DMSP-producing bacteria are more abundant in the surface microlayer than subsurface seawater of the East China Sea

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Abstract

Microbial production and catabolism of dimethylsulfoniopropionate (DMSP), generating the climatically-active gases dimethyl sulfide (DMS) and methanethiol (MeSH), have key roles in global carbon and sulfur cycling, chemotaxis and atmospheric chemistry. Microorganisms in the sea surface microlayer (SML), the interface between seawater and atmosphere, likely play an important role in the generation of DMS and MeSH and their exchange to the atmosphere, but little is known about these SML microorganisms. Here, we investigated the differences between bacterial community structure and the distribution and transcription profiles of the key bacterial DMSP synthesis (*dsyB* and *mmtN*) and catabolic (*dmdA* and *dddP*) genes in East China Sea SML and subsurface seawater (SSW) samples. Per equivalent volume, bacteria were far more abundant (~7.5-fold) in SML than SSW, as were those genera predicted to produce DMSP. Indeed, *dsyB* (~7-fold) and *mmtN* (~4-fold), robust reporters for bacterial DMSP production, were also far more abundant in SML than SSW. In addition, the SML had higher *dsyB* transcripts (~3-fold) than SSW samples, which may contribute to the significantly higher DMSP level observed in SML compared to SSW. Furthermore, the abundance of bacteria with *dmdA* and their transcription was higher in SML than SSW samples. Bacteria with *dddP* and transcripts were also prominent, but less than *dmdA* and presented at similar levels in both layers. These data indicate that the SML might be an important hotspot for bacterial DMSP production as well as generating the climatically-active gases DMS and MeSH, a portion of which are likely transferred to the atmosphere.

Key words: DMSP; biosynthesis and catabolism; surface microlayer; subsurface water; bacterial community; East China Sea

Introduction

Dimethyl sulfide (DMS), the most abundant sulfur-containing compound transferred from the seas to the air, has an important role in the global sulfur cycle [1]. DMS can act as a chemoattractant for seabirds and copepod crustaceans [2, 3] and its oxidation products nucleate and grow aerosols that can act as cloud condensation nuclei and potentially affect the climate [4, 5]. The major biological precursor of DMS is the osmolyte dimethylsulfoniopropionate (DMSP) [6, 7] that can also function as a signaling molecule [8] and nutrient [9-11] for marine organisms. DMSP was only thought to be synthesized by some marine phytoplankton [12, 13], macroalgae [14], corals [15] and angiosperms [16]. Many of these algae (some diatoms, haptophytes and dinoflagellates) and corals produce DMSP via the methionine (Met) transamination pathway and contain the DSYB gene encoding the key methylthiohydroxybutyrate (MTHB) *S*-methyltransferase enzyme of this pathway [17]. The diatom *Thalassiosira pseudonana* was shown to contain an isoform MTHB *S*-methyltransferase termed *TpMMT* gene [18].

Curson et al., 2017 discovered that many heterotrophic bacteria also produce DMSP via the same Met transamination pathway as marine algae [19]. The key MTHB S-methyltransferase gene *dsyB* was first identified in *Labrenzia aggrega* and was found to exist in many other marine Alphaproteobacteria [19, 20]. This bacterial DsyB enzyme is homologous to the algal DSYB enzyme and likely originated in bacteria [17]. Williams et al. (2019) isolated DMSP-producing bacteria from marine sediment and discovered that some Alphaproteobacteria, Gammaproteobacteria and Actinobacteria produce DMSP via

39 the Met methylation pathway similar to that existing in DMSP-producing angiosperms [20-22]. The
40 *mmtN* gene encoding the key Met *S*-methyltransferase in these organisms was identified in many of these
41 bacteria [20]. With the exception of *TpMMT* where no extensive studies have yet been carried out, the
42 DMSP synthesis genes have been shown to be robust reporters of an organisms potential to produce
43 DMSP and their transcription is indicative of the activity of the pathways [17, 19, 20]. By interrogating
44 the Ocean Microbial Reference Gene Catalogue Metagenomic Database (OM-RGC25; predominantly
45 surface seawater samples), *dsyB* was predicted to be present in up to 0.35% of bacteria [20], whereas
46 *mmtN* is far less abundant (~13-fold) [20]. Furthermore, there are many bacterial genera that produce
47 DMSP but which lack *dsyB* or *mmtN* in their genomes and likely have isoform enzymes or novel
48 pathways, e.g., *Marinobacter* [19, 20].
49 When released into the environment, DMSP is imported by a range of marine bacteria and algae as
50 an osmolyte, source of carbon, reduced sulfur and/or energy [9-11, 23]. There are three known DMSP
51 metabolic pathways: the demethylation pathway (mediated by Dmd enzymes) that can generate MeSH,
52 cleavage pathway (mediated by Alma1 and Ddd family DMSP lyase enzymes) that generates DMS and
53 a recently identified oxygenation pathway generating the novel compound
54 dimethylsulfoxoniumpropionate (no metabolic genes are currently known) [24]. The DMSP demethylase
55 'DmdA' can be divided into five clades (clade A, B, C, D, E) and 14 subclades, of which C/2 and D/1 are
56 the most abundant in the coastal areas and open oceans [25-27]. DmdA has been reported to occur in up
57 to 60% of marine bacteria (mainly Roseobacter, SAR11, SAR116 and Gammaproteobacteria), but most
58 recent estimates report 20% contain the genetic potential to demethylate DMSP [27-30]. DMSP cleavage
59 generating the climate-active gas DMS can be catalyzed by one of the eight known DMSP lyases, within
60 bacteria (*dddD*, *dddL*, *dddP*, *dddQ*, *dddW*, *dddY* and *dddK*) and algae (*Alma1*) [10, 11, 31-34]. *dddP* is
61 the most frequently detected of the *ddd* genes in marine environments (~ 8% of bacteria in the OM-RGC
62 marine samples) [17]. Functional DddP homologues are mainly found in Alphaproteobacteria,
63 predominantly in Roseobacter and SAR116 clade bacteria, but are also seen in some
64 Gammaproteobacteria, Ascomycete Fungi [35, 36] and in some bacteriophages [37]. *dddP* is used as a
65 key reporter of environmental DMSP cleavage due it being the most environmentally abundant bacterial
66 DMSP lyase and because gene probes exist for this gene [38]. Mostly containing *ddd* and/or *dmdA* genes,
67 Roseobacter, SAR11 and SAR116 clade bacteria are well known for their ability to catabolise DMSP
68 [39, 40]. The abundance and distribution of the *dddP* and *dmdA* genes have been well studied in the water
69 column from tropical to polar waters [27, 29, 41], but no study has focused on the abundance and/or
70 transcription of these genes or those bacterial DMSP synthesis in the sea surface microlayer (SML).
71 The SML, which is generally defined as the uppermost millimeters of the ocean (~10-250 μm of
72 the surface) [42], is the boundary layer between the atmosphere and the oceans, covering ~70% of the
73 Earth's surface. The SML is a sink for natural and anthropogenic material originating from the
74 atmosphere and the water column [43, 44], and is essential for the gaseous exchange and transport
75 mechanisms between these environments [45-48]. Physical, chemical and biological processes [10, 42,
76 49], bacterial communities [50, 51] and the metabolic characteristics [52] are quite different between
77 SML and the underlying waters. Several studies have looked at the DMSP and DMS concentrations in
78 the SML of oceans, coastal waters and bays during different seasons and these generally find that higher

DMSP levels and DMS production rates exist in the SML than in the subsurface water (SSW) [53-56]. Other studies have shown that bacteria including *Pseudoalteromonas*, *Pseudomonas* and *Vibrio*, are more abundant in SML than SSW of coastal seawater, estuarine and polluted seawater [50, 57]. Marine bacterial communities can be further divided into two fractions, the free-living (typically 0.22 -3 μm) fraction and particle-associated (>3 μm) fraction [58]. Several studies have found significant differences in the microbial community compositions and functional capacities of e.g. vibrios, Roseobacter group and some algal associated bacteria within these two fractions [59-61]. Indeed, some studies have investigated the abundance and transcription of DMSP catabolising genes in free-living and particle-associated bacteria [26, 62], but, none have investigated bacterial DMSP synthesis and catabolism together in SML compared to SSW samples.

Here, we investigated the differences in bacterial community structure between East China Sea SML and the corresponding SSW samples, and analysed the DMSP producing and catabolic bacteria present. Also, for the first time, we studied the distribution, diversity and transcription of key DMSP synthesis (*dsyB* and *mmtN*) and catabolic (*dddP* and *dmdA*) genes, together with DMSP and DMS standing stocks, and the influence of environmental factors on each layer. This study provides a better understanding of bacterial DMS and DMSP production in this boundary layer, and proposes bacteria as being more prominent DMSP-producers in this layer than in the SSW.

Materials and Methods

Sampling and environmental parameters

Samples were retrieved aboard the R/V *Dongfang Hong 2* from a total of 8 sites (Fig. 1 and Supplementary Table S1) of the East China Sea in April 3-10, 2017. SSW samples were collected using a Sealogger CTD (SBE25, Electronic Inc., USA) rosette water sampler (~4 m under the surface). The SML water samples were collected using the Garrett metal screen (MS) as described in Yang et al (2001) [63] within 3 minutes. It is possible that the 4 m of seawater below the SML is not a homogeneous mixed layer and that shallow density gradient may occur within this water. No measures were taken to assess these factors. Triplicate samples (1000 ml for SSW and 300 ml for SML) were filtered serially through 3 μm (Millipore Corporation, Billerica, MA, USA) and 0.22μm polycarbonate membranes (Millipore Corporation, Billerica, MA, USA). Membranes were then frozen in liquid nitrogen immediately (immersed in 200 μl RNAlater for RNA samples) and stored at -20°C on board before transferred into -80°C in laboratory. The 0.22-3 μm fraction was considered to contain free-living bacterioplankton, whereas the >3 μm was considered to contain larger and particle associated bacteria.

In situ hydrological parameters (temperature, salinity and depth) were monitored by CTD equipped on the water sampler. Chlorophyll *a* (Chl *a*) concentrations were measured as described previously [64]. In brief, seawater was collected on 0.7 μm pore size GF/F filters (Whatman), and then Chl *α* was extracted with 90% (v/v) acetone for 24 h in dark. The concentrations of Chl *α* in the extract were determined using a Turner-Designs Trilogy Laboratory® Fluorometer. DO was measured by Winkler method [65]. Waters were filtered with 0.45 μm cellulose acetate membranes and nutrients were analyzed by a nutrient auto-analyzer (AA3, Seal Analytical Ltd, UK) including PO_4^{3-}, NO_2^-, NO_3^-, SiO_3^{3-} and NH_4^+ [66].

117 **DMS and DMSP measurement**

118 *In situ*, DMS and DMSP concentrations were measured by Gui-peng Yang's group as previously
119 described in [67]. A cryogenic purge-and-trap preprocess system was used to capture DMS [68], and a
120 gravity-filtration method was applied to capture dissolved and particulate DMSP. Total DMSP ($DMSP_t$)
121 in water samples were obtained without filtration. Dissolved DMSP ($DMSP_d$) were from these waters
122 directly filtered through 0.45 μm membrane. Some bacteria containing DMSP will pass through the 0.45
123 μm membrane and will be apportioned to the $DMSP_d$ fraction. Particulate DMSP ($DMSP_p$) samples were
124 these above 0.45 μm membrane.

125 **Clone library of *dsyB* gene and phylogenetic analysis**

126 To determine the diversity of environmental *dsyB* sequences in our samples, we performed clone
127 library sequencing. Clone libraries were constructed from 10 samples (P3_m, P5_m, P7_m, E1_m, E3_m,
128 E5_m and S4_m of free-living bacteria and P5_m, P7_m, E1_s of particle-associated bacteria). All qPCR
129 products were separated by electrophoresis in 1% agarose gels and then purified using a DNA gel
130 extraction kit (Biomed, China). The purified *dsyB* gene amplicons were ligated into the pUCm-T vector
131 (Sangon, China) and transformed to *Escherichia coli* JM109. Transformants with correct inserts detected
132 by PCR were then sent for sequencing at the Sangon Biotech (Shanghai, China). The OTUs of *dsyB* were
133 determined with nucleotide similarity of 80 % by Mothur. Representative sequences of each OTU and
134 other known *dsyB* sequences [19] were used to construct phylogenetic tree by MEGA7.

135 **DNA/RNA extraction and qPCR (quantitative PCR) / RT-qPCR (reverse transcription**
136 **quantitative PCR)**

137 Total DNA was extracted from 3 μm (particle-associated) and 0.22 μm (free-living) membranes
138 using the phenol-chloroform method described by Yin et al. (2013) [69] with some modifications. The
139 mixture of membrane, 0.25 g silica and 600 μl sodium chloride-Tris-EDTA (STE) buffer was vigorously
140 beaten on a FastPrep-24 Homogenization System (MP Biomedicals, Irvine, California, USA) twice (60s
141 for each time at a speed of 6.0 m/s) to facilitate cell lysis. The extracted DNA was dissolved in 50 μl TE
142 buffer (100 mM Tris-HCl, 10 mM EDTA, pH 8.0) and stored at -80°C for further use. RNA was extracted
143 from 3 μm and 0.22 μm membranes as previous study [70]. RNA from some SML samples (P7 and S4)
144 were not extracted due to the loss of samples. Reverse transcription of RNA were performed according
145 to Williams et al. (2019) with some modification [20]. Absence of DNA in RNA samples was confirmed
146 by PCR using primers 338F/518R (Table 1), and 9 μl RNA were mixed with 1 μl 10 μM random reverse
147 primer for the RNA reverse transcription. Finally, resultant cDNA was stored at -80°C until use.

148 The qPCR and RT-qPCR were performed to quantify the abundance of total bacteria, the DMSP
149 catabolism genes *dddP* and *dmdA* (C/2, D/1 subclade) and producing genes *dsyB* and *mmtN*. All primers
150 and their annealing temperatures are listed in Table 1. The PCR reactions were conducted as follows: an
151 initial denaturation at 95°C for 3 min; then 35 cycles of 95°C for 20 s (30 s for 16S rRNA gene), primer
152 specific annealing temperature for 30 s, 72°C for 30 s. A melt curve was run after PCR as follows: 95°C
153 denaturation for 1 min, 0.5°C increment from annealing temperature (1°C for 16S rRNA gene from 55°C)
154 with signal collection. The qPCR standard curves were made using pUCm-T vector (Biotech, China) that

contained a single copy of the corresponding gene. Plasmids were extracted using Mini Plasmid Kit (TaKaRa, Tokyo, Japan), then linearized by restriction enzyme *Xho*I, purified by TIANgel Mini Purification Kit (TIANGEN Biotech, Beijing), and the concentrations of the products were quantified with a Nanodrop-1000 Spectrophotometer. The 10-fold serially diluted linearized plasmids were then used to generate the standard curves with all liner correlations showing $R^2 > 0.99$. The amplification efficiencies were between 95% and 105% (*dsyB* and *mmtN* with 83% to 96%). Three technical replicates were set for each sample. All samples were run on StepOne™ Real-time PCR System (Applied Biosystems) and the acquired data were analyzed by StepOne software (version 2.2). Bacterial abundance was represented by the copy numbers of the16S rRNA gene quantified by qPCR.

Bacterial community structure analysis

The 16S rRNA gene of total bacteria and plastids was amplified using primers 515modF and 806modR [71]. The PCR reaction contained 1 × Fast Pfu Buffer, 0.25 mM of dNTPs, 0.2 μM of each primer, 1U of FastPfu Polymerase, 10 ng of template DNA, and 0.2 μl of BSA (bovine serum albumin). PCR cycling condition was as follows: 35 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, 45 s for elongation at 72 °C. Amplified PCR products were purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's instruction. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300 bp) on Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

After subsampling each sample to an equal sequencing depth, operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using QIIME by usearch61 method. The taxonomic position of representative 16S rRNA gene sequence for each OTU was analyzed against the RDP 16S rRNA database (combined with the Silva 16S rRNA database) using confidence threshold of 70%. The plastid sequences were analysed against RDP 16S rRNA database (http://rdp.cme.msu.edu). The abundances of genera containing DMSP synthesis or catabolic genes were calculated according to the relative abundance of genera and absolute abundance of bacteria (qPCR).

Statistical analysis

The correlations between environmental factors and functional gene abundance were conducted using Pearson correlation test. The differences in DMS/DMSP concentrations, Shannon/Chao 1 indices, functional bacterial DMSP synthesis and catabolic gene abundance between SML and SSW were analysed using Wilcoxon signed rank test. All statistical analyses were performed by SPSS version 19.0 (SPSS Inc., Chicago, IL, USA), and significance level of $P < 0.05$ or 0.01 was adopted for all tests. Alpha diversity indices including Chao1 and Shannon were performed by Mothur to estimate the community richness and diversity. The relationship between bacterial community structure and environmental factors was evaluated by distance-based redundancy analysis (db-RDA) using Canoco software (Version 5.0, Microcomputer Power) with 9999 Monte Carlo permutation tests. The map of sampling sites was created using Ocean Data View (ODV, v5.1.7) and all the abundance figures were plotted by Origin 2018 (OriginLab Corporation, Northampton, USA)

193 **Data availability**

194 The raw reads of the high throughput sequencing were deposited into the NCBI Sequence Read
195 Archive (SRA) database with accession number SRP174872 under the BioProject PRJNA511511. The
196 partial sequences of *dsyB* gene from clone libraries were available in the GenBank database with
197 accession numbers MN232008 to MN232099.

198 **Results**

199 **Environmental parameters**

200 SML and SSW samples were taken from eight sites in the East China Sea (Fig. 1). The
201 environmental parameters are summarized in Supplementary Table S1. The salinity of the samples was
202 always > 30 PSU with the exception of those at site E1 (in nearshore water, Fig. 1). Wilcoxon signed
203 rank test was used to evaluate for significant difference in DMSP concentration between the two layers.
204 $DMSP_t$ and $DMSP_d$ concentrations were significantly higher ($P < 0.05$) in SML (means of 108.82 nM
205 and 14.02 nM) than SSW samples (means of 79.30 nM and 7.01 nM). However, there was no significant
206 difference between the levels of DMS detected in the SML and SSW samples, but it should be noted that
207 due to the SML sampling method, i.e. exposure of thin water layers to the air, there is inherently a larger
208 loss of DMS from the SML sample. Given many algae are known to produce high levels of DMSP and
209 DMS, the Chl *a* concentration of the samples was examined as a reporter of algal abundance. There was
210 no significant correlation between DMS and Chl *a* concentration in the SML and SSW samples
211 suggesting bacteria with DMSP lyase enzymes as important producers of DMS. In contrast, $DMSP_t$ and
212 $DMSP_p$ concentrations strongly correlated with Chl *a* concentration ($P < 0.01$ for both layers), but there
213 was no significant difference between the Chl *a* levels in the SML and SSW samples.

214 **Bacterial community diversity, richness and structure in SML and SSW**

215 The microbial communities within SML and SSW samples were examined to identify those
216 potentially producing and or catabolising DMSP. 16S rRNA gene amplicon and sequencing was carried
217 out, yielding 580,973 reads, ranging from 30,282 to 44,574 reads per sample with an average sequence
218 length of 273 bp (Supplementary Table S2). After subsampling, a total of 1556 OTUs were assigned at
219 cutoff level of 97% sequence similarity.

220 Eukaryotic plastid 16S rRNA genes, predominantly from Cryptomonadaceae (1.11% of total
221 community data [TCD] in SML and 0.12% in SSW, respectively) and Bacillariophyta (diatoms; 0.80%
222 of TCD in SML and 0.24% in SSW, respectively), were far more abundant in SML (~2.46% of TCD)
223 than in SSW samples (~0.49% of TCD, Supplementary Fig. S1). These Cryptomonadaceae,
224 Bacillariophyta and one unclassified chloroplast 16S rRNA sequences were ~9.3, 3.3 and 8.5-fold,
225 respectively, more abundant in the SML samples.

226 The numbers of bacterial OTUs detected in the SML and SSW 16S rRNA data were similar, with
227 307-617 OTUs in each sample (means of 449 and 407 in SML and SSW, respectively).The Good's
228 coverage values ranged from 99.6% to 99.9%, indicating good representativeness for most of the
229 bacterial communities in the samples studied. The Shannon and Chao 1 indices were used as proxies for
230 evaluating bacterial community diversity and richness, respectively (Supplementary Table S2). Both the

231 Chao 1 and Shannon diversity indices showed no remarkable differences between SML and SSW
232 samples.

233 The SML and SSW bacterial communities were both dominated by Gammaproteobacteria, followed
234 by Alphaproteobacteria, Actinobacteria and Flavobacteria (Supplementary Fig. S2). There were
235 significant differences between the SML and SSW community structures but they were not dramatic at
236 the class level (Supplementary Fig. S2 and S3). Compared with the SSW samples, the SML samples
237 generally possessed a higher proportion of Gammaproteobacteria ($P < 0.05$) (Supplementary Fig. S3).
238 *Pseudomonas* was the most abundant genus in all samples, whereas *Pseudoalteromonas*, unclassified
239 Flavobacteriaceae, unclassified Actinobacteria, *Candidatus* Pelagibacter, *Rhodococcus* and *Alteromonas*
240 were also abundant but were differentially present in SML and SSW samples. The relative abundance of
241 *Pseudoalteromonas*, *Cobetia*, *Nocardioides, Marinobacter* and *Halomonas* were higher in SML than
242 SSW samples, whilst the converse was true for unclassified Actinobacteria, *Formosa* and *Loktanella* (P
243 < 0.05, Supplementary Fig. S4). No significant differences were observed in the abundance of archaea
244 (0.52% of TCD in SML and 0.33% in SSW, respectively) between the SML and SSW samples ($P > 0.05$).
245 For some of these abundant genera, representative strains are known to contain DMSP synthesis and/or
246 catabolic genes [20, 72, 73] and/or carry out these processes. Perhaps some of these bacteria, e.g.
247 *Alteromonas* (with *mmtN*) [20], *Marinobacter* (that can produce DMSP) [20], *Halomonas* (which can
248 contain *dddD* and/or *dddP*) [38, 72] and *Loktanella* (with *dddL* and *dmdA*) [38], contribute to the
249 production and/or catabolism of DMSP in the SML and SSW samples.

250 **The abundance of bacterial 16S rRNA gene in free-living and particle-associated fractions in SML**
251 **and SSW**

252 Abundance of the 16S rRNA gene was quantified in free-living and particle-associated samples and
253 was used to estimate bacterial abundance in the SML and SSW samples (Supplementary Fig. S5). This
254 data was also used to estimate the absolute abundance of bacteria with the potential to produce and/or
255 catabolise DMSP, see below.

256 In all but one sample (from site E5) the 16S rRNA gene was more abundant in free-living (0.2-3
257 μm) than in the particle associated (>3 μm) fraction. Although some of these particle 16S rRNA
258 sequences likely come from archaea and plastids, most are bacterial (Supplementary Fig. S1 and S2).
259 The total abundance of the bacterial 16S rRNA gene (the sum of free-living and particle-associated
260 bacteria) in the SML samples (mean of 1.10×10^6 copies ml^{-1}) was ~7.5-fold higher than in the SSW
261 samples (mean of 1.46×10^5 copies ml^{-1}, $P < 0.01$). The maximal numbers of bacteria estimated were
262 located in site P7 for both SML and SSW (2.15×10^6 and 2.96×10^5 copies ml^{-1} for SML and SSW,
263 respectively), and the lowest abundance appeared in S4 (5.47×10^5 and 1.40×10^4 copies ml^{-1} for SML
264 and SSW, respectively).

265 The abundance of both the free-living and particle-associated and, thus, of the total bacterial 16S
266 rRNA gene in the SML samples was positively correlated with longitude ($P < 0.01$, Supplementary Table
267 S3 and S4), which might be due to the distance to the shore as longitude represents this gradient. In
268 addition, bacterial 16S rRNA gene abundance in both layers was negatively correlated with DMS
269 concentration ($P < 0.05$, Supplementary Table S3). The reasons for these correlations are unknown and

270 it is likely more informative to study correlations between DMSP and/or DMS concentration and the
271 abundance of the corresponding synthesis or catabolic genes (see below).

272 **Abundance of DMSP-producing genera and of Roseobacter and SAR11 clade bacteria in SML and**
273 **SSW samples**

274 To estimate the abundance of potential DMSP-producing and -catabolising bacteria in SML and
275 SSW, the relative abundance of these bacterial groups (from 16S rRNA gene amplicon data) was
276 normalized to the abundance of total bacteria (16S rRNA copy numbers via qPCR).
277 The genera *Labrenzia* and *Roseovarius*, some representatives contain both *dysB* and *mmtN* [20],
278 and *Ruegeria* and *Hoeflea* that can contain *dsyB* [20], were more abundant in SML than SSW samples
279 ($P < 0.05$, Fig. 2a and 2b). *Streptomyces* bacteria that can contain *mmtN* [20], were not abundant in any
280 samples and only existed in one near shore SML sample (E1). In contrast, *Alteromonas*, *Thalassospira*
281 and *Novosphingobium*, that can contain *mmtN* [20], were higher in SML than SSW ($P < 0.05$, Fig. 2b).
282 The genus *Marinobacter*, which can produce DMSP but the synthesis gene is not known [20], was higher
283 in SML than SSW samples ($P < 0.05$, Supplementary Fig. S4).
284 SAR11, which contain *dmdA* and ~25% of which likely have *dddK* [30, 33], SAR116 contains *dmdA*
285 and *dddP* [39, 74], and Roseobacter clade bacteria (in which *dmdA* and the varied *ddd* genes are common)
286 [34] were significantly more abundant in the SML than SSW samples (Fig. 3a, 3b and Supplementary
287 Table S2). The most abundant SML Roseobacter clade genera known to contain *dmdA* and/or *ddd* genes
288 were *Sulfitobacter*, *Paracoccus*, *Planktomarina*, *Rugeria*, *Labrenzia*, *Shimia* and *Marinovum* [38, 75, 76]
289 (Fig. 3a). Together these data show that bacteria associated with the capacity to synthesise and/or
290 catabolise DMSP are far more abundant in SML than in SSW samples. Given the likely inaccuracy of
291 these predictions due to the uncertainty of whether the named genera contain the functional genes or
292 carry out the process, qPCR methods were also utilized to assay the abundance and potential importance
293 of DMSP producers and consumers in the SML and SSW samples.

294 **Analysis of DMSP biosynthesis gene abundance and transcription in SML and SSW**

295 The DMSP producing genes, *dsyB* and *mmtN*, were used as the marker genes to quantify the
296 bacterial DMSP biosynthesis processes in water samples. The total abundances (particle-associated plus
297 free-living) of *dsyB* and *mmtN*, and thus bacteria with the potential to produce DMSP were higher in
298 SML (means of 4.49×10^2 and 1.62×10^1 copies ml^{-1}, respectively) than SSW (means of 6.43×10^1 and
299 3.99×10^0 copies ml^{-1}, respectively) ($P < 0.05$, Fig. 2c and 2d), and *dsyB* was ~10-fold higher than *mmtN*.
300 For each sample site both *dsyB* and *mmtN* genes were more abundant in the free-living fractions than the
301 particle-associated fractions in SML (Fig. 2c and 2d).
302 Importantly, *dsyB* and *mmtN* transcripts were detected in the majority of samples. Bacterial *dsyB*
303 transcripts were not detected in the middle of the East China Sea at site E3 in neither SML nor SSW
304 samples (E section, Fig. 4a). Indeed, the bacterial abundance indicated by 16S rRNA was also lower at
305 sites E1 and E3 than samples of the north East China Sea (sites P3, P5 and P7, Supplementary Fig. S5).
306 In all the other sites, *dsyB* transcripts were widespread in the two layers, and were far more abundant
307 than those for *mmtN* (Fig. 4a and 4b). The SML samples possessed ~3-fold higher *dsyB* transcript levels

308 ($P < 0.05$, mean of 2.02 copies ml^{-1}) than the SSW samples (mean of 5.44×10^{-1} copies ml^{-1}). However,
309 there was no significant difference in the transcript abundance of *mmtN* in the SML and SSW samples
310 (Supplementary Table S5).

311 To identify the most abundant and potentially important *dsyB* containing genera in the SML, we
312 performed *dsyB* clone libraries with the qPCR products. In total 92 clones were sequenced and clustered
313 into 16 OTUs (Fig. 5). All retrieved *dsyB* sequences had > 80 % nucleotide identity to ratified *dsyB*
314 sequences. OTUs that contained more than 10 sequences were OTU1, OTU2 and OTU3, which were
315 most homologous to *Salipiger bermudensis*, *Roseibacterium elongatum* and *Donghicola* sp., respectively.
316 All the OTUs fit into seven distinct clusters (Fig. 5), with cluster two possessing 2/3 of the dominant
317 OTUs. Organisms (OTU1) in cluster 2, with DsyB most homologous to *Salipiger bermudensis* DsyB,
318 were the most frequent and may be the main DMSP producing bacteria in this ECS sample set. Free-
319 living bacteria displayed a higher level of *dsyB* sequence diversity compared to those in particle-
320 associated bacteria (Fig. 5).

321 **Analysis of DMSP catabolic gene abundance and transcription in SML and SSW**

322 The abundance and transcription of key DMSP catabolic genes *dddP* and *dmdA* (C/2 and D/1) were
323 also investigated in the SML and SSW samples by qPCR. The DMSP lyase gene *dddP* and its transcripts
324 were detected in most samples confirming that bacteria with this gene are contributing to the DMS
325 production in the SML and SSW samples. However, there was no significant difference in *dddP*
326 abundance and transcript levels between the SML and SSW samples, which is consistent with the DMS
327 measurement data (Fig. 3c and Fig. 4c). As expected, the total abundance of *dmdA* in both SML and SSW
328 samples is higher (~3-fold) than that of *dddP*, but there are a few samples where the converse is true
329 (Sites S4 of SML and P7 of SSW). In contrast to *dddP*, *dmdA* (especially C/2), and thus the potential to
330 demethylate DMSP, was more abundant in SML than SSW samples ($P < 0.05$, Fig. 3d and Fig. 4d). The
331 transcripts of *dmdA* and C/2 subclade were also ~6.6-fold and ~8.2-fold higher in SML than SSW on
332 average (Fig. 4). As with the DMSP biosynthesis genes, both *dddP* and *dmdA* were more abundant in the
333 free-living fractions than the particle-associated fractions (Fig. 3c and 3d). The abundance of *dmdA* in
334 free-living bacteria was significantly higher in SML (mean of 1.36×10^{4} copies ml^{-1}) than SSW ($P <$
335 0.05, Fig. 3d and Supplementary Table S5).

336 No significant correlation was found between *dddP* and *dmdA* gene abundance and DMSP/DMS
337 concentrations in the SML and SSW samples. However, the transcripts of *dddP* in the free-living bacteria
338 were significantly correlated with $DMSP_t$ and $DMSP_p$ concentrations in the SML and SSW samples
339 (Supplementary Table S6). This correlation reveals the important role of the cleavage pathway in DMSP
340 catabolism in the ECS SML and SSW samples.

341 **Discussion**

342 Understanding microbial processes at the ocean surface, especially in the SML, is key to reduce
343 uncertainties regarding oceanic volatile exchange to the atmosphere and the potential climatic
344 implications [77]. The SML provides a habitat for diverse and abundant flora and fauna with much greater
345 biological activity than the SSW columns [78]. Although the SML has been investigated for its chemical

346 and biological properties [79-81], the role of SML bacteria in ocean-atmospheric interactions is relatively
347 underexplored. The marine osmolyte DMSP is synthesised by eukaryotes [14, 15, 82] and marine
348 heterotrophic bacteria [19], and is then catabolized largely by heterotrophic bacteria via the
349 demethylation (*dmd* genes) and cleavage (*ddd* family genes) pathways to generate the climate-active
350 gases MeSH and DMS, respectively. Studies on the abundance and activities of SML DMSP-producing
351 and -catabolising organisms are necessary to help evaluate environmental DMSP metabolism and DMS
352 emissions from the water columns to atmosphere. Our study showed that the abundance of bacteria,
353 particularly, DMSP-producing bacteria, their DMSP synthesis genes and *dsyB* transcripts in the SML
354 were significantly higher than in SSW water samples. Furthermore, bacteria with the potential to
355 demethylate DMSP and their *dmdA* transcripts were also higher in the SML. These data suggest that
356 bacterial DMSP production and consumption processes are greater in the SML than in the underlying
357 water in the East China Sea, which is consistent with previous process work [53, 54, 63].

358 **Bacteria are more abundant in the SML than in SSW**

359 The total abundance of bacteria in East China Sea SML samples was significantly higher (~7.5-fold)
360 than those in the SSW (Supplementary Fig. S5). This result is consistent with work on seawater from
361 close to Long Island, New York, which found a ~6-fold bacterial enrichment in SML compared to SSW
362 samples [80]. The higher SML bacterial abundance may be due to generally higher levels of available
363 nutrients in SML [57] and/or because the SML provides a physically more stable environment due to its
364 surface tension [78]. It is possible that variation in 16S rRNA gene copies in some bacteria [83] may
365 contribute to variations of 16S rRNA abundance in the SML and SSW samples.
366 Free-living bacteria were more abundant (~2-fold higher) than those particle-associated in both the
367 SML and SSW samples from the East China Sea (except the E1 SSW sample; Supplementary Fig. S5).
368 This data contradicts the findings of Cunliffe & Murrell (2009) studying samples from a freshwater pond
369 where particle-associated bacteria were more abundant in SML than in surface water (0.4 m of depth)
370 [84]. This discrepancy could be apportioned to differences between marine and freshwater SML samples
371 or more likely that the freshwater pond samples contained more suspended particles [60].
372 There were significant differences in the bacterial community composition between SML and SSW
373 samples. The relative abundance of Gammaproteobacteria was significantly higher in SML compared to
374 SSW samples, whereas Alphaproteobacteria were more abundant in the SSW samples (Supplementary
375 Fig. S3). In addition, many genera, such as *Pseudoalteromonas*, *Cobetia*, *Nocardioides, Marinobacter*,
376 *Halomonas* and *Vibrio* were significantly higher in SML compared to SSW samples (Supplementary Fig.
377 S4). Indeed, *Pseudoalteromonas* and *Vibrio* were also enriched to levels ~10.5-fold and ~22.6-fold higher,
378 respectively, in SML compared to SSW samples from the North Sea, UK [50]. In contrast, Agogue et al.
379 (2005) did not find any major bacterial community differences between SML and SSW samples from
380 coastal sites in France and Spain that were consistent with the findings presented here [85]. This
381 difference may be due to the variation in levels of available nutrients in different marine samples. Lastly,
382 the data presented here is consistent with that in Cunliffe et al. (2009) showing no significant difference
383 in the relative abundance of archaea in SML and SSW ($P > 0.05$), suggesting that bacteria and not archaea
384 favor the SML environment [86].

385 **DMSP-producing bacteria are more abundant in SML than SSW**

386 It is clear that the DMSP concentration was significantly higher (~1.5-fold) in SML of the East
387 China Sea than in SSW ($P < 0.05$, Supplementary Table S1), but there was no significant difference in
388 the DMS concentration. These results on SML DMSP enrichment are consistent with those carried out
389 on South China Sea, Atlantic and Pacific Ocean samples [53, 55, 87]. Not surprisingly DMSP
390 concentration was significantly correlated with Chl *a* both in SML and SSW (Supplementary Table S6),
391 highlighting the important role phytoplankton play in DMSP production [10]. Indeed, 16S rRNA plastid
392 sequences from Cryptomonadaceae, diatom and an unknown phytoplankton were shown to be more
393 abundant in the SML compared to SSW samples suggesting that these eukaryotes, with large cell volumes
394 compared to bacteria, may be responsible for the higher levels of DMSP detected in the SML samples.
395 The majority of Cryptomonadaceae phytoplankton tested in Keller et al. (1989) did not produce DMSP
396 and diatoms are generally known as low producers of DMSP (typically < 50 mM intracellular) [88].
397 Furthermore, the increased abundance of plastid sequences in the SML samples does not agree with the
398 finding of no significant difference in Chl *a* level between SML and SSW samples. Thus, the higher
399 DMSP concentration in SML could also be apportioned to other organisms, potentially heterotrophic
400 bacteria, which were far more abundant in these SML samples (Supplementary Fig. S5) [19].
401 Alternatively, phytoplankton in the SML exposed high UV levels may exhibit enhanced oxidative stress
402 that might enhance DMSP production [7] and thus the DMSP:Chl *a* ratio. Further work is required to
403 establish the significance of phytoplankton in DMSP production in these SML and SSW samples. It is
404 noteworthy for future studies that any DMSP measured in >3 μm fractions cannot be solely attributed to
405 eukaryotic algae since DMSP-producing bacteria and their *dsyB* and *mmtN* transcripts were detected in
406 these fractions.

407 Coincidently, the abundance of bacteria containing the DMSP synthesis genes *dsyB* and *mmtN*, and
408 thus the potential to produce DMSP, in SML was significantly higher than in SSW samples (Fig. 2,
409 Supplementary Table S5). This is consistent with the higher abundance of DMSP producing genera in
410 SML compared to SSW samples. For example, *Alteromonas*, *Ruegeria*, *Roseovarius, Hoeflea,*
411 *Thalassospira*, *Labrenzia* and *Novosphingobium* that can contain *dsyB* and/or *mmtN* genes were
412 significantly more abundant in SML (7.11% of TCD) compared to SSW samples (1.84% of TCD) (Fig.
413 2a and 2b). The *dsyB* gene was the major DMSP producing gene detected in the East China Sea samples,
414 its abundance being ~10-fold higher than *mmtN* both in SML and SSW (Fig. 2c and 2d). This was in
415 agreement with Williams et al. (2019) who found *dsyB* to be ~13-fold more abundant than *mmtN* in the
416 seawater samples [20]. Importantly, *dsyB* transcripts were ~3-fold higher in SML than in SSW samples
417 (Fig. 4a). *mmtN* transcripts were also detected in the sample but these were more evenly distributed
418 between SML and SSW samples. The *dsyB* and *mmtN* genes were more abundant in free-living than in
419 particle-associated bacteria, indicating that free-living bacteria may be the main DMSP producers.
420 Additionally, *Pseudoalteromonas*, the second most abundant genus in SML samples, is also a potential
421 DMSP producer [20], and its relative abundance were significantly higher in SML (11.62%) than in SSW
422 (0.17%) in the East China Sea (Supplementary Fig. S4). Some *Pseudoalteromonas* strains produce
423 DMSP but their mechanism of synthesis is unknown since their available genomes lack *dsyB* and *mmtN*
424 [20]. Indeed, *Pseudoalteromonas* was ~1.8-fold more abundant than all known DMSP producers that

425 contains *dsyB* and/or *mmtN*. Thus, it is possible that *Pseudoalteromonas* bacteria in the East China Sea
426 may be important contributors to the enhanced levels of DMSP observed in SML compared to SSW
427 samples.
428 Together these data provide strong evidence for heterotrophic bacteria with *dsyB*, *mmtN* or unknown
429 mechanisms (e.g. *Pseudoalteromonas*) significantly contributing to the DMSP levels observed in East
430 China Sea SSW and particularly in the SML, which is a hotspot for DMSP-producing bacteria and their
431 activity. It is likely that *Pseudoalteromonas* and/or bacteria with *dsyB* are key contributors to the higher
432 DMSP levels seen in SML versus SSW samples.

433 **Variations in abundance and transcription of DMSP demethylation and cleavage genes in SML**
434 **and SSW samples**

435 The *dmdA* (C/2 and D/1) primers targeting Roseobacter and SAR11 clade [28] and *dddP* primers
436 targeting the Roseobacter clade [89] were used to evaluate the abundance and transcription of the DMSP
437 demethylation and cleavage genes in the East China Sea SML and SSW. *dmdA* (sum of C/2 and D/1)
438 especially its C/2 subclade were significantly more abundant in the SML (~1.5-fold and ~3.2-fold)
439 compared to SSW samples (Fig. 3d). Consistently, their transcripts were also more abundant (~6.6-fold
440 for *dmdA* and ~8.2-fold for C/2) in SML compared to SSW samples (Fig. 4d). These data strongly suggest
441 that SML bacteria likely have higher DMSP demethylation activity than those in the SSW. In contrast,
442 there were no significant differences in *dddP* abundance or its transcript levels between the SML and
443 SSW samples (Fig. 3c and 4c), which is consistent with the observed DMS concentrations being similar
444 within these two environments. It is possible that other DMSP lyase genes are more abundant in the
445 tested samples than *dddP*, but this was not tested here. It is also possible that DMS consumption processes,
446 biological and abiotic, affect the SML samples more than the SSW samples, e.g. due to the higher DMS
447 flux to the atmosphere and potential losses during sampling. Future studies should consider DMSP and
448 DMS production and consumption rates. Moreover, the abundance and transcript levels of *dmdA* (C/2
449 and D/1 subclades) were higher than for *dddP* in both layers (Fig. 3c, 3d, 4c and 4d) potentially indicating
450 that bacterial DMSP demethylation have a more prominent role in the SML than in the SSW samples.
451 This data is consistent with the findings of Liu et al. (2018), and implies that DMSP demethylation
452 pathway (mediated by *dmdA*) may be the major DMSP catabolic process exceeding the cleavage
453 pathways in the East China Sea [38]. Unfortunately, we could not measure MeSH levels in the samples
454 or other indicators of DMSP demethylation, and further work is necessary to confirm this hypothesis.
455 The *dmdA* gene was significantly more abundant in free-living bacteria than in particle-associated
456 bacteria both in SML (~5.5-fold) and SSW (~5.3-fold) (Fig. 3d). This is in agreement with Morris et al.
457 (2002) who showed that SAR11 clade bacteria (containing *dmdA* gene and in many cases *dddK*) are
458 mainly free-living in Sargasso Sea and Oregon coastal surface waters [90]. The positive correlation
459 between *dddP* and *dmdA* genes in free-living bacteria and DMSP concentrations in SSW potentially
460 indicates that free-living bacteria may be the major DMSP consumers via the demethylation and cleavage
461 pathways (Supplementary Table S6).

462 **Conclusion**

463 This study focused on microbial DMSP production and catabolism in SML and SSW samples. It
464 showed that the total bacteria in SML were likely ~7.5-fold more abundant than in SSW. DMSP-
465 producing bacteria were also more abundant in SML than SSW samples, confirmed by the higher *dsyB*
466 (~7-fold) and *mmtN* (~4-fold) gene abundances in the former samples. Furthermore, SML samples
467 possessed ~3-fold higher *dsyB* transcripts than those from SSW, which is consistent with the higher
468 $DMSP_d$ and $DMSP_t$ concentration observed in the SML. Bacterial genera known to produce/potential
469 DMSP, e.g. *Pseudoalteromonas* and *Marinobacter*, were also more abundant in SML than in SSW and
470 potentially contribute to the DMSP produced. Regarding DMSP catabolism, the DMSP demethylase gene
471 *dmdA* (especially C/2) was more abundant in SML than SSW samples, but there was no difference in the
472 abundance of the DMSP lyase *dddP*. Both *dmdA* and *dddP* transcripts were detected in SML and SSW
473 samples. This study provides a case for bacteria being important contributors to both DMSP synthesis
474 and catabolism in SML and SSW samples, but reveals that, in particular, the SML may be an important
475 hotspot for bacterial DMSP production. Further studies are required to estimate the DMS emission and
476 the contributions of bacteria and phytoplankton to DMSP production in SML and SSW. It is important
477 to note that under a changing climate with measurable surface water temperatures rises predicted, such
478 changes will certainly enhance the DMS flux to the atmosphere. However, it is presently undetermined
479 what affects such changes will have on the microbial community composition and their metabolism, e.g.
480 on the production and cycling of DMSP and related compounds that can influence climate, in these
481 important marine environments. Depending on the specific effects, such changes could ameliorate or
482 exacerbate the situation. This is an important topic to address in future research.

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494 **Author contributions** X-HZ and JDT designed the experiments, analysed the data and wrote the
495 manuscript. HS collected samples, performed experiments, analysed the data and wrote the manuscript.
496 G-PY, YHZ and YFZ analysed the data. SZ performed statistical analysis. ST performed part of the qPCR
497 experiments. Q-YM performed the DMS and DMSP detection. All the authors edited and approved the
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769

770 **Table 1** Primers and amplification conditions for qPCR detection and high-through sequencing of bacteria.

Target gene	Primers	Sequences (5'-3')	Amplicon length (bp)	Annealing temp (°C)	Usage	References
16S rRNA	338F	ACTCCTACGGGAGGCAGCAG	180	53	qPCR	[69]
	518R	ATTACCGCGGCTGCTGG				
dsyB	dsyBF	CATGGGSTCSAAGGCSCTKTT	246	61		[20]
	dsyBR	GCAGRTARTCGCCGAAATCGTA				[20]
mmtN	mmtNF	CCGAGGTGGTCATGAAYTTYGG	301	54		[20]
	mmtNR	GGATCACGCACACYTCRTGRTA				[20]
dddP	874F	AAYGAAATWGTTGCCTTTGA	97	41		[89]
	971R	GCATDGCRTAAATCATATC				
dmdA(C/2)	291F	AGATGAAAATGCTGGAATGATAAATG	191	50		[89]
	482R	AAATCTTCAGACTTTGGACCTTG				[28]
dmdA(D/1)	268F	AGATGTTATTATTGTCCAATAATTGATG	89	49		[89]
	356R	ATCCACCATCTATCTTCAGCTA				[28]
16S rRNA	515modF	GTGYCAGCMGCCGCGGTAA	291	50	Amplicon sequencing	[71]
	806modR	GGACTACNVGGGTWTCTAAT				

771

772 **Titles and legends to figures**

773 **Fig. 1** East China Sea sampling site locations and their DMS and total DMSP ($DMSP_t$) concentration in
774 SML and SSW samples. The light blue and pink bars indicate the DMS concentration in SML and SSW
775 samples, respectively. The green and orange bars indicate the $DMSP_t$ concentration in SML and SSW
776 samples, respectively. The scales for DMS and DMSP concentration (nM) are indicated. The DMS and
777 $DMSP_t$ concentrations of P7 SML were not measured.

778

779 **Fig. 2** DMSP concentration (total and dissolved), the abundance of DMSP producing organisms and
780 genes in the SML and SSW samples from the East China Sea. (a), the abundance of genera with
781 representatives known to contain *dsyB*. (b), the abundance of genera with representatives known to
782 contain *mmtN*. (c), the abundance of *dsyB* and of *mmtN* (d) determined by qPCR. Three technical
783 replicates are set for each sample. The SML and SSW samples are coloured cyan or red and have "m" or
784 "s" in their sample names, respectively.

785

786 **Fig. 3** DMSP (dissolved) and DMS concentrations, the abundance of DMSP catabolic organisms and
787 genes in the SML and SSW samples from the East China Sea. (a), the abundance of Roseobacter clade
788 bacteria. (b), the abundance of SAR11 and SAR116 clade bacteria. (c), the abundance of *dddP* and of
789 *dmdA* (d) determined by qPCR. Three technical replicates are set for each sample. The SML and SSW
790 samples are coloured cyan or red and have "m" or "s" in their sample names, respectively.

791

792 **Fig. 4** DMSP (total and dissolved) and DMS concentrations and the abundance of DMSP producing and
793 catabolic gene transcripts in SML and SSW samples from the East China Sea. (a), the abundance of *dsyB*
794 transcripts. (b), the abundance of *mmtN* transcripts. (c), the abundance of *dddP* transcripts. (d), the
795 abundance of *dmdA* transcripts. Three technical replicates are set for each sample. The SML and SSW
796 samples are coloured cyan or red and have "m" or "s" in their sample names, respectively.

797

798 **Fig. 5** Neighbor-joining tree of representative *dsyB* OTU sequences in SML and SSW samples from the
799 East China Sea. 43 sequences were used to construct the nucleotide tree. The topologies of phylogenetic
800 trees were evaluated based on the bootstrap resampling method with 1000 replicates. Bootstrap
801 coefficients below 70% were not shown. OTUs of *dsyB* in free-living bacteria were marked with green
802 stars while particle-associated bacteria marked with cyan circles. Strains experimentally confirmed to
803 produce DMSP are marked with a red square. OTUs from SML samples were marked with blue font, and
804 OTUs from both SML and SSW samples were marked with red font.