, protein purifications, enzyme assays and genetic manipulations, see the Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions C.R.R. performed growth experiments, enzyme assays, protein purifications, substrate synthesis, phylogenetic analysis, and all reaction analysis except MALDI-FT-ICR. M.J.S. and I.J.A. performed MALDI-FT-ICR analysis. C.R.R. and V.A.V. performed genetic modifications of *R. pomeroyi* DSS-3. V.A.V. performed RT-qPCR. C.R.R. and M.A.M. conducted bioinformatic analyses. C.R.R., M.A.M. and W.B.W. designed the experiments and wrote the paper. All authors reviewed the manuscript before submission.

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METHODS

Preparation of *R. pomeroyi* DSS-3 for intracellular acyl-CoA analysis. To investigate the biochemical pathways used to assimilate DMSP and MMPA in *R. pomeroyi* DSS-3, a targeted extraction of CoA-containing intermediates, such as those in the methylmalonyl-CoA or ethylmalonyl-CoA pathways, was performed. These experiments used a rich medium to grow the cells to a high density in order to obtain high amounts of CoA-containing intermediates which could be detected by ultraviolet absorbance at 260 nm after HPLC separation. Wild-type *R. pomeroyi* DSS-3, *dmdA*$^-$ (SPO1913::Tn*5*), or *dmdC*$^-$ (SPO3804::Tn*5*) was grown overnight in half-strength YTSS medium[26]. Cells were collected, washed once, and resuspended in marine basal medium (MBM)[25] with 5 mM MMPA (wild-type) or DMSP (*dmdA*$^-$ and *dmdC*$^-$) After overnight incubation, cells were again collected and resuspended in 3 ml of MBM with 5 mM DMSP or MMPA. Cells were incubated for 1 h, and then coenzyme-A containing intermediates were extracted.

Coenzyme-A extraction and analysis. Cells were quenched by the addition of trichloroacetic acid to 5%. Cell debris was removed by centrifugation at 10,000*g* for 10 min. The supernatant was then passed through an oligo purification cartridge (OPC, Applied Biosystems), which had been pre-washed with 70% acetonitrile/30% 10 mM KH_2PO_4 and then pre-equilibrated with 10 mM KH_2PO_4 (ref. 27). The OPC was then washed with 3 ml of 10 mM KH_2PO_4 and flushed with 1 ml of air. Products retained on the cartridge were eluted with 0.5 ml of 70% acetonitrile/30% 10 mM KH_2PO_4. The eluent was diluted to 1.5 ml with dH_2O, frozen, and lyophilized.

Products retained by the OPC were resolved by reverse phase chromatography using a 4.6 × 250 mm, 5 µm particle size, Aquasil column (Thermo Fisher). The column was developed at a flow rate of 1 ml min^{-1} with a gradient of 2–20% acetonitrile in 50 mM ammonium acetate (pH 6.0) over 40 min. Products were detected by absorbance at 260 nm. Identification of acyl-CoAs was based on the elution time of known standards. Additionally, some products observed on the ultraviolet trace did not contain coenzyme-A and were not further identified.

Preparation of *R. pomeroyi* DSS-3 cell extracts. A 9 l culture of *R. pomeroyi* DSS-3 was grown in a 15 l fermenter with MBM and 3 mM MMPA as the sole source of carbon. The air flow was set to 10 l min^{-1}. Cells were collected after 2 days of growth at an attenuance D_{600} of 0.2 by centrifugation at 10,000*g* for 10 min and washed once with ice cold 50 mM Tris-HCl (pH 7.5). The pellet was resuspended in 5 ml of 50 mM Tris- HCl (pH 7.5). Cells were then lysed by passage through a French pressure cell at 100,000 kPa three times and centrifuged at 15,000*g* for 10 min. The supernatant was then centrifuged at 100,000*g* for 1 h at 4 °C. The supernatant at this stage was used for enzyme purifications.

***In vivo* production of MeSH.** *Burkholderia thailandensis* E264, *Pseudoalteromonas atlantica* T6C and *Ruegeria lacuscaerulensis* were grown in MBM with 4 mM acetate with and without 1 mM MMPA. *Pseudomonas aeruginosa* PAO1 and *Escherichia coli* BL21(DE3) were grown in M9 minimal medium with 4 mM acetate or 4 mM acetate supplemented with 1 mM MMPA. *Deinococcus radiodurans*[28] and *Myxococcus xanthus*[29] were grown in defined medium as described previously, with and without 1 mM MMPA. All experiments were performed with 5 ml of medium in 28 ml Balch tubes sealed with a Teflon-coated stopper.

SPO3805 (DmdD) purification. Q-Sepharose HP chromatography: the cell extract was applied to a Q-Sepharose HP (GE Healthcare) anion exchange column (1.6 × 10 cm) equilibrated with 50 mM Tris-HCl (pH 8.0) at a flow rate of 2 ml min^{-1}. Protein was eluted with a gradient from 0–1 M NaCl over 8 column volumes. Activity eluted over 20 ml between 19 and 30 mS cm^{-1}.

Phenyl-Superose chromatography: active fractions from Q-Sepharose chromatography were pooled and made 1.7 M $(NH_4)_2SO_4$ by addition of solid $(NH_4)_2SO_4$. The solution was applied to a phenyl-Superose HR (GE Healthcare) hydrophobic interaction column (1 × 10 cm) at a flow rate of 1 ml min^{-1}, and the column was washed with 1 column volume of 1.7 M $(NH_4)_2SO_4$ in 50 mM Tris-HCl (pH 7.5). Protein was eluted with a gradient of 1.7–0 M $(NH_4)_2SO_4$ in 50 mM Tris-HCl (pH 7.5). Activity eluted at 110–96 mS cm^{-1}. Active fractions were pooled and concentrated with an Amicon Ultra centrifugal filter (10 kDa). The final concentrate was suspended in 50 mM potassium phosphate buffer (pH 7.5).

HiTrap Blue chromatography: the concentrated protein solution was then applied to a HiTrap Blue column (6 ml, GE Healthcare) that was equilibrated with 50 mM potassium phosphate (pH 7.5). The column was washed with 4 column volumes of buffer, and protein was eluted with a 0–2 M gradient of KCl in buffer over 6 column volumes. Activity eluted after start of the gradient. The 4 ml fraction containing the highest activity was concentrated using an Amicon Ultra centrifugal filter (10 kDa).

Sephacryl S200 chromatography: the protein concentrate was applied to a Sephacryl S200 (GE Healthcare) gel filtration column (1.6 × 25 cm) that was pre-equilibrated with 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl. The protein was eluted with buffer at a flow rate of 1 ml min^{-1}. Fractions with activity were analysed on an SDS–PAGE gel and stained with GelCode Blue. The single protein band was excised with a razor blade and analysed by in-gel trypsin digestion and MALDI-TOF mass spectrometry at the UGA PAMS facility.

Native SPO2045 (DmdB) purification. Q-Sepharose HP chromatography: the cell extract was applied and eluted from the column as described above. Activity eluted over 20 ml between 33 and 42 mS cm^{-1}.

Phenyl-Superose chromatography: active fractions from Q-Sepharose chromatography were pooled, made 1.7 M $(NH_4)_2SO_4$, and chromatographed as described above. Activity eluted between 39 and 22 mS cm^{-1}. Active fractions were pooled and concentrated with an Amicon Ultra centrifugal filter (10 kDa). Final concentrate was suspended in 2 ml of 50 mM potassium phosphate (pH 7.5).

HiTrap Blue chromatography: the concentrated protein solution was then applied to a HiTrap Blue column and eluted as described above. Activity eluted after the start of the gradient. The 4 ml fraction containing the highest activity was concentrated using an Amicon Ultra centrifugal filter (10 kDa).

Sephacryl S200 chromatography: the protein concentrate was applied to a Sephacryl S200 gel filtration column and chromatographed as described above. Active fractions were analysed in duplicate on two SDS–PAGE gels. One gel was stained with silver stain and the second with GelCode Blue. Protein bands from the GelCode-Blue-stained gel were excised with a razor blade and analysed by in-gel trypsin digestion and MALDI-TOF mass spectrometry at the UGA PAMS facility.

Preparation of recombinant *E. coli*. Genes were amplified from *R. pomeroyi* DSS-3, *R. lacuscaerulensis*, *Burkholderia thailandensis* E264 and *Pseudomonas aeruginosa* PA01genomic DNA or from pUC57 plasmids containing the synthesized *P. ubique* genes by PCR. PCR product was cloned into the pET101 expression vector using the methods recommended by Invitrogen, constructing the plasmids summarized in Supplementary Table 6. All constructs were made using TOP10 *E. coli* and then introduced into BL21(DE3) or Rosetta (DE3) cells for protein expression. Cells for protein expression were grown overnight in Luria–Bertani (LB) broth. The culture was used to inoculate a flask containing 250–1,000 ml of LB broth, which was incubated at 37 °C until reaching an attenuance of 0.5–0.6. Isopropyl-β-D-thiogalactoside (IPTG) was then added to a concentration of 0.2 mM, and the culture was incubated overnight at room temperature. The culture was then collected by centrifugation at 10,000*g* for 10 min, washed with 50 mM Tris-HCl (pH 7.5) and again centrifuged. The pellet was resuspended in 4 ml of buffer and lysed by passing twice through a French pressure cell at 100,000 kPa. Cell debris was removed by centrifugation at 15,000*g* for 10 min. The soluble fraction was subsequently used for protein purification or enzyme assays.

MALDI-FT-ICR. Mass spectra were collected on a 7 tesla BioApex Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics) equipped with an intermediate pressure Scout100 MALDI source. Samples were overlain with a matrix consisting of saturated 2,5-dihydroxybenzoic acid.

Enzyme assays. HPLC analysis: analysis of DmdB, DmdC and DmdD reactions were performed using a 4.6 × 150 mm, 3 µm particle size, Hypersil Gold column (Thermo-Fisher) developed with a linear gradient of 2–20% acetonitrile in 50 mM ammonium acetate (pH 6) over 10 min.

Acetaldehyde dehydrogenase: acetaldehyde dehydrogenase activity was determined by measuring the increase in absorbance at 340 nm following the reduction of NAD to NADH using an extinction coefficient of 6,220 M^{-1}cm^{-1}. Enzyme assay mixture contained 50 mM HEPES (pH 7.5), 25 mM 2-mercaptoethanol, 1 mM NAD, 5 mM acetaldehyde, and 0.02–0.04 mg of cell extract. Reactions were initiated by the addition of acetaldehyde or cell extract.

Gas chromatography

Methanethiol was measured by headspace gas chromatography on an SRI 8610-C gas chromatograph with a Chromosil 330 column (Supelco) with N_2 carrier gas at a flow rate of 60 ml min^{-1}, an oven temperature of 60 °C, and a flame photometric detector. A standard curve for MeSH was obtained using a permeation tube (VICI Metronics).

Thiol quantification with DTNB. Free thiols resulting from the reaction of MTA-CoA were measured with 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) using an extinction coefficient of 14,150 M^{-1} cm^{-1}. DTNB was prepared at a concentration of 10 mM in 50 mM potassium phosphate buffer (pH 7.5). A reaction mixture containing 50 mM potassium phosphate (pH 7.5), 10 µM MTA-CoA, 0.5 mM DTNB and 2 µg of purified DmdD were mixed, and the absorbance at 412 nm was taken.

Acetaldehyde quantification. Acetaldehyde was detected and quantified after reaction with semicarbazide to form acetaldehyde semicarbazone and HPLC separation. A reaction, containing 0.1 mM MTA-CoA, 10 mM semicarbazide and 50 mM potassium phosphate (pH 7.5) in 90 µl, was initiated with addition of 2 µg purified recombinant DmdD in 5 µl of buffer and run to completion. Five microlitres of 1 M formic acid were then added to quench the reaction and convert any acetaldehyde hydrate to acetaldehyde[30]. The mixture was incubated at 37 °C for 2 h and centrifuged at 17,000*g* for 5 min. The supernatant was then analysed by

HPLC using a mobile phase of 2% acetonitrile with 0.5% acetic acid and ultraviolet detection at 224 nm (ref. 31).

Substrate synthesis. MMPA-CoA was synthesized by the mixed anhydride method[32] or enzymatically using purified recombinant DmdB with the same conditions described above. MMPA-CoA that was synthesized by either method was purified by reverse phase chromatography using an Ultrasphere ODS preparative column (10 × 250 mm). The column was developed with 50 mM ammonium acetate (pH 6) and a gradient of 2–20% acetonitrile. MMPA-CoA was detected at 254 nm. Fractions containing MMPA-CoA were lyophilized, resuspended in dH_2O and again lyophilized.

MTA-CoA was synthesized enzymatically by the dehydrogenation of MMPA-CoA using purified recombinant SAR11_0249 with the same reaction conditions described above. MTA-CoA was then purified by reverse phase chromatography as described for MMPA-CoA.

^{13}C-enriched substrates were synthesized enzymatically from ^{13}C-enriched DMSP. ^{13}C-enriched DMSP was synthesized as described previously[33] using ^{13}C-enriched acrylic acid and dimethylsulphide. The ^{13}C-enriched DMSP was then demethylated to MMPA using recombinant DmdA and tetrahydrofolate as the methyl acceptor as described previously[25]. MTA-CoA was produced enzymatically from MMPA using purified DmdB and DmdC and purified as described above.

Nuclear magnetic resonance. ^{1}H NMR was performed on a Varian Unity Inova 500 MHz spectrometer. A total of 1,000 scans were recorded with a relaxation delay of 2 s and a pulse angle of 45°. ^{13}C NMR was performed at 125 MHz with a 5 s relaxation delay, 45° pulse angle, and 2,000 scans.

Genetic modifications. Transposon mutagenesis was performed on *R. pomeroyi* DSS-3, mutants were screened for deficiency in MeSH production, and location of the transposon insertion was obtained by PCR and sequencing as described previously[3].

Targeted gene replacements were made by introduction of *tetAR* into *dmdB* (SPO2045) and *dmdD* (SPO3805). Up and downstream regions of homology 1,000–1,500 base pairs in length and the *tetAR* genes from pRK415 were PCR-amplified and cloned into the pCR2.1 vector, which cannot replicate in *R. pomeroyi*, by sequence and ligation-independent cloning (SLIC)[34]. Plasmid DNA was methylated by CpG methyltransferase as recommended by New England Biolabs and then introduced into *R. pomeroyi* DSS-3 cells by electroporation[35]. Mutants were selected for ability to grow on tetracycline but not kanamycin, and confirmed by PCR.

Plasmids for complementation were made by PCR amplifying genes SPO3804, SPO3805, and both SPO3804 and SPO3805 from *R. pomeroyi* DSS-3 genomic DNA and ligating into the broad host expression vector pRK415 (ref. 36). The plasmids constructed are summarized in Supplementary Table 6.

Growth curves. Three-millilitre cultures of wild-type *R. pomeroyi* DSS-3 and mutant strains were grown in MBM with 2 mM glucose as the sole source of carbon. After overnight growth, the cultures were used to inoculate fresh 3 ml cultures, and attenuance was recorded at 600 nm.

Chemostat cultures. *R. pomeroyi* DSS-3 was grown at 30 °C in a carbon-limited chemostat using 2 mM MMPA in a total volume of 144 ml and a dilution rate of 0.0416 h^{-1}. *R. lacuscaerulensis* was grown at 33 °C with 1 mM MMPA with the same volume and dilution rates as above. A portion of the culture, 100 ml, was removed and immediately centrifuged at 10,000*g* for 10 min, washed with 1.5 ml of ice cold 50 mM HEPES (pH 7.5). The cell pellet was resuspended in 1 ml of 50 mM HEPES (pH 7.5). Cells were then lysed by bead beating for 2 min. Cell debris was removed by centrifugation at 17,000 for 5 min.

Volatile organic sulphur consumption. Chemostat cultures of *R. pomeroyi* DSS-3 grown with DMSP were collected and 2 ml was immediately placed into a 70 ml vial and sealed with a Teflon-coated stopper. Approximately 125 nmol of MeSH was then added to the vials from the MeSH permeation tube, and samples were equilibrated for 10 min at 30 °C. Headspace samples were then analysed by gas chromatography at 30 min, 1 h and 19 h.

Quantitative RT–PCR. RNA was extracted from chemostat-grown cell cultures of *R. pomeroyi* DSS-3 with acetate, MMPA, or DMSP as the sole carbon source. Cell culture, 50 ml, was collected into a 5 ml solution of 5% phenol in ethanol and centrifuged at 8,000*g* for 10 min. The supernatant was decanted, and pellets stored at −80 °C. RNA was extracted using the RNeasy mini kit (Qiagen), and DNA was removed with TURBO DNA-free kit (Ambion). The primer sequences, amplicon sizes, and annealing temperatures are listed in Supplementary Table 7.

Reactions were performed with the Bio-Rad One-Step RT–PCR reaction mix with SYBR Green, 300 nM primer concentrations, and 0.5 µl iScript reverse transcriptase (RT) enzyme. DNA plasmid standards were constructed from a PCR product for each gene using the pCR 2.1-TOPO vector (Invitrogen). RT–qPCR was carried out on a Bio-Rad iCycler iQ with the following cycling conditions: 50 °C for 10 min, 95 °C for 5 min, 40 cycles of 95 °C for 15 s and 61 °C for 1.0 min, and a final step of 95 °C for 1.0 min and 61 °C for 1.0 min, followed by a melt curve analysis. Transcript copies were calculated from standard curves and normalized per ng of RNA.

Phylogenetic analysis. The DmdB (SAR11_0249), DmdC (SAR11_0248) and RecA (SAR11_0641) from *P. ubique* were used as query sequences for a BLASTp search against the genome sequences of *Burkholderia thailandensis*, *Pseudomonas aeruginosa*, *Pseudoalteromonas atlantica*, *Myxococcus xanthus*, *Deinococcus radiodurans*, *P. ubique* HTCC1062, HTCC1002, HTCC7211, HIMB5, HIMB59, marine γ-proteobacteria HTCC2143, *Puniceispirillum marinum* IMCC1322, *R. pomeroyi* DSS-3, *R. lacuscaerulensis* 1157, *Dinoroseobacter shibae* DFL-12, and *Escherichia coli* BL21(DE3). HIMB5 and HIMB59 were not included in the NCBI database at the time of analysis, the peptide sequences of the annotated genomes were obtained from JCVI. All hits with an expect value $< e^{-10}$ were compiled into separate databases for DmdB and DmdC, and then the sequences were aligned using the MUSCLE algorithm in MEGA 5. Sequences with poor alignment and annotated as unrelated proteins were removed. Phylogenetic trees were built using maximum likelihood method in MEGA 5 with 100 bootstraps. Tree topology was confirmed with maximum likelihood method using PHYLIP v3.69.

GOS bioinformatics analysis. A custom reference database was established with DmdB, DmdC and RecA homologues from the bacteria listed above. Reference sequences were designated as orthologues if they clustered in a phylogenetic tree with authentic DmdBs, DmdCs or RecAs, or were otherwise considered paralogues. Separately, the DmdB (SAR11_0249), DmdC (SAR11_0248) and RecA (SAR11_0641) sequences from *P. ubique* were used as query sequences in BLASTp analysis against the GOS metagenomic database, and homologues with an expect value $< e^{-20}$ for DmdB and DmdC and $< e^{-3}$ for RecA were retained. The GOS sequences were then blasted against the custom reference databases, and those with top hits to sequences in authentic clusters were summed while those with top hits to paralogous clusters were discarded. For DmdD, SPO3805 from *R. pomeroyi* DSS-3 was used as the query sequence for BLASTp analysis against the GOS metagenomic database.

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