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1 **Identification of dimethylamine monooxygenase in marine bacteria**
2 **reveals a metabolic bottleneck in the methylated amine degradation**
3 **pathway**

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17

18 **Conflict of Interest**

19 The authors declare no conflict of interest.

20

21 **Abstract**

22 Methylated amines (MAs) are ubiquitous in the marine environment and their
23 subsequent flux into the atmosphere can result in the formation of aerosols and
24 ultimately cloud condensation nuclei. Therefore, these compounds play a potentially
25 important role in climate regulation. Using *R. pomeroyi* as a model, we identified the
26 genes encoding dimethylamine (DMA) monooxygenase (*dmmABC*) and demonstrate
27 that this enzyme degrades DMA to monomethylamine (MMA). Although only
28 *dmmABC* are required for enzyme activity in recombinant *E. coli*, we found that an
29 additional gene, *dmmD*, was required for the growth of *R. pomeroyi* on MAs. The
30 *dmmDABC* genes are absent from the genomes of multiple marine bacteria, including
31 all representatives of the cosmopolitan SAR11 clade. Consequently, the abundance of
32 *dmmDABC* in marine metagenomes was substantially lower than the genes required
33 for other metabolic steps of the methylated amine degradation pathway. Thus, there is
34 a genetic and potential metabolic bottleneck in the marine MA degradation pathway.
35 Our data provide an explanation for the observation that DMA-derived secondary
36 organic aerosols (SOAs) are among the most abundant SOAs detected in fine marine
37 particles over the North and Tropical Atlantic Ocean.

38

39 **Introduction**

40 Methylated amines (MAs) form part of the marine dissolved organic nitrogen
41 (DON) pool and are ubiquitous in the marine environment. Their precursors,
42 trimethylamine *N*-oxide (TMAO), glycine betaine, choline, and carnitine are either
43 osmolytes or constituents of lipid membranes within eukaryotic cells (Ikawa and
44 Taylor, 1973; Treberg *et al.*, 2006). MAs (trimethylamine, TMA; dimethylamine,
45 DMA; monomethylamine, MMA) form part of a trace gas mix that is constantly
46 emitted from the oceans and collectively these trace gases have major implications for
47 the climate, largely through the production of particulate marine aerosols (Carpenter
48 *et al.*, 2012). Such aerosols can represent up to one fifth of the total gaseous base
49 compounds detected in the atmosphere over the oceans (Gibb *et al.*, 1999a). Their
50 global annual flux is estimated to be $\sim 80 \text{ Gg yr}^{-1}$ and their production in surface
51 seawater, and subsequent emission into the atmosphere, is thought to be largely
52 driven by biotic processes (Ge *et al.*, 2011). For example, over Cape Verde off the
53 coast of West Africa, the accumulation of MAs in fine marine particles was positively
54 correlated with algal blooms (Müller *et al.*, 2009). The flux of MAs into the
55 atmosphere is important since they can undergo a number of different reactions
56 resulting in a complex set of effects on the climate. For instance, they can influence
57 the absorption and scattering of UV radiation, the formation of cloud condensation
58 nuclei (CCN) (Ge *et al.*, 2011), and the cloud droplet number concentration (Rinaldi
59 *et al.*, 2010). Moreover, off the coast of California, during periods of elevated primary
60 production, a shift in the composition of secondary organic aerosols (SOAs) towards
61 amine-derived compounds resulted in an increase in CCN activity (Sorooshian *et al.*,
62 2009). Thus, as a component of marine aerosols, MAs can actively affect the climate
63 system.

64 Historically, the *in situ* quantification of MAs in the marine environment has
65 proven challenging. Consequently, there are only a few studies reporting their
66 standing stock concentrations (Carpenter *et al.*, 2012). Generally, in surface seawater
67 the concentration of MAs is in the nanomolar (nM) range whilst in marine sediments
68 it reaches low micromolar (μM) concentrations (Van Neste *et al.*, 1987; Gibb *et al.*,
69 1999b; Gibb and Hatton, 2004). Recent studies have identified a number of the key
70 genes and enzymes catalysing the degradation of TMA, TMAO, and MMA in the
71 marine environment (Chen *et al.*, 2010; 2011; Lidbury *et al.*, 2014) (Figure 1a). It is
72 now known that bacteria capable of degrading MAs are abundant in surface seawater
73 and are primarily related to the *Alphaproteobacteria* (Chen *et al.*, 2011; Sun *et al.*,
74 2011). Despite their low standing stock concentrations, expression of the key genes
75 and enzymes catalysing the degradation of MAs has been observed in surface
76 seawater from various oceanic regions (Lidbury *et al.*, 2014). Indeed, marine
77 *Alphaproteobacteria* often heavily transcribe the TMAO-specific transporter
78 suggesting that demethylation of TMAO to DMA may be a major process in surface
79 ocean waters (Sowell *et al.*, 2008; Ottesen *et al.*, 2011; 2013; Williams *et al.*, 2012;
80 Gifford *et al.*, 2013).

81 The marine *Roseobacter* clade (MRC) and SAR11 clade are two monophyletic
82 groups of *Alphaproteobacteria* that employ differing ecological strategies for growth
83 (Luo *et al.*, 2013). Both of these clades can catabolize MAs in order to generate
84 reducing power whereas the MRC can also utilize these compounds as a sole source
85 of both carbon and nitrogen (Chen, 2012). *Ruegeria pomeroyi* DSS-3, a member of
86 the MRC, has been used as a model organism to study the degradation of TMA,
87 TMAO, and MMA. However, how these marine bacteria degrade DMA remains
88 unknown. In the methylotrophic soil bacterium *Methylocella silvestris* BL2, a three-

89 gene cluster (*dmmABC*) is required for growth of this organism on DMA, as mutants
90 lacking *dmm* genes ceased to grow on DMA as sole nitrogen source (Zhu *et al.*,
91 2014). In addition, in another methylotrophic soil bacterium *Paracoccus aminophilus*
92 JCM 7686, mutants lacking a functional *dmmABC* or an additional gene (*dmmD*),
93 could no longer utilize DMA as a sole carbon source (Dziewit *et al.*, 2015).
94 Furthermore, a DMA monooxygenase (Dmm) has been purified from MA-grown
95 *Aminobacter aminovorans* cells and shown to be a NADPH-dependent enzyme that
96 produces MMA and formaldehyde with DMA being the most active substrate
97 (Alberta and Dawson, 1987). Dmm has a native molecular weight of ~210 kDa and
98 comprises three subunits 42 000, 36 000, and 24 000 Da in size, each of which are
99 essential for *in vitro* activity (Alberta and Dawson, 1987).

100 Here, we set out to determine the genes catalysing DMA demethylation in
101 marine bacteria using *R. pomeroyi* DSS-3 as the model organism. Dmm was
102 heterologously expressed in *Escherichia coli* and the function of the predicted three-
103 gene cluster, *dmmABC*, was confirmed for the first time by enzymatic, chemical, and
104 growth assays. We also demonstrate that, unlike the genes required for the catabolism
105 of TMA, TMAO, and MMA, the genes required for DMA catabolism are absent from
106 key marine bacterial taxa and are subsequently depleted in metagenomes derived from
107 oceanic surface waters.

108

109 **Materials and methods**

110 **Bacterial cultivation**

111 The strains used in this study are listed in Supplementary Table S1.
112 *R. pomeroyi* wild type (WT) and mutants were grown in a marine ammonium
113 minimal salts (MAMS) medium (Thompson *et al.*, 1995) with slight modifications
114 (Lidbury *et al.*, 2015) using 10 mM glucose as carbon source. TMA, TMAO, DMA,
115 and MMA (1 mM) were added as sole nitrogen source. To observe growth on
116 different nitrogen sources, cultures (n=3) were set up in 125 ml serum vials
117 containing 25 ml medium. Overnight starter cultures were harvested by centrifugation
118 (1 500 × g, 5 min) and washed three times in nitrogen-free MAMS prior to
119 inoculation (8% v/v). Cultures were kept under constant agitation (150 rpm) at 30°C.

120 **Overexpression of *dmmABC* and *dmmDABC* in a heterologous host**

121 All primers used in this study are listed in Supplementary Table S2. Either
122 *dmmABC* encoding the structural components of Dmm or the entire operon
123 *dmmDABC* were subcloned into the pGEM-T EASY vector (Promega, Southampton,
124 UK). Sequence integrity was checked prior to digestion using the restriction enzymes
125 *NheI* and *HindIII* and subsequent ligation into the expression vector pET28a, which
126 was transformed into *E. coli* BLR(DE3)pLysS (Promega). Transformed *E. coli* cells
127 were grown for 32 h at 25°C in the presence of 0.2 mM isopropyl
128 β-D-1-thiogalactopyranoside (IPTG) and 1 mM DMA.

129 **Mutagenic analysis and mutant complementation in *R. pomeroyi***

130 A *dmmD* disrupted mutant (*dmmD::Gm*) in *R. pomeroyi* DSS-3 was
131 constructed by cloning part of the gene (Spo1579) into the pGEM-T EASY vector. A

132 gentamicin resistance cassette (Dennis and Zylstra, 1998) was inserted into a naturally
133 occurring *SpeI* site located near the centre of the gene. The mutated construct was
134 cloned into the suicide vector, pk18mobsacB (Schäfer *et al.*, 1994), and mobilized
135 into *R. pomeroyi* via conjugation with *E. coli* S17-1 electrocompetent cells.
136 Transconjugants were streaked onto gentamicin plates containing MMA as the sole
137 nitrogen source to counterselect against *E. coli* (Lidbury *et al.*, 2014). Double
138 homologous recombination events were selected for by transconjugant sensitivity to
139 kanamycin. The mutation was confirmed by PCR and sequencing.

140 To complement the *dmmD::Gm* with *dmmDABC* plus its native promoter, the
141 entire gene cluster was amplified introducing the restriction sites *XbaI* and *KpnI* at the
142 5' and 3' ends, respectively. For complementation with the structural genes *dmmABC*,
143 the promoter alone was amplified introducing the restriction sites *XbaI* and *HindIII* at
144 the 5' and 3' ends, respectively. In addition, *dmmABC* was amplified introducing the
145 restriction sites *HindIII* and *KpnI* at the 5' and 3' ends, respectively. For
146 complementation using just *dmmD*, this gene (Spo1579) plus the promoter were
147 amplified introducing the restriction sites *BamHI* and *HindIII* at the 5' and 3' end,
148 respectively. All PCR fragments were subcloned into the pGEM-T EASY vector.
149 Sequence integrity was checked prior to cloning the construct into the broad-host
150 range plasmid pBBR1MCS-km (Kovach *et al.*, 1995) and mobilized into *dmmD::Gm*
151 via conjugation as before. Transconjugants were selected by growth on half-strength
152 Yeast Tryptone Sea Salts (1/2 YTSS) media (DMSZ) containing 80 µg ml⁻¹
153 kanamycin and 10 µg ml⁻¹ gentamicin. Complementation was confirmed by PCR and
154 sequencing.

155 **Quantification of methylated amines**

156 Cells were boiled for ≥ 10 min and debris was removed via centrifugation
157 ($17\,000 \times g$, 5 min). TMA, TMAO, DMA, and MMA were quantified on a cation-
158 exchange ion chromatograph (881 Compact IC pro, Metrohm, Runcorn, UK) supplied
159 with Metrosep C 4 guard and Metrosep C 4 - 250/4.0 separation column, and a
160 conductivity detector (Metrohm) using an external calibration (Lidbury *et al.*, 2014).

161 **Analysis of enzymes involved in MA metabolism in sequenced marine microbial** 162 **genomes**

163 Single amplified genomes used in this study derived from the Integrated
164 Microbial Genome (IMG) database of the Joint Genome Institute (JGI)
165 (<https://img.jgi.doe.gov/cgi-bin/m/main.cgi>). All available defined marine bacterial
166 genomes were screened for enzymes catalysing MA degradation using a BLASTP
167 analysis with Tmm (Spo1551), Tdm (Spo1562), DmmD (Spo1579), DmmA
168 (Spo1580), DmmB (Spo1581), DmmC (Spo1582), GmaS (Spo1573), and TmoX
169 (Spo1548) from *R. pomeroyi* DSS-3 as query sequences using a stringent cut-off
170 value of e^{-50} . Marine bacterial genomes containing genes encoding these proteins are
171 listed in Supplementary Table S3. Taxonomy information at the phylum, class, and
172 order level was exported from the IMG/JGI database. For phylogenetic analysis,
173 amino-acid sequences of *dmmD*, *dmmA*, *dmmB*, and *dmmC* from 36 taxa were aligned
174 individually by MUSCLE (Edgar, 2004), trimmed at either end and combined to one
175 alignment. Evolutionary analysis was conducted in MEGA7 (Kumar *et al.*, 2016) on a
176 total of 1043 positions remaining in the dataset after exclusion of gaps and missing
177 data. A phylogenetic tree was inferred by a maximum likelihood approach applying
178 the WAG model (Whelan and Goldman, 2001) with 999 bootstrap replicates and
179 using a maximum parsimony tree derived from Neighbor-Joining as the initial tree.

180 **Analysis of enzymes involved in MA metabolism in marine metagenomes and**
181 **metatranscriptomes**

182 The metagenomes used in this study and the abundances of MA degradation
183 genes are listed in Supplementary Table S4. Metagenomes were chosen from the
184 IMG/JGI database and predominantly consisted of sites used in the global ocean
185 sampling (GOS) expedition (Rusch *et al.*, 2007). A BLASTP analysis was performed
186 using a stringency of >30% identity and a cut-off value of e-50. Query sequences
187 were identical to those described above. The number of retrieved sequences for each
188 protein was normalized by dividing the length of the query by the length of RecA.
189 Finally, the normalized hits were divided by the number of hits retrieved for two
190 single copy genes (*recA* and *gyrB*) to obtain the percentage of MA-utilising marine
191 bacteria present at each site. For phylogenetic analysis, hits were clustered using CD-
192 HIT (Huang *et al.*, 2010) at a similarity cut-off of 0.8. Representative sequences were
193 then used as query in BLASTP (multiple query function) searches using the National
194 Centre for Bioinformatics (NCBI) database (nr). The best hit was used to assign
195 taxonomy at the family level.

196 The metatranscriptomes used in this study are listed in Supplementary
197 Table S5. Metatranscriptomes deposited in the IMG/JGI database were used for a
198 BLASTP analysis with a stringency level of >40% similarity and a cut-off value of
199 e-20. Query sequences were identical to those used above and data normalized by the
200 length of RecA as described above.

201

202 **Results**

203 **Identification of a four-gene cluster in *R. pomeroyi* DSS-3**

204 *R. pomeroyi* can utilize TMA, DMA, and MMA as a sole nitrogen source
205 (Lidbury *et al.*, 2015). Therefore, a BLASTP analysis on *R. pomeroyi* was performed
206 to identify candidate genes involved in DMA catabolism using the three-gene cluster
207 identified as *dmmABC* (Msil_3607, Msil_3608, Msil_3609) from *M. silvestris* as the
208 query sequences (Zhu *et al.*, 2014). Three open reading frames (ORFs), Spo1580,
209 Spo1581, Spo1582 shared good homology with Msil_3607 (E-value, 4.0e-32;
210 38.92%), Msil_3608 (E-value, 4.0e-75; 41.07%), Msil_3609 (E-value, 4.20e-157;
211 62.24%), respectively (Figure 1b, c). Another ORF, Spo1579, found in an apparent
212 operon with the other three ORFs, shared homology with Msil_3605 (E-value,
213 2.0e-67; 35.75%), both of which contain a conserved tetrahydrofolate (H₄F)-binding
214 domain (GcvT). The GcvT domain is highly conserved in DmmD homologues (Zhu
215 *et al.*, 2014) and is also found in bacterial TMAO demethylase (Tdm) (Lidbury *et al.*,
216 2014). Spo1579, Spo1580, Spo1581, and Spo1582 are hereafter referred to as *dmmD*,
217 *dmmA*, *dmmB*, and *dmmC*, respectively. Unlike in *M. silvestris*, *dmmD* was always
218 co-located with *dmmABC* in the genomes of various MRC isolates screened
219 (Supplementary Table S3), suggesting that its expression is tightly coordinated to that
220 of *dmmABC*. Interestingly, *dmmDABC* was absent from the genome of *Candidatus*
221 *Pelagibacter ubique* HTCC1062 (Figure 1d), a member of the SAR11 clade that can
222 utilize TMA and MMA (Sun *et al.*, 2011).

223 **DmmABC forms a functional DMA monooxygenase**

224 To determine if all four subunits of Dmm were essential for DMA
225 demethylation, both *dmmDABC* and *dmmABC* were cloned into the expression vector

226 pET28a and transformed into *E. coli* BLR(DE3)pLysS. In the *E. coli* strain
227 harbouring *dmmABC*, complete degradation of DMA (1 mM) occurred within 8 h
228 whilst the concentration of MMA increased in a stoichiometric 1:1 manner (Figure 2).
229 In the *E. coli* strain harbouring *dmmDABC*, DMA degradation in accordance with
230 MMA production still occurred, albeit at a slower rate, again, stoichiometrically in a
231 1:1 ratio (Figure 2). In cultures complemented with the empty pET28a vector, no
232 DMA degradation and thus no MMA production was observed (Figure 2), while
233 cultures grew comparably (Supplementary Figure S1). Together, these results show
234 that the three-subunit cluster alone forms a functional Dmm.

235 ***dmmD* is essential for growth on DMA and other methylated amines in**
236 ***R. pomeroyi***

237 To determine the function of *dmmD* in *R. pomeroyi*, the gene was disrupted by
238 insertion of a gentamicin resistance marker (Dennis and Zylstra, 1998) and the
239 *dmmD::Gm* mutant subsequently grown on MAs including DMA as a sole nitrogen
240 source. Disruption of the *dmmD* gene resulted in an inability of the mutant to grow on
241 TMA, TMAO, or DMA as a sole nitrogen source (Figure 3a-c). However, growth on
242 MMA and NH_4^+ was unaffected (Figure 3d, Supplementary Figure S2a).
243 Complementation with *dmmD* did not restore growth in comparison to the wild type
244 (WT) (Figure 3a-c). *dmmDABC* forms a single operon and therefore deletion of
245 *dmmD* may have affected the downstream expression of *dmmABC*. When grown on
246 TMA and TMAO, the *dmmD* mutant accumulated DMA in the culture medium
247 revealing a bottleneck in the MA degradation pathway (Supplementary Figure S3).
248 However, when grown on DMA as the sole nitrogen source DMA degradation was
249 slightly enhanced by complementation (Figure 3c), suggesting that *dmmD* may be

250 required for DMA degradation in *R. pomeroyi*.

251 Due to the potential polar effect on *dmmABC* by deletion of *dmmD*, the *dmmD*
252 mutant was complemented with either the four-gene cluster *dmmDABC*
253 (*dmmD::Gm+dmmDABC*) or the three subunits of Dmm, *i.e.* *dmmABC*
254 (*dmmD::Gm+dmmABC*). To achieve this, these two gene clusters were cloned into the
255 broad-host range plasmid pBBR1MCS-km (Kovach *et al.*, 1995) together with the
256 putative promoter located at the 5' untranslated region upstream of *dmmD*. For the
257 *dmmD::Gm+dmmDABC* complemented mutant, growth on TMA and TMAO as a
258 sole nitrogen source was restored whilst for *dmmD::Gm+dmmABC*, missing an intact
259 *dmmD*, the complemented mutant failed to grow on either TMA or TMAO
260 (Figure 4a, b). Consequently, in the *dmmD::Gm+dmmABC* complemented mutant,
261 DMA accumulated in the medium as TMA or TMAO degradation occurred
262 (Supplementary Figure S4). However, both complemented strains could degrade and
263 subsequently grow on DMA, MMA, and NH_4^+ as sole nitrogen sources (Figure 4c, d,
264 Supplementary Figure S5a), suggesting that *dmmD* is essential for TMA and TMAO
265 degradation but not for growth on DMA or MMA in this bacterium.

266 **The distribution of DmmDABC in marine bacterial genomes and metagenomes**

267 The distribution of genes encoding DmmDABC was investigated using
268 BLASTP analysis among marine bacterial genomes deposited in the Integrated
269 Microbial Genomes database of the Joint Genome Institute (IMG/JGI). In parallel, the
270 distribution of genes encoding the other enzymes required for growth on MAs (e.g.
271 Tmm, Tdm, TmoX, and GmaS) was also determined using *R. pomeroyi* homologs as
272 the query sequences. The *dmmDABC* gene cluster was identified in 30 isolates related
273 to *Alphaproteobacteria* and 6 related to *Gammaproteobacteria* (Figure 5a). The

274 majority of *Alphaproteobacteria* homologs were related to the MRC (27/30). In
275 addition, *dmmDABC* homologs were retrieved from *Candidatus Puniceispirillum*
276 *marinum* IMCC1322 (IMCC1132), a member of the cosmopolitan SAR116 clade (Oh
277 *et al.*, 2010; Giovannoni and Vergin, 2012) and clustered with the MRC homologs
278 suggesting horizontal gene transfer has occurred (Figure 5a). A number of *dmmDABC*
279 homologs were also found in the genomes of largely uncultivated pelagic *Roseobacter*
280 (Figure 5a, Supplementary Table S3), some of which have been previously reported
281 to possess features of a free-living life-style (e.g. *Rhodobacterales* sp. HTCC2255)
282 (Billerbeck *et al.*, 2016; Zhang *et al.*, 2016). Notably, all representatives of the
283 *Pelagibacterales* (SAR11 clade) lack homologs of the genes encoding DmmDABC
284 (Figure 5, Supplementary Table S3), whereas genes encoding GmaS, Tmm, Tdm, and
285 TmoX were ubiquitous within the genomes of strains related to this clade (Figure 5b,
286 Supplementary Table S3).

287 Previous studies have shown that *tmm*, *tdm*, and *gmaS* are abundant in marine
288 metagenomes primarily due to their occurrence in SAR11 clade bacteria (Chen *et al.*,
289 2011; Lidbury *et al.*, 2014). We hypothesized that the abundance of *dmmDABC* in
290 marine metagenomes would be lower than that of *tmm*, *tdm*, and *gmaS*, reflecting
291 their absence from the genomes of SAR11 clade bacteria. To test this hypothesis, a
292 number of metagenomes deposited in the IMG/JGI database, predominantly from the
293 global ocean sampling (GOS) expedition (Rusch *et al.*, 2007) were screened
294 (stringency, e-50) for the presence of *dmmDABC* as well as *tmm*, *tdm*, *tmoX*, and
295 *gmaS* using the *R. pomeroyi* homologs as the query sequences. To determine the
296 percentage of marine bacteria possessing MA degradation genes present at each site,
297 counts were normalized against the average counts of two single copy genes (*recA*
298 and *gyrB*). As expected *tmm*, *tdm*, *tmoX*, and *gmaS* were present in 20-25% of marine

299 bacteria (Figure 6a). However, *dmmDABC* was found at a much lower abundance
300 (Figure 6a, Supplementary Table S4). To rule out the possibility that the under-
301 representation of *dmmDABC* genes in marine metagenomes was due to the use of a
302 high stringency cut-off value (e-50), we re-analysed metagenomes from the GOS
303 dataset with a range of stringency thresholds (e-40, e-20, e-10, e-8) and the number of
304 hits related to *dmmDABC* did not increase relative to that of *tmm* and *tdm*
305 (Supplementary Figure S6). *dmmDABC* were also retrieved from metagenomes
306 associated with high primary productivity, e.g. a photosynthetic picoeukaryote bloom
307 in the Norwegian Sea (IMG genome ID 3300002186), albeit at a lower abundance
308 than other MA-degrading genes (Supplementary Table S4). Phylogenetic analysis
309 revealed that *dmmDABC* sequences retrieved from marine metagenomes were
310 primarily related to the MRC (Figure 6b). It should be noted that several *tmm* and *tdm*
311 sequences were related to the newly identified gammaproteobacterium, *Candidatus*
312 *Thioglobus singularis* (Marshall and Morris, 2015). A similar pattern was also
313 observed when scrutinizing metatranscriptomes (Supplementary Table S5). No
314 transcripts related to *dmmDABC* could be detected from various open ocean and
315 coastal ocean waters, whilst transcripts related to various other genes involved in the
316 MA degradation pathway (*tmm*, *tdm*, *gmaS*, or *tmoX*) were readily detected
317 (Supplementary Table S5, Ottesen *et al.*, 2011; 2013; Gifford *et al.*, 2013).
318

319 Discussion

320 Recently, the genes involved in DMA degradation were identified in
321 methylotrophic soil bacteria (Zhu *et al.*, 2014; Dziewit *et al.*, 2015). However, neither
322 study conclusively demonstrated the functionality of Dmm at the protein level. By
323 identifying *R. pomeroyi dmmDABC* homologs similar to those found in *M. silvestris*
324 and *P. aminophilus* we were able to confirm that *dmmABC* does indeed encode a
325 functional Dmm, an enzyme originally described in *A. aminovorans* (Alberta and
326 Dawson, 1987). In both *M. silvestris* and *P. aminophilus*, *dmmD* was not essential for
327 growth on MAs, but disruption of this gene did affect their growth rates on TMA,
328 DMA, and TMAO (the latter substrate was shown for *M. silvestris* only) (Zhu *et al.*,
329 2014; Dziewit *et al.*, 2015). These findings, alongside the data presented here
330 (Figures 2-4), further suggest that *dmmD* is required for normal growth on MAs.
331 Since DmmD possesses a H₄F-binding domain, its primary role is likely to be
332 involved in the conjugation of free formaldehyde, released from the demethylation of
333 DMA, with the one carbon (C1) carrier molecule H₄F (Zhu *et al.*, 2014). Unlike
334 *M. silvestris* and *P. aminophilus*, marine bacteria only possess the genes for C1
335 oxidation via the H₄F pathway, lacking the genes required for C1 oxidation through
336 either the tetrahydromethanopterin (H₄MPT), glutathione (GSH)-linked pathway or
337 the formaldehyde activating enzyme (Fae) (Chistoserdova, 2011; Dziewit *et al.*,
338 2015). Thus, there is a greater dependency of the H₄F-linked C1 oxidation pathway to
339 deal with formaldehyde stress. The consistently tight genetic arrangement of
340 *dmmDABC* in marine bacteria coupled with the non-essential function of *dmmD* in
341 DMA or MMA degradation further strengthens the hypothesis that *dmmD* serves a
342 key role in reducing formaldehyde toxicity. Furthermore, conjugation with H₄F also
343 allows the C1 unit to be fully oxidized to CO₂ and thus generate reducing power

344 (Lidbury *et al.*, 2015).

345 The absence of *dmmDABC* from members of the SAR11 clade as well as
346 abundant marine *Gammaproteobacteria* and *Deltaproteobacteria* is intriguing.
347 *C. Pelagibacter* ubique HTCC1062 has been shown to oxidize TMA, TMAO, and
348 MMA in order to generate ATP (Sun *et al.*, 2011). However, currently there is no
349 evidence that this bacterium or any other member of the SAR11 clade can oxidize
350 DMA. Furthermore, there is no evidence that SAR11 clade bacteria can grow on MAs
351 as a source of nitrogen, which would require the complete demethylation of MAs,
352 including DMA (Lidbury *et al.*, 2015). During N-limitation *C. Pelagibacter* ubique
353 HTCC1062 does express a protein that is predicted to be a general amine oxidase
354 (Smith *et al.*, 2013), but its role in DMA oxidation has not been confirmed
355 experimentally. In contrast to the *Pelagibacteriales*, *dmmDABC* is found in pelagic
356 *Roseobacters* (Figure 5a, Supplementary Table S3), thus, ruling out an affiliation of
357 its absence with a pelagic life style. Representatives possessing the *dmm* genes have
358 been found in the streamlined, largely non-cultivated pelagic *Roseobacter* lineages
359 DC5-80-3 and NAC11-7 (Zhang *et al.*, 2016), while the other globally abundant
360 pelagic *Roseobacter* CHAB-I-5 lineage (Billerbeck *et al.*, 2016; Zhang *et al.*, 2016)
361 only shows genetic evidence for oxidation of TMA, TMAO, and MMA, but not DMA
362 (*i.e.* no *dmm* genes found in their genomes).

363 The flux of MAs from surface seawaters is important since these compounds
364 can lead to the formation of aerosols and thus CCN (Ge *et al.*, 2011). Due to the
365 scarcity of labile organic nitrogen in marine surface waters, biological consumption of
366 MAs as a nitrogen source is likely to be a major limitation on the air-sea exchange of
367 these compounds (Balch, 1985; Carpenter *et al.*, 2012; Chen, 2012). In addition,

368 *R. pomeroyi* and *C. Pelagibacter* ubiquely rapidly turn over MAs as an energy source
369 (Sun *et al.*, 2011; Lidbury *et al.*, 2015), further reducing the amount of MAs available
370 for air-sea exchange. The lack of *dmmDABC* homologs relative to other MA-
371 degradation genes (*tmm*, *tdm*, *gmaS*) in marine metagenomes suggests that DMA may
372 accumulate in surface waters and therefore be susceptible to a greater amount of air-
373 sea exchange. In support of this hypothesis, besides methanesulfonic acid (MSA),
374 DMA amine salts were often the most abundant SOAs detected in fine marine
375 particles at sites located in the North and Tropical Atlantic Ocean (Facchini *et al.*,
376 2008; Müller *et al.*, 2009). In these studies, a link between elevated concentrations of
377 amine-derived SOAs detected in fine marine particles and elevated levels of primary
378 production was observed and thought to be of biological origin. In another study, a
379 shift towards amine-derived SOAs and the subsequent accumulation of CCN was
380 correlated with elevated periods of primary production (Sorooshian *et al.*, 2009). In
381 this context, metagenomic data collected during a photosynthetic picoeukaryote
382 bloom in the Norwegian Sea revealed that *dmmDABC* homologs were substantially
383 reduced (5.95% of total bacteria) compared to those of *tmm*, *tdm*, and *gmaS* (42.83%
384 of total bacteria) (Supplementary Table S4). Similarly, in the North Sea where
385 members of the MRC are often numerically abundant during phytoplankton blooms
386 (Teeling *et al.* 2012; Wemheuer *et al.* 2015), *dmmDABC* homologs were again under-
387 represented (6% of total bacteria) relative to other MA degradation genes (21% of
388 total bacteria) (an average of 41 metagenomes, Supplementary Table S4). Therefore,
389 a lack of DMA-degrading bacteria relative to other MA-degrading bacteria in the
390 euphotic zone, especially during periods of elevated primary production, may be an
391 explanation for the higher abundance of DMA-containing SOAs.

392 In conclusion, this study has confirmed the genes and enzyme catalysing

393 DMA degradation in marine bacteria and revealed a potential bottleneck in the MA
394 degradation pathway in surface seawaters. We propose that this metabolic bottleneck
395 likely explains the elevated abundance of DMA-derived amine salts detected in fine
396 marine particles. Further research on the environmental cycling of MAs, especially
397 DMA, is required to better understand the air-sea exchange of these climatically
398 important compounds.

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402 with the construction of the *dmmD::Gm* mutant used in this study.

403 **Conflict of Interest**

404 The authors declare no conflict of interest.

405

406 Supplementary Information is available at The ISME Journal's website.

407

408

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587

588 **Figure Legends**

589 **Figure 1.** Scheme of (a) the proposed pathway of methylated amine (MA) catabolism
590 in *Ruegeria pomeroyi* DSS-3 and related marine *Roseobacter* clade (MRC) bacteria
591 and (b) genomic regions encompassing the genes (*dmmDABC*) encoding the DMA
592 monooxygenase (Dmm) in *Ruegeria pomeroyi* DSS-3, and (c) *Methylocella silvestris*
593 BL2. (d) *Candidatus Pelagibacter ubique* HTCC1062 does not possess *dmmDABC* in
594 its genome despite containing all other genes required for TMA, TMAO, and MMA
595 degradation. *amtB*, ammonia transporter gene B; CH₂=H₄F, 5,10-methylene
596 tetrahydrofolate; DMA, dimethylamine; DmmA, DmmB, DmmC, DmmD, DMA
597 monooxygenase subunit A, B, C, or D; GMA, gamma-glutamylmethylamide; GmaS,
598 gamma-glutamylmethylamide synthetase; MgdABCD, *N*-methylglutamate
599 dehydrogenase; MgsABC, *N*-methylglutamate synthase; MMA, monomethylamine;
600 NMG, *N*-methylglutamate; Tdm, trimethylamine *N*-oxide demethylase; TMA,
601 trimethylamine; TMAO, trimethylamine *N*-oxide; Tmm, trimethylamine
602 monooxygenase; *tmoP*, TMAO permease gene, TmoXVW, ATP-dependent TMAO
603 transporter.

604

605 **Figure 2.** Assessment of (a) DMA degradation and (b) MMA accumulation in
606 recombinant *E. coli* following heterologous expression of either the complete
607 *dmmDABC* gene cluster from *R. pomeroyi* or just the structural genes (+ *dmmABC*),
608 or of the expression vector pET28a as a negative control (C-). Results presented are
609 the mean of triplicates and error bars denote standard deviation.

610

611 **Figure 3.** Growth of *R. pomeroyi* DSS-3 wild-type (WT), *dmmD* mutant
612 (*dmmD::Gm*), and its complementation with *dmmD* (*dmmD::Gm+dmmD*) on (a)

613 TMA, (b) TMAO, (c) DMA, and (d) MMA as the sole nitrogen source. Solid lines
614 represent cell growth. Dashed lines represent the degradation of the appropriate
615 substrate with the concentrations of TMA, TMAO, DMA, and MMA being quantified
616 throughout the whole experiment. Results presented are the mean of triplicates and
617 error bars denote standard deviation.

618

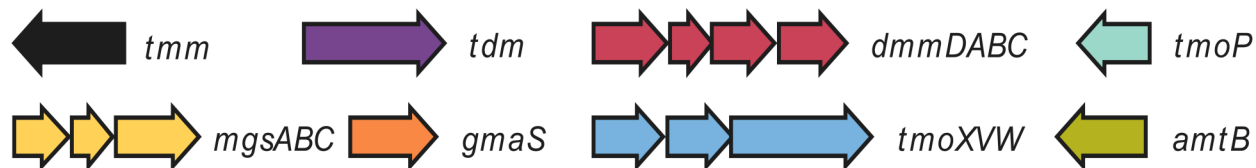
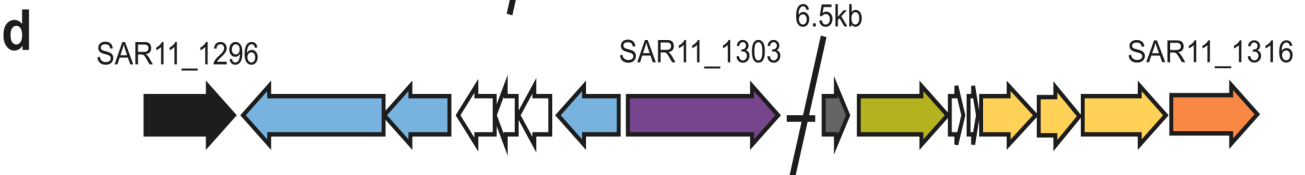
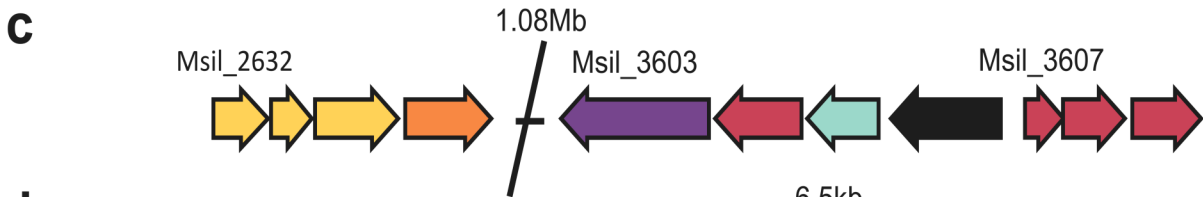
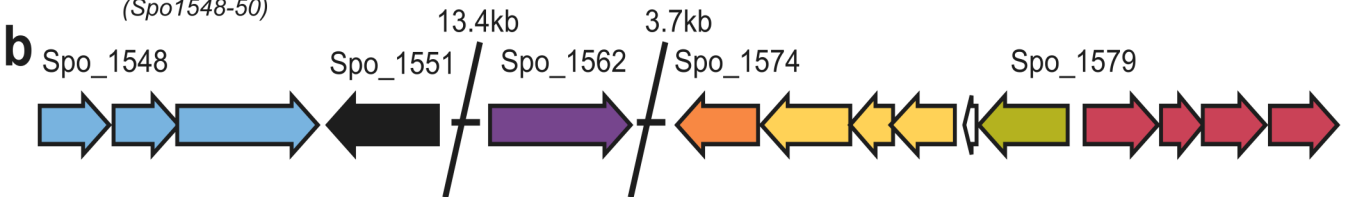
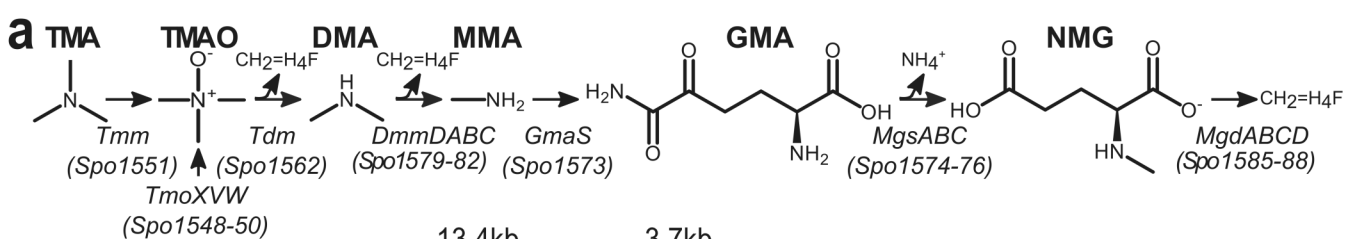
619 **Figure 4.** Growth of *R. pomeroyi* DSS-3 wild-type (WT), and the *dmmD* mutant
620 (*dmmD::Gm*) complemented with either the four-gene cluster *dmmDABC*
621 (*dmmD::Gm+dmmDABC*) or only the structural genes *dmmABC*
622 (*dmmD::Gm+dmmABC*) along with the promoter on different nitrogen sources.
623 Nitrogen was supplied in the form of (a) TMA, (b) TMAO, (c) DMA, and (d) MMA.
624 Solid lines represent cell growth. Dashed lines represent the degradation of the
625 appropriate substrate with the concentrations of TMA, TMAO, DMA, and MMA
626 being quantified throughout the whole experiment. Results presented are the mean of
627 triplicates and error bars denote standard deviation.

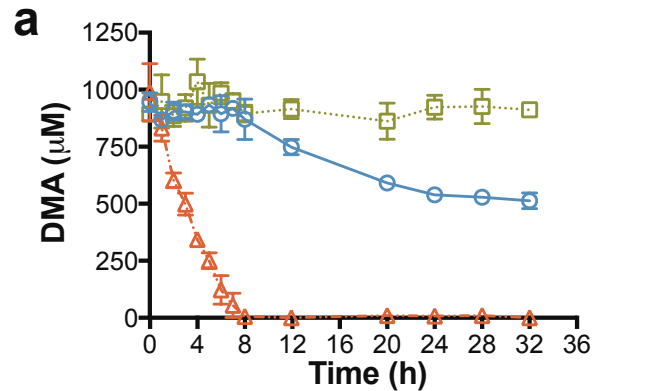
628

629 **Figure 5.** Distribution of genes for MA metabolism in marine bacterial isolates. (a)
630 Maximum likelihood phylogenetic tree of *dmmDABC* homologues in marine bacterial
631 isolates. For each node bootstrap values (999 replicates) greater than 50% are given.
632 MRC are marked in orange. An asterisk indicates pelagic *Roseobacter*, with the
633 affiliation of two representatives to the largely uncultivated pelagic *Roseobacter*
634 lineages according to Zhang *et al.* (2016) given in brackets. (b) Phylogenetic
635 distribution of the genes encoding the enzymes involved in MA metabolism. TmoX,
636 substrate-binding protein of the TMAO transporter, other abbreviations are as
637 described in Figure 1.

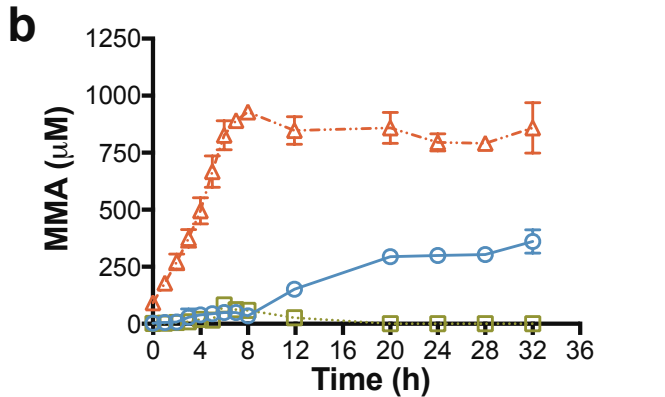
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639 **Figure 6.** Distribution of genes encoding proteins for MA metabolism in selected
640 marine metagenomes. **(a)** Abundance of selected genes in marine bacteria, and **(b)**
641 their phylogenetic affiliation. In the box-whisker plot whiskers represent the 5 and 95
642 percentiles and the line corresponds to the median. Circles represent outliers with all
643 high-range outliers of Tmm, Tdm, GmaS, and TmoX deriving from the same
644 Sargasso Sea metagenome sample (Supplementary Table S4). The phylogenetic
645 composition represents the normalised relative abundances of MA-degrading genes
646 using metagenomes primarily retrieved from the global ocean sampling (GOS) dataset
647 (Rusch *et al.*, 2007) (see Supplementary Table S4). Abbreviations are as described in
648 Figure 1.





—○— + *dmmDABC* DMA —△— + *dmmABC* DMA —□— C- DMA



—○— + *dmmDABC* MMA —△— + *dmmABC* MMA —□— C- MMA

