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Seasonal changes in the abundance of bacterial genes related to dimethylsulfoniopropionate catabolism in seawater from Ofunato Bay revealed by metagenomic analysis

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Running title: Marine bacteria-derived DMSP catabolic genes

ABSTRACT

Ofunato Bay is located in the northeastern Pacific Ocean area of Japan, and it has the highest biodiversity of marine organisms in the world, primarily due to tidal influences from the cold *Oyashio* and warm *Kuroshio* currents. Our previous results from performing shotgun metagenomics indicated that *Candidatus Pelagibacter* *ubique* and *Planktomarina temperata* were the dominant bacteria (Reza et al., 2018a, b). These bacteria are reportedly able to catabolize dimethylsulfoniopropionate (DMSP) produced from phytoplankton into dimethyl sulfide (DMS) or methanethiol (MeSH). This study was focused on seasonal changes in the abundances of bacterial genes (*dddP*, *dmdA*) related to DMSP catabolism in the seawater of Ofunato Bay by BLAST+ analysis using shotgun metagenomic datasets. We found seasonal changes among the *Candidatus Pelagibacter* *ubique* strains, including those of the HTCC1062 type and the Red Sea type. A good correlation was observed between the chlorophyll *a* concentrations and the abundances of the catabolic genes, suggesting that the bacteria directly interact with phytoplankton in the marine material cycle system and play important roles in producing DMS and MeSH from DMSP as signaling molecules for the possible formation of the scent of the tidewater or as fish attractants.

KEY WORDS: Bacterial functions, DMSP catabolic genes, Metagenomics, Ofunato Bay

1. Introduction

The Sanriku *Rias Coast* is located in northeastern Japan off the Pacific Ocean, and it is one of the most productive coastal areas in the world. This area is continuously under tidal influence from the cold *Oyashio* and warm *Kuroshio* currents (Ishizaki, 1998) along with the warm *Tsugaru* current (Hanawa and Mitsudera, 1987), resulting in remarkable changes in the levels of salinity, dissolved oxygen, chlorophyll-*a* (chl-*a*) and various nutrient components in a season-dependent manner (Ishizaki, 1998). The high productivity of the Sanriku *Rias Coast* has attracted the extensive aquaculture of many filter feeder species of marine invertebrates and has likely brought about a substantial change in the extent of the genetic diversity within these communities.

Ofunato Bay in Iwate Prefecture is located almost at the center of the Sanriku *Rias Coast*. The size of the bay is 7 km in length and 2.5 km in width, with an average depth of 20 m. Chl-*a* concentrations have been reported to be high in the bay, especially during the spring (JAXA, 2004; Yamada et al., 2017), which has attracted buoy-and-rope type scallop and oyster culture facilities (Komatsu et al., 2012). A number of studies have reported on the planktonic populations of the bay (Ogata et al., 1982; Sakamoto et al., 1992), but detailed analyses on its microbial communities are not available, and thus their functions have remained unclear.

Several metagenomic studies have demonstrated the taxonomic diversity of the microbial communities in marine and freshwater ecosystems from different parts of the world (Venter et al., 2004; Rusch et al., 2007; Oh et al., 2011; Grzymiski et al., 2012; Ghai et al., 2013) by using direct whole-genome sequencing (WGS) and amplicon-based metagenomic sequencing, with WGS being able to analyze the microbial community composition more accurately (Shah et al., 2011; Logares et al.,

2014). Due to the lack of availability of information on microbial diversity and its related functions in Ofunato Bay, we performed metagenomic analyses on the bay using the WGS approach for the first time along the Sanriku *Rias Coast*, and we provided a comprehensive understanding of the microbial ecosystem and function within this environment (Reza et al., 2018a, b). According to this survey, the most frequently recovered bacterial sequences were those of Proteobacteria, predominantly comprising *Candidatus Pelagibacter* (Family Pelagibacterales) and *Planktomarina* (Family Rhodobacteraceae).

Candidatus Pelagibacter ubique HTCC1062 belongs to Alphaproteobacteria, is the first member of this phylum to be cultured and has the smallest genome size among free-living microorganisms (Giovannoni et al., 2005). It originates from temperate and higher productivity ocean regions. *Candidatus Pelagibacter* ubique strains have also been isolated from the Red Sea, and they have been analyzed by single cell genomics (the NCBI database: <https://www.ncbi.nlm.nih.gov/genome/genomes/491>). *Planktomarina temperata* RCA23 also belongs to the Alphaproteobacteria and inhabits temperate to polar marine waters. These bacteria, the *Candidatus Pelagibacter* and *Planktomarina temperata* RCA23, can reportedly catabolize dimethylsulfoniopropionate (DMSP) to dimethyl sulfide (DMS) or methanethiol (MeSH) (Fig. 1) (Voget et al., 2015; Sun et al., 2016). DMSP is globally one of the most abundant organosulfur molecules; it is produced by phytoplankton and is known as a signaling molecule and the major precursor for DMS and MeSH (Seymour et al., 2010). DMSP catabolism in marine bacteria has two pathways, cleavage and demethylation (Fig. 1). DMSP lyases that are encoded by *dddP* or *dddK* degrade DMSP to DMS in the cleavage pathway, and DMSP demethylase encoded by *dmdA* degrades DMS into

3-methylthiopropionate (MMPA). This MMPA is further degraded into MeSH through several steps. DMS provides the scent of the tide, acting as another infochemical in the signaling pathway, and it affects Earth's albedo and potentially its climate via the production of sulfate aerosol and cloud condensation nuclei (Nevitt, 2011). MeSH plays similar roles to those of DMS (Bentley et al., 2004).

This study was undertaken to determine the seasonal changes in the abundance of bacterial genes encoding *dddP* and *dmdA* in the seawater at Ofunato Bay. These genes are related to DMSP catabolism, as determined by the NCBI BLAST Program (BLAST+) analysis using the shotgun metagenomic datasets. Seasonal changes were observed in the abundances of the DMSP catabolic genes in association with those of *Candidatus* Pelagibacter ubique strains, including the HTCC1062-type and Red Sea-type strains. A good correlation was also observed between the *chl-a* concentrations and the abundances of these catabolic genes. We will discuss the roles of these catabolic compounds as signaling molecules in forming the scent of the tidewater or as fish attractants.

2. Materials and methods

2.1 Sampling sites

The study area is located in Ofunato Bay, Iwate Prefecture, Japan. We selected three sampling locations for collecting seawater samples as in our previous paper (Reza et al., 2018a) as follows: KSt. 1 (141.73457E, 39.063370N; avg. depth 10.3 m), KSt. 2 (141.73245E, 39.044612N; avg. depth 25.3 m) and KSt. 3 (141.72820E, 39.019030N; avg. depth 38.5 m), where KSt. 1 and KSt. 3 are located at the innermost northeast part

and the southwest entrance part of the bay, respectively, while KSt. 2 is located at the center of the bay.

2.2 Sample collection and processing

Seawater sampling and processing were performed according to Reza et al. (2018a). In brief, seawater samples were collected once a month from January 2015 to October 2016 using a vertical water sampler from a 1 m depth at all three sampling stations, at a 10 m depth at KSt. 2 and KSt. 3 and at an 8 m depth at KSt. 1 due to the limited depth from the sea bottom. The corresponding seawater quality parameters including the temperature, salinity and dissolved oxygen (DO) were also measured on the spot using a water quality profiler (RINKO AAQ176, JFE Advanced Co. Ltd., Nishinomiya, Japan).

Approximately 8 L of the collected seawater was first pre-filtered through 100- μ m filters to remove debris. Approximately 8 L of seawater was first pre-filtered through a 100- μ m filter to remove debris and 250 mL was used for nutrient and chl-*a* measurements; the rest was filtered sequentially through a 20- μ m pore-sized Nylon Net Filters NY20 with a 47 mm diameter (Merck Millipore Ltd., Tullagreen, Ireland) and 5, 0.8 and 0.2- μ m pore-sized Isopore Membrane Filters with 142 mm diameters (Merck Millipore Ltd.) using a peristaltic pump. After the filtrations, all the filters were stored at -80°C until DNA extraction.

2.3 DNA extraction and shotgun metagenomic sequencing

Genomic DNA extraction and shotgun metagenomic sequencing were performed as described in our previous report (Reza et al, 2018a). In brief, DNA was extracted from the 0.2- μ m filters using a PowerWater® DNA Isolation Kit (MoBio Laboratories Inc.,

Carlsbad, CA, USA) according to the manufacturer's recommendations. DNA samples of 1 ng each were prepared from the three sampling stations for metagenomics by employing a Nextera XT DNA Sample Preparation Kit (Illumina Inc., San Diego, CA, USA) and sequenced as paired-end reads using an Illumina MiSeq platform and a MiSeq Reagent Kit v3 (600 cycles) (each containing approximately 0.05 ng of DNA).

2.4 Sequence processing and analyses

The acquired Illumina paired reads from each library as described above were first joined by overlapping forward and reverse reads from the same DNA fragment (paired-end sequences) using FLASH software (Magoc and Salzberg, 2011) with the default parameters (overlap minimum, 10 nt; maximum allowed ratio between the number of mismatched base pairs and the overlap length, 0.25). The WGS reads were then quality-filtered by removing <50 bp and quality-trimmed to Phred 20 using Genomics Workbench (CLCbio, Cambridge, MA). BLAST+ was used to select the hit reads (counts) and to produce significant alignments with DMSP catabolic genes. The WGS raw reads have been deposited into the DDBJ Sequence Read Archive under the accession numbers DRA005744.

2.5 Strains, genomes and genes used in this study

The strains and genes used in this study are summarized in Tables 1 and 2. SAR11 strain *Candidatus Pelagibacter ubique* HTCC1062 was isolated from the coastal region of the Northeast Pacific and a temperate environment (Giovannoni et al., 2005, Grote et al., 2012). *Candidatus Pelagibacter ubique* HTCC7211 was isolated from the Sargasso Sea, the Atlantic and a subtropical environment (Grote et al., 2012). *Candidatus Pelagibacter*

ubique HIMB59 was isolated from the coastal region of the North Pacific and a tropical environment (Grote et al., 2012). *Planktomarina temperata* RCA23 is a type of Alphaproteobacteria that inhabits temperate to polar marine regions (Voget et al., 2015). Single-cell genomics data from the *Candidatus* Pelagibacter ubique strains isolated from the Red Sea were obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov/genome/genomes/491>) (Table 2).

3. Results and discussion

3.1. Seasonal changes in concentrations of chlorophyll *a* and extracted DNA

Ofunato Bay in Iwate Prefecture, Japan, is a deep coastal bay located at the center of the Sanriku *Rias Coast*, and it is considered as an economically and environmentally important asset. Fig. 2 shows seasonal changes in the concentrations of chlorophyll *a* in the bay seawaters at KSt. 1 (innermost area), KSt. 2 (center area) and KSt. 3 (bay entrance), with each at a 1 m depth for the three sampling stations and from 8 m (KSt. 1) and 10 m (KSt. 2 and KSt. 3) from January 2015 to October 2016. The large peaks were observed in March at KSt. 1 for the 8 m depth water irrespective of the 2015 and 2016 results, while there were only small peaks for a few months during the year. It is known that the chl-*a* concentrations reflect the abundance of phytoplankton in the seawaters including those of Ofunato Bay (Ogata et al., 1982; Yamada et al., 2017).

Fig. 3 shows the DNA concentrations of DNA that was extracted from 0.2- μ m filters for the seawater collected from the three sampling stations from January 2015 to October 2016. The seasonal changes were similar between KSt. 2 and KSt. 3 at a 1 m depth, showing the largest values in July of 2015 (approximately 3.5 and 2.5 ng/g

seawater, respectively), while the peaks during 2016 were much smaller than those of 2015. The DNA concentrations for KSt. 1 at two depths and KSt. 2 and KSt. 3 at the 10 m depth showed no apparent changes. There was no apparent relationship between these seasonal changes and those of chl-*a*, suggesting the inflow of freshwater-containing nutrients for bacteria from rivers around Ofunato Bay, where the Sakari River is the largest supplier around KSt. 1 (Reza et al., 2017a).

3.2. Characteristics of the metagenomic datasets

Our previous study described the first WGS study on the microbial communities in Ofunato Bay, where surface water samples were collected from the three stations along its length to cover the entire bay. DNA was extracted through 0.2- μ m filters and sequenced, targeting all the microbes (Reza et al., 2018a) and the free-living bacterial fraction alone (Reza et al., 2018b). In this study, we focused on the bacterial genes associated with DMSP from the 0.2- μ m filters.

The normalization of the gene counts in the extracted DNAs from the 0.2- μ m filters was examined to target *Planktomarina temperata dddP*. The seasonal changes in the gene fragment read counts/ng DNA from the seawaters collected from KSt. 1 at two depths are shown in Supplementary Fig. S1A, while those multiplied by the DNA concentrations are shown in Supplementary Fig. S1B. The total gene fragment reads/g seawater were then normalized to the read counts/one million reads in the datasets, and the obtained values were then multiplied by the DNA concentrations, providing the read counts/g seawater (Supplementary Fig. S1C). These normalized read counts/g seawater were used for further analyses and hereafter defined as the read counts/g seawater.

Our previous study demonstrated that the most frequently recovered bacterial

sequences were Proteobacteria, predominantly comprising *Planktomarina* (Family Rhodobacteraceae) and *Candidatus Pelagibacter* (Family Pelagibacterales) (Reza et al., 2018a, b). Many reports are available about *Candidatus Pelagibacter* ubique HTCC1062 (Giovannoi et al., 2005, Grote et al., 2012), *Candidatus Pelagibacter* ubique strains from the Red Sea (NCBI data) and *Planktomarina temperata* RCA23 (Voget et al., 2015). As mentioned in the introduction, these bacteria contain genes that catabolize DMSP to DMS/MeSH. As shown in Fig. 1, DMSP catabolism in marine bacteria has two pathways, cleavage and demethylation. DMSP lyases that are encoded by *dddP* and *dddK* degrade DMSP to DMS through cleavage and then, DMSP demethylase encoded by *dmdA* degrades DMSP to MMPA, which is subsequently degraded to MeSH through several steps associated with the demethylases encoded by *dmdB* and *C*. This study focused on the abundances of these genes, which function in the DMSP catabolic pathway of Ofunato Bay seawater, and their seasonal changes occur in association with environmental factors, especially with those of chl-*a*.

Fig. 4 shows the seasonal changes in the read counts/g seawater of *dddP* from *Planktomarina temperata* in the three sampling stations at the two depths, where large peaks were observed in 1 m depth samples collected in July of 2015 and between July and August of 2016. In addition, a large peak was observed in July at an 8 m depth of KSt. 1. Small peaks were observed at KSt. 1 for the 1 m depth in November of 2015, at the 8 m depth in March, 2016, and at KSt. 3 for the 1 m depth, in 2016. It is noted that the peak was considerably smaller in 2016 than in 2015. The read counts of *dmdA1* in the datasets at the 1 m depth in July were almost the same as those of *dddP* (data not shown).

Fig. 5 shows the read counts/g seawater of *dmdA* encoding the demethylation enzyme

of *Candidatus Pelagibacter ubique* HTCC1062. The seawater samples in KSt. 1 showed a large peak in May at an 8 m depth and between April and June in 2015. However, the corresponding peaks in 2016 were not apparent. The changes in KSt. 2 were slightly different from those at KSt. 1. One large peak was observed in March at a 10 m depth in 2015, while another smaller peak occurred in June. The seawater samples at the 1 m depth showed a large peak in June and another smaller peak in April. However, only small peaks were observed in March and July of 2016. KSt. 3 showed similar patterns in comparison to those of KSt. 2, although the read counts at KSt. 3 were lower than those in KSt. 2. It appears that large peaks between March and June in 2015 are considered to correspond to the spring bloom as revealed by high concentrations of chl-*a* (Fig. 2). It has been reported that the DMSP lyase encoded by *dddK* in HTCC1062 was much less abundant than that encoded by *dmdA*, *B* and *C*, and it was tandemly clustered on the genome (Sun et al., 2016). In this study, the read counts/g seawater of HTCC1062 *dddK* from KSt. 2 at the 10 m depth were almost one-tenth of those of HTCC1062 *dmdA* in Ofunato Bay (unpublished data). Single-cell genomics data from the *Candidatus Pelagibacter ubique* strains isolated from the Red Sea are available from the NCBI database. We searched the homologs of the genes encoding DMSP catabolic enzymes associated with the cleavage and demethylation pathways along with their downstream enzyme homologs. We found cleavage enzyme homologs that were encoded by *dddP* in *Candidatus Pelagibacter ubique* HTCC7211 and *dddP1* in *Candidatus Pelagibacter ubique* HIMB59 strains (Tables 1 and 2). Some strains were found to contain both gene homologs. *DmdA*, *B*, and *C* homolog genes were located in approximately 5 kb DNA regions in this order. It has been reported that *dmdA* is localized in the genomes of the *Candidatus Pelagibacter ubique* strains isolated from the

Red Sea (NCBI database). These results suggest that the *Candidatus Pelagibacter* ubiquitous strains isolated from the Red Sea contain the DMSP catabolic pathway, and the *dmdA*, *B* and *C* homologs form a cluster in the genome.

Fig. 6 shows the read counts of an O19-*dddP* gene homolog, from the *dddP1*-HIMB59 type, in *Candidatus Pelagibacter* ubiquitous. Peaks were observed between September and December in both 2015 and 2016, irrespective of the three sampling stations and two water depths. Similar seasonal changes were observed in the read counts of an O19-*dmdA* gene homolog in *Candidatus Pelagibacter* ubiquitous at KSt. 2 (Fig. 7). Seasonal changes have also been observed in the *dmdA* gene homologs of other *Candidatus Pelagibacter* ubiquitous strains that were isolated from the Red Sea (unpublished data). These results indicate that seasonal changes occurred among the *Candidatus Pelagibacter* ubiquitous strains, that is, the HTCC1062-type strains and the Red Sea-type strains. A phytoplankton bloom reportedly occurs in the Red Sea between mid-October and mid-March in the following year (from autumn to the following spring) (Raitos et al., 2013). It is interesting to analyze the changes in the abundances of genes that participate in the DMSP catabolic pathway of *Candidatus Pelagibacter* ubiquitous strains in the Red Sea.

Fig. 8 shows a correlation between the *chl-a* concentrations and the read counts of the DMSP catabolic genes in KSt. 1. The *chl-a* concentrations were low in January and February, and they were high between March and May (see peaks I, IV), indicating a phytoplankton bloom, while there were intermittent small peaks between June and December, as described before in this paper (see II, III, V and VI).

A large peak I in the read counts of *dmdA* in *Candidatus Pelagibacter* ubiquitous HTCC1062 appeared immediately after the *chl-a* peak at the 8 m depth in KSt. 1 and at

the same time for the 1 m depth. Similarly, the peaks from II to VI of the read counts for the bacterial genes were considered to be related to those from II to VI of phytoplankton, respectively. Similar tendencies were obtained for KSt. 2 (Fig. 9) and KSt. 3 (Fig. 10) except those of IV and A at KSt. 3. At KSt. 3, peak IV was observed in March 2016 for *dmdA* in HTCC1062 and *dddP* in *Planktomarina temperata*, but not for *chl-a*, suggesting that these peaks are related to the *chl-a* peak in KSt. 1. However, a small peak A was observed in *chl-a* at KSt. 3 in May 2016, which may correspond to a very small peak for *dddP* in *Planktomarina temperata*, suggesting the existence of unknown catabolic genes. These results suggested that a correlation was observed between the *chl-a* concentration and the counts of the normalized hit reads to catabolic genes. They also suggest that the bacteria have a close correlation with phytoplankton in the marine material cycle system.

4. Conclusions

Seasonal changes in 2015 and 2016 were investigated in relation to the bacterial genes that participated in DMSP metabolism within Ofunato Bay. Peaks in the abundances of *dmdA* in *Candidatus Pelagibacter ubique* HTCC1062 were observed between March and June, suggesting its correlation with the spring phytoplankton bloom. The *dddP* peaks in *Planktomarina temperata* were observed in July, while those of O19-*dddP* and its homologs (*dmdA*) in *Candidatus Pelagibacter ubique* were observed between September and December, respectively. We found similar seasonal changes in the *Candidatus Pelagibacter ubique* HTCC1062-type and the Red Sea-type strains.

A good correlation was observed between the *chl-a* concentrations and the abundances of the DMSP catabolic genes. The DMSP catabolic pathway and the clustering of *dmdA*,

B, *C* homologs in the genome were also found in *Candidatus Pelagibacter ubique* strains isolated from the Red Sea. These bacteria have the gene homologs to catabolize the DMSP produced by phytoplankton, suggesting that these bacteria have a close relationship with the phytoplankton in the marine material cycle system. DMSP, DMS and MeSH have been reported as signaling molecules, suggesting that they have functional roles in attracting bacteria, algae, animal plankton, fishes and seabirds. These results suggest that the molecules are important in the possible formation of the scent of the tide or fish attractants.

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Figure Legends

Fig. 1. DMSP catabolic pathways in marine bacteria. The abbreviations used here are as follows: DMSP, dimethylsulfoniopropionate; DMS, dimethyl sulfide; methanethiol, MeSH; and 3-methylthio propanoate (MMPA).

Fig. 2. Seasonal changes in the concentrations of chlorophyll *a* (chl-*a*) in seawater collected from KSt. 1 (innermost bay), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from 2015 to 2016.

Fig. 3. DNA isolated from seawater collected from KSt. 1 (innermost bay), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from 2015 to 2016.

Fig. 4. Seasonal changes in the read counts of *dddP* from *Planktomarina temperata* in seawater collected from KSt. 1 (innermost bay), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from 2015 to 2016.

Fig. 5. Seasonal changes in the read counts of the *dmdA* gene encoding a demethylation enzyme in *Candidatus Pelagibacter ubique* HTCC1062 in seawater collected from KSt. 1 (innermost bay), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from

2015 to 2016.

Fig. 6. Seasonal changes in the read counts of the O19-*dddP* homolog (*dddP1*-HIMB59 type) in *Candidatus Pelagibacter ubique* in seawater collected from KSt. 1 (bay innermost), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from 2015 to 2016.

Fig. 7. Seasonal changes in the read counts of an O19-*dmdA* gene homolog in *Candidatus Pelagibacter ubique* in seawater collected from KSt. 1 (innermost bay), KSt. 2 (bay center) and KSt. 3 (bay entrance) at 1 m (KSt. 1, KSt. 2 and KSt. 3) and 8 m (KSt. 1) or 10 m depths (KSt. 2 and KSt. 3) in Ofunato Bay from 2015 to 2016.

Fig. 8. Correlation between the chlorophyll *a* (chl-*a*) concentrations and read counts of the genes participating in the catabolic pathways of dimethylsulfoniopropionate (DMSP), including the *Candidatus Pelagibacter ubique* HTCC1062 *dmdA*, *Planktomarina temperata* *dddP* and *Candidatus Pelagibacter ubique* O19-*dddP* gene homolog (*dddP1*-HIMB59 type) from KSt. 1 at 1 m and 8 m depths in Ofunato Bay from 2015 to 2016.

Fig. 9. Correlation between the chlorophyll *a* (chl-*a*) concentrations and read counts of the genes participating in the catabolic pathways of dimethylsulfoniopropionate (DMSP) including the *Candidatus Pelagibacter ubique* HTCC1062 *dmdA*, *Planktomarina temperata* *dddP* and *Candidatus Pelagibacter ubique* O19-*dddP* gene

homolog (*dddP1*-HIMB59 type) from KSt. 2 at 1 m and 10 m depths in Ofunato Bay from 2015 to 2016.

Fig. 10. Correlation between the chlorophyll *a* (*chl-a*) concentrations and read counts of the genes participating in the catabolic pathways of dimethylsulfoniopropionate (DMSP) including the *Candidatus Pelagibacter ubique* HTCC1062 *dmdA*, *Planktomarina temperata dddP* and *Candidatus Pelagibacter ubique* O19-*dddP* gene homolog (*dddP1*-HIMB59 type) from KSt. 3 at 1 m and 10 m depths in Ofunato Bay from 2015 to 2016.

Table 1

SAR11 strains used in this study.

SAR11 strain	Characteristics	Reference
<i>Candidatus Pelagibacter</i> ubique HTCC1062	Alphaproteobacteria, the first cultured member of this clade. The smallest genome for a free-living microorganism (NCBI data). It originates from temperate and higher-productivity ocean regions.	Giovannoni et al. (2005), Grote et al. (2012)
<i>Candidatus Pelagibacter</i> ubique HTCC7211	Isolated from the Sargasso Sea, Atlantic and subtropic environments. Small genome.	Grote et al. (2012)
<i>Candidatus Pelagibacter</i> ubique HIMB59	Isolated from coastal regions of North Pacific and tropic environments.	Grote et al. (2012)
<i>Candidatus Pelagibacter</i> ubique strain O19	Isolated from the Red Sea. Single-cell genomics data from the NCBI database.	www.ncbi.nlm.nih.gov/genome/genomes/49
<i>Planktomarina temperata</i> RCA23	Alphaproteobacteria located in temperate to polar marine regions.	Voget et al. (2015)

Table 2

Enzyme genes related to the DMSP catabolic pathways that were analyzed or discussed in this study.

Enzyme gene	Pathway	Strain	Reference
<i>dmdA</i>	demethylation	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>acuK</i>	cleavage	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>dddA</i>	cleavage	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>prpE</i>	cleavage	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>acuI</i>	cleavage	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>dmdB</i>	demethylation	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>dmdC</i>	demethylation	<i>Candidatus</i> Pelagibacter ubique HTCC1062	Sun et al. (2016)
<i>dddP</i>	cleavage	<i>Planktomarina temperate</i> RCA23	Voget et al. (2015)
<i>dddP</i>	cleavage	<i>Candidatus</i> Pelagibacter ubique strain O19-dddP homolog	www.ncbi.nlm.nih.gov/genome/genomes/49
<i>dmdA</i>	demethylation	<i>Candidatus</i> Pelagibacter ubique strain O19	www.ncbi.nlm.nih.gov/genome/genomes/49

Table 3

Homologs of the genes encoding DMSP catabolic enzymes found in single-cell genomics results for *Candidatus Pelagibacter ubique* strains isolated from the Red Sea.

Strain name	Size (Mb)	HTCC7211 <i>dddP</i>	HIMB59 <i>dddPI</i>	HTCC1062 <i>acuK</i>	HTCC1062 <i>dddA</i>	HTCC1062 <i>prpE</i>	HTCC1062 <i>acul</i>	HTCC1062 <i>dmdA</i>	HTCC1062 <i>dmdB</i>	HTCC1062 <i>dmdC</i>	<i>dmdA</i> *
I19-CVST	1.02	+	+	+	+	–	+	+	+	+	+
J21-CVSK	0.87	+	–	–	+	–	–	+	–	–	+
P11-CVS	0.98	+	–	+	+	–	+	+	+	+	+
O20-CVSS	1.01	+	–	+	+	+	+	+	+	+	+
O19-CVSQ	0.80	–	+	+	+	–	+	+	+	+	+
A20-CVSB	1.14	–	+	+	+	+	+	–	–	–	–
D22-CVSF	1.01	+	–	–	+	+	–	+	+	+	+
E22-CVSH	0.80	–	+	+	–	+	+	+	+	+	+
F16-CVSI	0.95	+	–	+	+	+	+	+	+	+	+
L23-CVSM	0.83	–	–	–	+	+	+	+	–	–	–
M22-CVSO	0.86	–	–	–	–	+	–	+	+	+	+
M18-CVSN	1.05	+	+	+	+	+	+	+	–	–	+
K18-CVSL	0.73	+	+	+	+	+	+	+	+	+	+
N17-CVSR	0.61	–	–	–	–	–	–	–	–	–	
N8-CVSP	0.56	+	–	–	+	–	–	–	–	–	
G15-CVSJ	0.29	–	–	–	–	–	+	–	–	–	

(+) denotes the presence of homolog with more than 65% identity and (–) denotes no homolog in the NCBI database.

*Cited from the NCBI database.

Dimethyl sulfide (DMS)

DMSP lyase
(*dddP*, *dddK*)

Dimethylsulfoniopropionate
(DMSP)

DMSP demethylase
(*dmdA*)

3-Methylthiopropionate
(MMPA)

dmdB,C

Figure 1

Chl-*a* concentration ($\mu\text{g/L}$)

Figure 2

DNA concentration (ng/g seawater)

Figure 3

Read counts/g seawater

Figure 4

Read counts/g seawater

Figure 5

Read counts/g seawater

Figure 6

Read counts/g seawater

Figure 7

Figure 8

Figure 9

Figure 10