Supporting Information

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SI Materials and Methods

Construction of Marker-Exchange Mutants in *Ruegeria pomeroyi* DSS-3. To construct a TMAO demethylase (*tdm*) mutant, we amplified a region toward the 5′end (with PstI/EcoRI and XbaI sites engineered in) and a region toward the 3′ end (with a HindIII and XbaI engineered in) of the target gene (SPO1562). The two regions were subcloned, along with a gentamycin gene cassette, amplified from p34S-Gm (1), and inserted at an XbaI site between the two regions, into the cloning vector pGEM-T (Promega). The entire construct was ligated into the suicide vector pK18mobsacB at sites PstI and HindIII. The plasmid was transformed into *Escherichia coli* S17.1 via electroporation and mobilized into *R. pomeroyi* via conjugation, using 1/2 YTSS as the medium (DSMZ). Transconjugants were selected for on the sea salts minimal medium as described in ref. 2 with gentamicin (10 $\mu g \cdot mL^{-1}$) and monomethylamine (MMA) (3 mM) as a sole N source. Double-crossover mutants were selected by their sensitivity to kanamycin and homologous recombination was confirmed by PCR and DNA sequencing.

To construct a TMA monooxygenase (*tmm*) mutant, a 770-bp region of SPO1551 (*tmm*) was amplified by PCR (primers used are listed in Table S4) and subsequently cloned into pK18mobsacB via XbaI and HindIII sites. A gentamycin gene cassette was released from plasmid p34S-Gm using SalI, which was then inserted into pK18mobsacB. The resulting plasmid was transformed into *E. coli* S17.1 via electroporation and mobilized into *R. pomeroyi* DSS-3 via conjugation. Double-crossover mutants were selected as described above and confirmed by PCR and DNA sequencing.

Complementation of the *tdm* Mutant with the Native *tdm* of *R. pomeroyi* DSS-3 and the *tdm* of *Pelagibacter* sp. Strain HIMB59. The promoter of the *tdm* in *R. pomeroyi* was amplified with an XbaI and an NdeI site engineered at 5′ and 3′ end, respectively, and subcloned into pGEM-T vector. The construct was released from pGEM-T and inserted into the pET28a containing the *tdm* from either *R. pomeroyi* or strain HIMB59. The promoter and gene were released and inserted into the broad-host-range plasmid pBBR1MCS-km at sites XbaI/EcoRI for the native *tdm* of *R. pomeroyi* and sites XbaI/BamHI for *tdm* of strain HIMB59. The plasmid was transformed via electroporation into *E. coli* S17.1 and then mobilized into the *tdm* mutant via conjugation. Transconjugants were selected for as described above, but replacing the gentamicin with kanamycin (80 $\mu g \cdot mL^{-1}$).

Complementation of *tmoX* Mutant in *R. pomeroyi* with Its Native *tmoX*. Because of substrate-binding protein of the TMAO transporter (*tmoX*) seemed to be toxic to the *E. coli* JM109 competent cells, we amplified the promoter and *tmoX* gene separately. We amplified the promoter (250 bp upstream region) of *tmoX* (engineering the sites HindIII and BamHI at the 5′ and 3′ end, respectively) and subcloned it into pGEM-T. We then amplified the *tmoX* gene (engineering the sites BamHI and XbaI at the 5′ and 3′ end, respectively) and subcloned into pGEM-T. The *tmoX* gene was released from pGEM-T and ligated into the plasmid pBBR1MSC-km using the engineered restriction sites. The promoter was subsequently ligated in at sites HindIII and BamHI. The ligation mixture was desalted using the NucleoSpin Gel and PCR clean-up kit (Macherey–Nagel) and transformed into *R. pomeroyi* via electroporation. The settings used were 2.5 kV/mm, 200 A resistance, and 25 Ω capacitance. The time constant varied between 3.9 and 4.5 ms.

Electrocompetent cells were prepared by modifying the protocol in ref. 3. Briefly, *R. pomeroyi* was grown in a minimal medium with glucose (10 mM) as the C source and ammonium (16 mM) as the N source. Cells (50 mL) were incubated at 30 °C until the cultures reached an OD_{540} of ∼0.4. Cells were washed four times with ice-cold, sterile 10% (vol/vol) glycerol to remove salts and then resuspended in a final volume of 2 mL and 50-μL aliquots were rapidly frozen in dry ice/ethanol. Aliquots were stored at −80 °C until use.

Overexpression of Tdm in *E. coli*. The *tdm* gene from *R. pomeroyi* DSS-3 was amplified by PCR (primers used are listed in Table S4) and subcloned into the pGEM-T vector (Promega). The *tdm* gene was then excised using the NdeI/EcoRI sites and ligated into the expression vector pET28a (Merck Biosciences). The *tdm* gene from *Pelagibacteraceae* strain HIMB59 was chemically synthesized with *E. coli* codon use (GenScript Corporation). The synthesized gene was inserted into the expression vector pET28a using the NdeI/BamHI sites. The resulting plasmids were then transformed into the expression host *E. coli* BLR(DE3) pLysS (Merck Biosciences). To overexpress Tdm, *E. coli* cells were grown at 37 °C to an OD_{600} of 0.6. Isopropyl β-D-1-thiogalactopyranoside was then added to a final concentration of 0.2 mM and trimethylamine *N*-oxide (TMAO) was added to a final concentration of 1 mM. Cultures were then incubated at 25 °C for 20 h before assaying supernatant for dimethylamine (DMA) production from TMAO on a cation-exchange ion chromatograph equipped with a Metrosep C4/250-mm separation column and a conductivity detector (Metrohm).

1. Dennis JJ, Zylstra GJ (1998) Plasposons: Modular self-cloning minitransposon derivatives for rapid genetic analysis of gram-negative bacterial genomes. *Appl Environ Microbiol* 64(7):2710–2715.
2. Chen Y, Patel NA, Crombie A, Scrivens JH, Murrell JC (2011) Bacterial flavin-containing monooxygenase is trimethylamine monooxygenase. *Proc Natl Acad Sci USA* 108(43): 17791–17796.
3. Sebastian M, Ammerman JW (2009) The alkaline phosphatase PhoX is more widely distributed in marine bacteria than the classical PhoA. *ISME J* 3(5):563–572.

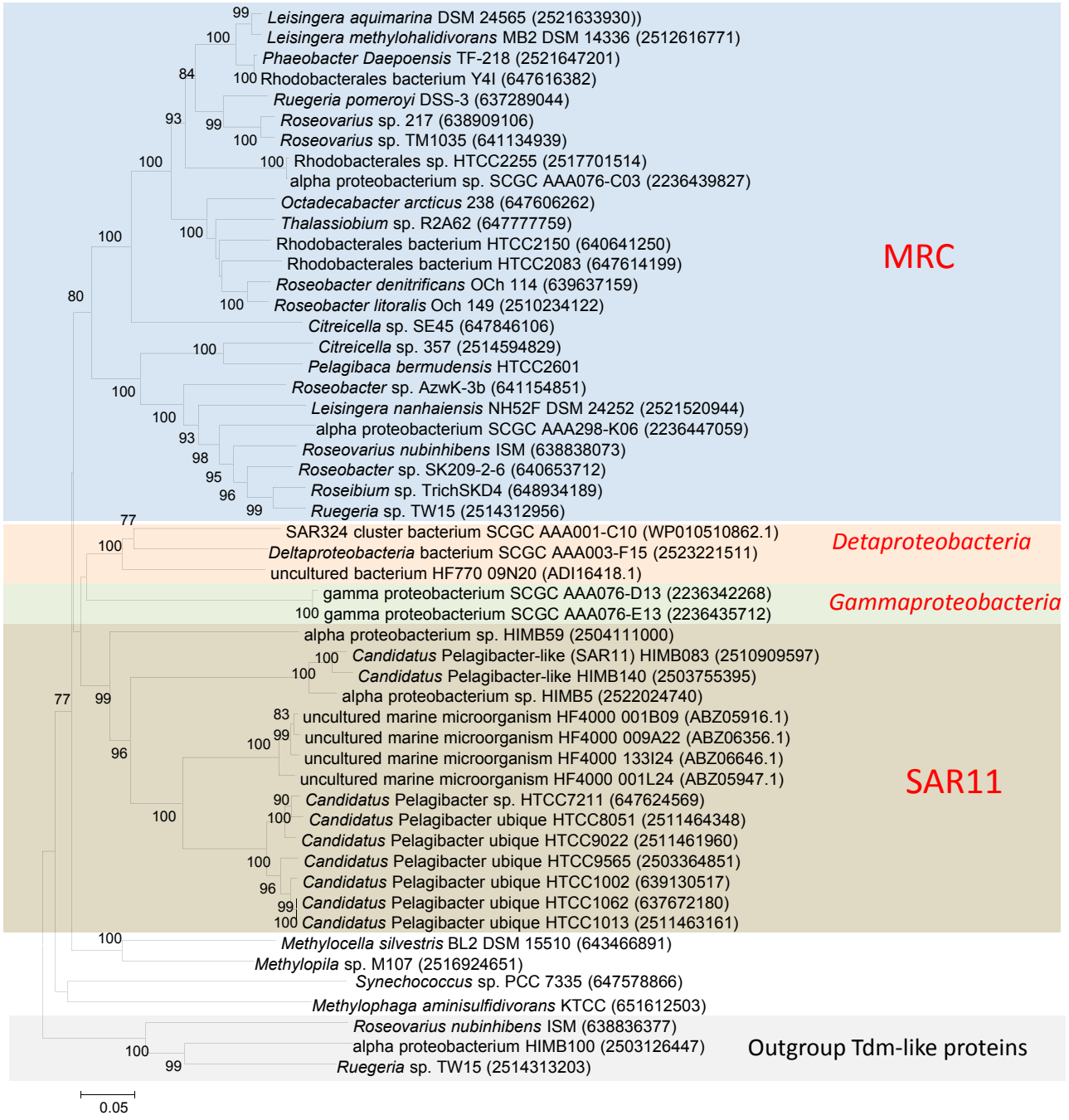

Fig. S1. Phylogenetic distribution of the *tdm* gene among marine bacteria. Nodes at some of the major branched with high bootstrap values (500 replicates) are indicated.

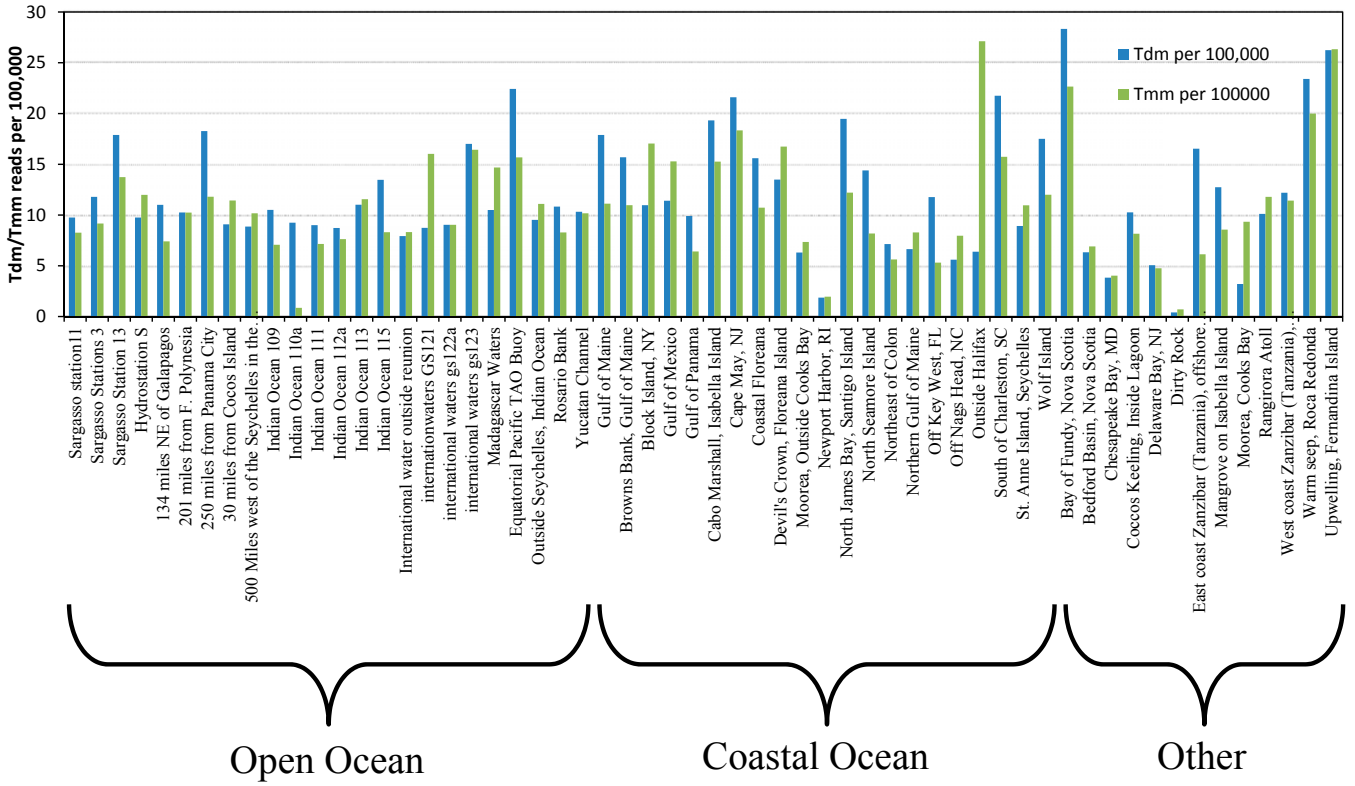

Fig. S2. Abundance of Tmm and Tdm at sites throughout the Global Ocean Survey. Protein abundances are given per 100,000 reads. Protein sizes were normalized against RecA.

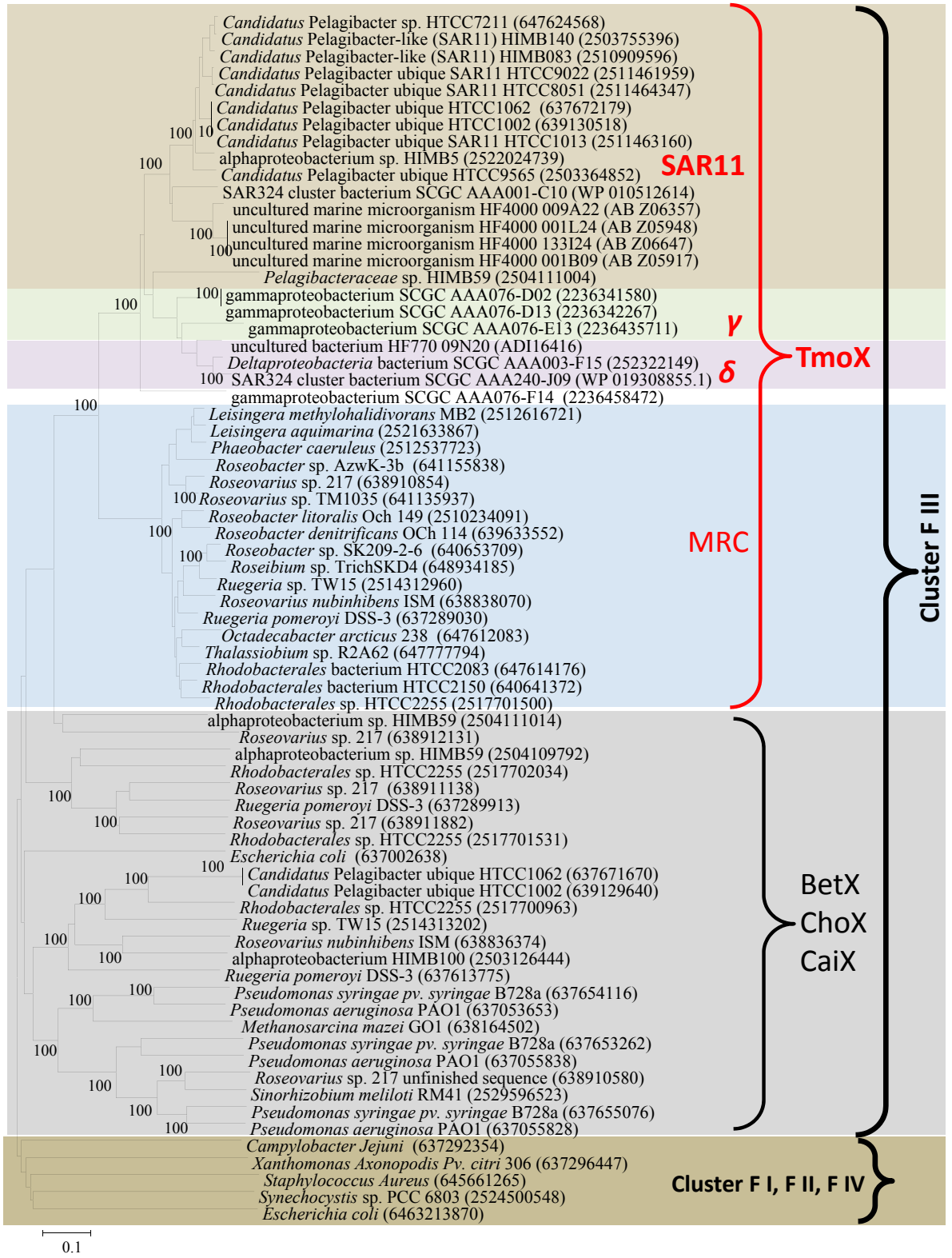

Fig. S3. Neighbor-joining phylogenetic tree showing the distribution of *tmoX* among marine bacteria. High bootstrap values (500 replicates) at some of the major nodes are shown. Integrated Microbial Genomes (IMG) gene numbers are given in brackets. Where no IMG gene number is possible, the National Center for Biotechnology Information accession number was used.

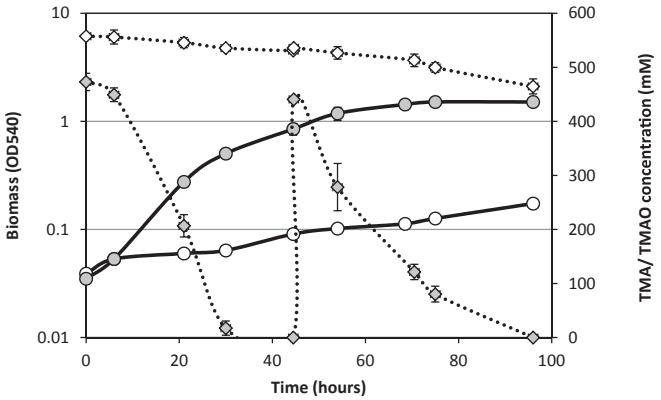

Fig. S4. Growth of the TMAO transporter mutant of *R. pomeroyi* (Δ*tmoXW*::*Gm*) on trimethylamine (TMA) (gray circles) and TMAO (white circles) as a sole N source. TMA (gray diamonds) and TMAO (white diamonds) concentrations were quantified throughout the growth. Cultures were grown in triplicate and error bars denote SD.

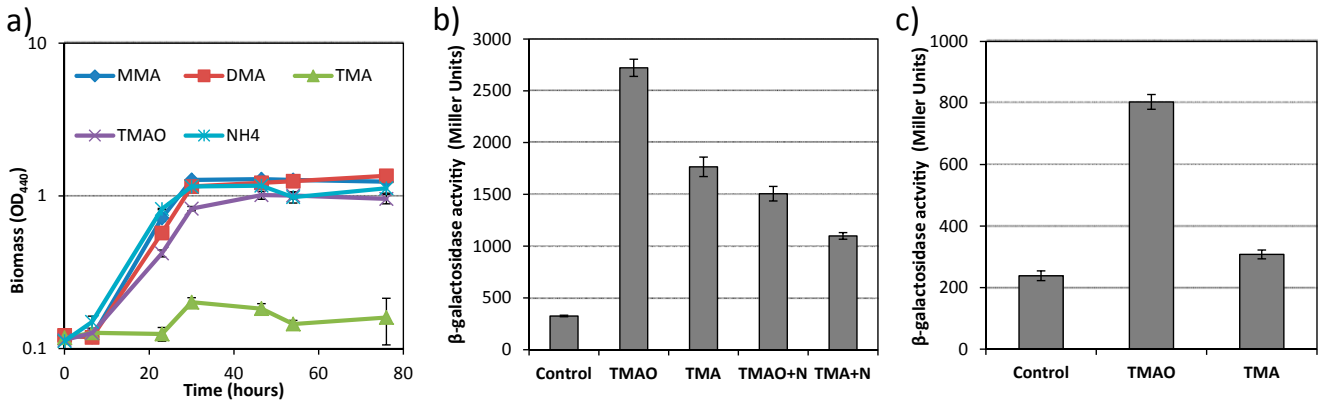

Fig. S5. (*A*) Growth of Δ*tmm*::*Gm* mutant on methylated amines as a sole N source. (*B*) Wild-type *R. pomeroyi* DSS-3 containing the *tmoX–lacZ* fusion plasmid pBIL101 were grown in a defined medium in the presence of TMA (1 mM) or TMAO (1 mM) with succinate (8 mM) as the C source, or in the presence of TMA (0.5 mM) or TMAO (0.5 mM) with additional ammonium (4 mM) (TMA+N and TMAO+N, respectively) and succinate (8 mM). β-Galactosidase activities were assayed in triplicate and error bars denote SDs. (*C*) The *R. pomeroyi* DSS-3 mutant (Δ*tmm*::*Gm*) containing pBIL101 was grown for 16 h in the presence of additional TMA (0.5 mM) or TMAO (0.5 mM) and β-galactosidase activities were assayed in triplicate. Growth was performed in a defined medium with succinate (8 mM) as the C source and ammonium as the N source (4 mM). Error bars denote SDs.

Table S1. Growth of *R. pomeroyi* genotypes on methylated amines

Strain	MMA	DMA	TMAO	TMA	NH_4^+
Wild type	+	+	+	+	+
Δ*tmm*::*Gm*	+	+	+	−	+
Δ*tdm*::*Gm*	+	+	−	−	+
Δ*tmoX*::*Gm*	+	+	−	+	+
Δ*tmoXW*::*Gm*	+	+	−	+	+

Table S2. Distribution of *tmm* and *tdm* among isolates from the marine *Roseobacter* clade and growth of marine *Roseobacter* clade isolates on TMA and TMAO

Strain	*tmm*	*tdm*	TMA	TMAO
Citreicella sp. E45	+	+	+	+
Citreicella sp. 357	+	+	NT	NT
Dinoroseobacter shibae DFL12	–	–	–	–
Jannashia sp. CCS1	–	–	NT	NT
Labrenzia aggregata IAM 12614	–	–	NT	NT
Labrenzia alexandrii DFL-11	–	–	NT	NT
Loktanella sp. CCS2	–	–	NT	NT
Loktanella vestfoldensis SKA53	–	–	NT	NT
Maritimibacter alkaliphilus HTCC2654	–	–	NT	NT
Nautella italic R11	–	–	NT	NT
Oceanibulbus indolifex HEL-45	–	–	NT	NT
Oceanicola batsensis HTCC2597	–	–	–	–
Oceanicola granulosus HTCC2516	–	–	NT	NT
Octadecabacter antarcticus 238	+	+	NT	NT
Octadecabacter antarcticus 307	–	–	NT	NT
Pelagibaca bermudensis HTCC2601	+	+	NT	NT
Phaeobacter gallaeciensis 2.10	–	–	NT	NT
Phaeobacter gallaeciensis BS107	–	–	–	–
Roseibium sp. TrichSKD4	–	+	NT	NT
Roseobacter denitrificans OCh 114	+	+	+	+
Roseobacter litoralis Och 149	+	+	+	+
Roseobacter sp. AzwK-3b	+	+	NT	NT
Roseobacter sp. MED193	–	–	–	–
Roseobacter sp. SK209-2–6	–	+	–	+
Roseovarius nubinhibens ISM	+	+	+	+
Roseovarius sp. 217	+	+	+	+
Roseovarius sp. TM1035	+	+	+	+
Ruegeria lacuscaerulensis ITI-1157	–	–	NT	NT
Ruegeria pomeroyi DSS-3	+	+	+	+
Ruegeria sp. TW15	–	+	NT	NT
Ruegeria sp. TM1040	–	–	NT	NT
Ruegeria sp. TrichCH4B	–	–	NT	NT
Sagittula stellata E-37	–	–	–	–
Sulfitobacter sp. EE-36	–	–	–	–
Sulfitobacter sp. GAI101	–	–	NT	NT
Sulfitobacter sp. NAS-14.1	–	–	NT	NT
Thalassobium sp. R2A62	+	+	NT	NT
Rhodobacterales bacterium HTCC2083	+	+	NT	NT
Rhodobacterales bacterium HTCC2150	+	+	NT	NT
Rhodobacterales bacterium Y4I	+	+	NT	NT
Rhodobacteraceae bacterium KLH11	–	–	NT	NT
Rhodobacterales sp. HTCC2255	+	+	NT	NT

NT, not tested.

Table S3. Bacterial strains and plasmids used in this study

Plasmids/strains	Description/use	Source
E. coli BLR(DE3) pLysS	Host for heterologous protein expression	Promega
E. coli S17.1	Electrocompetent cells used for conjugation	Lab collection
E. coli JM109	Routine host for cloning	Promega
R. pomeroyi DSS-3	Wild type	1
R. pomeroyi DSS-3 Δ*tmm*::*Gm*	Wild type with disrupted *tmm*	This study
R. pomeroyi DSS-3 Δ*tdm*::*Gm*	Wild type with disrupted *tdm*	This study
R. pomeroyi DSS-3 Δ+DSS-3	*tdm* mutant complemented with pBIL001	This study
R. pomeroyi DSS-3 Δ+HIMB59	*tdm* mutant complemented with pBIL002	This study
R. pomeroyi DSS-3 Δ*tmoXW*::*Gm*	Wild type with disrupted *tmoXW*	This study
R. pomeroyi DSS-3 Δ*tmoX*::*Gm*	Wild type with disrupted *tmoX*	This study
R. pomeroyi DSS-3 Δ+*tmoX*	*tmoX* mutant complemented with pBIL101	This study
p34S-Gm	Source of a gentamycin gene cassette	2
pK18mobsacB	Suicide vector for *R. pomeroyi*, Kan^R	3
pBBR1MCS-km	Broad-host-range plasmid (Kan^R)	4
pBIO1878	Spc^R derivative of pMP220 with *lacZ* reporter gene	5
pBIL001	SPO1562 (*tdm*) and its promoter cloned into pBBR1MCS-km	This study
pBIL002	*tdm* of *Pelagibacter* strain HIMB59 and the promoter of SPO1562 cloned into pBBR1MCS-km	This study
pBIL101	SPO1548 (*tmoX*) and its promoter cloned into pBBR1MCS-km	This study
pKIL101	Internal fragment of SPO1562 (*tdm*) and the Gm^R cassette cloned into pK18mobsacB	This study
pKIL201	Internal fragment of SPO1548 (*tmoX*) and SPO1549 (*tmoXV*) and the Gm^R cassette cloned into pK18mobsacB	This study
pKIL202	Internal fragment of SPO1548 (*tmoX*) and the Gm^R cassette cloned into pK18mobsacB	This study
pBIOIL101	SPO1548 (*tmoX*) promoter cloned into pBIO1878	This Study

1. González JM, et al. (2003) *Silicibacter pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. *Int J Syst Evol Microbiol* 53(Pt 5):1261–1269.
2. Dennis JJ, Zylstra GJ (1998) Plasposons: Modular self-cloning minitransposon derivatives for rapid genetic analysis of gram-negative bacterial genomes. *Appl Environ Microbiol* 64(7): 2710–2715.
3. Schäfer A, et al. (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: Selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* 145(1):69–73.
4. Kovach ME, et al. (1995) Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene* 166(1):175–176.
5. Todd JD, Kirkwood M, Newton-Payne S, Johnston AWB (2012) DddW, a third DMSP lyase in a model *Roseobacter* marine bacterium, *Ruegeria pomeroyi* DSS-3. *ISME J* 6(1):223–226.

Table S4. All PCR primers used in this study

Primer	Sequence	Used for
Tdm_AF1_EcoRI	ATCAGGAATTCACCGTGTGAGATCGTCTGTG	Cloning region A of SPO1562 (*tdm*)
Tdm_AR1_XbaI	AATGCTCTAGAACACTGGAAATCGGTGCATT	Cloning region A of SPO1562 (*tdm*)
Tdm_BF1_XbaI	AATGCTCTAGAGTCTATACCGCCATGTGCT	Cloning region B of SPO1562 (*tdm*)
Tdm_BR1_PstI	CAATGCTGCAGTAGCCGGCAAAGATCAACC	Cloning region B of SPO1562 (*tdm*)
Tdm_CONF_F1	GAACGGAACGCTATGTGGTT	Confirmation of Δ*tdm*:*Gm*
Tdm_CONF_F2	TCTCCATCCGGTCGTAAAAG	Confirmation of Δ*tdm*:*Gm*
Tmm_F_XbaI	GTTACGTCTAGACGCTGGATCGACTACAATGA	Cloning of SPO1551 (*tmm*)
Tmm_R_HindIII	GTTACGAAGCTTGCCACCAGTTCCTTGACGTA	Cloning of SPO1551 (*tmm*)
Tmm_CONF	TCTGGAATTCGCCGACTATT	Confirmation of Δ*tmm*:*Gm*
Tmm_CONR	AGATACGCCTCCATGCTGTC	Confirmation of Δ*tmm*:*Gm*
TmoX_AF_HindIII	CAATAAGCTTTCGCTCTGCTTTGACATGAG	Cloning region A of SPO1548 (*tmoX*)
TmoX_AR_XbaI	CAATTCTAGAAAAGGCCCCTTCCCACAC	Cloning region A of SPO1548 (*tmoX*)
TmoX_BF_XbaI	CAATTCTAGAACTTTGCCGAAGCGGTCT	Cloning region B of SPO1548 (*tmoX*)
TmoX_BR_PstI	CAATCTGCAGGCGCGAATATCGTCGAAC	Cloning region B of SPO1548 (*tmoX*)
TmoX_CONF_F1	ATCTGCGCGAGGAACATAAC	Confirmation of Δ*tmoX*:*Gm*
TmoX_CONF_R1	AAAGGACTGGAACACCATGC	Confirmation of Δ*tmoX*:*Gm*
TmoXW_AF_HindIII	CAATAAGCTTGAAATCGCTGCAAATGATCC	Cloning region A of SPO1548 (*tmoX*)
TmoXW_AR_XbaI	CAATTCTAGAACCGGACCATCCAGATAGC	Cloning region A of SPO1548 (*tmoX*)
TmoXW_BF_XbaI	CAATTCTAGAGGGCGCGAGGATTATTTC	Cloning region B of SPO1549 (*tmoW*)
TmoXW_BR_PstI	CAATCTGCAGGCTTGCCTTCAACAGGATGT	Cloning region B of SPO1549 (*tmoW*)
TmoXW_CONF_F1	CCGTTCGATTTGGTCGTATT	Confirmation of Δ*tmoXW*:*Gm*
TmoXW_CONF_R1	ATGTCCCATTGTCCGATCAT	Confirmation of Δ*tmoXW*:*Gm*
Tdm_DSS-3_F1_NdeI	CAATCATATGATGCTGGATACCAAATATCCCGAGAT	Cloning SPO1562 (*tdm*)
Tdm_DSS-3_R1_EcoRI	CAATGAATTCTCAAGAGCGGGGTCTGGTTTTCTGCG	Cloning SPO1562 (*tdm*)
Tdm_prom_F1_XbaI	CAATCATATGGTTGCCACTCCGGTCATTTG	Cloning the promoter of SPO1562 (*tdm*)
Tdm_prom_R1_NdeI	CAATTCTAGAAACCCCAGCCCGGTCGCCAG	Cloning the promoter of SPO1562 (*tdm*)
TmoX_Prom_F_KpnI	CAATGGTCCAATTCAAAATCAACGCGCAAT	Cloning the TmoXWV promoter *lac* fusion
TmoX_Prom_R_PstI	CAATCTGCAGGCCGCCGAACCTGGAGAGAGTG	Cloning the TmoXWV promoter for *lac* fusion
TmoX_F1_BamHI	CAGAGGATCCGTGCGATTGTTTCGAGAAATCGC	Cloning SPO1548 (*tmoX*)
TmoX_R1_XbaI	CAATTCTAGAGATTAGCCGTCCAGCCAGGGGCG	Cloning SPO1548 (*tmoX*)
TmoX_Prom_F2_HindIII	CAATAAGCTTATTCAAAATCAACGCGCAAT	Cloning the promoter of SPO1548 (*tmoX*)
TmoX_Prom_R2_BamHI	CAATGGATCCGCCGCCGAACCTGGAGAGAGTG	Cloning the promoter of SPO1548 (*tmoX*)