Supporting Information

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SI Materials and Methods

Cultivation of Methylocella silvestris. M. silvestris was grown at 25 °C in either a 4-L fermentor or 125-mL serum vials using diluted mineral salt medium as described previously (1). The following compounds were used as sole carbon and nitrogen sources, trimethylamine hydrochloride (10 mM), dimethylamine hydrochloride (10 mM), monomethylamine hydrochloride (10 mM), trimethylamine N-oxide (TMAO, 10 mM), and methanol (10 mM) plus ammonium chloride (2 mM). For enzyme assays, cells were harvested from the fermentor at late exponential phase, resuspended in 10 mM 1,4-piperazine diethanesulfonic acid (Pipes) buffer (pH 7.6), and then stored at -80 °C before assays were carried out. To test if the trimethylamine monooxygenase (tmm) mutant could grow on methylated amines, all growth experiments were set up in triplicate using 120-mL serum vials containing 20 mL medium with an inoculum size of 10%. The serum vials were incubated at 25 °C in a shaker (150 rpm, Sanyo-Gallenkamp Inc).

Cultivation of Ruegeria pomeroyi DSS-3 and Roseovarius sp. 217. *Ruegeria pomeroyi* DSS-3 and *Roseovarius* sp. 217 were cultivated in shake flasks in triplicate using a defined medium containing artificial sea salts from Sigma-Aldrich (S9883) 40 g·L⁻¹, sodium phosphate 0.2 mM (pH 8.0), Hepes 10 mM (pH 8.0), FeCl₃ 50 μ M, and malate (10 mM). The vitamins were added as described previously (2). The nitrogen sources used were trimethylamine (2.0 mM) or ammonium chloride (2.0 mM). Negative controls, without added N compounds, were also set up to evaluate contamination of N in the chemicals used for growth. Samples were taken at various time points to determine the optical density at 540 nm. Subsets of samples were taken at late exponential phase, and cell-free crude extracts were prepared. These were then used to determine the NADPH-dependent Tmm activities using the assay as described below.

Quantitative Comparative Proteomics. A total of 700 μ g of soluble protein extract from MMA, TMA, and methanol grown *M. silvestris* cells were resuspended in 1 mL of 0.1% RapiGest (Waters Corporation) and concentrated using a 5-kDa cutoff spin column. The solution was then heated at 80 °C for 15 min, reduced with 100 mM DTT at 60 °C for 15 min, alkylated in the dark with 200 mM iodoacetamide at ambient temperature for 30 min, and digested with 1:50 (wt/wt) sequencing-grade trypsin (Promega) at 37 °C overnight. RapiGest was hydrolyzed by the addition of 2 μ L of 15 M HCl, vortexed, and centrifuged in a 0.22- μ m Nylon membrane filter (Corning Inc.) to remove particulate matter. Each sample was diluted 1:1 with 100 fmol/ μ L rabbit glycogen phosphorylase B standard tryptic digest in 0.1% (vol/vol) formic acid, to give a final protein concentration of 500 mg/ μ L and an internal standard concentration of 50 fmol/ μ L. Nanoscale liquid

chromatography (LC) separations of tryptic peptides were performed with a nanoACQUITY ultrapressure liquid chromatograph system (Waters Corporation) and mass analyzed on a Synapt HDMS (Waters Corporation) in a data-independent manner (MS^E). Details of LC and mass spectrometer configurations are described elsewhere (3).

Raw data files were processed using ProteinLynx Global Server v.2.4 and searched against a *M. silvestris* database. The sequence for rabbit phosphorylase B and contaminants from the common Repository of Adventitious Proteins proteins were added to the database, along with randomized sequences for each database entry. A fixed modification of carbamidomethyl-C was specified, in addition to variable modifications acetyl N terminus, deamidation N, deamidation Q, and oxidation M. Automatic settings for mass accuracy were used, and one missed tryptic cleavage site was allowed. The protein identification criteria included the detection of at least three fragment ions per peptide, a minimum of one peptide determined per protein, and identification of the protein in at least two of the four technical replicates.

A label-free method was used to allow simultaneous identification and quantification of proteins in a LC-MS workflow. This method is based on the relationship between peptide peak area and intensity observed by electrospray ionization MS and protein concentration (4). With the use of an internal standard, this relationship was used to calculate a universal response factor. Absolute quantification of a protein was determined by the application of the universal response factor to its corresponding peptides.

Enzyme Assays and Kinetics. For cell-free crude extract, Tmm and DMA monooxygenase activities were measured by following the decrease in absorbance at 340 nm of substrate-dependent oxidation of NADPH (Sigma-Aldrich). The following concentrations of substrates were used: TMA or DMA (1 mM) and NADPH (0.1 mM). TMAO demethylase activity was measured by quantifying the substrate-dependent production of formaldehyde using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald reagent; Sigma) as described previously (1).

Overexpressed TMA monooxygenases were purified using a Histag protein purification kit as described in the manufacturer's protocol (Merck), except that the column was washed using a wash buffer containing 80 mM imidazole before elution to remove trace protein contaminants. Kinetic assays were performed in triplicate at 22 °C on a UV-visible spectrophotometer. A 1-mL mixture contained 0.8 mg purified enzyme, 80 mM Pipes (pH 7.6), and 0.25 mM NADPH. The reaction was initiated by adding substrate, and the decrease in absorbance at 340 nm was recorded continuously for 3 min. All substrates were dissolved in water except *N*,*N*dimethylaniline, which was dissolved in ethanol.

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	*	20	*	40	*	60	*	80	*	100
aFMO3 :	MYGNNNKKSINI	TSMFQNLIPEGS	DIFSRRCIWV	IGPV <mark>IVGAG</mark> PSC	LAVAAGLE	REG	-VPFIILERA	NCIAS WQNR		
aFMO2 :	MEF\	/TETLGKRIHD-E	YVEETRCLMI	PGPI <mark>IVG</mark> SGPSC	LATAACLE	KSRD	-IPSLILERS	TCIAS DWQHK		
aFMO1 :	N	IESHPHNKTDQ	TQHIILVE	IGPIIIGAGPSG	LATSACLS	SRG	-VPSLILERS	DSIAS MKSK		
hFMO2 :			MAH	KKVAVIGAG <mark>V</mark> SG	LISLKCCV	/DEG	-LEPTCFER	EDIGGVWRFK		
hFMO5 :			MTKH	KRIAVIG <mark>G</mark> GVSG	LSSIKCCV	/EEG	-LEPVCFERI	DDIGGLWRFQ		
hFMO3 :			MGH	KVAIIGAG <mark>V</mark> SG	LASIRSCI	SEEG	-LEPTCFEKS	NDIGGL <mark>W</mark> KFS		
oFMO3 :			MGH	KVAIIGAG <mark>I</mark> SG	LASIRSCI	JEEG	-LEPTCFEMS	DDIGGLWKFS		
hFMO4 :			MAH	KVAVIGAG <mark>V</mark> SG	LSSIKCCV	/DED	-LEPTCFERS	DDIGGLWKFT		
sp217 :			MTKH	KRIAIIGAG <mark>P</mark> SG	LAQLRAFO	SAAAKGAE	IPEIVCFEKÇ	DNWGGLWNYTWF	RTG	
DSS-3 :			MTTSH	KRVAIIGAGPSG	LAQLRAFO	SAAAKGEE	IPEIVCFEK <mark>Ç</mark>	DNWGGLWNYTWF	RTG	
Mpha :			MA	RIAILGAG <mark>P</mark> SG	MAQLRAFO	SAQEKGAE	IPELVCFEK,	ADWGGQ <mark>W</mark> NYTWF	RTG	
Msil :			M	RVAIIGAG <mark>P</mark> SG	LAQLRAFO	SAGKKGAA	IPELVCFEKÇ	SDWGGLWNYTWF	RTG	
HTCC1002 :			M	KVAIIGAGPCG	LSALRSFE	EQAEKNGEK	IPEIVCEDKÇ	DWGGLWNY SWF	RTG	
HTCC7211 :			MS	SKVAIIGAGPCG	LSILRAFE	EHLEKKGEK	IPEIVCFEKÇ	E SWGGLWNYNWF	RTG	
yFMOsc :			MTVNDKH	KRLAIIG <mark>G</mark> GPGG	LAAARVFS	SQSLPN	-FEIEIFVKI	YDIGGVWHYP		
yFMOsp :			MCLPTIN	RKIAIIGAGESC	IVTARALI	JAEKA	FDQVTLFERF	GSPGGVMNYTS1	LSNKLPVPS	TNPILTTEP
	*	220	*	240	*	260	*	280	*	300
aEMO3 :	CEFEYICRW	WATGENAEKVVP	DEEGLEDE-	GEDVLHAG	DYK-SGGE	RYOGEKVIN	/VGCGNSGME	SLDLYNHGANE	SMVVR	
aFMO2 :	-TMEYVSRWLM	VATGENAEEVMP	EIDGIPDF-	GEPILHTS	SYK-SGEI	IF SEKKTLA	VGCGNSGME	CLDLCNENALE	SLVVR	
aFMO1 :	TEYLSKWL	VATGENADEYEP	EIPGRKKES	GEKIVHAS	EYK-SGER	RROKVL	VGCGNSGME	ISLDLVRHNASP	HLVVR	
hEMO2 :	KEOSAVEDAVM	VCSGHHILPHIP	LKSEPGMERE-	KCOYFHSE	OYK-HPDO	GEGERIL	/IGMGNSGSD	IAVELSKNAAOV	FISTRHGTW	VMSRISEDGY
hFMO5 :	KKEMNVEDGVM	VCTGHHTNAHLP	LESEPGIEKE-	KCOYFHSF	DYK-NPEC	GF TGKRVII	IIGIGNSGGD	AVEISOTAKOV	FLSTRRGAW	ILNRVGDYGY
hFMO3 :	KKESAVEDAVM	VCSGHHVYENLE	KESFPGLNHF-	KCKCFHSF	DYK-EPGV	VENGKRVLV	VGLGNSGCD	TATELSRTAEOV	MISSRSGSW	VMSRVWDNGY
oFMO3	KKETAVEDAVM	ICSCHHVYENLE	KDSF PGLKHF-	Keksfusf	EYK-EPGI	IF KGKRVLV	/IGLGNSGCD	IATELSHTAEOV	VISSRSGSW	VMSRVWDDGY
hFMO4 :	KONRAVEDAVM	VCTGHFLNPHLP	LEAF PGI HKF-	KCOILHSC	EYK-IPE	GEOGKRVLV	/IGLGNTGGD	IAVELSRTAAOV	LLSTRTGTW	VLGRSSDWGY
sp217 :	RTYTEEFDHVI	VCSGHESTENVE	YFPGFENF-	KERVLHAH	DFR-DAL	FRONDILI	IVGTSYSAED	IGSOCWKYGCKS	VTVS	HRTAPMGE
DSS-3	RVYSEEFDHVI	CASCHESTENVE	HYEGFETE-	NGRLVHAH	DFR-DAR	FAGEDIL	VGSSYSAED	IGSOCWKYGAKS	ITSC	YRSAPMGE
Mpha :	TIYSEEFDYVV	CCTGHFSTPYVP	EFEGFEKF-	GERILHAH	DFR-DAL	SEKDKTVLI	LVGSSYSAED	IGSOCYKYGAKK	LISC	YRTAPMGY
Msil :	ELYSETFDYVV	VASGHESTENVE	HFPGIEVF-	PCRVLHAH	DFR-DANE	FVGKNLLV	/VGSSYSAED	LASQCYKYGAKS	ITFS	YRSKPLNE
HTCC1002 :	KISKDIFDYVI	VSTGHFSVPFIP	EYPGMKAF-	PCRIMHSH	IDFR-DAEF	EFRGENVV	/LGSSYSAED	VALQCHKYGAKS	VTIG	YRHN PMGF
HTCC7211 :	KTLSDTEDYLV	VSTGHFSVPFIP	EYEGMSSF-	PCRIMHSH	DFR-DAE	FRGENVIV	/LGSSYSAED	VALQCNKYGAKS	VTIG	YRHN PMGF
yFMOsc :	RTTKSDFDFVI	VASGHYSVPKLP	-TNIAGLDLWE	DNKGAFHSF	DFK-NCER	AREKVVIV	VGNGSSGQD	IANQLTTVAKKV	YNSIKEP	ASNQLKA
yFMOsp :	PISKDIFDAVS	I CNGHYEV PYI P	NIKGLDEYA	AKAVPESVL <mark>H</mark> SS	LFR-EPEI	LEVGESVLV	VGGASSAND	LVRHLT PVAKHP	IYQSLLGGG	DIQNESLQQV

Fig. S1. Alignment of the sequences of *Methylocella silvestris* FMO (Msil), *Methylopha* sp. SK1 FMO (Mpha), *Homo sapiens* FMOs (hFMO2, hFMO3, hFMO4, hFMO5), *Oryctolagus cuniculus* FMO3 (oFMO3), *Arabidopsis thaliana* FMOs (aFMO1, aFMO2, aFMO3), *Saccharomyces cerevisiae* FMO (yFMOsc), *Schizosaccharomyces pombe* FMO (yFMOsp), and FMOs from *Roseovarius* sp. 217 (sp217), *Ruegeria pomeroyi* DSS-3 (DSS-3), and *Pelagibacter ubique* HTCC1002 and HTCC7211. The black bars indicate the FAD binding domain, the fingerprint sequence for FMOs, and the NADP binding domain, respectively. The alignment was performed with ClustalX and displayed with Genedoc.







Fig. S3. One-dimensional gel electrophoresis of crude extract of TMA-grown and ammonium-grown *Roseovarius* sp. 217 and *Ruegeria pomeroyi* DSS-3, respectively. The expression of Tmm homologs in TMA cultures was confirmed by MALDI/MS analyses.



Fig. S4. A phylogenetic tree showing the Tmm homologs from marine Roseobacter clade bacteria (A) and marine SAR11 clade bacteria (B), respectively.





Fig. S5. A phylogenetic tree showing the GmaS homologs from sequenced bacterial genomes and environmental sequences retrieved from the GOS data set.

Table S1. Oligonucleotides used in this study

	Primer seque		Annealing		
Target	Forward primer	Reverse primer	Product size, bp	temperature, °C	
Msil3604	CGCCAGCCAATGCTATAAAT	ATTGTCCTGCCAGAAAATGC	286	55	
Msil3604/3605	GCGCCAGAATTCAAGTCATT	GTCGGACTGTTTTTCGAAGC	305	55	
Msil3603/3604	GTGGGAGGAAGACAAGGTCA	CCCCCTCGAGATTTGTAATG	360	55	
Construction of tmm	CCCTCTTGTTCTTCCCCTTC	TTGACCGTCACCGTAAACAA	888	54	
mutant of <i>M. silvestris</i>	CGCCAGCCAATGCTATAAAT	GCGATATCCCATGATGCTCT	601	54	
Confirmation of <i>tmm::kan</i> mutant of <i>M. silvestris</i>	ATCGCGCTACTTCGAGCAT	GAGCCTTTTCAATGCCTGTC	2,077 (WT) 3,054 (mutant)	53 53	
tmm of M. silvestris	CATATGACTCGTGTTGCAATTA TTGGC	AAGCTTTTATTCCGCGGCGGC TTCCG	1356	54	
tmm of Roseovarius sp. 217	CATATGACCAAAAAACGAAT TGCGATC	AAGCTTTTAGTTCTGGAGGTA AACTTCGAG	1338	54	
tmm of R. pomeroyi DSS-3	CATATGACCACCAGCAAACGTG TGGC	AAGCTTTTAGTTGCGCAGATA CGCCTCCATGC	1353	54	

	Organism	tmm [†]	TMA^{\ddagger}
1	Rhodobacterales bacterium HTCC2150	+	NT
2	Maritimibacter alkaliphilus HTCC2654	—	NT
3*	Citreicella sp. SE45	+	+
4*	Dinoroseobacter shibae DFL 12		_
5	Jannaschia sp. CCS1		NT
6	Labrenzia aggregata IAM 12614	—	NT
7	Labrenzia alexandrii DFL-11		NT
8	Loktanella vestfoldensis SKA53	—	NT
9	Oceanibulbus indolifex HEL-45	_	NT
10*	Oceanicola batsensis HTCC2597	_	_
11	Oceanicola granulosus HTCC2516	_	NT
12	Octadecabacter antarcticus 238	+	NT
13	Octadecabacter antarcticus 307	_	NT
14	Phaeobacter gallaeciensis 2.10		NT
15	Phaeobacter gallaeciensis BS107	_	NT
16	Rhodobacteraceae bacterium KLH11		NT
17*	Roseobacter denitrificans OCh 114	+	+
18*	Roseobacter litoralis Och 149	+	+
19	Roseobacter sp. AzwK-3b	+	NT
20	Roseobacter sp. CCS2		NT
21	Roseobacter sp. GAI101	_	NT
22	Roseobacter sp. MED193		NT
23*	Roseobacter sp. SK209-2-6		_
24*	Roseovarius nubinhibens ISM	+	+
25*	Roseovarius sp. 217	+	+
26	Roseovarius sp. HTCC2601	+	NT
27*	Roseovarius sp. TM1035	+	+
28	Ruegeria lacuscaerulensis ITI-1157	_	NT
29*	Ruegeria pomeroyi DSS-3	+	+
30	Ruegeria sp. R11		NT
31	Ruegeria sp. TM1040	_	NT
32	Silicibacter sp. TrichCH4B		NT
33*	Sagittula stellata E-37	_	_
34	Sulfitobacter sp. EE-36		NT
35	Sulfitobacter sp. NAS-14.1	_	NT
36	Thalassobium sp. R2A62	+	NT
37	Rhodobacterales bacterium HTCC2083	+	NT
38	Rhodobacterales bacterium Y4I	+	NT
39	Rhodobacterales sp. HTCC2255	+	NT

Table S2. Presence of tmm homologs and growth on TMA inRoseobacter clade bacteria

*Strains that have been tested for their growth on TMA in this study. [†]Presence (+) or absence (-) of *tmm* genes in the genome sequences. [‡]Growth test on TMA as sole nitrogen source. NT, not tested.

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Table S3.	Steady-state	e kinetic assay	s on purified	Tmm from	recombinant E. coli
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	Substrate	<i>K</i> _m , μΜ	V _{max} , nmol∙min ⁻¹ ∙mg ⁻¹
Methylocella silvestris BL2	TMA	9.4 ± 2.1	29.4 ± 3.2
	DMA	89.7 ± 13.2	6.9 ± 0.1
	DMS	10.3 ± 0.7	34.6 ± 0.2
	DMSO	3,575 ± 151	4.8 ± 1.5
Ruegeria pomeroyi DSS-3	TMA	20.8 ± 2.9	267.7 ± 52.2
	DMA	1,119.7 ± 55.3	83.7 ± 4.0
	DMS	97.3 ± 8.8	374.5 ± 83.2
	DMSO	16,424.5 ± 1,033.2	70.4 ± 10.5
Roseovarius sp. 217	TMA	21.6 ± 1.9	1,133.6 ± 58.6
	DMA	864.2 ± 35.3	358.0 ± 12.3
	DMS	25.7 ± 4.1	577.4 ± 75.7
	DMSO	16,340.8 ± 1155.2	179.4 ± 41.2
Pelagibacter ubique HTCC7211	TMA	28.5 ± 4.4	67.3 ± 3.2
	DMA	306.1 ± 51.3	41.4 ± 2.7
	DMS	26.4 ± 7.2	97.2 ± 6.9
	DMSO	7,456.0 ± 907.8	41.3 ± 4.7
Pelagibacter ubique HTCC1002	TMA	27.5 ± 4.2	70.8 ± 7.7
	DMA	1,237.7 ± 98.5	41.2 ± 5.6
	DMS	33.2 ± 5.6	50.8 ± 5.0
	DMSO	19,334.3 ± 1,870.4	29.9 ± 8.9

DMA, dimethylamine; DMS, dimethylsulfide; DMSO, dimethyl sulfoxide; TMA, trimethylamine.

Dataset S1. Polypeptides detected in each grown culture of Methylocella silvestris BL2

Dataset S1 (XLSX)

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The numbers show the relative abundance (in percentages) of each polypeptide in the soluble proteome for each growth condition.