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

Ian Lidbury[1*], Michaela A Mausz[1*], David J Scanlan[1], Yin Chen[1¶]

[1] School of Life Sciences, Gibbet Hill Campus, University of Warwick, Coventry, CV4 7AL, United Kingdom

[*] These authors contributed equally to this study.

[¶] Correspondence to Y Chen, School of Life Sciences, University of Warwick, CV4 7AL, Coventry, United Kingdom Email: Y.chen.25@warwick.ac.uk Phone: 00442476528976;

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Conflict of Interest

The authors declare no conflict of interest.

Abstract

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

Introduction

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

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

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

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

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

Materials and methods

Bacterial cultivation

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

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

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

Mutagenic analysis and mutant complementation in *R. pomeroyi*

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

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

To complement the *dmmD::Gm* with *dmmDABC* plus its native promoter, the entire gene cluster was amplified introducing the restriction sites *Xba*I and *Kpn*I at the 5' and 3' ends, respectively. For complementation with the structural genes *dmmABC*, the promoter alone was amplified introducing the restriction sites *Xba*I and *Hin*dIII at the 5' and 3' ends, respectively. In addition, *dmmABC* was amplified introducing the restriction sites *Hin*dIII and *Kpn*I at the 5' and 3' ends, respectively. For complementation using just *dmmD*, this gene (Spo1579) plus the promoter were amplified introducing the restriction sites *Bam*HI and *Hin*dIII at the 5' and 3' end, respectively. All PCR fragments were subcloned into the pGEM-T EASY vector. Sequence integrity was checked prior to cloning the construct into the broad-host range plasmid pBBR1MCS-km (Kovach *et al.*, 1995) and mobilized into *dmmD::Gm* via conjugation as before. Transconjugants were selected by growth on half-strength Yeast Tryptone Sea Salts (1/2 YTSS) media (DMSZ) containing 80 μg ml^{-1} kanamycin and 10 μg ml^{-1} gentamicin. Complementation was confirmed by PCR and sequencing.

Quantification of methylated amines

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

Analysis of enzymes involved in MA metabolism in sequenced marine microbial genomes

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

Analysis of enzymes involved in MA metabolism in marine metagenomes and metatranscriptomes

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

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

Results

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

R. pomeroyi can utilize TMA, DMA, and MMA as a sole nitrogen source (Lidbury *et al.*, 2015). Therefore, a BLASTP analysis on *R. pomeroyi* was performed to identify candidate genes involved in DMA catabolism using the three-gene cluster identified as *dmmABC* (Msil_3607, Msil_3608, Msil_3609) from *M. silvestris* as the query sequences (Zhu *et al.*, 2014). Three open reading frames (ORFs), Spo1580, Spo1581, Spo1582 shared good homology with Msil_3607 (E-value, 4.0e-32; 38.92%), Msil_3608 (E-value, 4.0e-75; 41.07%), Msil_3609 (E-value, 420e-157; 62.24%), respectively (Figure 1b, c). Another ORF, Spo1579, found in an apparent operon with the other three ORFs, shared homology with Msil_3605 (E-value, 2.0e-67; 35.75%), both of which contain a conserved tetrahydrofolate (H_4F)-binding domain (GcvT). The GcvT domain is highly conserved in DmmD homologues (Zhu *et al.*, 2014) and is also found in bacterial TMAO demethylase (Tdm) (Lidbury *et al.*, 2014). Spo1579, Spo1580, Spo1581, and Spo1582 are hereafter referred to as *dmmD, dmmA, dmmB*, and *dmmC*, respectively. Unlike in *M. silvestris*, *dmmD* was always co-located with *dmmABC* in the genomes of various MRC isolates screened (Supplementary Table S3), suggesting that its expression is tightly coordinated to that of *dmmABC.* Interestingly, *dmmDABC* was absent from the genome of *Candidatus* Pelagibacter ubique HTCC1062 (Figure 1d), a member of the SAR11 clade that can utilize TMA and MMA (Sun *et al.*, 2011).

DmmABC forms a functional DMA monooxygenase

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

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

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

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

required for DMA degradation in *R. pomeroyi*.

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

The distribution of DmmDABC in marine bacterial genomes and metagenomes

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

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

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

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

Discussion

Recently, the genes involved in DMA degradation were identified in methylotrophic soil bacteria (Zhu *et al.*, 2014; Dziewit *et al.*, 2015). However, neither study conclusively demonstrated the functionality of Dmm at the protein level. By identifying *R. pomeroyi dmmDABC* homologs similar to those found in *M. silvestris* and *P. aminophilus* we were able to confirm that *dmmABC* does indeed encode a functional Dmm, an enzyme originally described in *A. aminovorans* (Alberta and Dawson, 1987). In both *M. silvestris* and *P. aminophilus*, *dmmD* was not essential for growth on MAs, but disruption of this gene did affect their growth rates on TMA, DMA, and TMAO (the latter substrate was shown for *M. silvestris* only) (Zhu *et al.*, 2014; Dziewit *et al.*, 2015). These findings, alongside the data presented here (Figures 2-4), further suggest that *dmmD* is required for normal growth on MAs. Since DmmD possesses a H_4F-binding domain, its primary role is likely to be involved in the conjugation of free formaldehyde, released from the demethylation of DMA, with the one carbon (C1) carrier molecule H_4F (Zhu *et al.*, 2014). Unlike *M. silvestris* and *P. aminophilus*, marine bacteria only possess the genes for C1 oxidation via the H_4F pathway, lacking the genes required for C1 oxidation through either the tetrahydromethanopterin (H_4MPT), glutathione (GSH)-linked pathway or the formaldehyde activating enzyme (Fae) (Chistoserdova, 2011; Dziewit *et al.*, 2015). Thus, there is a greater dependency of the H_4F-linked C1 oxidation pathway to deal with formaldehyde stress. The consistently tight genetic arrangement of *dmmDABC* in marine bacteria coupled with the non-essential function of *dmmD* in DMA or MMA degradation further strengthens the hypothesis that *dmmD* serves a key role in reducing formaldehyde toxicity. Furthermore, conjugation with H_4F also allows the C1 unit to be fully oxidized to CO_2 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 *Pelagibacterales*, *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 ubique 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

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

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Conflict of Interest

The authors declare no conflict of interest.

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

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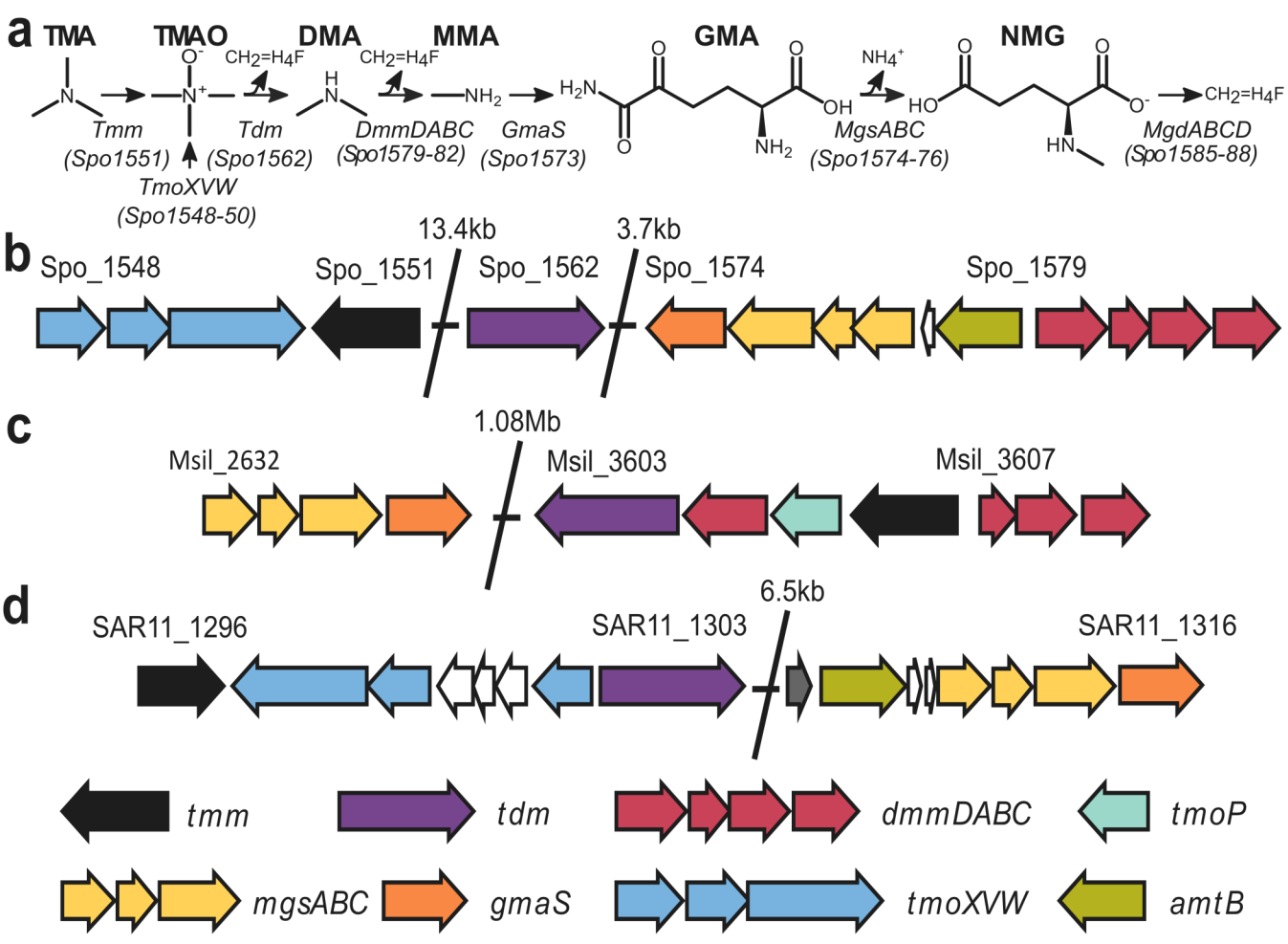
588 **Figure Legends**

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; CH_2=H_4F, 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.

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


a
TMA
TMAO
DMA
MMA
GMA
NMG
CH2=H4F
NH4+
Tmm
(Spo1551)
TmoXVW
(Spo1548-50)
Tdm
(Spo1562)
DmmDABC
(Spo1579-82)
GmaS
(Spo1573)
MgsABC
(Spo1574-76)
MgdABCD
(Spo1585-88)
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Spo_1579
c
1.08Mb
Msil_2632
Msil_3603
Msil_3607
d
6.5kb
SAR11_1296
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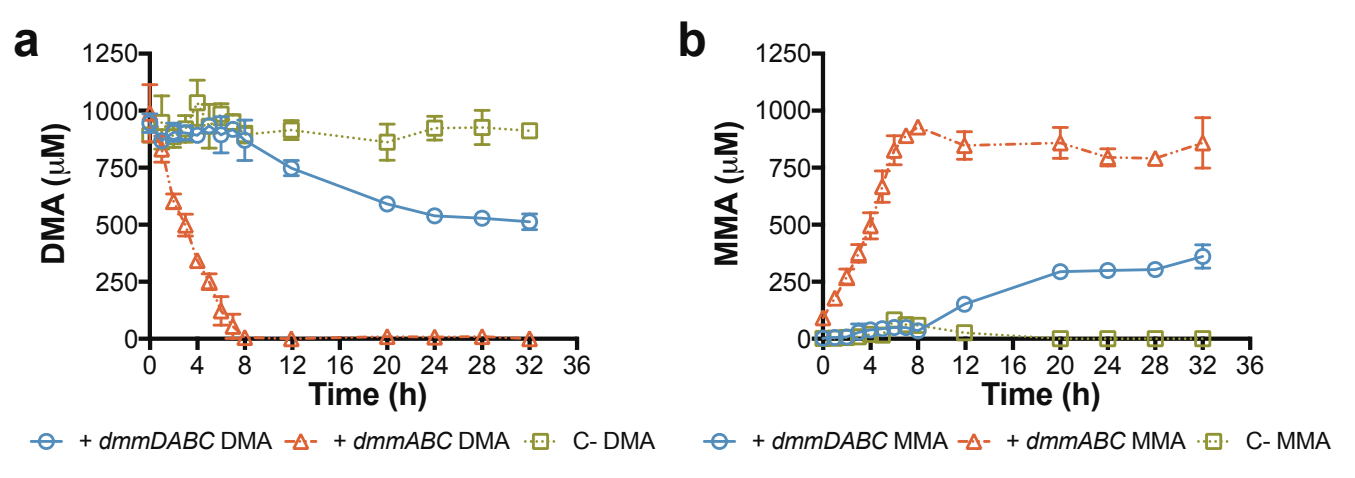
a
DMA (μM)
1250
1000
750
500
250
0
0 4 8 12 16 20 24 28 32 36
Time (h)
+ dmmDABC DMA
+ dmmABC DMA
C- DMA
b
MMA (μM)
1250
1000
750
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250
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Time (h)
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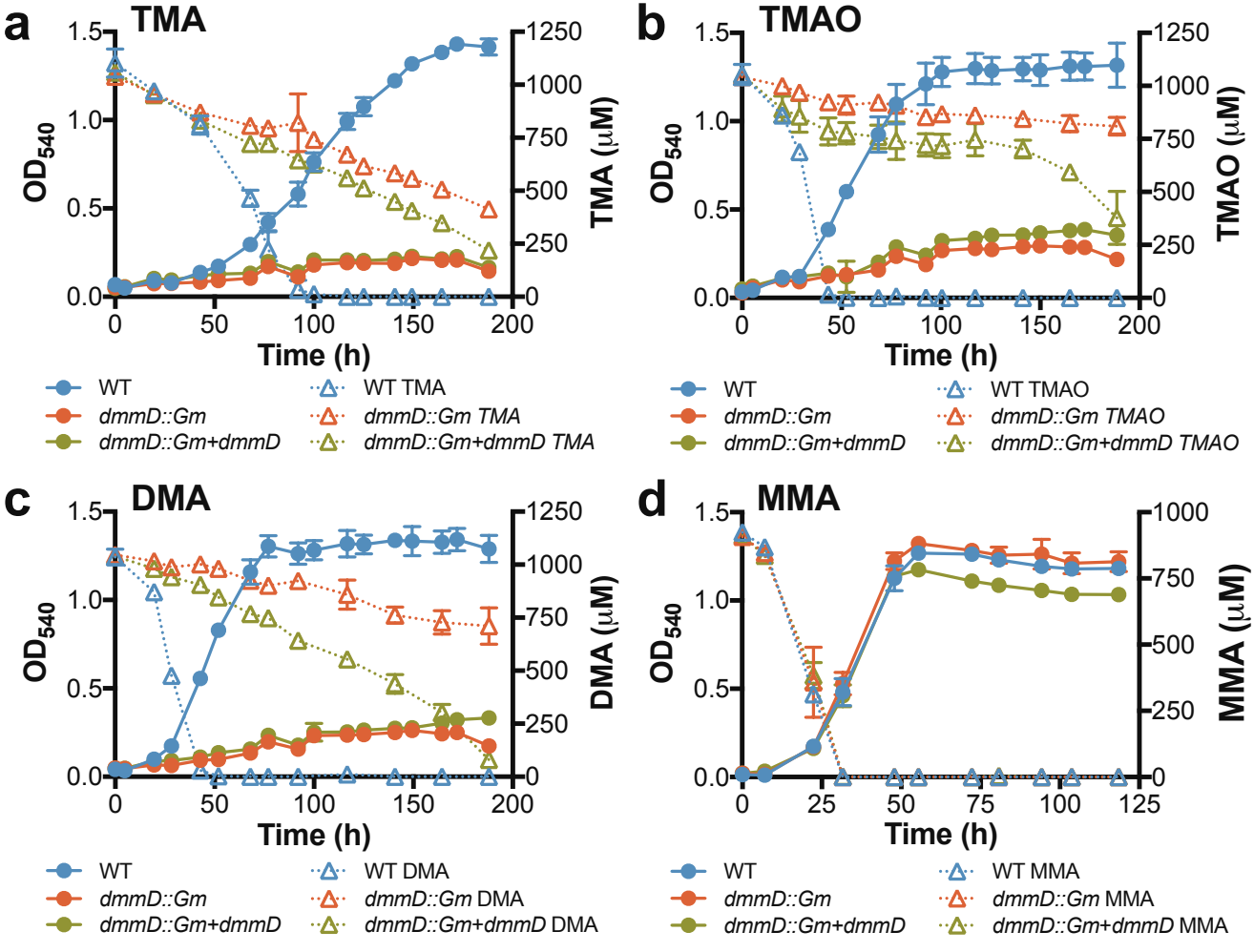
+ dmmABC MMA
C- MMA


a
TMA
OD540
TMA (μM)
Time (h)
WT
dmmD::Gm
dmmD::Gm+dmmD
WT TMA
dmmD::Gm TMA
dmmD::Gm+dmmD TMA
b
TMAO
OD540
TMAO (μM)
Time (h)
WT
dmmD::Gm
dmmD::Gm+dmmD
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c
DMA
OD540
DMA (μM)
Time (h)
WT
dmmD::Gm
dmmD::Gm+dmmD
WT DMA
dmmD::Gm DMA
dmmD::Gm+dmmD DMA
d
MMA
OD540
MMA (μM)
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dmmD::Gm+dmmD
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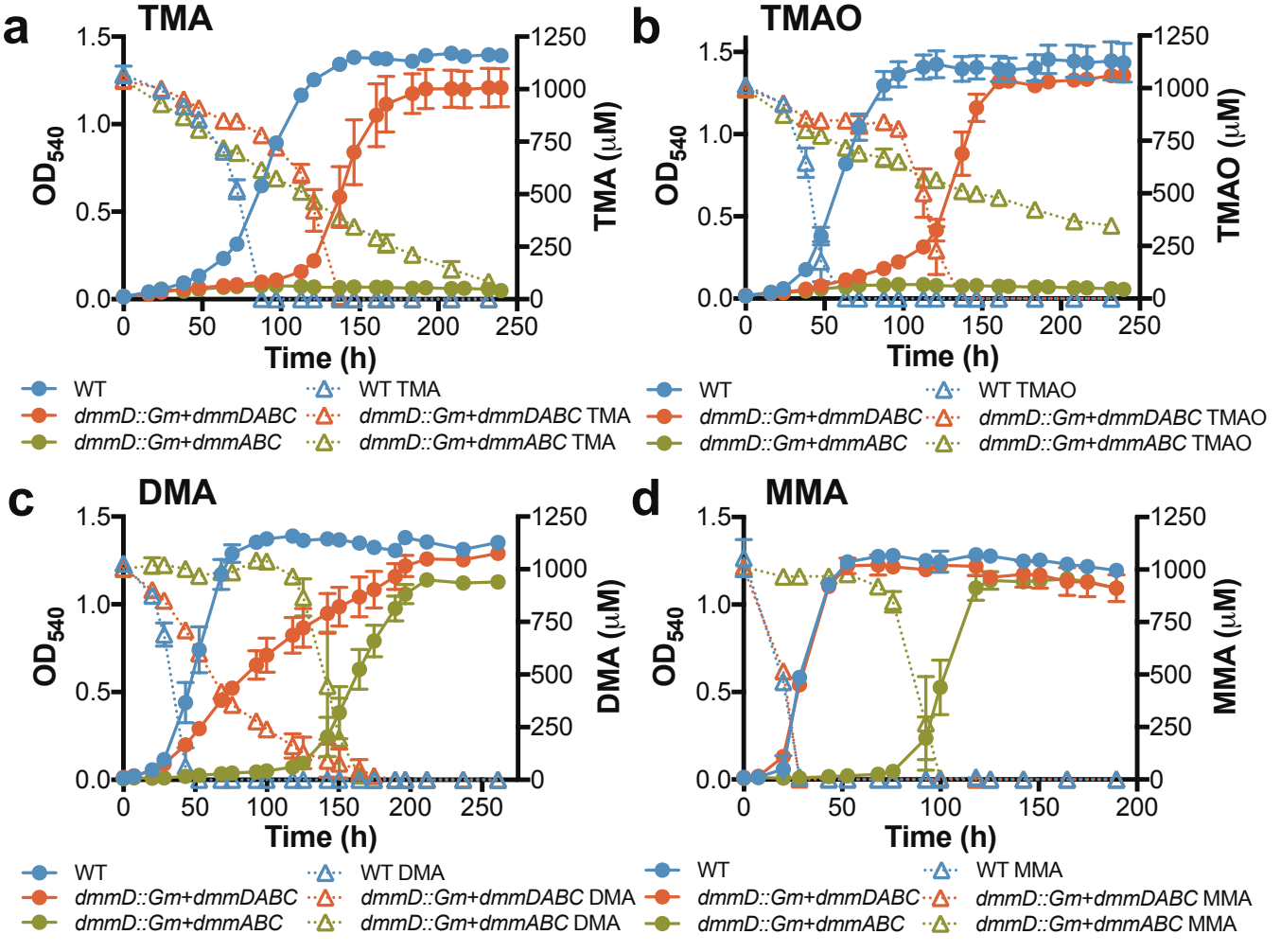
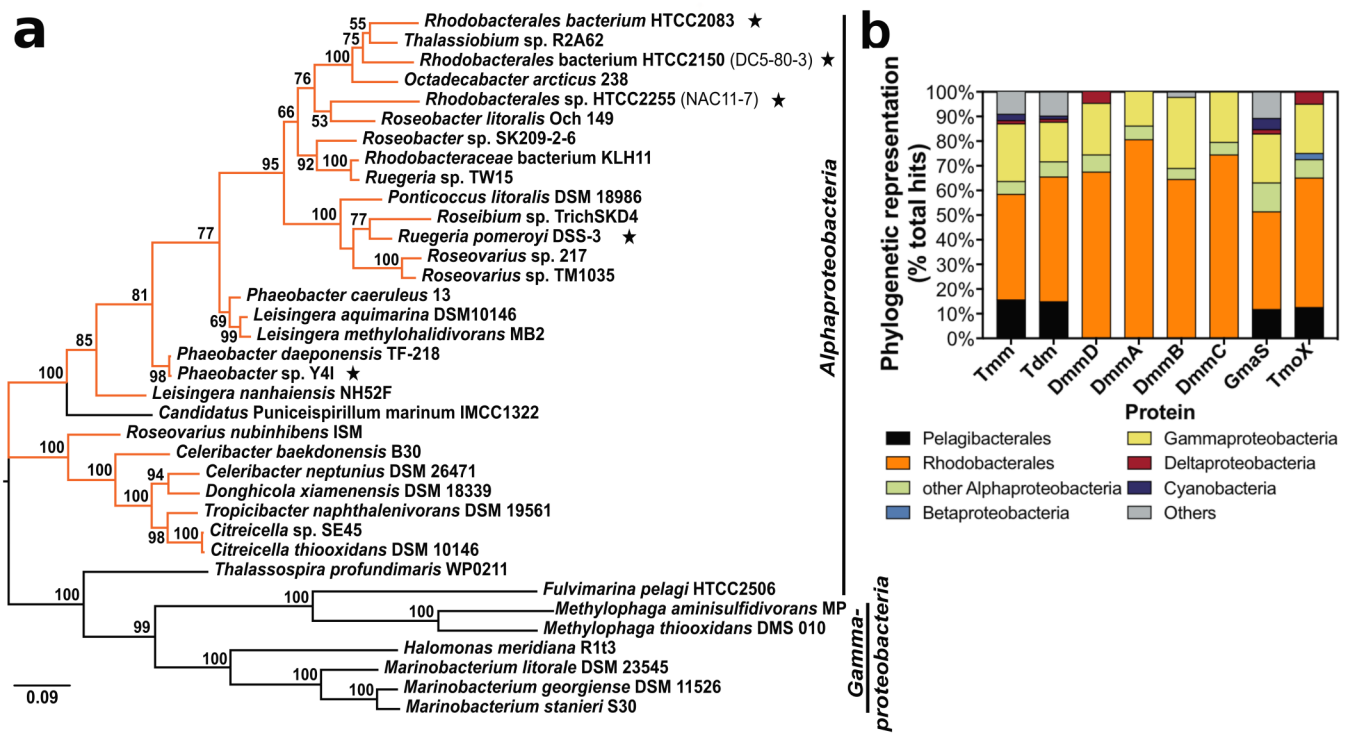
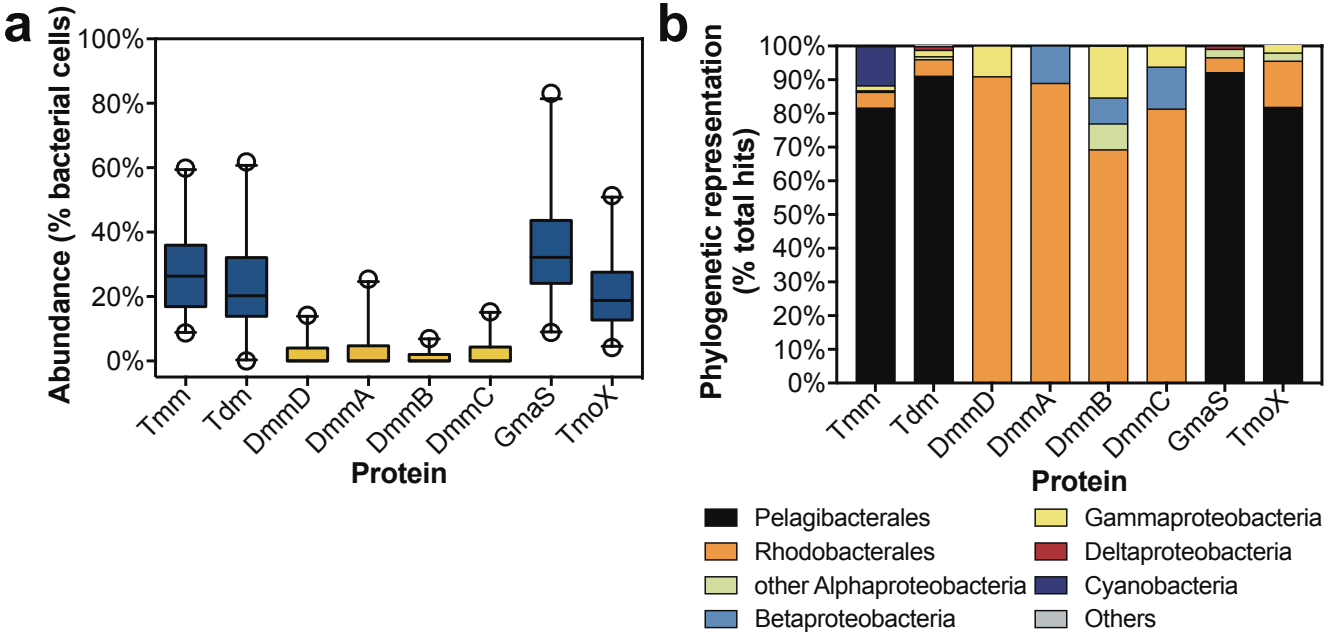
dmmD::Gm+dmmD MMA


a
TMA
OD540
TMA (μM)
Time (h)
WT
dmmD::Gm+dmmDABC
dmmD::Gm+dmmABC
WT TMA
dmmD::Gm+dmmDABC TMA
dmmD::Gm+dmmABC TMA
b
TMAO
OD540
TMAO (μM)
Time (h)
WT
dmmD::Gm+dmmDABC
dmmD::Gm+dmmABC
WT TMAO
dmmD::Gm+dmmDABC TMAO
dmmD::Gm+dmmABC TMAO
c
DMA
OD540
DMA (μM)
Time (h)
WT
dmmD::Gm+dmmDABC
dmmD::Gm+dmmABC
WT DMA
dmmD::Gm+dmmDABC DMA
dmmD::Gm+dmmABC DMA
d
MMA
OD540
MMA (μM)
Time (h)
WT
dmmD::Gm+dmmDABC
dmmD::Gm+dmmABC
WT MMA
dmmD::Gm+dmmDABC MMA
dmmD::Gm+dmmABC MMA


a
Rhodobacterales bacterium HTCC2083 ★
Thalassiobium sp. R2A62
Rhodobacterales bacterium HTCC2150 (DC5-80-3) ★
Octadecabacter arcticus 238
Rhodobacterales sp. HTCC2255 (NAC11-7) ★
Roseobacter litoralis Och 149
Roseobacter sp. SK209-2-6
Rhodobacteraceae bacterium KLH11
Ruegeria sp. TW15
Ponticoccus litoralis DSM 18986
Roseibium sp. TrichSKD4
Ruegeria pomeroyi DSS-3 ★
Roseovarius sp. 217
Roseovarius sp. TM1035
Phaeobacter caeruleus 13
Leisingera aquimarina DSM10146
Leisingera methylohalidivorans MB2
Phaeobacter daeponensis TF-218
Phaeobacter sp. Y4I ★
Leisingera nanhaiensis NH52F
Candidatus Puniceispirillum marinum IMCC1322
Roseovarius nubinhibens ISM
Celeribacter baekdonensis B30
Celeribacter neptunius DSM 26471
Donghicola xiamenensis DSM 18339
Tropicibacter naphthalenivorans DSM 19561
Citreicella sp. SE45
Citreicella thiooxidans DSM 10146
Thalassospira profundimaris WP0211
Fulvimarina pelagi HTCC2506
Methylophaga aminisulfidivorans MP
Methylophaga thiooxidans DMS 010
Halomonas meridiana R1t3
Marinobacterium litorale DSM 23545
Marinobacterium georgiense DSM 11526
Marinobacterium stanieri S30
0.09
Alphaproteobacteria
Gamma-proteobacteria
b
Phylogenetic representation (% total hits)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Tmm
Tdm
DmmD
DmmA
DmmB
DmmC
GmaS
TmoX
Protein
Pelagibacterales
Rhodobacterales
other Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Cyanobacteria
Others


a
Abundance (% bacterial cells)
100%
80%
60%
40%
20%
0%
Tmm
Tdm
DmmD
DmmA
DmmB
DmmC
GmaS
TmoX
Protein
b
Phylogenetic representation (% total hits)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Tmm
Tdm
DmmD
DmmA
DmmB
DmmC
GmaS
TmoX
Protein
Pelagibacterales
Rhodobacterales
other Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Deltaproteobacteria
Cyanobacteria
Others