



# Microbial Dimethylsulfoniopropionate Cycling in Deep Sediment of the Mariana Trench

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**ABSTRACT** Dimethylsulfoniopropionate (DMSP) and related organic sulfur compounds play key roles in global sulfur cycling. Bacteria have been found to be important DMSP producers in seawater and surface sediments of the aphotic Mariana Trench (MT). However, detailed bacterial DMSP cycling in the Mariana Trench subseafloor remains largely unknown. Here, the bacterial DMSP-cycling potential in a Mariana Trench sediment core (7.5 m in length) obtained at a 10,816-m water depth was investigated using culture-dependent and -independent methods. The DMSP content fluctuated along the sediment depth and reached the highest concentration at 15 to 18 cm below the seafloor (cmbsf). *dsyB* was the dominant known DMSP synthetic gene, existing in 0.36 to 1.19% of the bacteria, and was identified in the metagenome-assembled genomes (MAGs) of previously unknown bacterial DMSP synthetic groups such as *Acidimicrobiia*, *Phycisphaerae*, and *Hydrogenedentia*. *dddP*, *dmdA*, and *dddX* were the major DMSP catabolic genes. The DMSP catabolic activities of DddP and DddX retrieved from *Anaerolineales* MAGs were confirmed by heterologous expression, indicating that such anaerobic bacteria might participate in DMSP catabolism. Moreover, genes involved in methanethiol (MeSH) production from methylmercaptopropionate (MMPA) and dimethyl sulfide (DMS), MeSH oxidation, and DMS production were highly abundant, suggesting active conversions between different organic sulfur compounds. Finally, most culturable DMSP synthetic and catabolic isolates possessed no known DMSP synthetic and catabolic genes, and actinomycetes could be important groups involved in both DMSP synthesis and catabolism in Mariana Trench sediment. This study extends the current understanding of DMSP cycling in Mariana Trench sediment and highlights the need to uncover novel DMSP metabolic genes/pathways in extreme environments.

**IMPORTANCE** Dimethylsulfoniopropionate (DMSP) is an abundant organosulfur molecule in the ocean and is the precursor for the climate-active volatile gas dimethyl sulfide. Previous studies focused mainly on bacterial DMSP cycling in seawater, coastal sediment, and surface trench sediment samples, but DMSP metabolism in the Mariana Trench (MT) subseafloor sediments remains unknown. Here, we describe the DMSP content and metabolic bacterial groups in the subseafloor of the MT sediment. We found that the tendency for vertical variation of the DMSP content in the MT was distinct from that of the continent shelf sediment. Although *dsyB* and *dddP* were the dominant DMSP synthetic and catabolic genes in the MT sediment, respectively, both metagenomic and culture methods revealed multiple previously unknown DMSP metabolic bacterial groups, especially anaerobic bacteria and actinomycetes. The active conversion of DMSP, DMS, and methanethiol may also occur in the MT sediments. These results provide novel insights for understanding DMSP cycling in the MT.

**KEYWORDS** DMSP, deep sediment, Mariana Trench, bacteria

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Dimethylsulfoniopropionate (DMSP) is an abundant organic sulfur compound in the global ocean, with an estimated level of biogenic production of  $10^9$  tons annually (1, 2). Marine algae, corals, plants, and heterotrophic bacteria can produce DMSP, which serves as a key nutrient (3), a signaling molecule (4, 5), or an antistress compound (6–9) in diverse organisms. Most importantly, bacterial cleavage of DMSP is the predominant source of dimethyl sulfide (DMS), which potentially plays a vital role in the regulation of the Earth's climate (10). Once released into the atmosphere, the oxidation products of DMS aggregate into aerosols that can affect cloud formation, resulting in the backscatter of solar radiation and affecting the global temperature (11, 12). Another important volatile sulfur compound produced by DMSP demethylation is methanethiol (MeSH) (13), which can be converted to DMS by diverse bacteria (14).

The biosynthesis of DMSP is initiated from methionine (Met) through three known pathways, namely, the transamination pathway (15–19), the methylation pathway (20–26), and the decarboxylation pathway (27). In the well-studied transamination pathway, three key methylthiohydroxybutyrate (MTHB) *S*-methyltransferase enzymes were identified from different organisms: DsyB in some *Alphaproteobacteria* (15), DSYB in green algae and corals (16), and TpMMT in the diatom *Thalassiosira pseudonana* (28). Homologs of DsyB are widespread in many marine *Alphaproteobacteria* (predominantly *Rhodobacterales*), some *Gammaproteobacteria*, and *Actinobacteria* (9, 15, 25). For the methylation pathway, only one key Met methyltransferase, MmtN, was identified in *Alphaproteobacteria*, *Gammaproteobacteria*, and some Gram-positive bacteria (25, 29, 30). However, bacteria possessing *mmtN* are commonly less abundant than those with *dsyB* in seawater and sediments (25). The decarboxylation pathway was found in the heterotrophic microalga *Cryptocodinium cohnii* (30), but the key DMSP biosynthetic enzyme in this pathway remains unknown.

DMSP can be catabolized through the cleavage and demethylation pathways. In the cleavage pathway, DMS is produced as a coproduct with acrylate, hydroxypropionate, or acryloyl-CoA. Several bacterial DMSP lyases (DddD, DddL, DddP, DddQ, DddY, DddW, DddK, and DddX) and an algal DMSP lyase (Alma1) have been identified in this pathway (10, 31–36). Among these bacterial DMSP lyases, DddP is the most abundant environmental DMSP lyase and is often found in the marine *Roseobacter* clade (MRC) (9, 37). However, the major pathway for the degradation of DMSP is the demethylation pathway, and it is estimated that 50 to 90% of DMSP is metabolized by marine bacteria in this way (38). The key demethylase DmdA first converts DMSP to methylmercapto-propionate (MMPA), which is subsequently degraded by the MMPA-CoA ligase DmdB, the MMPA-CoA dehydrogenase DmdC, the methylthioacryloyl-CoA (MTA-CoA) hydratase DmdD, or the acrylate utilization hydratase AcuH to generate MeSH in diverse bacteria (39). DmdA has been reported to be prevalent in the MRC and the SAR11 clade (40, 41), which are considered the main bacterial groups for DMSP catabolism in the oceans (42).

The conversion between other organic sulfur compounds related to DMSP/DMS is also important and highly active in marine environments. The transformation of MeSH to DMS contributes to DMS production in both marine and terrestrial environments (14, 43), catalyzed by the key *S*-adenosylmethionine (SAM)-dependent methyltransferase Mdda (14). On the other hand, MeSH can be oxidized to formaldehyde by MTO found in *Thiobacillus*, *Rhodococcus*, and *Hyphomicrobium* strains (37, 43). DMS can also be produced through the reduction of dimethyl sulfoxide (DMSO) by the bacterial DMSO reductase DorA (44). Conversely, DMS oxidation to DMSO represents a major sink of DMS in surface seawater (45). The multicomponent monooxygenase DsoABCDEF (46), the DMS dehydrogenase DdhAB (47), and the flavin-containing trimethylamine (TMA) monooxygenase Tmm (48) are responsible for DMS oxidation. In addition, the two-component DMS monooxygenase DmoAB can oxidize DMS to MeSH (49). The *megL* gene could encode a Met gamma lyase (MegL) that cleaves Met into MeSH (50).

The DMSP concentration in surface marine sediments can be up to 3 orders of magnitude higher than that in most seawater samples, but these levels were reported to

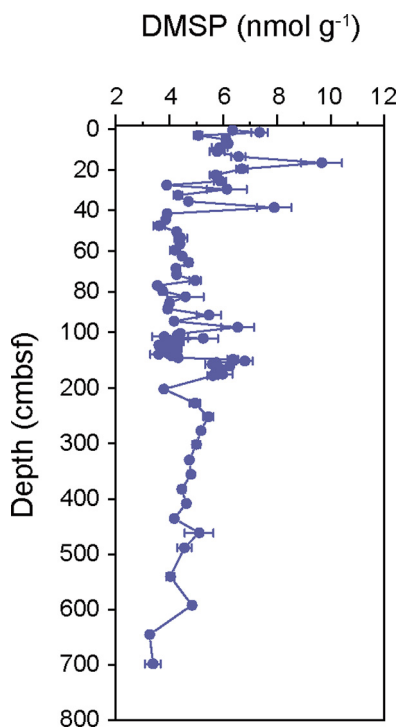
decrease with sediment depth and oxygen availability (25, 41, 51). Microbial cycling of DMSP in sediment has been revealed recently, and bacteria are considered the key DMSP producers in these aphotic sediments, with *dsyB* being the dominant DMSP biosynthetic gene (9, 25, 37). The microbial DMSP catabolic potential seems to vary between different sediments (52). In surface salt marsh sediments, DMSP lyase genes (*dddD*, *dddL*, and *dddP*) were far more abundant than *dsyB* (25), but the abundances of *dmdA* and *dddP* were higher than that of *dsyB* in Mariana Trench (MT) deep-sea sediment, where *dsyB* far outnumbered both *dmdA* and *dddP* (9). In the hydrothermal sediment of Bohai Sea and Yellow Sea sediment (BYSS) samples, the abundance of DMSP degradation genes was low, and only the *dddP* gene was detected (37). For other genes involved in DMSP cycling, *mddA* was found mainly in *Rhodopseudomonas* and *Thioalkalivibrio* in salt marsh sediment (43) as well as in *Desulfospira*, *Thioalkalivibrio*, *Crocospaera*, and *Pseudomonas* in BYSS samples (37). *dmoA* was predicted to be found mainly in *Alcaligenes* and *Pseudomonas* within BYSS samples (37). These results indicate that active DMSP cycling may occur in sediment environments.

The Mariana Trench is the deepest site in the ocean, with a depth of 11,000 m at the Challenger Deep (53). Despite the extreme environmental conditions in the Mariana Trench, there is still a high prokaryotic biomass (approximately  $2.01 \mu\text{g C g}^{-1}$ ) and high activity in the sediments of this region (54). Bacterial DMSP synthesis and catabolism were examined in seawater (0 to 10,500 m) and surface sediment samples from the Challenger Deep of the Mariana Trench (9), demonstrating that bacteria are important DMSP producers in marine aphotic environments and that DMSP may be involved in hydrostatic pressure protection. However, the DMSP concentration, DMSP-cycling microbes, and their importance in deep Mariana Trench subseafloor sediments remain unexplored. In this study, we obtained a sediment core (~8 m) from a 10,816-m depth in the Challenger Deep and investigated the detailed vertical distribution of the DMSP content. The abundance of bacterial genes involved in DMSP cycling was analyzed by quantitative PCR (qPCR) and metagenome sequencing. Culture-dependent and -independent methods were employed to identify previously unknown DMSP metabolic bacteria and genes in Challenger Deep sediments.

## RESULTS

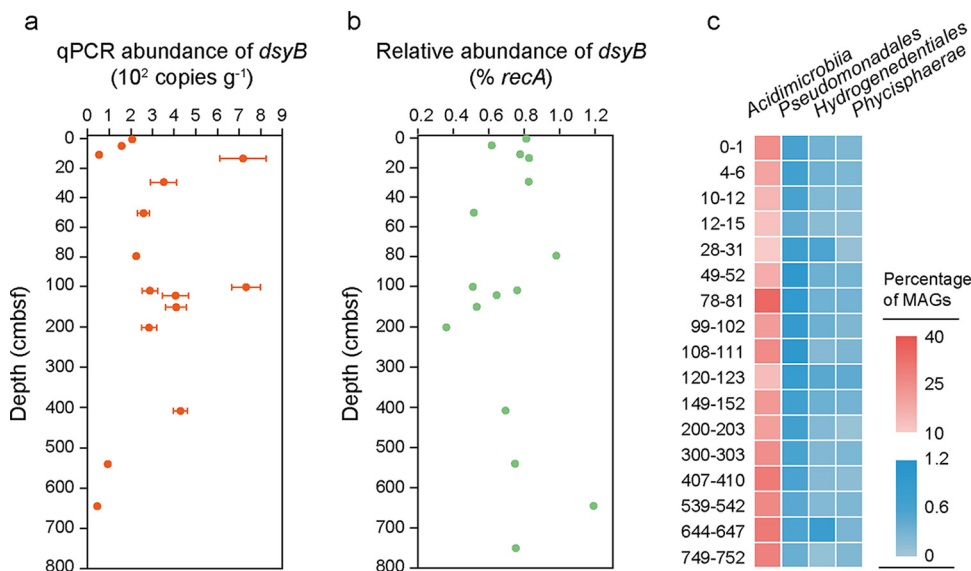
**DMSP content in the Mariana Trench deep sediment.** The total DMSP concentrations in the sediment samples from the Challenger Deep (10,816 m) ranged from  $3.26 \pm 0.37$  to  $9.66 \pm 0.76 \text{ nmol g}^{-1}$  wet sediment (Fig. 1) and varied with sediment depth. The DMSP concentration in the surface sediment samples was  $6.35 \pm 0.09 \text{ nmol g}^{-1}$ , whereas higher concentrations of DMSP were quantified at 15 to 18 cm below the seafloor (cmbsf) ( $9.66 \pm 0.76 \text{ nmol g}^{-1}$ ) and 37 to 40 cmbsf ( $7.90 \pm 0.63 \text{ nmol g}^{-1}$ ). The DMSP concentration was lower at 40 to 96 cmbsf ( $3.55$  to  $5.47 \text{ nmol g}^{-1}$ ) than at the above layers and fluctuated between 96 cmbsf and 200 cmbsf, with a higher value at ~150 cmbsf ( $6.80 \pm 0.31 \text{ nmol g}^{-1}$ ). In the deep sediment below 250 cmbsf, the concentration of DMSP reduced gradually from  $5.45 \pm 0.18 \text{ nmol g}^{-1}$  to  $3.26 \pm 0.37 \text{ nmol g}^{-1}$ . Generally, DMSP in the Challenger Deep sediment was present at levels similar to those reported previously for the surface sediment of the Mariana Trench (3.15 to  $6.14 \text{ nmol g}^{-1}$  wet sediment) (9) and lower than those in marine surface sediment ( $11.25$  to  $20.90 \text{ nmol g}^{-1}$  wet sediment) (52). However, the variations in DMSP concentrations along the sediment depth were more fluctuant in the Challenger Deep than in other subseafloor sediments (52).

**Vertical distribution of DMSP synthesis genes.** To quantify the abundance of bacterial DMSP biosynthetic genes, we performed qPCR analysis on the DMSP biosynthetic genes *dsyB* and *mmtN*. The abundance of the major bacterial DMSP biosynthetic gene *dsyB* ranged between  $0.45 \times 10^2$  copies  $\text{g}^{-1}$  and  $7.31 \times 10^2$  copies  $\text{g}^{-1}$  (Fig. 2a; see also Table S1 in the supplemental material). Corresponding to the DMSP content, *dsyB* was highly abundant at 12 to 15 cmbsf ( $3.59 \times 10^2$  copies  $\text{g}^{-1}$ ) and 99 to 102 cmbsf ( $3.66 \times 10^2$  copies  $\text{g}^{-1}$ ). The abundance of *dsyB* in samples from below 500 cmbsf was extremely low ( $<0.1 \times 10^2$  copies  $\text{g}^{-1}$ ). The *mmtN* gene could not be detected by qPCR at all depths of the Challenger Deep sediment (Table S1).

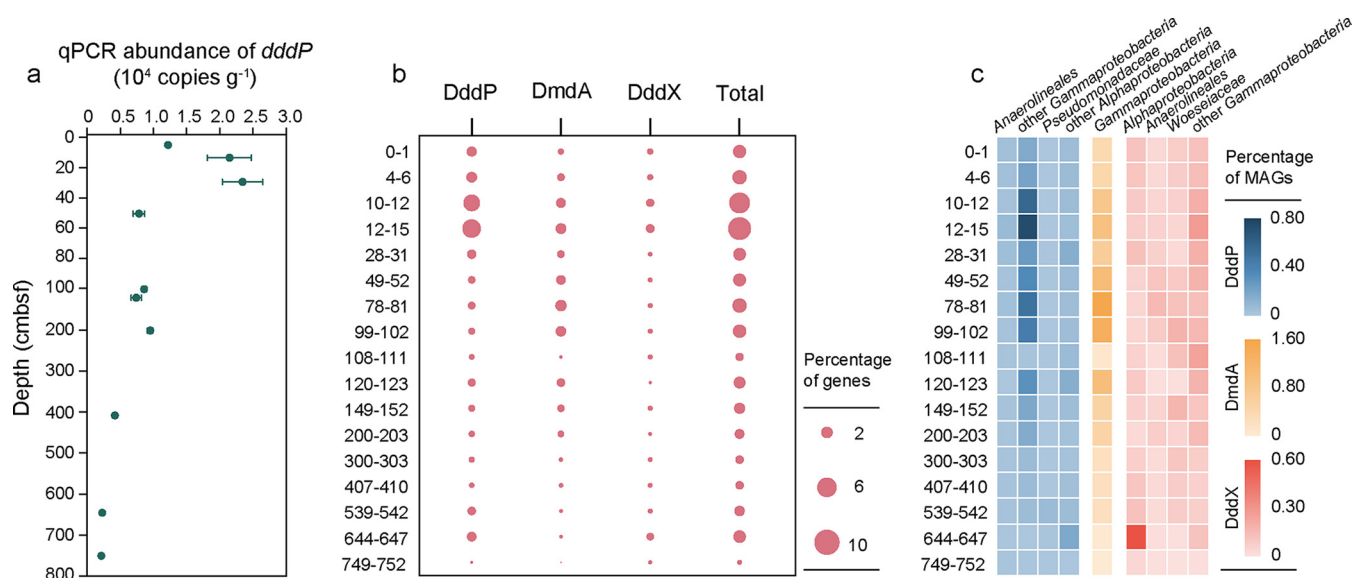


**FIG 1** DMSP concentrations in the deep sediments from the Mariana Trench. Data are presented as means ± standard deviations (SD).

In the metagenomes from different depths, six protein homologs of DsyB were obtained, while homologs of MmtN (Fig. 2b, Table S2, and Fig. S1) were not found, which was consistent with the qPCR results, suggesting that bacteria synthesizing DMSP through the Met methylation pathway were scarce in these sediment samples. DsyB homologs were retrieved from all depths, and higher percentages of bacteria with DsyB existed at 12 to 15 cmbsf (0.83%), 28 to 31 cmbsf (0.83%), 78 to 81 cmbsf (0.98%), and 644 to 647 cmbsf (1.19%). Twelve high-quality metagenome-assembled genomes (MAGs) with *dsyB* (completeness of >50% and contamination of <10%) were obtained (Fig. 2c, Table S3, and Fig. S1), which were annotated as *Acidimicrobiia* (8/12), *Gammaproteobacteria* (*Pseudomonadales* order)



**FIG 2** Abundance of the DMSP synthesis gene *dsyB* in Mariana Trench sediment. (a) Absolute abundance of *dsyB* determined by qPCR. Data are presented as means ± SD. (b) Relative abundance of *dsyB* in metagenomes. (c) Relative abundance of MAGs with *dsyB* and their taxonomy information.

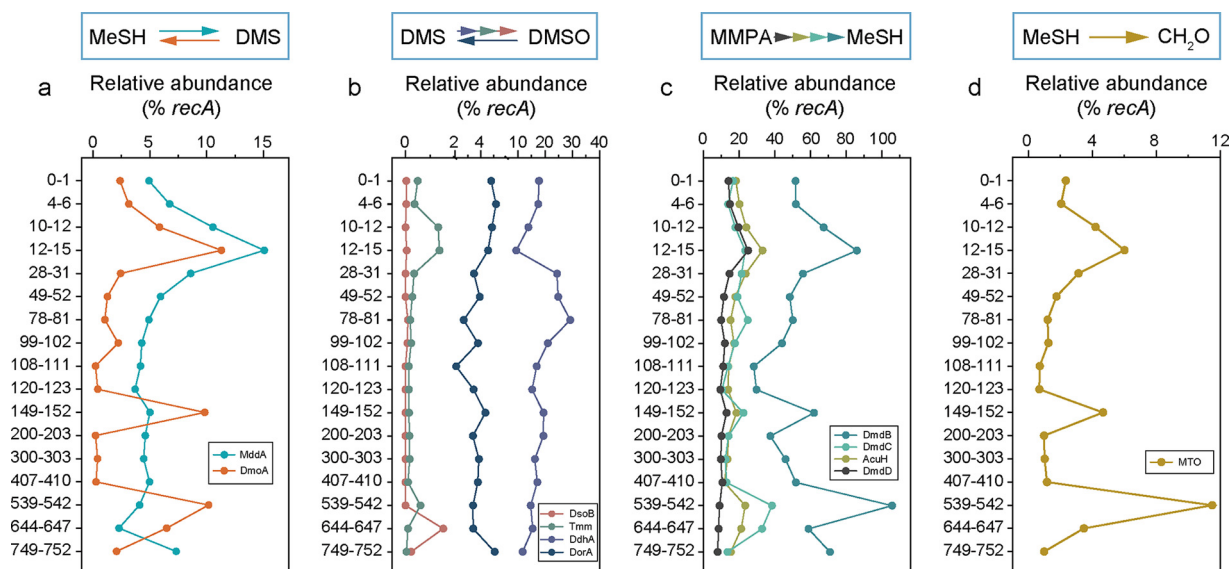


(2/12), *Phycisphaerae* (1/12), as well as *Hydrogenedentia* (*Hydrogenedentiales* order) (1/12). Among these MAGs, only a few bacteria belonging to *Gammaproteobacteria* were previously reported to possess *dsyB* (25); no *DsyB* homologs were reported in the acidophilic actinomycetes *Acidimicrobiia* (55) as well as anaerobic *Phycisphaerae* (*Planctomycetota*) (56, 57) and *Hydrogenedentia* (58) bacteria. *Acidimicrobiia* MAGs harboring *dsyB* were present at high abundances across all depths, accounting for 1.25% to 3.09% in the sediment environment, and showed the highest abundance at 78 to 81 cmbsf (3.09%).

**Vertical distribution of DMSP catabolism genes.** Quantification of the major DMSP catabolic genes *dddP* and *dmdA* (C2/D1 clades) showed that only *dddP* could be detected by qPCR analysis (Fig. 3a and Table S1), indicating that *dddP* might be the most abundant DMSP catabolic gene in these sediments. The abundance of *dddP* was highest in shallow sediment at 28 to 31 cmbsf ( $2.34 \times 10^4$  copies  $g^{-1}$ ) and was reduced to approximately  $0.23 \times 10^4$  copies  $g^{-1}$  below 600 cmbsf. Although the key gene *dmdA* has been reported to be the dominant DMSP catabolic gene, with a higher abundance than those of the *ddd* genes (9), the levels of both the C2 and D1 clades of *dmdA* genes were below the qPCR detection threshold (Table S1).

Correspondingly, 17 protein homologs of DddP, 5 of DmdA, and 11 of DddX were retrieved from the metagenomes of the Challenger Deep sediment samples, but homologs of DddD, DddQ, DddL, DddK, DddW, DddY, and Alma1 were not found (Fig. 3b, Table S2, and Fig. S2 to S4). These homologs of DddP, DddX, and DmdA were distributed at all depths, and these DMSP catabolic genes were also more abundant at depths with higher DMSP concentrations, especially in samples at 10 to 12 cmbsf and 12 to 15 cmbsf. The percentage of bacteria with DddP reached 5.37% at 12 to 15 cmbsf, higher than those of DmdA (1.74%) and DddX (1.10%). In the deep seafloor, the percentage of bacteria with DddP and DddX increased at 649 to 752 cmbsf (1.40% for DddP and 0.86% for DddX), while at 49 to 102 cmbsf, the relative abundance of DmdA (1.36% to 1.94%) seemed to be higher than those of DddP (0.72% to 0.85%) and DddX (0.32% to 0.35%). Further phylogenetic analysis indicated that the *dmdA* genes in the metagenomes clustered with *dmdA* subclades A, B, and E (Fig. S3), and this possibly explained why no *dmdA* was detected with primers for C2 and D1. However, *dddP* remained the most abundant DMSP catabolic gene in the shallow sediment above 15 cmbsf.

In total, 29 MAGs were annotated as possessing at least one DMSP catabolic gene (Table S3 and Fig. S2 to S4). Most MAGs with *dddP* belonged to *Pseudomonadaceae* and other *Gammaproteobacteria* as well as some *Alphaproteobacteria* (Fig. 3c). Surprisingly, one



**FIG 4** Abundance of genes involved in other processes of DMS/DMSP cycling in Mariana Trench sediment. (a) Relative abundances of the MeSH transmethylation gene *mddA* and the DMS oxidation gene *dmoA*. (b) Relative abundances of DMS oxidation-related genes (*dsoB*, *tmm*, and *ddhA*) and the DMSO reduction gene *dorA*. (c) Relative abundances of genes involved in the conversion of MMPA to MeSH (*acuH*, *dmdB*, *dmdC*, and *dmdD*). (d) Relative abundance of the MeSH oxidation gene *mtoX*.

DddP homolog and three DddX homologs were found in anaerobic *Anaerolineales* MAGs (Table S3), indicating that such anaerobic bacteria may be involved in DMSP catabolism in Challenger Deep sediment. Homologs of DddX were also identified in MAGs from *Woeseiaceae* and other *Gammaproteobacteria*. The relative abundance of DddX-containing MAGs from *Alphaproteobacteria* was extremely high at 644 to 647 cmbsf (Fig. 3c). MAGs with *DmdA* were all from *Gammaproteobacteria*, with the highest abundance at 78 to 81 cmbsf (17.81%).

**Genes involved in other DMS/MeSH production and oxidation processes.** To better depict DMSP/DMS cycling in Challenger Deep sediment, we examined the relative abundances of other genes involved in these processes in metagenomes. In total, 51 *mddA*, 56 *dmoA*, 7 *tmm*, 178 *ddhA*, and 58 *dorA* homologs were obtained. For conversion between DMS and MeSH, the MeSH-dependent DMS-producing gene *mddA* was identified in 2.29 to 15.06% of bacteria and reached the highest abundance at 12 to 15 cmbsf (Fig. 4a). Bacteria with the DMS monooxygenase enzyme *DmoA* were generally less abundant than bacteria with *MddA*, accounting for 0.23 to 11.30% of the sediment metagenomes. However, the percentages of bacteria with *DmoA* were significantly higher at 12 to 15 cmbsf (11.30%), 149 to 152 cmbsf (9.83%), and 539 to 542 cmbsf (10.18%). Fifty-two MAGs harboring *mddA* were retrieved from *Bacteroidota* (1/52), *Planctomycetota* (4/52), *Proteobacteria* (6/52), *Gemmatimonadota* (14/52), *Marinisomatota* (16/52), *Myxococcota* (7/52), and *Hydrogenedentota* (2/52) (Table S4), which were not reported in previous studies except for those from *Proteobacteria*. *DmoA* was distributed in MAGs of *Acinetobacter* (4/28), *Microbacterium* (2/28), and *Pseudomonas* (1/28), as reported in a previous study on marine sediment samples (37). Additionally, *DmoA* homologs were also identified in MAGs from *Burkholderiaceae* (11/28), *Dehalococcoidia* (8/28), and *Brevibacillus* (2/28), which have not been reported previously.

For conversion between DMS and DMSO (Fig. 4b), *ddhA* was the dominant DMS oxidation gene throughout the sediment, compared with the other DMS oxidation genes *tmm* and *dsoB*. The relative abundance of *ddhA* ranged from 9.43% to 29.22% of bacteria, which increased at 28 to 81 cmbsf (>24.38%). The percentages of bacteria with *Tmm* were higher at 10 to 15 cmbsf (1.32%), 12 to 15 cmbsf (1.37%), and 539 to 542 cmbsf (0.60%). Only one *dsoB* homolog was found in the sediment metagenomes and reached the highest relative abundance of 1.52% at 644 to 647 cmbsf. A total of 146 MAGs, mainly from *Actinobacteriota*, *Bacteroidota*, *Chloroflexota*, *Gemmatimona-*

*dota*, *Hydrogenedentota*, *Marinisomatota*, *Planctomycetota*, and *Proteobacteria*, harbored *ddhA*, and more than one *ddhA* hit was found in 57 (out of 146) MAGs. A total of 3 (out of 1,350) MAGs harbored *DsoB*, which were retrieved only from *Acinetobacter pittii*, while *Tmm* was identified in only two *Gammaproteobacteria* MAGs. The DMSO reduction gene *dorA* was found in approximately 5% of bacteria in the sediment metagenomes, with a lower proportion than that of *ddhA*. Moreover, *DorA* predominantly existed in MAGs of *Dehalococcoidia* (17/31), *Acidimicrobiia* (5/31), *Gammaproteobacteria* (4/31), *Rhodospirillales* (1/31), and *Anaerohalospaeraceae* (1/31).

Similar to *mddA* and *dmoA*, the MMPA-dependent MeSH-producing genes *dmdB*, *dmdC*, *dmdD*, and *acuH* and the MeSH oxidation gene *mtoX* reached higher abundances at 12 to 15 cmbsf, 149 to 152 cmbsf, and 539 to 542 cmbsf. The abundance of *DmdB* ranged from 28.12% to 105.75% (Fig. 4c) and was higher at 12 to 15 cmbsf (85.97%), 149 to 152 cmbsf (61.93%), and 539 to 542 cmbsf (105.75%). *AcuH* (33.04%) and *DmdD* (24.95%) were more abundant at 12 to 15 cmbsf, while the abundance of *DmdC* (38.35%) was higher at 539 to 542 cmbsf. Alternately, the abundance of *MTO* (Fig. 4d) was higher at 12 to 15 cmbsf (6.00%), 149 to 152 cmbsf (4.66%), and 539 to 542 cmbsf (11.50%). A total of 651 (out of 1,350) MAGs harbored *DmdB*, *DmdC*, *DmdD*, and *AcuH*, while 51 MAGs were found to harbor *MTO* (Table S4). Among them, *DmdB* was identified in 411 MAGs, which were from *Acidimicrobiia* (108 MAGs), *Chloroflexota* (77), *Gammaproteobacteria* (77), *Gemmatimonadetes* (33), *Alphaproteobacteria* (23), *Myxococcota* (16), *Desulfobacteriales* (14), *Elusimicrobiota* (13), *Thermodesulfovibrionia* (11), *Planctomycetota* (9), *Hydrogenedentiales* (6), *Acidobacteriota* (3), *Brevibacillus agri* (2), “*Candidatus* *Omnitrphota*” (1), and unknown bacteria (18). A total of 33 (out of 51) MAGs possessing *MTO* were from *Gammaproteobacteria*, including *Pseudomonadales* (15 MAGs), *Cupriavidus gilardii* (10), *Woeseiaceae* (3), and other *Gammaproteobacteria* (5). Additionally, other MAGs with *MTO* were from *Myxococcota* (8/51), *Thermodesulfovibrionia* (6/51), *Alphaproteobacteria* (2/51), *Elusimicrobiota* (1/51), and *Anaerolineales* (1/51).

**Isolates involved in DMSP cycling in surface sediment.** Surface sediment samples (0 to 1 cmbsf) were used to enrich and isolate DMSP-producing and -catabolizing bacteria. In total, 69 strains were obtained from enrichment cultures with Met, and 156 strains were obtained from enrichment cultures with DMSP. We further examined the DMSP synthetic and catabolic activities of these isolates as well as their ability to produce DMS or MeSH independent of DMSP.

Ten isolates, belonging to *Alphaproteobacteria* (3 isolates), *Gammaproteobacteria* (1), *Actinomycetia* (5), and *Bacilli* (1), could use Met to produce DMSP through the transamination pathway, accounting for 19.2% of all representative strains. At the genus level, these isolates were from *Alcanivorax* (1 isolate), *Qipengyuania* (3), *Dietzia* (3), *Brachybacterium* (2), and *Mammaliococcus* (1). Among these DMSP-producing isolates, *Qipengyuania citrea* CHJ019 and *Brachybacterium paraconglomeratum* CHJ016 showed the highest DMSP concentrations in their cultures (>10 nmol DMSP/mL) (Table S5). So far, none of the culturable actinomycetes and bacillus isolates have been reported to produce DMSP in previous studies. Although multiple isolates from *Qipengyuania* (which used to be classified as *Erythrobacter*) have been found to produce DMSP, no known DMSP biosynthesis genes were identified in these isolates (9, 52). Indeed, *dsyB* and *mmtN* were not detected in these 10 strains by PCR amplification, suggesting that there may be previously unknown DMSP-producing genes in these isolates.

Moreover, all 10 of these isolates could produce MeSH and DMS when Met was added, indicating the existence of *MegL* and *Mdd* enzymes in these bacteria (14). In addition to the above-mentioned DMSP-producing isolates, *Glutamicibacter mysorens* CHJ159, *Brachybacterium ginsengisoli* CHJ148, and *Arthrobacter crystallopoietes* CHJ137 could also metabolize MeSH to produce DMS and may possess the MeSH transmethylation pathway (Table S5). This MeSH-dependent DMS-producing pathway was reported previously in diverse actinomycetes as well as in proteobacteria (37, 43).

For DMSP catabolism, 39 isolates were found to degrade DMSP to DMS, while 9 of them could also degrade DMSP to MeSH. These isolates, belonging to *Alphaproteobac-*

teria (9 isolates), *Gammaproteobacteria* (5), *Actinomycetia* (20), *Bacilli* (4), and *Sphingobacteriia* (1), exhibited varied levels of DMSP catabolism activity (Table S6). Surprisingly, more than one-half of these culturable DMSP catabolic isolates (51.3%) producing DMS were from *Actinomycetia*, including *Glutamicibacter* (9 isolates), *Microbacterium* (3), *Brachybacterium* (4), *Agrococcus* (1), *Arthrobacter* (1), *Corynebacterium* (1), and *Brevibacterium* (1) at the genus level. However, the DMSP catabolic gene in these actinomycetes remained unknown (34). DMSP catabolic isolates from *Alphaproteobacteria* accounted for 23%, including those from *Qipengyuania* (4 isolates), *Tritonibacter* (2), *Aliihoeflea* (1), *Paracoccus* (1), and *Erythrobacter* (1). DMSP catabolic isolates from *Gammaproteobacteria* were *Psychrobacter* (2 isolates), *Pseudomonas* (1), *Alcanivorax* (1), and *Luteimonas* (1). Additionally, two *Bacilli* isolates, from *Staphylococcus* and *Mammaliococcus*, were also found to catabolize DMSP. DMSP catabolic isolates in *Bacteroidetes* have been identified in Mariana Trench samples, as described previously by Zheng et al. (9), but those from *Sphingobacteriia* were reported for the first time in this study. Isolates that could degrade DMSP to MeSH were mainly from *Alphaproteobacteria* (3 isolates), *Gammaproteobacteria* (1), *Actinomycetia* (2), *Bacilli* (2), and *Sphingobacteriia* (1). None of these DMSP catabolic isolates harbored *dddP*, *dddD*, *dddL*, and *dmdA* subclades (C2/D1) when detected by PCR. Although it could also be possible that the degenerate primers of known DMSP metabolic genes may not fully cover all divergent homologous genes in these strains, previously unknown DMSP catabolic and biosynthetic genes may exist in Mariana Trench sediments.

**Enzyme activity of the DMSP metabolic proteins from MAGs.** To confirm whether the annotated DMSP metabolic enzymes from MAGs were active, we synthesized and heterologously expressed representative sequences to detect their activity, including homologs of *dsyB* (4 sequences), *dddP* (2 sequences), and *dddX* (1 sequence) (Table S7). Four *DsyB* sequences (690 bp to 1,014 bp) were retrieved from MAGs annotated as belonging to *Acidimicrobiia*, *Gammaproteobacteria*, *Hydrogenedentia* (*Hydrogenedentiales* order), and *Phycisphaerae*. However, none of the heterologously expressed *dsyB* genes showed DMSP synthetic activity in *Escherichia coli* BL21. This result indicated that these putative *DsyB* proteins may not be active MTHB S-methyltransferases. However, it is also possible that this may be due to the lack of an appropriate cofactor for the *DsyB* protein to be active in *E. coli*, as described previously by Li et al. (59). Further experiments are needed to confirm the activity of these putative *DsyB* proteins. For DMSP catabolism, two *dddP* sequences (1,161 bp and 1,305 bp) were chosen from *Anaerolineales* and *Pseudomonadales* MAGs, respectively, and one *dddX* sequence (2,127 bp) was chosen from an *Anaerolineales* MAG. The overexpressed *DddP* protein from *Pseudomonas* showed the highest DMSP catabolic enzyme activity ( $270,773.45 \pm 18,002.42$  nmol/mg protein) (Fig. S5). Surprisingly, we confirmed that both *DddX* and *DddP* from the *Anaerolineales* MAG can degrade DMSP to DMS, with activities of  $138.91 \pm 25.23$  nmol/mg protein and  $70.95 \pm 4.17$  nmol/mg protein, respectively.

## DISCUSSION

As an important compound involved in global sulfur cycling, the process for the metabolism of DMSP in marine sediments has gradually been revealed in recent years. Although photosynthetic eukaryotes were thought to be dominant DMSP producers in the photic zones, bacteria were demonstrated to be significant contributors of DMSP in diverse marine sediments (25, 37, 52) as well as in the aphotic waters of Earth's deepest ocean site: the Challenger Deep (9). Our previous study of the Mariana Trench revealed that bacteria are key DMSP producers in deep seawater and sediment, and a physiological function for DMSP in hydrostatic pressure protection has been proposed (9). However, the detailed and complete bacterial DMSP-cycling process through the depth profile of the Challenger Deep sediment remains to be further explored.

The DMSP concentrations in the surface sediment of the Mariana Trench at 5,000- to 10,500-m water depths varied between 3.15 and 6.14 nmol g<sup>-1</sup> wet sediment, as



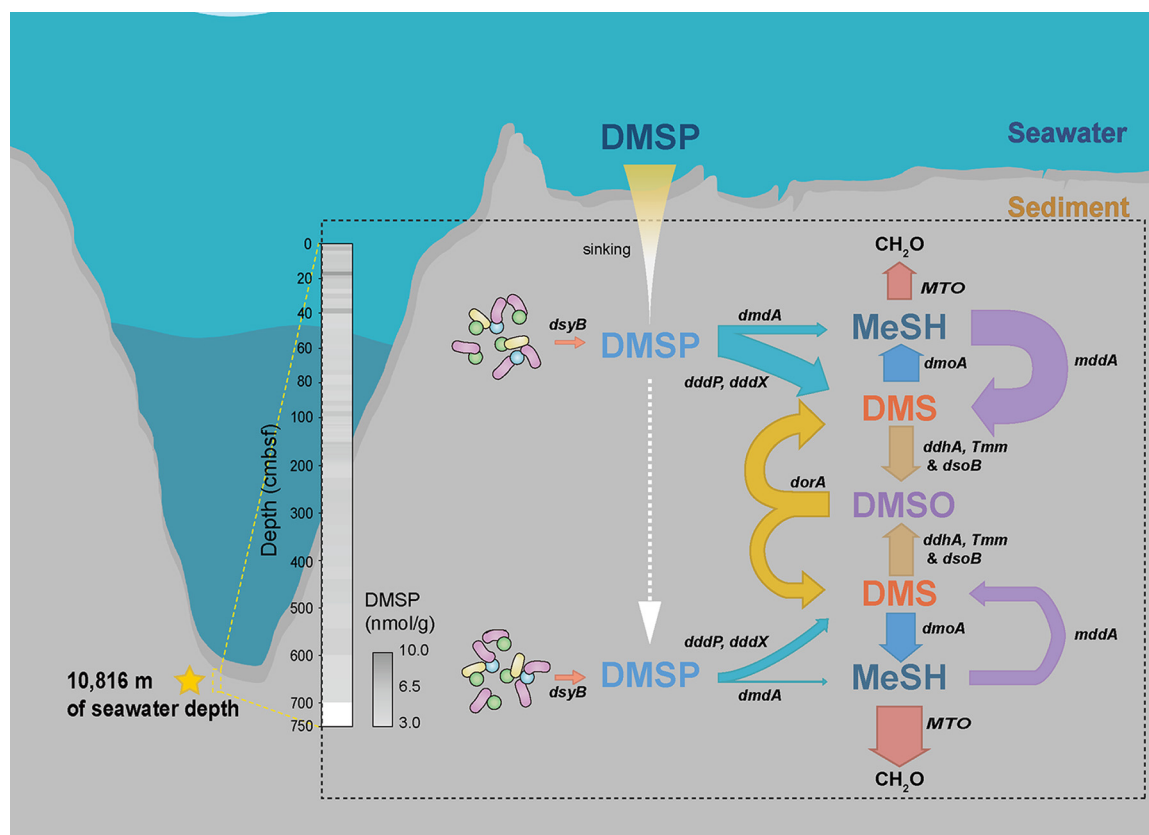
reported previously by Zheng et al. (9). In the current study, the DMSP content in the surface sediment at 10,816 m is even slightly higher ( $6.35 \pm 0.09 \text{ nmol g}^{-1}$ ). Indeed, combined with the results of Zheng et al. (9), we suggest that the DMSP concentrations in the surface sediment are not correlated with the water depth in the Mariana Trench. In addition, the DMSP concentrations in hadal Mariana Trench surface sediment were lower than those in the South China Sea (SCS) continent slope sediment (11.25 to  $20.90 \text{ nmol g}^{-1}$ ) (60) and even lower than those in coastal sediment ( $\sim 100 \text{ nmol g}^{-1}$ ) (25), ferruginous sediment ( $\sim 100 \text{ nmol g}^{-1}$ ), and sulfidic sediment ( $\sim 600 \text{ nmol g}^{-1}$ ) (61). Most importantly, we found that the depth profile of the DMSP content from the surface to the seafloor sediments showed distinct variation tendencies compared with those of the SCS sediment. There was no dramatic reduction in the DMSP concentrations in the top 30 cmbsf, and two extremely high DMSP concentrations were detected at 15 to 18 cmbsf and 37 to 40 cmbsf, which were even higher than those in the surface sediment. Such fluctuations in the DMSP content may indicate a nonhomogeneous process of sedimentation possibly affected by special topography and frequent tectonic activities (62). Notably, although the surface DMSP concentration in the Mariana Trench sediment is lower than that in the SCS sediment, the DMSP concentration in the deep seafloor ( $\sim 700$  cmbsf) of the Mariana Trench ( $3.26 \pm 0.37 \text{ nmol g}^{-1}$ ) is even higher than that in the SCS seafloor sediment (0.56 to  $2.08 \text{ nmol g}^{-1}$ ). Such depth profiles of the DMSP content in the deep Mariana Trench sediment suggested that there might be active microbial cycling processes for DMSP and other organic sulfur compounds. Although some DMSP in the sediment is expected to arise from sinking particles of dead algae and/or fecal pellets, it seems that bacterial DMSP synthesis is likely an important source of DMSP considering the high DMSP turnover rates in photic seawater (9).

For DMSP biosynthesis, *dsyB* was the only gene detected by qPCR and metagenomic sequencing. This is reasonable since the abundance of *mmtN* was consistently lower than that of *dsyB* in diverse sediments (9, 52). However, as the dominant DMSP biosynthetic gene, the abundance of *dsyB* at 10,816 m in this study ( $10^2$  copies  $\text{g}^{-1}$ ) was lower than those in the Mariana Trench surface sediment at 5,000 to 10,910 m ( $10^3$  to  $10^5$  copies  $\text{g}^{-1}$ ) (9). Corresponding to the DMSP concentrations, peaks of *dsyB* abundance were observed at several depths (12 to 15 cmbsf and 99 to 102 cmbsf), and there seemed to be no obvious decline in either the absolute or relative abundance of *dsyB* along sediment depths. The metagenomic analysis predicted that approximately 1% of bacteria contain *dsyB* in deep sediment samples, which is comparable to that in Stiffkey salt marshes ( $\sim 1\%$  of bacteria) (25). MAGs containing *dsyB* in these samples were from *Gammaproteobacteria* (*Pseudomonadales* order), *Acidimicrobiia*, *Hydrogenedentia* (*Hydrogenedentiales* order), and *Phycisphaerae*, which are distinct from the commonly known *dsyB*-containing bacteria belonging to *Alphaproteobacteria* (9, 25). DsyB was also previously reported in some bacteria of the *Gammaproteobacteria* (all were unassigned), *Betaproteobacteria*, and *Actinobacteria* in coastal sediments, such as *Flammeovirgaceae*, *Actinomycetospora*, and *Ponticoccus* (25). However, anaerobic bacteria of the *Acidimicrobiia* (55), *Phycisphaerae* (56, 57), and *Hydrogenedentia* (*Hydrogenedentiales* order) (58), which were commonly found in deep anoxic sediment, have never been reported to possess *dsyB* or produce DMSP. Although we attempted to heterogeneously express these *dsyB* homologs from the above-mentioned MAGs, no DMSP-producing activity was detected in the recombinant *E. coli* strains. This result indicated that these putative DsyB proteins may not be active MTHB S-methyltransferases; however, this may also be due to the lack of certain cofactors in the *E. coli* strain, as indicated previously by Li et al. (59). Further experiments are required to confirm the activity of the DsyB homologs found in these MAGs. Moreover, culturable isolates from the *Alcanivorax*, *Qipengyuania*, *Dietzia*, *Brachybacterium*, and *Mammaliococcus* genera were considered potential DMSP producers without *dsyB* or *mmtN* homologs. These isolates might contain previously unknown DMSP biosynthetic genes and pathways, and they could also possibly be the dominant DMSP producers instead of those bacteria with *dsyB* in Mariana Trench sediment.

For DMSP catabolism, 0.33 to 8.22% of the total bacteria in Mariana Trench sediment were predicted to contain at least one DMSP catabolic gene. The DMSP lyase DddP, instead of the demethylase DmdA, was the dominant enzyme, and higher abundances of DddP were found at ~20 and 40 cmbsf, corresponding to the DMSP content. *dddP* was identified in MAGs from *Alphaproteobacteria*, *Gammaproteobacteria*, and *Anaerolineae*, and MAGs from these groups with *dddP* were also retrieved from seawater of the Mariana Trench (9). *dmdA* genes were reported in *Alphaproteobacteria*, such as in the Changjiang Estuary (63), the Sanriku Coastal Region of Japan (41), and Eastern China Marginal Seas seawater and sediment (37, 64). However, MAGs containing *dmdA* genes in Mariana Trench deep sediment were mainly from *Gammaproteobacteria*. Furthermore, the *dmdA* genes in this study were clustered in *dmdA* subclades A, B, and E, while the *dmdA* genes in seawater and other sediments were mainly from subclades D1 and C2 (41, 65). For other *ddd* genes, only *dddX* was identified in the metagenomes and was predicted to be present in 0.12 to 1.10% of bacteria, which was identified in an *Anaerolineales* MAG. Both DddX and DddP retrieved from an *Anaerolineales* MAG were shown to be active in DMSP catabolism, indicating for the first time that anaerobes from *Anaerolineales* might be responsible for DMSP degradation in deep sediment. It should also be noted that *Actinomyetia* isolates accounted for a large proportion (51%) of the culturable DMSP catabolic bacteria, suggesting that actinobacteria can be important DMSP catabolic groups in Mariana Trench deep sediment. Although the DMSP catabolic capacity of many other Gram-positive actinobacteria has been previously reported (66), the DMSP catabolic gene and pathway remain unknown in these actinobacteria. Overall, we found that the known DMSP catabolic genes in deep Mariana Trench sediment were possibly from distinct bacterial groups compared with those in the seawater and other sediment environments, and potential previously unknown DMSP catabolic genes in actinomycetes and some anaerobic bacterial groups might be important for DMSP catabolism in such sediments. However, it should be noted that degenerate PCR may not fully cover the homologous genes of known DMSP metabolic enzymes in the obtained culturable isolates, considering the high level of divergence of these genes. Further experiments such as genome sequencing may be needed to confirm the existence of previously unknown active DMSP metabolic genes in these isolates.

For other genes involved in DMSP cycling, we found that the relative abundances of several genes were extremely high, especially the *dmdB* gene, encoding the MMPA-CoA ligase, as well as *dmdCD* and *acuH*, which are involved in the conversion of MMPA (resulting from DMSP catabolism by *dmdA*) to MeSH. A similarly high *dmdB* abundance was observed in Eastern China Marginal Seas sediments (37). Interestingly, we found that the relative abundances of genes related to MeSH production from MMPA and DMS, as well as MeSH oxidation to formaldehyde, consistently reached peaks at 12 to 15 cmbsf, 149 to 152 cmbsf, and 539 to 542 cmbsf, indicating that there might be active production and oxidation of MeSH at these depths for an unknown reason. The abundance of *mddA*, involved in the transformation of MeSH to DMS, was also the highest at 12 to 15 cmbsf. Therefore, except for DMSP biosynthesis and catabolism, active transformation between MeSH, DMS, and other compounds may occur in the deep sediment. Indeed, we found that many culturable isolates, including all of the DMSP-producing isolates, could use Met to produce DMS and MeSH and could use MeSH to produce DMS.

The whole DMSP-cycling processes in the deep sediment of the Mariana Trench are depicted in Fig. 5. In this study, we discuss the similarities and differences in bacterial DMSP metabolism in the Mariana Trench compared with other sediment environments. It seems that under extreme physical-chemical conditions, such as the low temperature and extremely high hydrostatic pressure in the trench sediment, there might be active but distinct bacterial groups involved in DMSP cycling. Further research is needed to reveal the potential previously unknown DMSP metabolic genes and



**FIG 5** Proposed bacterial DMSP cycling in Mariana Trench subseafloor sediment. The thickness of the arrows indicates the relative abundance of genes involved in DMSP cycling. The arrowheads indicate the flow directions of organic sulfur compounds.

pathways in these sediments, and the collection of more deep-sediment cores may help to provide a comprehensive understanding of DMSP metabolism in the Mariana Trench.

**Conclusion.** In this study, we outlined the complete bacterial DMSP-cycling processes in the deep sediment of the Mariana Trench. Both the DMSP contents and the DMSP metabolic gene levels fluctuated with the sediment depths. *dsyB* was the dominant DMSP biosynthesis gene, and *dddP* was the most abundant DMSP catabolic gene. Importantly, special bacterial groups in such deep sediments were involved in the DMSP biosynthetic pathway, such as *Acidimicrobiia*, *Phycisphaerae*, and *Hydrogenedentia*. Similarly, homologs of DddP and DddX were found in anaerobic *Anaerolineales*, and their DMSP catabolic activities were confirmed for the first time. More than one-half of the culturable bacteria involved in DMSP catabolism were actinomycetes, suggesting the significance of the potential novel DMSP catabolic gene in actinomycetes for DMSP catabolism in deep sediment. Moreover, highly abundant genes that participate in the conversion of MeSH, DMS, and other related organic compounds were identified.

## MATERIALS AND METHODS

**Sample collection and DNA preparation.** A sediment gravity core was collected from the Challenger Deep of the Mariana Trench (11°19.904', 142°12.083') during a cruise in July 2020 conducted on the R/V *Dong Fang Hong 3*. The depth of seawater was 10,816 m at the sampling site. The length of the sediment core reached approximately 750 cmbsf, and the core was sliced with a stainless steel cutter for subsampling. A total of 81 samples were obtained along the sediment core. The samples were transferred to sterilized plastic tubes and stored at  $-80^{\circ}\text{C}$  before DNA extraction. Metagenomic DNA was extracted from 10 to 12 g (wet weight) of representative sediment samples from 17 depths, as previously described by Zhou et al. (67). Briefly, each 1-g sample was washed with 3.3 mL of extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM sodium EDTA [pH 8.0], 100 mM sodium phosphate [pH 8.0], 1.5 M NaCl, 1% cetyltrimethylammonium bromide [CTAB]) and then centrifuged at  $6,000 \times g$  for 20 min at

**TABLE 1** Primers and amplification conditions for qPCR detection and target gene amplicon sequencing for bacteria

Target gene	Primer	Sequence (5'–3')	Amplicon length (bp)	Annealing temp (°C)	Reference
<i>dsyB</i>	dsyBF	CATGGGSTCSAAGGCSCTKT	246	58	25
	dsyBR	GCAGRTARTCGCCGAAATCGTA			
<i>mntN</i>	mntNF	CCGAGGTGGTCATGAAYTTYGG	301	54	25
	mntNR	GGATCACGCACACYTCRTGRTA			
<i>dddP</i>	874F	AAYGAAATWGTTCCTTTGA	97	41	68
	971R	GCATDGCRTAAATCATATC			
<i>dmdA</i> (C2)	291F	AGATGAAAATGCTGGAATGATAAATG	191	50	68
	482R	AAATCTTCAGACTTTGGACCTTG			
<i>dmdA</i> (D1)	268F	AGATGTTATTATTGTCCAATAATTGATG	89	49	68
	356R	ATCCACCATCTATCTTCAGCTA			
<i>dddD</i>	dddDF	ACCAACGTCATTGCAGGACC	63	56	69
	dddDR	TGTGCGTGTCTCCGGTG			
<i>dddL</i>	dddLF	CTGGGAATACGGCTACGAGA	239	53	69
	dddLR	GTTCAAGATCAGCGATCCGG			

room temperature. The concentrated biomass was ground in liquid nitrogen, 122  $\mu$ g proteinase K and 0.37 mL 20% SDS were added, and the mixture was incubated at 37°C for 30 min and at 65°C for 2 h, respectively. After phenol-chloroform extraction (ratio of 24:1, 15 volumes), DNA was precipitated with 0.6 volumes of isopropanol, washed with 70% ethanol, and air dried. Finally, the PowerSoil DNA isolation kit (MoBio Lab) was used for DNA purification to improve the DNA quality and satisfy the sequencing quality threshold.

**Quantification of DMSP in sediment.** The DMSP content was measured in samples from 82 depths, as described previously by Zheng et al. (9). One hundred microliters of purified water and 100  $\mu$ L of NaOH (10 M) were added to 0.1-g samples placed into 2-mL vials, and the samples were then stored in the dark at room temperature for 24 h. The DMS released from DMSP cleavage was quantified by a purge-and-trap gas chromatography (GC) system or GC autoinjection using a flame photometric detector (Agilent 7890B GC system fitted with a 7693A autosampler) and an HP-INNOWax 30-m by 0.320-mm capillary column (Agilent Technologies, J&W Scientific). An eight-point calibration curve of DMS standards was used (60), and the detection limit for headspace DMS was 0.015 nmol. All experiments described here were performed using three biological replicates.

**Quantitative PCR.** The abundance of genes involved in DMSP synthesis and degradation was quantified using qPCR in 17 samples from different depths, including *dsyB*, *mntN*, as well as *dddP* and *dmdA* subclades (C2 and D1) (11, 40). The primers (5' to 3') used in this assay and their annealing temperatures are displayed in Table 1 (25, 40, 68, 69). A qPCR standard was prepared, and standard melt curves were conducted as described in previous reports (11, 40). The PCRs were conducted as follows: an initial denaturation step at 95°C for 3 min and then 35 cycles of 95°C for 30 s, the corresponding annealing temperature for 30 s, and 72°C for 30 s. A melt curve was run after PCR as follows: denaturation at 95°C for 1 min and 0.5°C increments from the annealing temperature with signal collection. Each sample was run with negative controls conducted in triplicate using a QuantStudio 5 system (Thermo Fisher Scientific). A 10-fold serial dilution for PCR amplification of plasmids containing each target gene fragment was used to construct the standard curves. The amplification curves exhibited clear linear relationships ( $R^2 > 0.999$ ) and yielded an amplification efficiency of 0.90 for each gene.

**Metagenomic sequencing and binning.** A total of 17 sediment DNA samples were used for the metagenomic analysis. Fifteen DNA samples (0 to 542 cmbsf, each about 1  $\mu$ g) were sent to BGI Tech Solutions (Beijing Liuhe) Co., Ltd., and two DNA samples (644 to 647 cmbsf [0.4  $\mu$ g] and 749 to 752 cmbsf [0.2  $\mu$ g]) were sent to Majorbio BiFo-Pharm Technology Co., Ltd., Shanghai, China, for metagenomic sequencing. Libraries were prepared without any amplification step for each sample. Metagenomic shotgun sequencing was performed on the Illumina HiSeq X-Ten platform, with 2 $\times$  150-bp paired-end reads.

All of the raw reads containing >10% undefined bases and >40% low-quality bases and that had >15 bases matching the adapters were removed. The construction of genes was performed using MetaWrap-megahit (70), and gene prediction was performed by prodigal with default parameters (71). A total of 15,802,294 sequences were clustered at 95% identity into a set of 40,154,822 nonredundant sequences using cd-hit-est with the command cd-hit-est -c 0.95 -n 10 -d 0 -M 0 -T 1 -aS 0.9, and sequences of <100 bp were discarded (72). However, to retrieve more high-quality MAGs, the construction of MAGs was performed using MetaWrap-metaspades, -assembly, and -reassemble\_bins with default parameters (70), and protein prediction for MAGs was performed using prokka with default parameters (73). MAGs with >50% completeness and <10% contamination were retained for further metagenomic analysis. The completion and contamination of each MAG were evaluated by using CheckM version 1.0.12 (74). The taxonomy of these MAGs was annotated by using GTDB-Tk version 1.6.0 (75).

**Bioinformatic analyses of genes involved in DMS/DMSP cycling.** To explore the genetic potential for DMSP/DMS cycling, hidden Markov model (HMM)-based searches for homologs in metagenome data sets and MAGs were performed using HMMER 3.3.2 (76). Ratified MmtN, DsyB, DddD, DddP, DddK, DddQ, DddW, DddL, Dddy, DddX, DmdA, Alma1, Mdda, DmoA (the catalytic subunit of the DMS monooxygenase DmoAB), DorA, Tmm, DsoB (a key catalytic subunit of the monooxygenase DsoABCDEF), DdhA (the catalytic subunit of the DMS dehydrogenase DdhABC), DmdB, DmdC, DmdD, and MTO protein sequences were obtained from the National Center for Biotechnology Information (NCBI) database. HMMs were created for each enzyme using protein sequences that have been biochemically or structurally characterized, and the cutoff values used were selected based on established stringency cutoff values from previous reports (see Table S8 in the supplemental material) (10, 15, 16, 25, 33–36, 39, 48, 77–80). Separate cutoff E values were confirmed by BLAST analysis between functionally verified protein sequences (37). However, homologs of DmdD, DsoB, and DmoA in metagenomes were obtained using BLASTp with an E value cutoff of  $10^{-30}$  and an identity cutoff of 40% (39, 49). All of the predicted DMSP/DMS cycling proteins were placed into phylogenetic trees to further identify functional homologs. Phylogenetic trees were constructed using IQ-TREE version 1.6.12 (81) and included nonfunctional sequences used as outgroups. Also, these amino acid sequences were aligned using MAFFT version 7 with default settings (82, 83). Sequences that clustered with nonfunctional sequences were removed. In addition, the DmdA sequences in the metagenomic analysis were used to build the neighbor-joining phylogenetic tree, as described previously by Varaljay et al. (40).

As previously described by Zheng et al. (9), to compare the counts of bacterial cells among samples, the percentages of bacteria harboring genes were normalized using the single-copy housekeeping gene *recA*. The HMM profile for *RecA* was downloaded from FunGene (<http://fungene.cme.msu.edu/>), and sequences with an E value of  $\leq 10^{-50}$  were retained. The percentage of cells containing a particular gene of interest was calculated as (gene homologs  $\times$  100)/*recA*. Their RPKM (reads per kilobase per million mapped reads) values were confirmed using BWA-MEM (bwa version 0.7.17-r1188, using default settings) and samtools version 1.10 (84). Reads with coverages of  $<80\%$  were filtered and discarded using covm filter version 0.4.0 (Ben Woodcroft, CMR, QUT, UK).

**Heterologous expression and activity of potential DMSP metabolic enzymes.** To further validate the activity of the environmental sequences retrieved from these marine metagenomes predicted in MAGs, four DsyB sequences, two DddP sequences, and one DddX sequence (Table S7) were chosen for further experiments. These candidate sequences were synthesized by Sangon Biotech (Shanghai, China), cloned, and overexpressed using the pET24a plasmid in *E. coli* BL21(DE3) to test their activity. The recombinant *E. coli* strains were incubated in LB medium at 37°C for 3 h, and 0.1 mM isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) was added before culturing for 20 h at 16°C. For DMSP catabolic proteins, 200  $\mu$ L of an *E. coli* culture with 0.1 mM DMSP was mixed in a sealing vial, and the reaction mixture was incubated at 37°C at 170 rpm. After 24 h, DMS was measured to calculate the enzyme activities monitored by GC (see the section on DMSP concentration measurement, above). In addition, 200  $\mu$ L of the culture was centrifuged to collect the cells, and the cell pellet was then resuspended in 1 mL Tris-HCl buffer (50 mM; pH 7) and sonicated (3 times for 10 s each) using a Scientz JY92-IIN ultrasonic homogenizer. The DMSP catabolic activity was normalized to the cellular protein content estimated by the Bradford method (Bio-Rad). *E. coli* BL21 containing the original pET24a plasmid was used as a negative control. For DMSP biosynthetic proteins, a 20- $\mu$ L *E. coli* culture was mixed with 180  $\mu$ L of LB medium containing 0.05 mM MTHB and 0.2 mM IPTG in a sealing vial at 37°C at 170 rpm for 12 h. Subsequently, 100  $\mu$ L of 10 M NaOH was added to each sealing vial, and the reaction mixture was incubated at 28°C at 170 rpm for 2 h. As described above, the DMS concentration was then measured to calculate the enzyme activities monitored by a GC assay (see the section on DMSP concentration measurement, above). The DMSP biosynthetic activity was normalized to the cellular protein content, as mentioned above. All experiments described here were performed using three biological replicates.

**Bacterial isolation and identification of DMSP-producing and -degrading isolates.** Culturable DMSP-producing and -degrading bacteria were isolated from the enrichment culture of a sample from 0 to 1 cmbsf. For the enrichment experiment, minimal marine basal medium (MBM) (salinity of 35 PSU [practical salinity units]) (85) supplemented with a mixed carbon source and either 0.5 mM L-Met (for DMSP-producing bacteria) or 0.5 mM DMSP (for DMSP-degrading bacteria) was used. The mixed carbon source contained succinate, glucose, pyruvate, sucrose, and glycerol (with final concentrations of 2 mM each). The enrichment process was carried out at 28°C for over 2 months, with supplementation with fresh medium each week. The resulting enriched cultures were serially diluted and spread onto marine agar (MA) (1 g of yeast extract, 5 g of peptone, and 0.01 g of ferric phosphate [pH 7.6] per L of seawater) plates and incubated at 28°C for 5 to 7 days. A single colony was picked and subsequently purified three times by streaking. The 16S rRNA genes of isolates were amplified using the primer set B8F (5'-AGAGTTTGATCCTGGCTCAG-3') and B1510R (5'-GGTACCTGTTCAGACTT-3') (86) and sequenced. Taxonomy information identifying these cultivated strains was obtained from the Ezbiocloud server (<http://www.ezbiocloud.net/identify>).

All of these obtained isolates were tested for their DMSP production or catabolism capacity. For DMSP biosynthesis, bacterial isolates were cultivated in MBM supplemented with 0.5 mM L-Met at 28°C for 24 h. Two hundred microliters of the culture and 100  $\mu$ L of NaOH (10 M) were mixed in 2-mL vials. The vials were crimped immediately and incubated at 170 rpm for 2 h in the dark, and headspace DMS was monitored by GC as described above. For DMSP catabolism, bacterial isolates were cultivated in MBM supplemented with 0.5 mM DMSP at 28°C for 24 h. The resulting DMS and MeSH were monitored directly by GC. To detect the ability to produce DMS directly from L-Met or MeSH, bacterial isolates were cultivated in MBM supplemented with 0.5 mM L-Met or MeSH at 28°C for 24 h in sealed vials, and the resulting DMS was monitored by GC. All experiments were conducted with three biological replicates.

Degenerate primers of several known DMSP biosynthetic and catabolic genes (Table S7) were used to detect these genes in the obtained DMSP metabolic isolates by PCR. The PCR system and amplification conditions were described previously by Williams et al. (25).

**Data availability.** The metagenome sequences and MAGs from the current study have been submitted to the NCBI under BioProject accession numbers [PRJNA957232](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA957232) and [PRJNA957236](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA957236), respectively.

## SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

**SUPPLEMENTAL FILE 1**, DOCX file, 2.8 MB.

**SUPPLEMENTAL FILE 2**, XLSX file, 0.04 MB.

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We declare no competing interests.

## REFERENCES

- Gali M, Devred E, Levasseur M, Royer SJ, Babin M. 2015. A remote sensing algorithm for planktonic dimethylsulfoniopropionate (DMSP) and an analysis of global patterns. *Remote Sens Environ* 171:171–184. <https://doi.org/10.1016/j.rse.2015.10.012>.
- Ksionzek KB, Lechtenfeld OJ, McCallister SL, Schmitt-Kopplin P, Geuer JK, Geibert W, Koch BP. 2016. Dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory. *Science* 354:456–459. <https://doi.org/10.1126/science.aaf7796>.
- Seymour JR, Simo R, Ahmed T, Stocker R. 2010. Chemoattraction to dimethylsulfoniopropionate throughout the marine microbial food web. *Science* 329:342–345. <https://doi.org/10.1126/science.1188418>.
- Damm E, Nomura D, Martin A, Dieckmann GS, Meiners KM. 2016. DMSP and DMS cycling within Antarctic Sea ice during the winter-spring transition. *Deep Sea Res 2 Top Stud Oceanogr* 131:150–159. <https://doi.org/10.1016/j.dsr2.2015.12.015>.
- Seyedsayamdost MR, Case RJ, Kolter R, Clardy J. 2011. The Jekyll-and-Hyde chemistry of *Phaeobacter gallaeciensis*. *Nat Chem* 3:331–335. <https://doi.org/10.1038/nchem.1002>.
- Raina JB, Tapiolas DM, Forest S, Lutz A, Abrego D, Ceh J, Seneca FO, Clode PL, Bourne DG, Willis BL, Mottl CA. 2013. DMSP biosynthesis by an animal and its role in coral thermal stress response. *Nature* 502:677–680. <https://doi.org/10.1038/nature12677>.
- Sunda W, Kieber DJ, Kiene RP, Huntsman S. 2002. An antioxidant function for DMSP and DMS in marine algae. *Nature* 418:317–320. <https://doi.org/10.1038/nature00851>.
- Thume K, Gebser B, Chen L, Meyer N, Kieber DJ, Pohnert G. 2018. The metabolite dimethylsulfoxonium propionate extends the marine organosulfur cycle. *Nature* 563:412–415. <https://doi.org/10.1038/s41586-018-0675-0>.
- Zheng Y, Wang J, Zhou S, Zhang Y, Liu J, Xue C-X, Williams BT, Zhao X, Zhao L, Zhu X-Y, Sun C, Zhang H-H, Xiao T, Yang G-P, Todd JD, Zhang X-H. 2020. Bacteria are important dimethylsulfoniopropionate producers in marine aphotic and high-pressure environments. *Nat Commun* 11:4658. <https://doi.org/10.1038/s41467-020-18434-4>.
- Curson ARJ, Todd JD, Sullivan MJ, Johnston AWB. 2011. Catabolism of dimethylsulphoniopropionate: microorganisms, enzymes and genes. *Nat Rev Microbiol* 9:849–859. <https://doi.org/10.1038/nrmicro2653>.
- Todd JD, Rogers R, Li YG, Wexler M, Bond PL, Sun L, Curson ARJ, Malin G, Steinke M, Johnston AWB. 2007. Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. *Science* 315:666–669. <https://doi.org/10.1126/science.1135370>.
- Vallina SM, Simo R. 2007. Strong relationship between DMS and the solar radiation dose over the global surface ocean. *Science* 315:506–508. <https://doi.org/10.1126/science.1133680>.
- Sievert SM, Kiene RP, Schulz-Vogt HN. 2007. The sulfur cycle. *Oceanography* 20:117–123. <https://doi.org/10.5670/oceanog.2007.55>.
- Carrion O, Pratscher J, Curson ARJ, Williams BT, Rostant WG, Murrell JC, Todd JD. 2017. Methanethiol-dependent dimethylsulfide production in soil environments. *ISME J* 11:2379–2390. <https://doi.org/10.1038/ismej.2017.105>.
- Curson ARJ, Liu J, Bermejo Martinez A, Green RT, Chan Y, Carrion O, Williams BT, Zhang S-H, Yang G-P, Bulman Page PC, Zhang X-H, Todd JD. 2017. Dimethylsulfoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process. *Nat Microbiol* 2:17009. <https://doi.org/10.1038/nmicrobiol.2017.9>.
- Curson ARJ, Williams BT, Pinchbeck BJ, Sims LP, Martinez AB, Rivera PPL, Kumaresan D, Mercade E, Spurgin LG, Carrion O, Moxon S, Cattolico RA, Kuzhiumparambil U, Guagliardo P, Clode PL, Raina J-B, Todd JD. 2018. DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nat Microbiol* 3:430–439. <https://doi.org/10.1038/s41564-018-0119-5>.
- Zhang X-H, Liu J, Liu J, Yang G, Xue C-X, Curson ARJ, Todd JD. 2019. Biogenic production of DMSP and its degradation to DMS—their roles in the global sulfur cycle. *Sci China Life Sci* 62:1296–1319. <https://doi.org/10.1007/s11427-018-9524-y>.
- Gage DA, Rhodes D, Nolte KD, Hicks WA, Leustek T, Cooper AJ, Hanson AD. 1997. A new route for synthesis of dimethylsulphoniopropionate in marine algae. *Nature* 387:891–894. <https://doi.org/10.1038/43160>.
- Summers PS, Nolte KD, Cooper AJ, Borgeas H, Leustek T, Rhodes D, Hanson AD. 1998. Identification and stereospecificity of the first three enzymes of 3-dimethylsulfoniopropionate biosynthesis in a chlorophyte alga. *Plant Physiol* 116:369–378. <https://doi.org/10.1104/pp.116.1.369>.
- James F, Paquet L, Sparace SA, Gage DA, Hanson AD. 1995. Evidence implicating dimethylsulfoniopropionaldehyde as an intermediate in dimethylsulfoniopropionate biosynthesis. *Plant Physiol* 108:1439–1448. <https://doi.org/10.1104/pp.108.4.1439>.
- Kocsis MG, Nolte KD, Rhodes D, Shen T-L, Gage DA, Hanson AD. 1998. Dimethylsulfoniopropionate biosynthesis in *Spartina alterniflora*: evidence that S-methylmethionine and dimethylsulfoniopropylamine are intermediates. *Plant Physiol* 117:273–281. <https://doi.org/10.1104/pp.117.1.273>.
- Kocsis MG, Hanson AD. 2000. Biochemical evidence for two novel enzymes in the biosynthesis of 3-dimethylsulfoniopropionate in *Spartina alterniflora*. *Plant Physiol* 123:1153–1161. <https://doi.org/10.1104/pp.123.3.1153>.
- Otte ML, Wilson G, Morris JT, Moran BM. 2004. Dimethylsulphoniopropionate (DMSP) and related compounds in higher plants. *J Exp Bot* 55:1919–1925. <https://doi.org/10.1093/jxb/erh178>.
- Lyon BR, Lee PA, Bennett JM, DiTullio GR, Janech MG. 2011. Proteomic analysis of a sea-ice diatom: salinity acclimation provides new insight into the dimethylsulfoniopropionate production pathway. *Plant Physiol* 157:1926–1941. <https://doi.org/10.1104/pp.111.185025>.
- Williams BT, Cowles K, Bermejo Martinez A, Curson ARJ, Zheng Y, Liu J, Newton-Payne S, Hind AJ, Li C-Y, Rivera PPL, Carrion O, Liu J, Spurgin LG, Brearley CA, Mackenzie BW, Pinchbeck BJ, Peng M, Pratscher J, Zhang X-H, Zhang Y-Z, Murrell JC, Todd JD. 2019. Bacteria are important

- dimethylsulfoniopropionate producers in coastal sediments. *Nat Microbiol* 4:1815–1825. <https://doi.org/10.1038/s41564-019-0527-1>.
26. Hanson AD, Rivoal J, Paquet L, Gage DA. 1994. Biosynthesis of 3-dimethylsulfoniopropionate in *Wollastonia biflora* (L.) DC. (evidence that S-methylmethionine is an intermediate). *Plant Physiol* 105:103–110. <https://doi.org/10.1104/pp.105.1.103>.
  27. Kiene RP. 1996. Biological and environmental chemistry of DMSP and related sulfonium compounds. Plenum Press, New York, NY.
  28. Kageyama H, Tanaka Y, Shibata A, Waditee-Sirisattha R, Takabe T. 2018. Dimethylsulfoniopropionate biosynthesis in a diatom *Thalassiosira pseudonana*: identification of a gene encoding MTHB-methyltransferase. *Arch Biochem Biophys* 645:100–106. <https://doi.org/10.1016/j.abb.2018.03.019>.
  29. Liao C, Seebeck FP. 2019. *In vitro* reconstitution of bacterial DMSP biosynthesis. *Angew Chem Int Ed Engl* 58:3553–3556. <https://doi.org/10.1002/anie.201814662>.
  30. Uchida A, Ooguri T, Ishida T, Kitaguchi H, Ishida Y. 1996. Biosynthesis of dimethylsulfoniopropionate in *Cryptothecodinium cohnii* (Dinophyceae), p 97–107. In Kiene RP, Visscher PT, Keller MD, Kirst GO (ed), Biological and environmental chemistry of DMSP and related sulfonium compounds. Springer, Boston, MA.
  31. Todd JD, Curson ARJ, Dupont CL, Nicholson P, Johnston AWB. 2009. The *dddP* gene, encoding a novel enzyme that converts dimethylsulfoniopropionate into dimethyl sulfide, is widespread in ocean metagenomes and marine bacteria and also occurs in some ascomycete fungi. *Environ Microbiol* 11:1376–1385. <https://doi.org/10.1111/j.1462-2920.2009.01864.x>.
  32. Todd JD, Curson AR, Kirkwood M, Sullivan MJ, Green RT, Johnston AW. 2011. DddQ, a novel, cupin-containing, dimethylsulfoniopropionate lyase in marine roseobacters and in uncultured marine bacteria. *Environ Microbiol* 13:427–438. <https://doi.org/10.1111/j.1462-2920.2010.02348.x>.
  33. Curson ARJ, Sullivan MJ, Todd JD, Johnston AWB. 2011. DddY, a periplasmic dimethylsulfoniopropionate lyase found in taxonomically diverse species of Proteobacteria. *ISME J* 5:1191–1200. <https://doi.org/10.1038/ismej.2010.203>.
  34. Li C-Y, Wang X-J, Chen X-L, Sheng Q, Zhang S, Wang P, Quareshy M, Rihtman B, Shao X, Gao C, Li F, Li S, Zhang W, Zhang X-H, Yang G-P, Todd JD, Chen Y, Zhang Y-Z. 2021. A novel ATP dependent dimethylsulfoniopropionate lyase in bacteria that releases dimethyl sulfide and acryloyl-CoA. *Elife* 10:e64045. <https://doi.org/10.7554/eLife.64045>.
  35. Alcolombri U, Ben-Dor S, Feldmesser E, Levin Y, Tawfik DS, Vardi A. 2015. Identification of the algal dimethyl sulfide-releasing enzyme: a missing link in the marine sulfur cycle. *Science* 348:1466–1469. <https://doi.org/10.1126/science.aab1586>.
  36. Schnicker NJ, De Silva SM, Todd JD, Dey M. 2017. Structural and biochemical insights into dimethylsulfoniopropionate cleavage by cofactor-bound DddK from the prolific marine bacterium *Pelagibacter*. *Biochemistry* 56:2873–2885. <https://doi.org/10.1021/acs.biochem.7b00099>.
  37. Song D, Zhang Y, Liu J, Zhong H, Zheng Y, Zhou S, Yu M, Todd JD, Zhang X-H. 2020. Metagenomic insights into the cycling of dimethylsulfoniopropionate and related molecules in the Eastern China Marginal Seas. *Front Microbiol* 11:157. <https://doi.org/10.3389/fmicb.2020.00157>.
  38. Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu D, Paulsen I, Nelson KE, Nelson W, Fouts DE, Levy S, Knap AH, Lomas MW, Nealson K, White O, Peterson J, Hoffman J, Parsons R, Baden-Tillson H, Pfannkoch C, Rogers Y-H, Smith HO. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science* 304:66–74. <https://doi.org/10.1126/science.1093857>.
  39. Reisch CR, Stoudemayer MJ, Varaljay VA, Amster IJ, Moran MA, Whitman WB. 2011. Novel pathway for assimilation of dimethylsulphoniopropionate widespread in marine bacteria. *Nature* 473:208–211. <https://doi.org/10.1038/nature10078>.
  40. Varaljay VA, Howard EC, Sun S, Moran MA. 2010. Deep sequencing of a dimethylsulfoniopropionate-degrading gene (*dmdA*) by using PCR primer pairs designed on the basis of marine metagenomic data. *Appl Environ Microbiol* 76:609–617. <https://doi.org/10.1128/AEM.01258-09>.
  41. Cui Y, Suzuki S, Omori Y, Wong S-K, Ijichi M, Kaneko R, Kameyama S, Tanimoto H, Hamasaki K. 2015. Abundance and distribution of dimethylsulfoniopropionate degradation genes and the corresponding bacterial community structure at dimethyl sulfide hot spots in the tropical and subtropical Pacific Ocean. *Appl Environ Microbiol* 81:4184–4194. <https://doi.org/10.1128/AEM.03873-14>.
  42. Howard EC, Sun S, Reisch CR, del Valle DA, Burgmann H, Kiene RP, Moran MA. 2011. Changes in dimethylsulfoniopropionate demethylase gene assemblages in response to an induced phytoplankton bloom. *Appl Environ Microbiol* 77:524–531. <https://doi.org/10.1128/AEM.01457-10>.
  43. Carrion O, Pratscher J, Richa K, Rostant WG, Farhan UI Haque M, Murrell JC, Todd JD. 2019. Methanethiol and dimethylsulfide cycling in Stiffkey saltmarsh. *Front Microbiol* 10:1040. <https://doi.org/10.3389/fmicb.2019.01040>.
  44. Bray RC, Adams B, Smith AT, Richards RL, Lowe DJ, Bailey S. 2001. Reactions of dimethylsulfoxide reductase in the presence of dimethyl sulfide and the structure of the dimethyl sulfide-modified enzyme. *Biochemistry* 40:9810–9820. <https://doi.org/10.1021/bi010559r>.
  45. Lidbury I, Krober E, Zhang Z, Zhu Y, Murrell JC, Chen Y, Schafer H. 2016. A mechanism for bacterial transformation of dimethylsulfide to dimethylsulfoxide: a missing link in the marine organic sulfur cycle. *Environ Microbiol* 18:2754–2766. <https://doi.org/10.1111/1462-2920.13354>.
  46. Horinouchi M, Yoshida T, Nojiri H, Yamane H, Omori T. 1999. Polypeptide requirement of multicomponent monooxygenase DsoABCDEF for dimethyl sulfide oxidizing activity. *Biosci Biotechnol Biochem* 63:1765–1771. <https://doi.org/10.1271/bbb.63.1765>.
  47. McDevitt CA, Hanson GR, Noble CJ, Cheesman MR, McEwan AG. 2002. Characterization of the redox centers in dimethyl sulfide dehydrogenase from *Rhodovulum sulfidophilum*. *Biochemistry* 41:15234–15244. <https://doi.org/10.1021/bi026221u>.
  48. Chen Y, Patel NA, Crombie A, Scrivens JH, Murrell JC. 2011. Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase. *Proc Natl Acad Sci U S A* 108:17791–17796. <https://doi.org/10.1073/pnas.1112928108>.
  49. Boden R, Murrell JC, Schafer H. 2011. Dimethylsulfide is an energy source for the heterotrophic marine bacterium *Sagittula stellata*. *FEMS Microbiol Lett* 322:188–193. <https://doi.org/10.1111/j.1574-6968.2011.02349.x>.
  50. Tanaka H, Esaki N, Yamamoto T, Soda K. 1976. Purification and properties of methioninase from *Pseudomonas ovalis*. *FEBS Lett* 66:307–311. [https://doi.org/10.1016/0014-5793\(76\)80528-5](https://doi.org/10.1016/0014-5793(76)80528-5).
  51. Zhuang G-C, Lin Y-S, Bowles MW, Heuer VB, Lever MA, Elvert M, Hinrichs K-U. 2017. Distribution and isotopic composition of trimethylamine, dimethylsulfide and dimethylsulfoniopropionate in marine sediments. *Mar Chem* 196:35–46. <https://doi.org/10.1016/j.marchem.2017.07.007>.
  52. Zhang Y, Sun K, Sun C, Shi X, Todd JD, Zhang X-H. 2021. Dimethylsulfoniopropionate biosynthetic bacteria in the seafloor sediments of the South China Sea. *Front Microbiol* 12:731524. <https://doi.org/10.3389/fmicb.2021.731524>.
  53. Huang C, Xie Q, Wang D, Shu Y, Xu H, Xiao J, Zu T, Long T, Zhang T. 2018. Seasonal variability of water characteristics in the Challenger Deep observed by four cruises. *Sci Rep* 8:11791. <https://doi.org/10.1038/s41598-018-30176-4>.
  54. Manea E, Dell'Anno A, Rastelli E, Tangherlini M, Nunoura T, Nomaki H, Danovaro R, Corinaldesi C. 2019. Viral infections boost prokaryotic biomass production and organic C cycling in hadal trench sediments. *Front Microbiol* 10:1952. <https://doi.org/10.3389/fmicb.2019.01952>.
  55. Itoh T, Yamanoi K, Kudo T, Ohkuma M, Takashina T. 2011. *Aciditerrimonas ferrireducens* gen. nov., sp. nov., an iron-reducing thermoacidophilic actinobacterium isolated from a solfataric field. *Int J Syst Evol Microbiol* 61:1281–1285. <https://doi.org/10.1099/ijs.0.023044-0>.
  56. Fukunaga Y, Kurahashi M, Sakiyama Y, Ohuchi M, Yokota A, Harayama S. 2009. *Phycisphaera mikurensis* gen. nov., sp. nov., isolated from a marine alga, and proposal of *Phycisphaeraeaceae* fam. nov., *Phycisphaerales* ord. nov. and *Phycisphaerae* classis nov. in the phylum *Planctomycetes*. *J Gen Appl Microbiol* 55:267–275. <https://doi.org/10.2323/jgam.55.267>.
  57. Lage OM, van Niftrik L, Jogler C, Devos DP. 2019. *Planctomycetes*, p 614–626. In Schmidt TM (ed), *Encyclopedia of microbiology*, 4th ed. Academic Press, Oxford, United Kingdom. <https://doi.org/10.1016/B978-0-12-809633-8.90689-7>.
  58. Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng J-F, Darling A, Malfatti S, Swan BK, Gies EA, Dodsworth JA, Hedlund BP, Tsiamis G, Sievert SM, Liu W-T, Eisen JA, Hallam SJ, Kyrpides NC, Stepanauskas R, Rubin EM, Hugenholtz P, Woyke T. 2013. Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499:431–437. <https://doi.org/10.1038/nature12352>.
  59. Li C-Y, Crack JC, Newton-Payne S, Murphy ARJ, Chen X-L, Pinchbeck BJ, Zhou S, Williams BT, Peng M, Zhang X-H, Chen Y, Le Brun NE, Todd JD, Zhang Y-Z. 2022. Mechanistic insights into the key marine dimethylsulfoniopropionate synthesis enzyme DsyB/DSYB. *Mlife* 1:114–130. <https://doi.org/10.1002/mlf2.12030>.
  60. Liu J, Zhang Y, Liu J, Zhong H, Williams BT, Zheng Y, Curson ARJ, Sun C, Sun H, Song D, Mackenzie BW, Bermejo Martinez A, Todd JD, Zhang X-H. 2021. Bacterial dimethylsulfoniopropionate biosynthesis in the East China Sea. *Microorganisms* 9:657. <https://doi.org/10.3390/microorganisms9030657>.
  61. Wilkening JV, Turcyn AV, Redeker KR, Mills JV, Antler G, Carrion O, Todd JD. 2019. The production and fate of volatile organosulfur compounds in sulfidic

- and ferruginous sediment. *J Geophys Res Biogeosci* 124:3390–3402. <https://doi.org/10.1029/2019JG005248>.
62. Ichino MC, Clark MR, Drazen JC, Jamieson A, Jones DOB, Martin AP, Rowden AA, Shank TM, Yancey PH, Ruhl HA. 2015. The distribution of benthic biomass in hadal trenches: a modelling approach to investigate the effect of vertical and lateral organic matter transport to the seafloor. *Deep Sea Res 1 Oceanogr Res Pap* 100:21–33. <https://doi.org/10.1016/j.dsr.2015.01.010>.
  63. Sun H, Liu J, Tan S, Zheng Y, Wang X, Liang J, Todd JD, Zhang X-H. 2021. Spatiotemporal distribution of bacterial dimethylsulfoniopropionate producing and catabolic genes in the Changjiang Estuary. *Environ Microbiol* 23:7073–7092. <https://doi.org/10.1111/1462-2920.15813>.
  64. Sun H, Zhang Y, Tan S, Zheng Y, Zhou S, Ma Q-Y, Yang G-P, Todd JD, Zhang X-H. 2020. DMSP-producing bacteria are more abundant in the surface microlayer than subsurface seawater of the East China Sea. *Microb Ecol* 80:350–365. <https://doi.org/10.1007/s00248-020-01507-8>.
  65. Varaljay VA, Gifford SM, Wilson ST, Sharma S, Karl DM, Moran MA. 2012. Bacterial dimethylsulfoniopropionate degradation genes in the oligotrophic North Pacific subtropical gyre. *Appl Environ Microbiol* 78:2775–2782. <https://doi.org/10.1128/AEM.07559-11>.
  66. Liu J, Liu J, Zhang S-H, Liang J, Lin H, Song D, Yang G-P, Todd JD, Zhang X-H. 2018. Novel insights into bacterial dimethylsulfoniopropionate catabolism in the East China Sea. *Front Microbiol* 9:3206. <https://doi.org/10.3389/fmicb.2018.03206>.
  67. Zhou J, Bruns MA, Tiedje JM. 1996. DNA recovery from soils of diverse composition. *Appl Environ Microbiol* 62:316–322. <https://doi.org/10.1128/aem.62.2.316-322.1996>.
  68. Levine NM, Varaljay VA, Toole DA, Dacey JW, Doney SC, Moran MA. 2012. Environmental, biochemical and genetic drivers of DMSP degradation and DMS production in the Sargasso Sea. *Environ Microbiol* 14:1210–1223. <https://doi.org/10.1111/j.1462-2920.2012.02700.x>.
  69. Raina JB, Tapiolas D, Willis BL, Bourne DG. 2009. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Appl Environ Microbiol* 75:3492–3501. <https://doi.org/10.1128/AEM.02567-08>.
  70. Uritskiy GV, DiRuggiero J, Taylor J. 2018. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. *Microbiome* 6:158. <https://doi.org/10.1186/s40168-018-0541-1>.
  71. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>.
  72. Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* 22:1658–1659. <https://doi.org/10.1093/bioinformatics/btl158>.
  73. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
  74. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
  75. Parks DH, Chuvpochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil P-A, Hugenholtz P. 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 36:996–1004. <https://doi.org/10.1038/nbt.4229>.
  76. Kudo T, Kobiyama A, Rashid J, Reza MS, Yamada Y, Ikeda Y, Ikeda D, Mizusawa N, Ikeo K, Sato S, Ogata T, Jimbo M, Kaga S, Watanabe S, Naiki K, Kaga Y, Segawa S, Mineta K, Bajic V, Gojobori T, Watabe S. 2018. Seasonal changes in the abundance of bacterial genes related to dimethylsulfoniopropionate catabolism in seawater from Ofunato Bay revealed by metagenomic analysis. *Gene* 665:174–184. <https://doi.org/10.1016/j.gene.2018.04.072>.
  77. Teng Z-J, Qin Q-L, Zhang W, Li J, Fu H-H, Wang P, Lan M, Luo G, He J, McMinn A, Wang M, Chen X-L, Zhang Y-Z, Chen Y, Li C-Y. 2021. Biogeographic traits of dimethyl sulfide and dimethylsulfoniopropionate cycling in polar oceans. *Microbiome* 9:207. <https://doi.org/10.1186/s40168-021-01153-3>.
  78. Carrion O, Curson ARJ, Kumaresan D, Fu Y, Lang AS, Mercade E, Todd JD. 2015. A novel pathway producing dimethylsulphide in bacteria is widespread in soil environments. *Nat Commun* 6:6579. <https://doi.org/10.1038/ncomms7579>.
  79. Eyice O, Myronova N, Pol A, Carrion O, Todd JD, Smith TJ, Gurman SJ, Cuthbertson A, Mazard S, Mennink-Kersten MA, Bugg TDH, Andersson KK, Johnston AWB, Op den Camp HJM, Schafer H. 2018. Bacterial SBP56 identified as a Cu-dependent methanethiol oxidase widely distributed in the biosphere. *ISME J* 12:145–160. <https://doi.org/10.1038/ismej.2017.148>.
  80. Cao H-Y, Wang P, Xu F, Li P-Y, Xie B-B, Qin Q-L, Zhang Y-Z, Li C-Y, Chen X-L. 2017. Molecular insight into the acryloyl-CoA hydration by AcuH for acrylate detoxification in dimethylsulfoniopropionate-catabolizing bacteria. *Front Microbiol* 8:2034. <https://doi.org/10.3389/fmicb.2017.02034>.
  81. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>.
  82. Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30:3059–3066. <https://doi.org/10.1093/nar/gkf436>.
  83. Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780. <https://doi.org/10.1093/molbev/mst010>.
  84. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, Li H. 2021. Twelve years of SAMtools and BCFtools. *Gigascience* 10:giab008. <https://doi.org/10.1093/gigascience/giab008>.
  85. Starr MP. 1981. *The prokaryotes: a handbook on habitats, isolation, and identification of bacteria*. Springer-Verlag, Berlin, Germany.
  86. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>.