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[Review]

Enzymes and Their Reaction Mechanisms in Dimethylsulfoniopropionate Cleavage and Biosynthesis of Dimethylsulfide by Marine Bacteria

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In marine ecosystems, the biosynthesis and catabolism of dimethylsulfoniopropionate (DMSP) by marine bacteria is critical to microbial survival and the ocean food chain. Furthermore, these processes also influence sulfur recycling and climate change. Recent studies using emerging genome sequencing data and extensive bioinformatics analysis have enabled us to identify new DMSP-related genes. Currently, seven bacterial DMSP lyases (DddD, DddP, DddY, DddK, DddL, DddQ and DddW), two acrylate degrading enzymes (DddA and DddC), and four demethylases (DmdA, DmdB, DmdC, and DmdD) have been identified and characterized in diverse marine bacteria. In this review, we focus on the biochemical properties of DMSP cleavage enzymes with special attention to DddD, DddA, and DddC pathways. These three enzymes function in the production of acetyl coenzyme A (CoA) and CO_2 from DMSP. DddD is a DMSP lyase that converts DMSP to 3-hydroxypropionate with the release of dimethylsulfide. 3-Hydroxypropionate is then converted to malonate semialdehyde by DddA, an alcohol dehydrogenase. Then, DddC transforms malonate semialdehyde to acetyl-CoA and CO_2 gas. DddC is a putative methylmalonate semialdehyde dehydrogenase that requires nicotinamide adenine dinucleotide and CoA cofactors. Here we review recent insights into the structural characteristics of these enzymes and the molecular events of DMSP degradation.

Keywords: Dimethylsulfide, Dimethylsulfoniopropionate, Molecular modeling, Methylmalonate semialdehyde dehydrogenase, Sulfur recycle

1. Introduction

The movement and cycling of sulfur through the geosphere and biosphere is facilitated by weathering, microbes, and plants. Sulfur is released from the geosphere by volcanic activity and then flows into the ocean with rain. In marine ecosystems, sulfur exists mainly in its dimethylsulfoniopropionate (DMSP) form in microalgae, macroalgae, and fish (Fig. 1) (Kiene et al., 2000; Simo, 2001; Howard et al., 2008; Carslaw et al., 2010). DMSP functions as an osmolyte and antioxidant and its degraded products are major sources of carbon and sulfur for marine microbes. The biosynthesis of DMSP occurs primarily in marine phytoplankton and macroalgae. The sulfur-containing amino acid methionine is the DMSP precursor. The backbone for methionine is derived from the aspartate family, but sulfur may also be derived from cysteine, methanethiol, and hydrogen sulfide (Fig. 2) (Kiene et al., 2000). Additional studies are required to understand the specific mechanism of DMSP biosynthesis at the molecular level.

Several marine bacteria can degrade DMSP into dimethylsulfide (DMS), a volatile sulfur compound that contributes significantly to the global sulfur cycle. Specifically, DMS molecules influence cloud formation by acting as cloud condensation nuclei (CCN), and they move into the atmosphere, thereby returning to land with rain (Reisch et al., 2011; Moran et al., 2012; Song et al., 2020). Moreover, DMS may be involved in the food web. DMS dissolved in the ocean as well as its gaseous form attracts sea birds and copepod crustaceans so that sea animals can locate food sources such as marine phytoplankton and macroalgae (Kiene et al., 2000; Simo, 2001; Nevitt and Haberman, 2003).

Marine bacteria species have their own unique DMSP degrad-

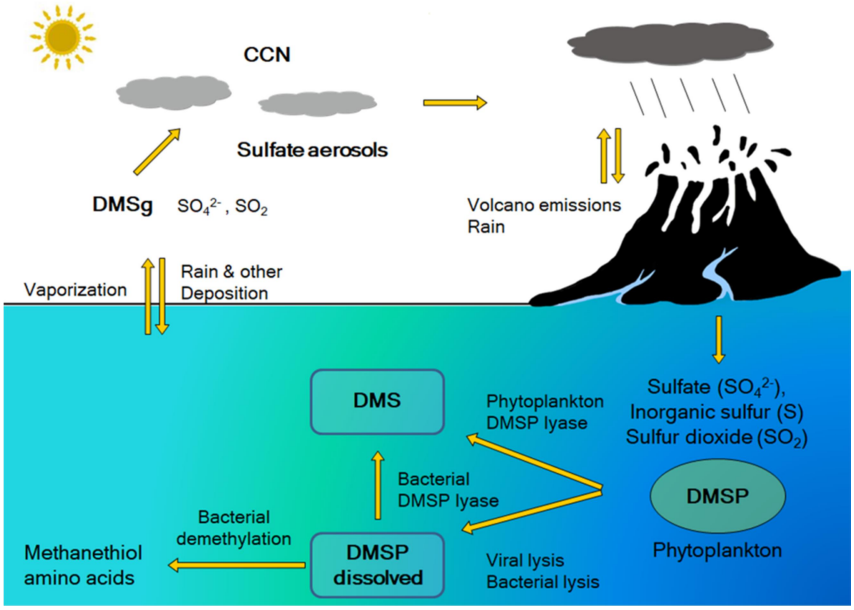

Fig. 1. Overview of global sulfur cycling mediated by marine bacteria. Dissolved dimethylsulfoniopropionate (DMSP) is degraded via two pathways: demethylation and cleavage. Dimethylsulfide (DMS) is exchanged at the ocean surface affecting weather changes. Abbreviations: CCN, cloud condensation nuclei.

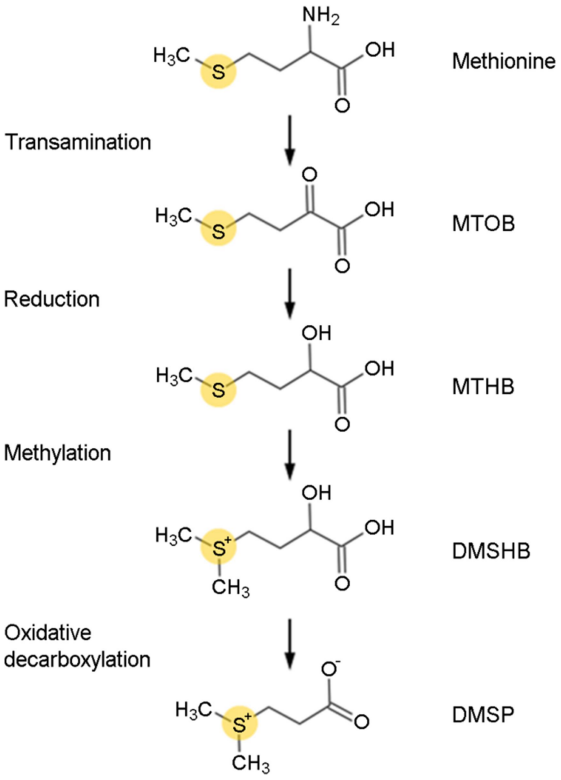

Fig. 2. Dimethylsulfoniopropionate (DMSP) synthesis in marine algae. Abbreviations: DMSHB, 4-dimethylsulfonio-2-hydroxybutyrate; MTOB, 4-methylthio-2-oxobutyrate; MTHB, methylthio-2-hydroxybutyrate.

ation pathways. The degradation of DMSP by marine bacteria can be categorized into two competing pathways: the DMSP cleavage pathway and the DMSP demethylation pathway. To date, seven bacterial DMSP lyases (DddD, DddP, DddY, DddK, DddL, DddQ and DddW) have been identified as participants in the DMSP cleavage pathway (Reisch et al., 2011; Moran et al., 2012; Schnicker et al., 2017; Peng et al., 2019). Notably, Alma1 is a phytoplankton specific DMSP lyase but it is not found in marine bacteria. Thus, it is not the subject of this study. DMSP lyase activity has been observed in crude extracts of the macrophyte *Polysiphonia lanosa* and the heterotrophic dinoflagellate *Gyrodinium cohnii* and, more recently, in whole cells of axenic *Phaeocystis* sp. cultures (Anderson and Cantoni, 1956; Van Boekel and Stefels, 1993). DMSP lyase cleaves DMSP into DMS and acrylate, and the latter is further degraded by the DddA and DddC enzymes. In the DMSP demethylation pathway, DMSP demethylases (DmdA, DmdB, DmdC, and DmdD) convert DMSP into methyl mercaptopropionate with tetrahydrofolate as the methyl acceptor (Fig. 3) (Reisch et al., 2011; Moran et al., 2012; Schuller et al., 2012). Oceans are the main reservoir and source of atmospheric DMS. Many studies have focused on the DMSP lyases because they produce DMS and 3-hydroxypropionate (3HP) directly from DMSP.

Despite the importance of DMSP in marine ecosystems, the details of its enzymatic reactions are largely uncharacterized. However, recent genome-wide studies have rapidly improved understanding of the bacterial genes involved in DMSP degrad-

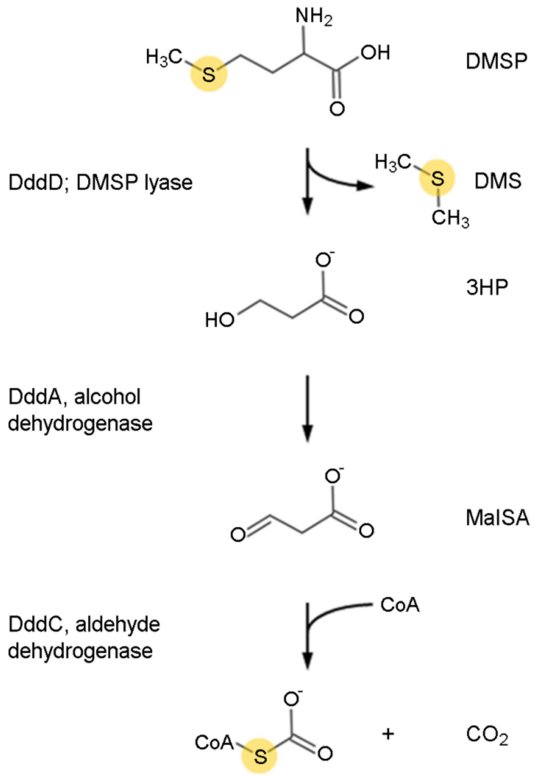

Fig. 3. Marine bacterial enzymes and their reaction pathways involved in dimethylsulfoniopropionate (DMSP) catabolism. Abbreviations: CoA, coenzyme A; DMS, dimethylsulfide; 3HP, 3-hydroxypropionate; MalSA, malonate semialdehyde.

ation. Metagenomic methodology has also enabled easy identification of DMSP-degrading marine bacteria. In addition, the results of X-ray crystallography studies have revealed the three-dimensional structure and substrate-binding site of DMSP at the molecular level (Schuller et al., 2012; Tan et al., 2013; Hehemann et al., 2014; Li et al., 2014; Do et al., 2016).

In this review, we describe the DMSP-degrading enzymes involved in the biosynthesis of DMS and summarize the current understanding of their mechanisms of action. We focus specifically on the protein function and enzymatic reaction mechanisms of DddD, DddA, and DddC, which degrade DMSP and produce DMS, acetyl-coenzyme A (CoA), and CO_2.

2. Catabolism and Cleavage of DMSP

DMSP can be catalyzed with enzymes for the cleavage or demethylation pathways, which are found in phytoplankton or marine bacteria. Bacterial activity is the main contributor to DMS production in seawater (Kiene et al., 2000). DMSP can be converted into acrylic acid or acrylate and DMS by DMSP lyases. Seven DMSP lyases—DddD, DddP, DddY, DddK, DddL, DddQ and DddW—have been identified, four of which—DddY, DddP, DddQ, and DddW—have been purified and characterized for their activities in DMS production (Kirkwood et al., 2010; Curson et al., 2011; Hehemann et al., 2014; Li et al., 2014; Brummett et al., 2015; Schnicker et al., 2017; Peng et al., 2019). Even though the functions of these enzymes are similar, their genes show low sequential similarity (Reisch et al., 2011; Moran et al., 2012). Among them, the structures of DddQ (Li et al., 2014) and DddP (Hehemann et al., 2014) were recently resolved with X-ray crystallography, and their DMSP binding sites and reaction mechanisms were investigated.

dddQ, the most abundant gene in marine metagenomes, is a DMSP lyase gene found in diverse microorganisms (Todd et al., 2009; Todd et al., 2011). DddQ has been identified as a zinc metalloenzyme and, compared with non-metal ion-binding enzymes, shows four- or five-fold increased activity when Mn or Co is added, respectively. Recently, a high-resolution structure of the DddQ from *Ruegeria lacuscaerulensis* was shown to include five α-helices and eight β-strands (Li et al., 2014). These eight antiparallel β-strands form a β-barrel fold structure, which is typical for members of the cupin superfamily. Incidentally, Li et al. (2014) also found the active site through co-crystallization with 2-(N-morpholino)-ethanesulfonic acid, which is derived from the crystallization buffer. 2-(N-Morpholino)ethanesulfonic acid has a sulfate and carbon backbone structure similar to that of DMSP. Furthermore, the authors confirmed this active site with a DMSP complex structure and a single mutagenesis experiment. DMSP binds to the cupin motif in the active pocket. The entrance of the active site tunnel is formed by two main loops called loop 1 (residues 74~78) and loop 2 (residues 184~180). DMSP may migrate through the tunnel into the active pocket and bind with zinc ion. Notably, the Tyr131 residue has two conformations and may have an important role in the cleavage of DMSP (Li et al., 2014).

The crystal structure of DddP from *Roseobacter denitrificans* (RdDddP) also has been determined at a resolution of 2.15 Å (Hehemann et al., 2014). RdDddP is a homodimeric metalloprotein, and each monomer can be divided into N- and C-terminal domains. The N-terminal domain consists of six β-strands surrounded by five α-helices. In the C-terminal domain, six antiparallel β-strands are surrounded by four α-helices (Hehemann et al., 2014). The metal ions bind closely to the substrate-binding site, and the entrance of the substrate binding pocket is surrounded by loops: residues 116~120, 135~139, 364~369, and 383~401

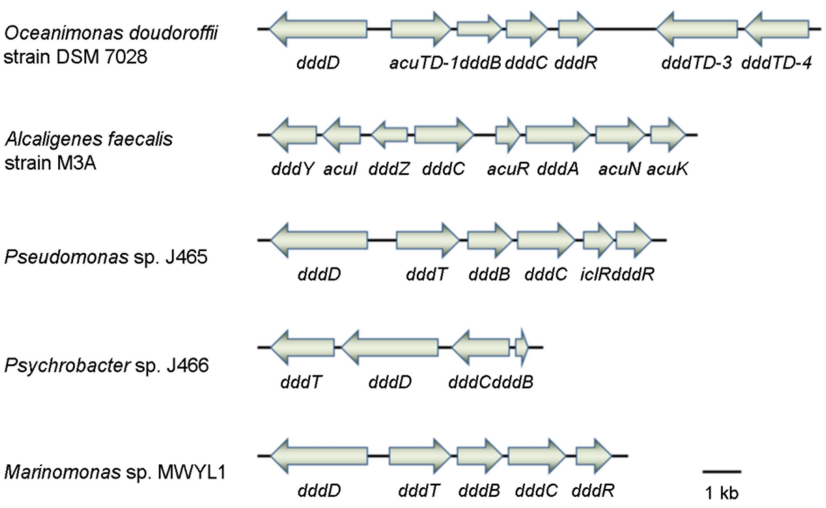

Fig. 4. Arrangement and localization of dimethylsulfoniopropionate (DMSP) catabolite genes in *Oceanimonas doudoroffii* strain DSM 7028, *Alcaligenes faecalis* strain M3A, *Pseudomonas* sp. J465, *Psychrobacter* sp. J466, and *Marinomonas* sp. MWYL1. The scale bar shows a 1-kb gene size.

(Hehemann et al., 2014). Even with a structure for RdDddP in hand, the specific mechanism at the molecular level remains unclear. The results of this study raise the question of whether the mechanism of metalloprotein DddP or DMSP demethylation is metal dependent.

3. DddD (DMSP lyase)

DddD is sporadically found in α- and β-proteobacteria and is more frequently observed in γ-proteobacteria (Curson et al., 2012; Alcolombri et al., 2014). This enzyme cleaves DMSP to DMS and 3HP. Recently, the biochemical characterization of DddD (UniProtKB code A6W2K8, 837 amino acids) from the marine bacterium *Marinomonas* sp. MWYL1 was reported by Alcolombri et al. (2014) (Fig. 4). The authors suggested that this DddD protein has dual enzymatic functions as a CoA transferase and DMSP lyase. The authors also demonstrated that DddD preferentially uses acetyl-CoA as a CoA donor and produces 3HP-CoA.

When the sequence of *Alcaligenes faecalis* DddD (UniProtKB code E7DDH7) was used to search databases of microbial genomes, close homologs of DddD (>75% identical at the amino acid level) were found in a large number of divergent marine bacteria. The highly conserved putative active site Asp602 was identified with multiple sequence alignment and homology modeling, and its mutation to alanine significantly reduced the DMSP lyase activity of DddD. This result indicated that Asp602 is a critical and plausible active site for DddD enzyme activity (Alcolombri et al., 2014). DMS, one of the reaction products of DddD, is released into the marine environment as a gas, whereas another product, 3HP-CoA, is further processed by two additional enzymes, DddA and DddC.

4. DddA (putative 3HP dehydrogenase)

DddA is a member of the alcohol dehydrogenase superfamily. This enzyme catalyzes the oxidation of 3HP-CoA in the DMSP catabolism pathway, forming malonate semialdehyde (Reisch et al., 2011; Moran et al., 2012). Multiple sequence alignments of DddA from *Alcaligenes faecalis* (AfaDddA; UniProtKB code E7DDH7) and other dehydrogenases including DddA from *Halomonas* sp. HTNK1 (UniProtKB code C8YX89) and a choline oxidase from *Arthrobacter globiformis* (UniProtKB code Q7X2H8, PDB code 2JBV) (Quaye et al., 2008) were determined using the ClustalX program (Fig. 5A). The results showed that AfaDddA has the conserved and catalytically important residues H381 and H491. In the *Arthrobacter globiformis* choline oxidase structure, the E312 residue is involved in substrate binding (Quaye et al., 2008). However, AfaDddA and *Halomonas* sp. HTNK1 DddA proteins have a glutamine residue in the corresponding position, thereby supporting the hypothesis that compared with choline oxidases, DddA proteins have different substrate binding sites such as 3HP.

Until now, no structural information of DddA enzyme is available. Thus, the homology model structure of the full-length AfaDddA was constructed using the Swiss Model program server (Arnold et al., 2006). The crystal structure of the choline oxidase from *Arthrobacter globiformis* (Quaye et al., 2008) was used as a template because its amino acid sequence has the highest level of identity to AfaDddA (39%; Fig. 5B). The quality of the homology model was tested using the program MolProbity (Davis et al.,

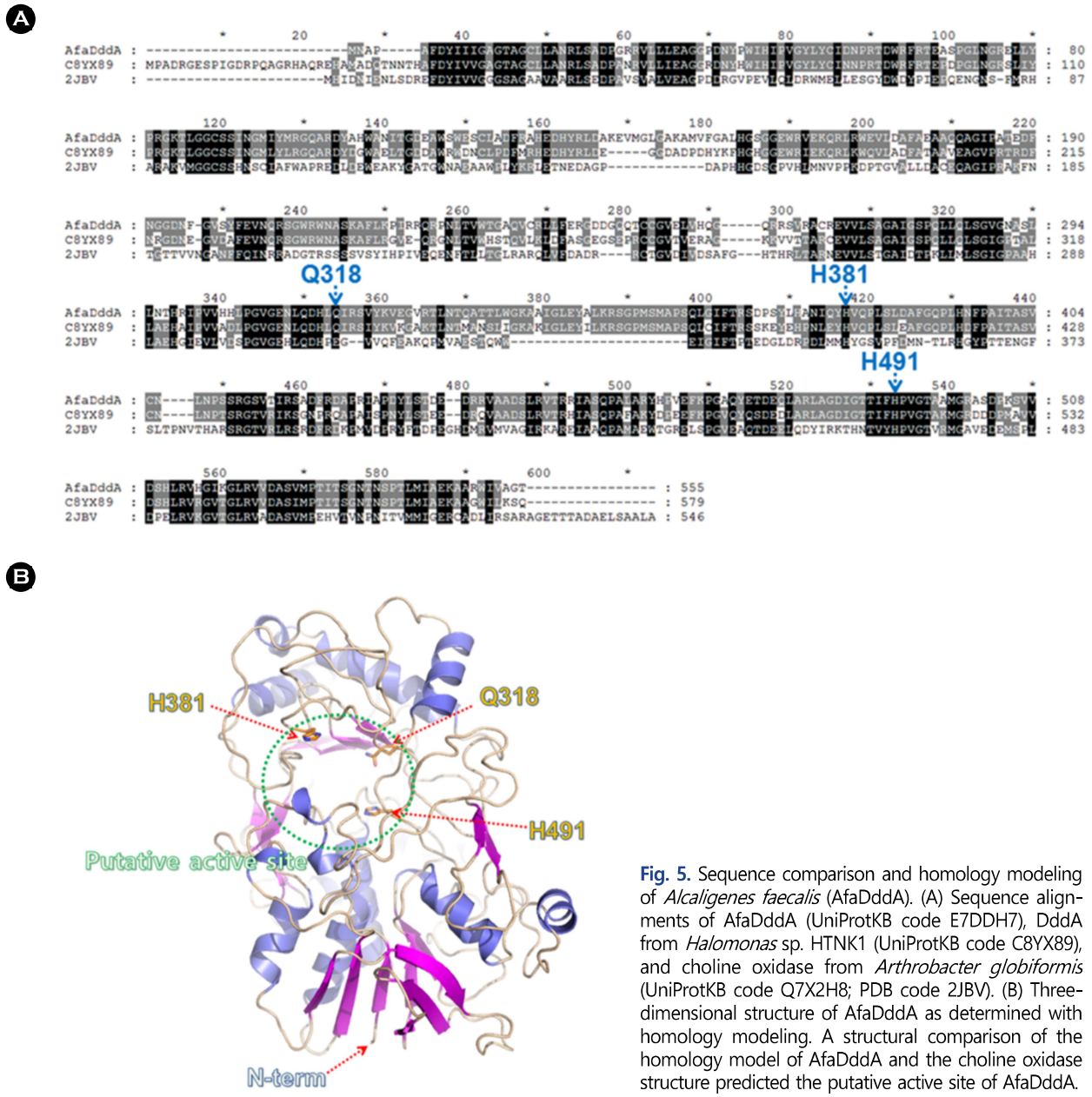

Fig. 5. Sequence comparison and homology modeling of *Alcaligenes faecalis* (AfaDddA). (A) Sequence alignments of AfaDddA (UniProtKB code E7DDH7), DddA from *Halomonas* sp. HTNK1 (UniProtKB code C8YX89), and choline oxidase from *Arthrobacter globiformis* (UniProtKB code Q7X2H8; PDB code 2JBV). (B) Three-dimensional structure of AfaDddA as determined with homology modeling. A structural comparison of the homology model of AfaDddA and the choline oxidase structure predicted the putative active site of AfaDddA.

2007), and a Ramachandran plot of the DddA model revealed that 95.2% of the residues were located in allowed regions and only 4.8% (26 of the total 546 amino acids) were in disallowed regions.

5. DddC (methylmalonate semialdehyde dehydrogenase)

The *dddC* gene is located near the DMSP lyase genes and encodes methylmalonate semialdehyde dehydrogenase (MMSDH) (Curson et al., 2012). MMSDH belongs to the CoA-dependent aldehyde dehydrogenase subfamily and is involved in the decarboxylation of malonate semialdehyde downstream of the DMSP cleavage pathway (Todd et al., 2010). DddC, an MMSDH, catalyzes the transformation of malonate semialdehyde to acetyl-CoA and CO_2 gas. The newly classified gram-negative marine γ -proteobacterium *Oceanimonas doudoroffii* (ATCC 27123) was isolated from seawater and has multiple DMSP lyase genes (Fig. 4).

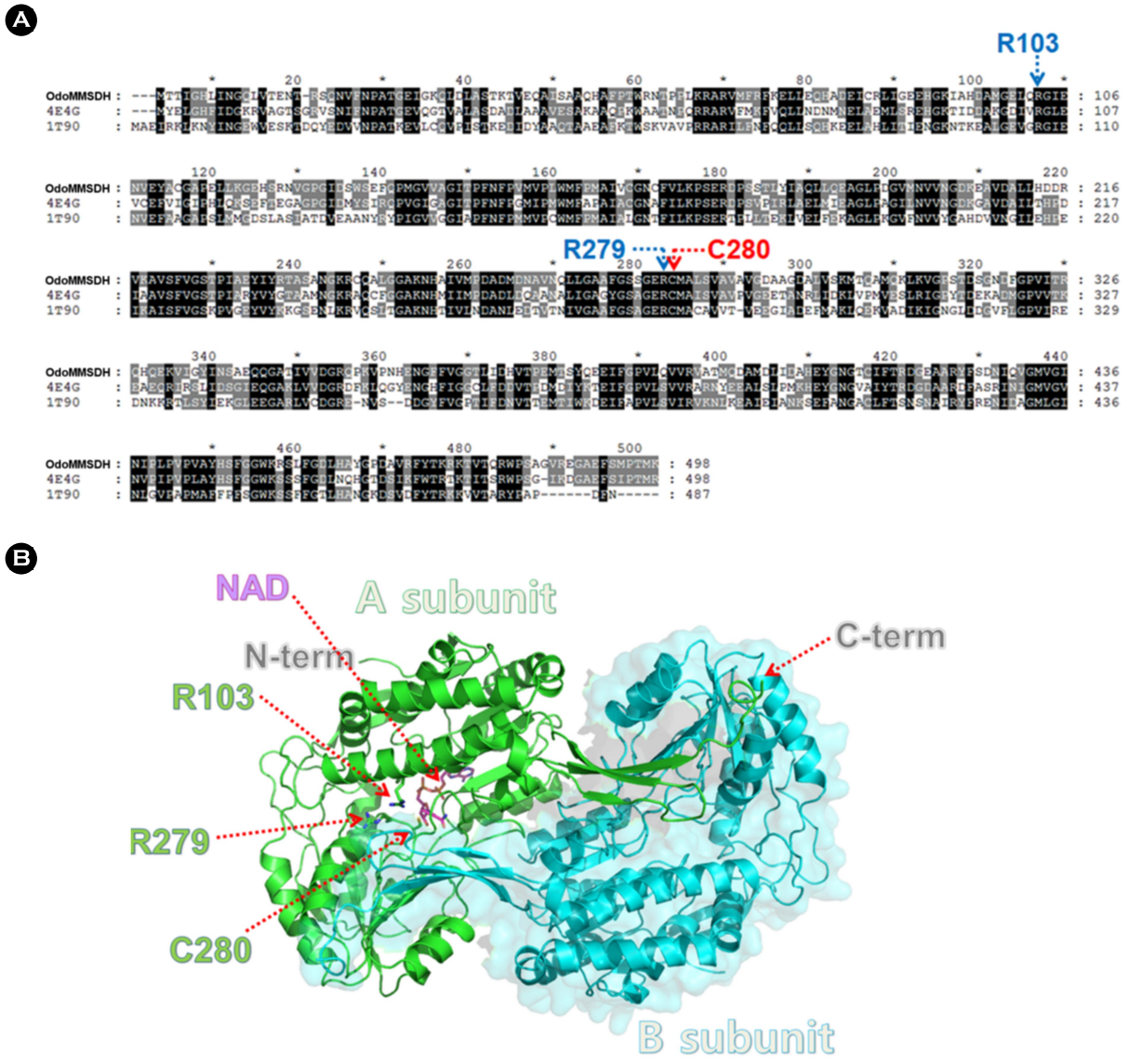

Fig. 6. Structural information of DddC from *Oceanimonas doudoroffii* (*Odo*MMSDH; UniProtKB code G5CZI2). (A) Sequence alignments of *Odo*MMSDH, methylmalonate semialdehyde dehydrogenase from *Sinorhizobium meliloti* strain 1021 (UniProtKB code Q92RW4; PDB code 4E4G), and methylmalonate semialdehyde dehydrogenase from *Bacillus subtilis* strain 168 (UniProtKB code P42412; PDB code 1T90). The two highly conserved Arg residues and catalytic active Cys residue are indicated with blue and red arrows, respectively. (B) Dimeric structure of the *Odo*MMSDH (PDB code 4ZZ7) is shown as a cartoon representation.

Recently, our group successfully determined the crystal structure of the *dddC* (UniProtKB code G5CZI2) product from *O. doudoroffii* (*Odo*MMSDH). The molecular weight of *Odo*MMSDH was estimated to be approximately 120 kDa according to analytic size-exclusion chromatography. These results revealed that *Odo*MMSDH in solution undergoes self-association to form a dimer because the calculated molecular weight of the monomer was ~54 kDa (Do et al., 2016).

The results of multiple sequence alignments of *Odo*MMSDH with other MMSDH enzymes revealed that the R103, R279, and C280 residues are highly conserved (Fig. 6A). The conservation of amino acid residues across several species suggests that these residues have critical roles in enzyme activity or stability. The results of sequence and structural comparison studies suggested that the C280 residue is a catalytic site and that the highly conserved arginine residues may be crucial for substrate binding (Talfournier

et al., 2011; Bchini et al., 2012; Do et al., 2016). Recent structural studies combined with the result of size-exclusion chromatography have shown that *Odo*MMSDH is a dimeric structure and have determined the important residues involved in the substrate binding (Do et al., 2016) (Fig. 6B).

Conclusively, this review summarizes and discusses the structure, function, and enzymatic mechanisms of DddD, DddA, and DddC as participants in the pathways of DMSP catabolism. Additional structural and functional studies with site-directed mutagenesis may elicit additional information about key residues in these important enzymes.

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Competing Interests

The authors declare no conflicts of interest.

References

Alcolombri U, Laurino P, Lara-Astiaso P, Vardi A, Tawfik DS. 2014. DddD is a CoA-transferase/lyase producing dimethyl sulfide in the marine environment. Biochemistry 53: 5473-5475.

Anderson D, Cantoni G. 1956. Enzymatic cleavage of dimethylpropiothetin by *Polysiphonia lanosa*. J Biol Chem 222: 171-177.

Arnold K, Bordoli L, Kopp J, Schwede T. 2006. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22: 195-201.

Bchini R, Dubourg-Gerecke H, Rahuel-Clermont S, Aubry A, Branlant G, Didierjean C, Talfournier F. 2012. Adenine binding mode is a key factor in triggering the early release of NADH in coenzyme A-dependent methylmalonate semialdehyde dehydrogenase. J Biol Chem 287: 31095-31103.

Brummett AE, Schnicker NJ, Crider A, Todd JD, Dey M. 2015. Biochemical, Kinetic, and Spectroscopic Characterization of *Ruegeria pomeroyi* DddW—A Mononuclear Iron-Dependent DMSP Lyase. PloS One 10: e0127288.

Carslaw K, Boucher O, Spracklen D, Mann G, Rae J, Woodward S, Kulmala M. 2010. A review of natural aerosol interactions and feedbacks within the Earth system. Atmos Chem Phys 10: 1701-1737.

Curson AR, Fowler EK, Dickens S, Johnston AW, Todd JD. 2012. Multiple DMSP lyases in the γ-proteobacterium *Oceanimonas doudoroffii*. Biogeochemistry 110: 109-119.

Curson AR, Sullivan MJ, Todd JD, Johnston AW. 2011. DddY, a periplasmic dimethylsulfoniopropionate lyase found in taxonomically diverse species of Proteobacteria. ISME J 5: 1191-1200.

Davis IW, Leaver-Fay A, Chen VB, Block JN, Kapral GJ, Wang X, Murray LW, Arendall WB, Snoeyink J, Richardson JS. 2007. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res 35: W375-W383.

Do H, Lee CW, Lee SG, Kang H, Park CM, Kim HJ, Park H, Park H, Lee JH. 2016. Crystal structure and modeling of the tetrahedral intermediate state of methylmalonate-semialdehyde dehydrogenase (MMSDH) from *Oceanimonas doudoroffii*. J Microbiol 54: 114-121.

Hehemann J-H, Law A, Redecke L, Boraston AB. 2014. The Structure of Rd DddP from *Roseobacter denitrificans* Reveals That DMSP Lyases in the DddP-Family Are Metalloenzymes. PloS One 9: e103128.

Howard EC, Sun S, Biers EJ, Moran MA. 2008. Abundant and diverse bacteria involved in DMSP degradation in marine surface waters. Environ Microbiol 10: 2397-2410.

Kiene RP, Linn LJ, Bruton JA. 2000. New and important roles for DMSP in marine microbial communities. J Sea Res 43: 209-224.

Kirkwood M, Le Brun NE, Todd JD, Johnston AW. 2010. The dddP gene of *Roseovarius nubinhibens* encodes a novel lyase that cleaves dimethylsulfoniopropionate into acrylate plus dimethyl sulfide. Microbiology 156: 1900-1906.

Li C-Y, Wei T-D, Zhang S-H, Chen X-L, Gao X, Wang P, Xie B-B, Su H-N, Qin Q-L, Zhang X-Y. 2014. Molecular insight into bacterial cleavage of oceanic dimethylsulfoniopropionate into dimethyl sulfide. Proc Natl Acad Sci 111: 1026-1031.

Moran MA, Reisch CR, Kiene RP, Whitman WB. 2012. Genomic insights into bacterial DMSP transformations. Ann Rev Mar Sci 4: 523-542.

Nevitt GA, Haberman K. 2003. Behavioral attraction of Leach's storm-petrels (*Oceanodroma leucorhoa*) to dimethyl sulfide. J Exp Biol 206: 1497-1501.

Peng M, Chen XL, Zhang D, Wang XJ, Wang N, Wang P, Todd JD, Zhang YZ, Li CY. 2019. Structure-Function Analysis Indicates that an Active-Site Water Molecule Participates in Dimethylsulfoniopropionate Cleavage by DddK. Appl Environ Microbiol

85: e03127-e03118.

Quaye O, Lountos GT, Fan F, Orville AM, Gadda G. 2008. Role of Glu312 in Binding and Positioning of the Substrate for the Hydride Transfer Reaction in Choline Oxidase. Biochemistry 47: 243-256.

Reisch CR, Moran MA, Whitman WB. 2011. Bacterial catabolism of dimethylsulfoniopropionate (DMSP). Front Microbiol 2: 172.

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.

Schuller DJ, Reisch CR, Moran MA, Whitman WB, Lanzilotta WN. 2012. Structures of dimethylsulfoniopropionate-dependent demethylase from the marine organism *Pelagabacter ubique*. Protein Sci 21: 289-298.

Simo R. 2001. Production of atmospheric sulfur by oceanic plankton: biogeochemical, ecological and evolutionary links. Trends Ecol Evol 16: 287-294.

Song D, Zhang Y, Liu J, Zhong H, Zheng Y, Zhou S, Yu M, Todd JD, Zhang XH. 2020. Metagenomic Insights Into the Cycling of Dimethylsulfoniopropionate and Related Molecules in the Eastern China Marginal Seas. Front Microbiol 11: 157.

Talfournier F, Stines-Chaumeil C, Branlant G. 2011. Methylmalonate-semialdehyde dehydrogenase from *Bacillus subtilis* substrate specificity and coenzyme A binding. J Biol Chem 286: 21971-21981.

Tan D, Crabb WM, Whitman WB, Tong L. 2013. Crystal structure of DmdD, a crotonase superfamily enzyme that catalyzes the hydration and hydrolysis of methylthioacryloyl-CoA. PloS One 8: e63870.

Todd JD, Curson AR, Dupont CL, Nicholson P, Johnston AW. 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.

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.

Todd JD, Curson AR, Nikolaidou-Katsaraidou N, Brearley CA, Watmough NJ, Chan Y, Page PC, Sun L, Johnston AW. 2010. Molecular dissection of bacterial acrylate catabolism-unexpected links with dimethylsulfoniopropionate catabolism and dimethyl sulfide production. Environ Microbiol 12: 327-343.

Van Boekel J, Stefels W. 1993. Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species *Phaeocystis* sp. Mar Ecol Prog Ser 97: 11-18.