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SUPPLEMENTARY INFORMATION

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Bacteria are important dimethylsulfoniopropionate producers in coastal sediments

Beth T. Williams[1], Kasha Cowles[1], Ana Bermejo Martínez[1], Andrew R. J. Curson[1], Yanfen Zheng[1,2], Jingli Liu[1,2], Simone Newton-Payne[1], Andrew J. Hind[3], Chun-Yang Li[2], Peter Paolo L. Rivera[1], Ornella Carrión[3], Ji Liu[1,2], Lewis G. Spurgin[1], Charles A. Brearley[1], Brett Wagner Mackenzie[4], Benjamin J. Pinchbeck[1], Ming Peng[5], Jennifer Pratscher[6], Xiao-Hua Zhang[2], Yu-Zhong Zhang[5], J. Colin Murrell[3] and Jonathan D. Todd[1]*

[1]School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, UK. [2]College of Marine Life Sciences, Ocean University of China, Qingdao, China. [3]School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, UK. [4]Department of Surgery, University of Auckland, Auckland, New Zealand. [5]State Key Laboratory of Microbial Technology, Shandong University, Qingdao, China. [6]The Lyell Centre, Heriot-Watt University, Edinburgh, UK. *e-mail: jonathan.todd@uea.ac.uk

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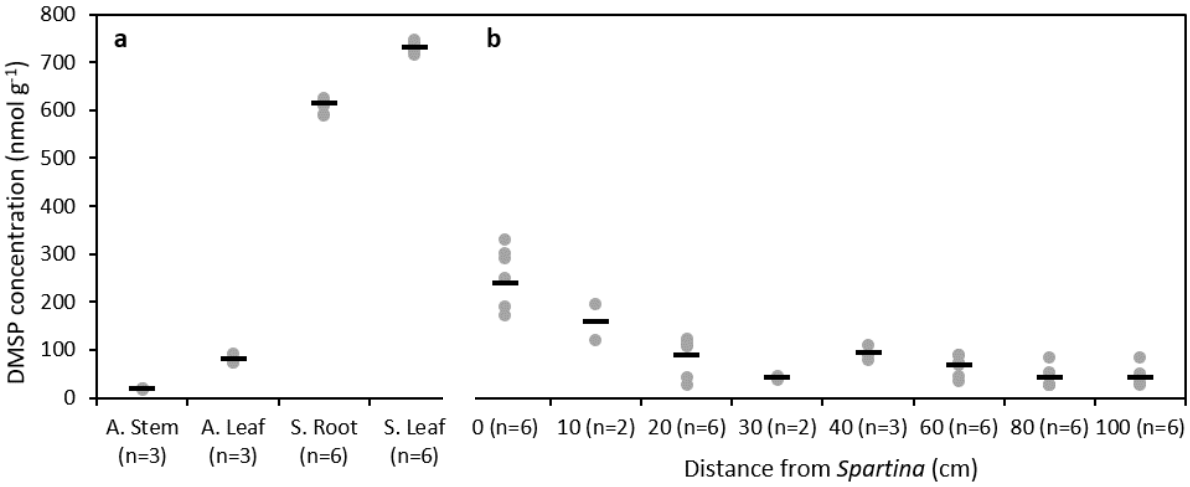
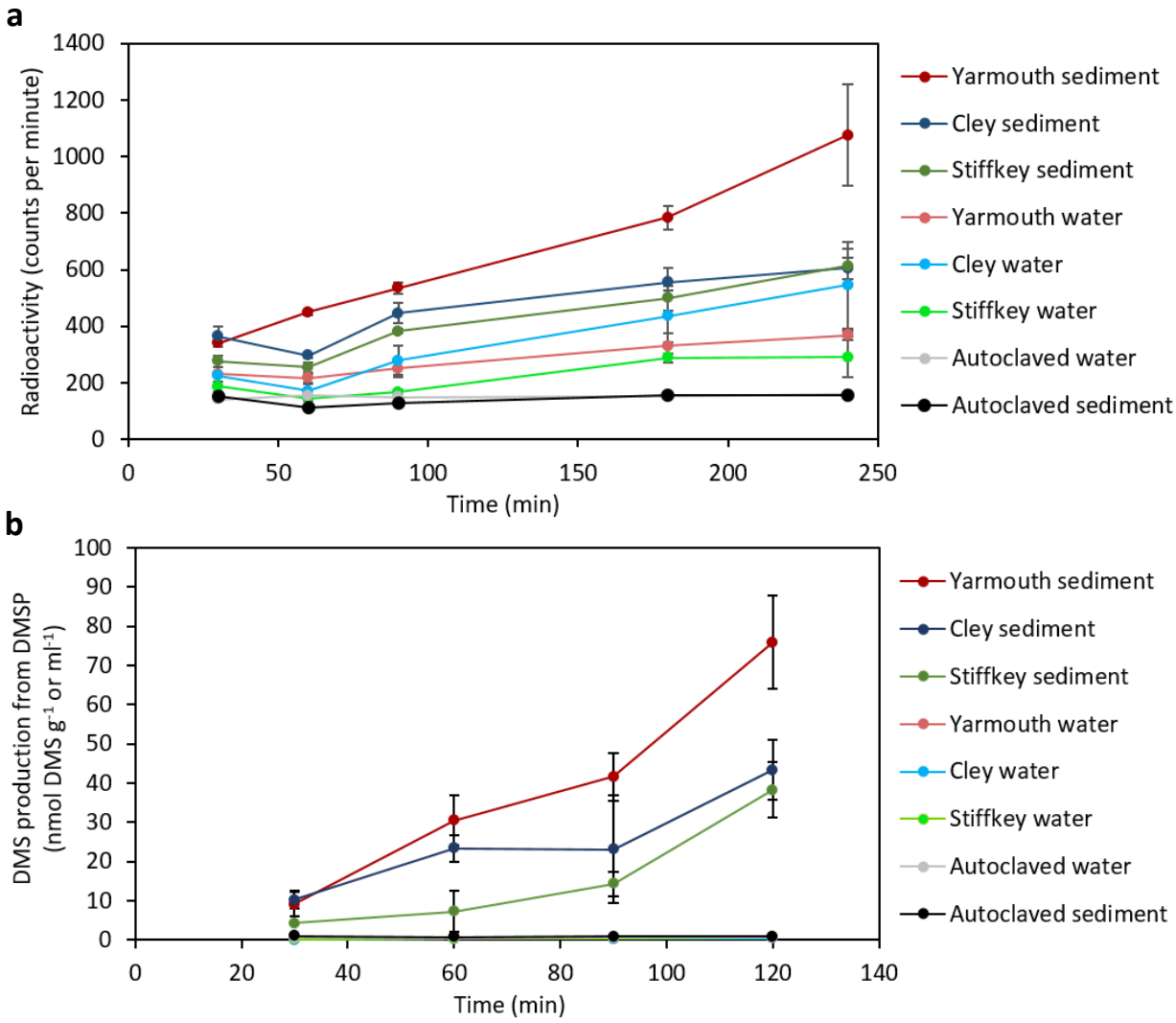
Supplementary Information

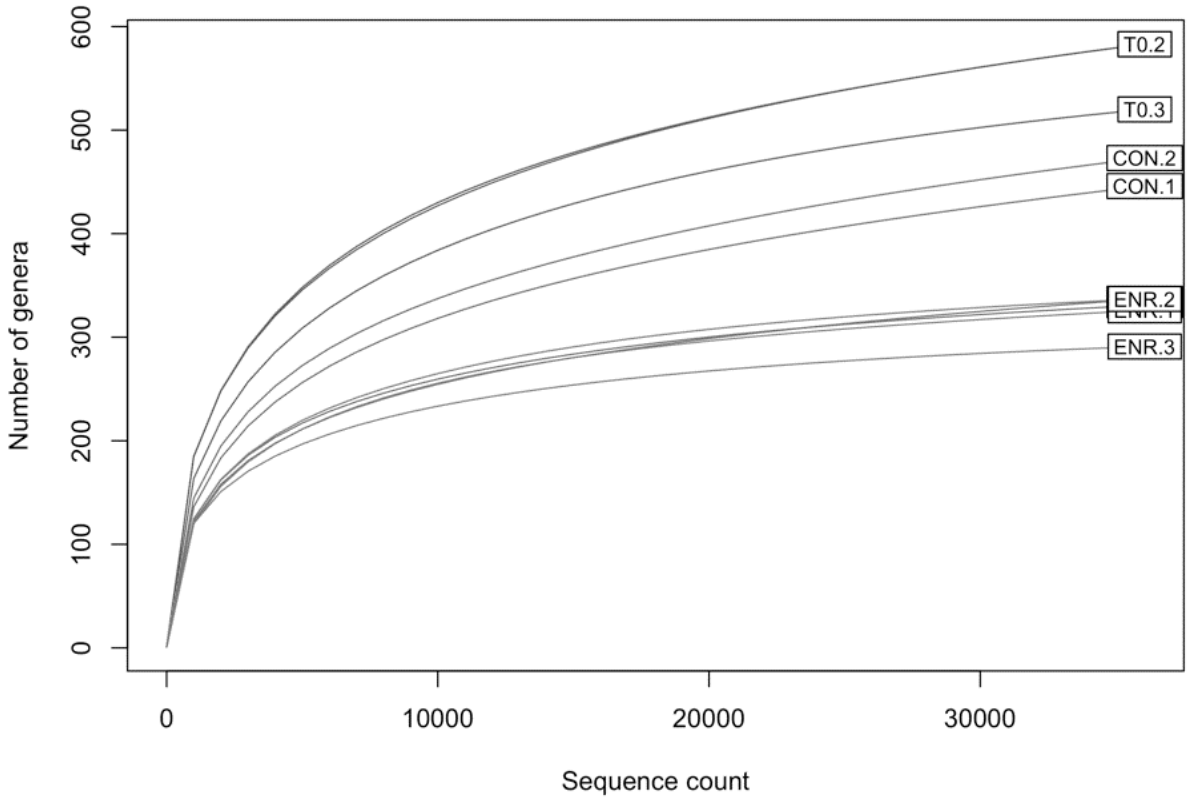
Supplementary Figures:



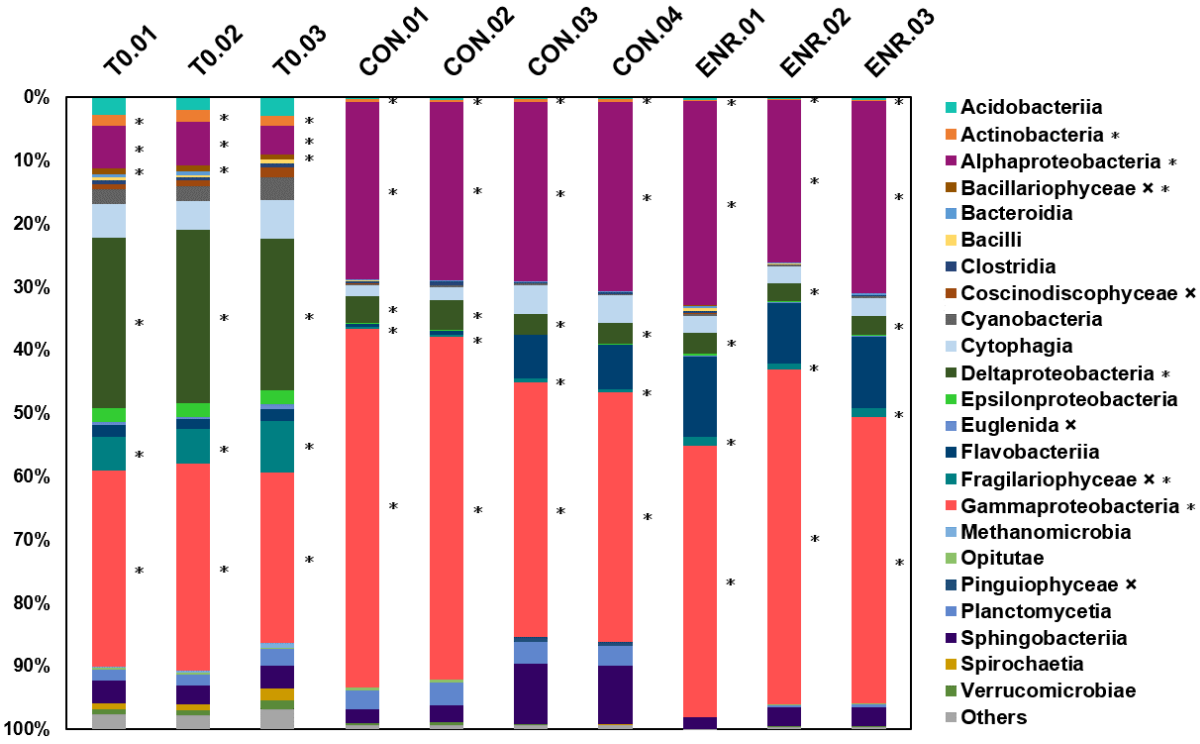
Supplementary Figure 1. (a) DMSP concentration in leaf, root and stem material from *Aster tripolium* (A.) and *Spartina anglica* (S.) growing around the edges of sampled Great Yarmouth estuary and Stiffkey saltmarsh ponds, respectively (n=3 and n=6 biologically independent samples respectively, the black line represents the mean value). (b) DMSP concentration in Stiffkey saltmarsh surface sediment samples moving away from the *S. anglica* from 0 cm to 100 cm (n=2, n=3 and n=6 biologically independent samples, the black line represents the mean value).



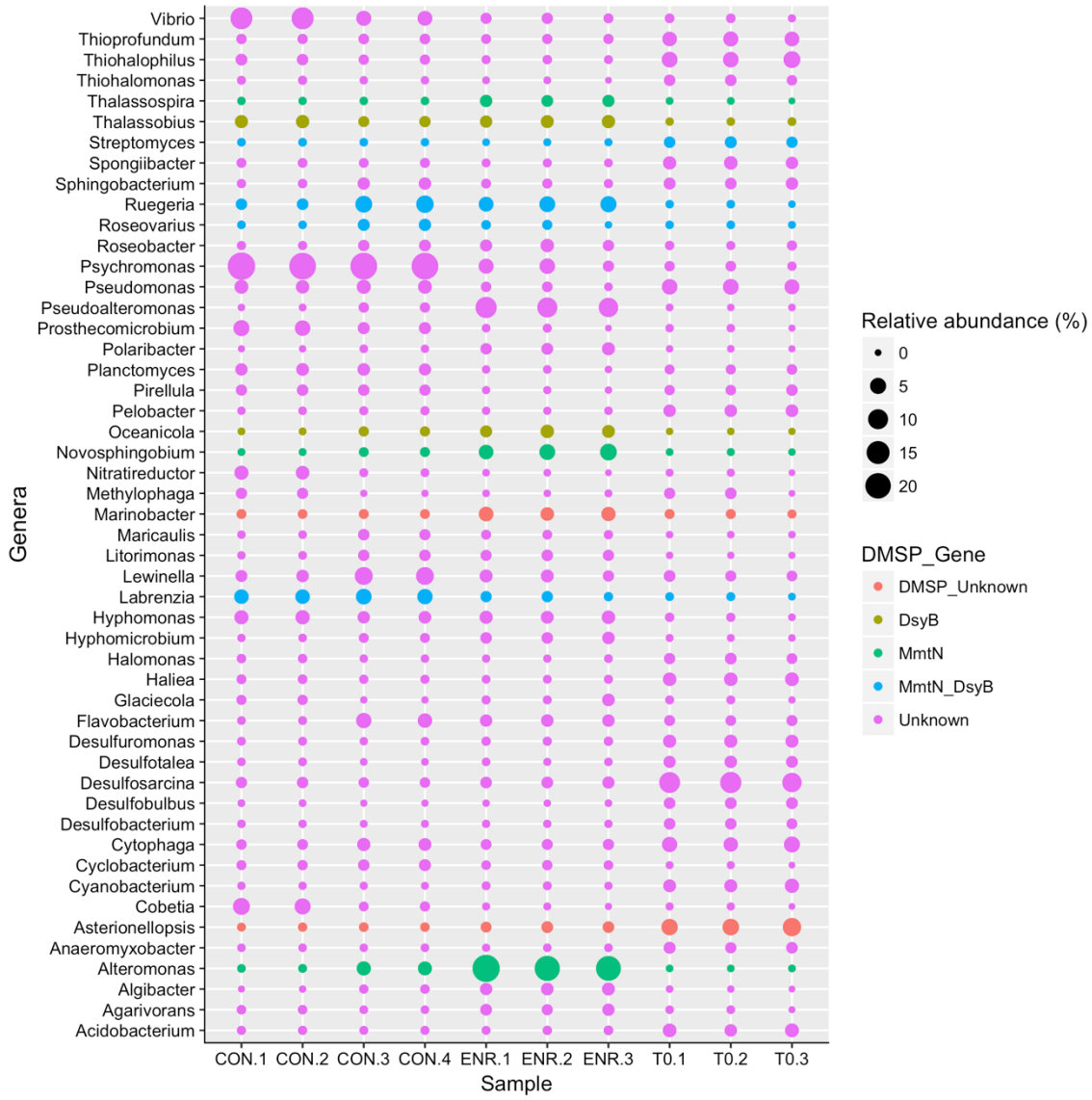
Supplementary Figure 2. (a) DMSP production from varied marine sediment and overlying seawater samples when incubated with L-[methyl-^{3}H]-methionine, measured on the basis of the amount of radioactivity (counts per minute) due to ^{3}H-DMS release from DMSP following alkaline lysis (n=3 biologically independent samples). Autoclaved sediment and seawater samples were used as negative controls. (b) DMS produced from varied marine sediment and overlying seawater samples when incubated with 0.1 mM DMSP (n=3 biologically independent samples). Autoclaved sediment and seawater samples were used as negative controls. Error bars represent standard deviation from the mean value.



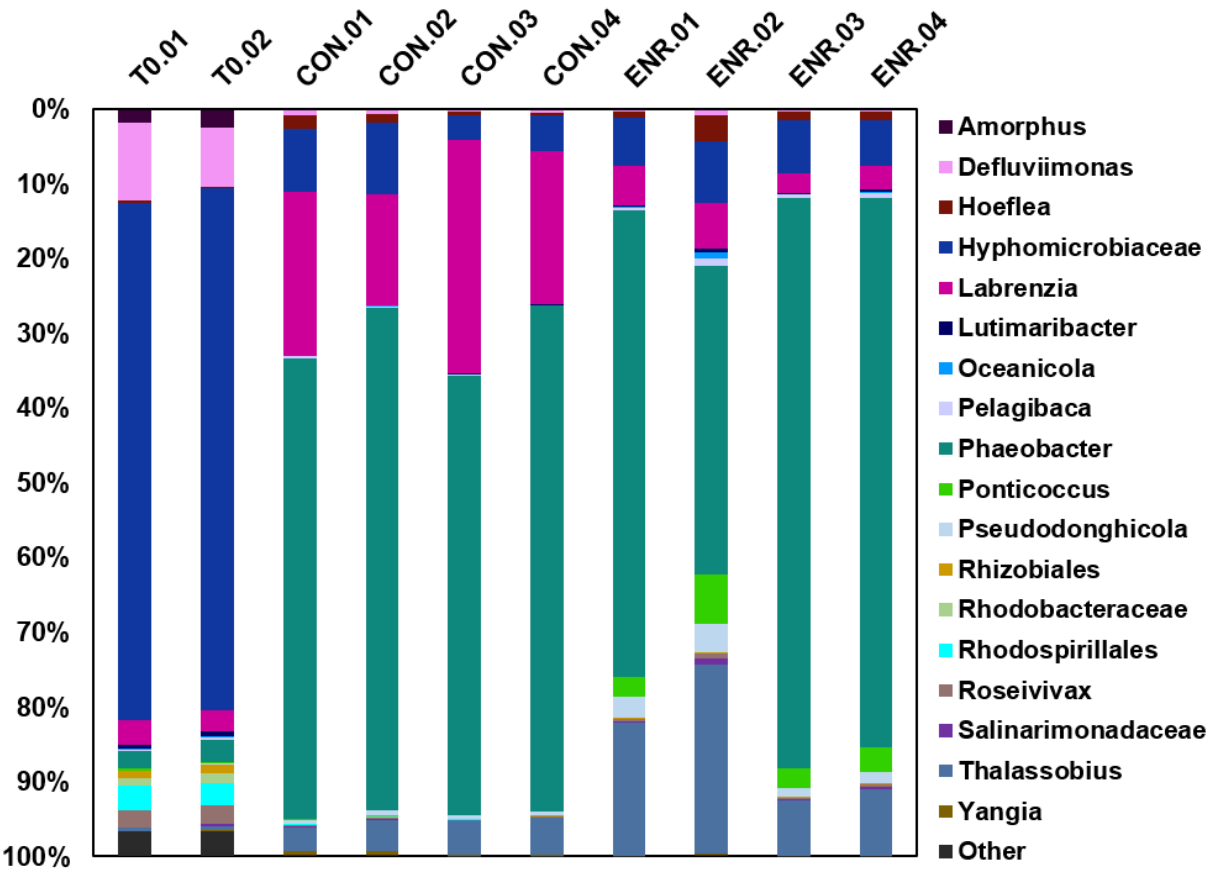
Supplementary Figure 3. Alpha diversity rarefaction curves of the 16S rRNA gene amplicon sequencing data denoted as the number of genera at an even sample depth of 36,066 sequences per sample. Samples are labelled by treatment (T0 (n=3 biologically independent samples), CON (n=4 biologically independent samples), ENR (n=3 biologically independent samples)). The mean number of genera ± standard deviation for each group are as follows: Time zero = 562 ± 36.4, Control = 396.8 ± 73.3, Enriched = 318 ± 24.



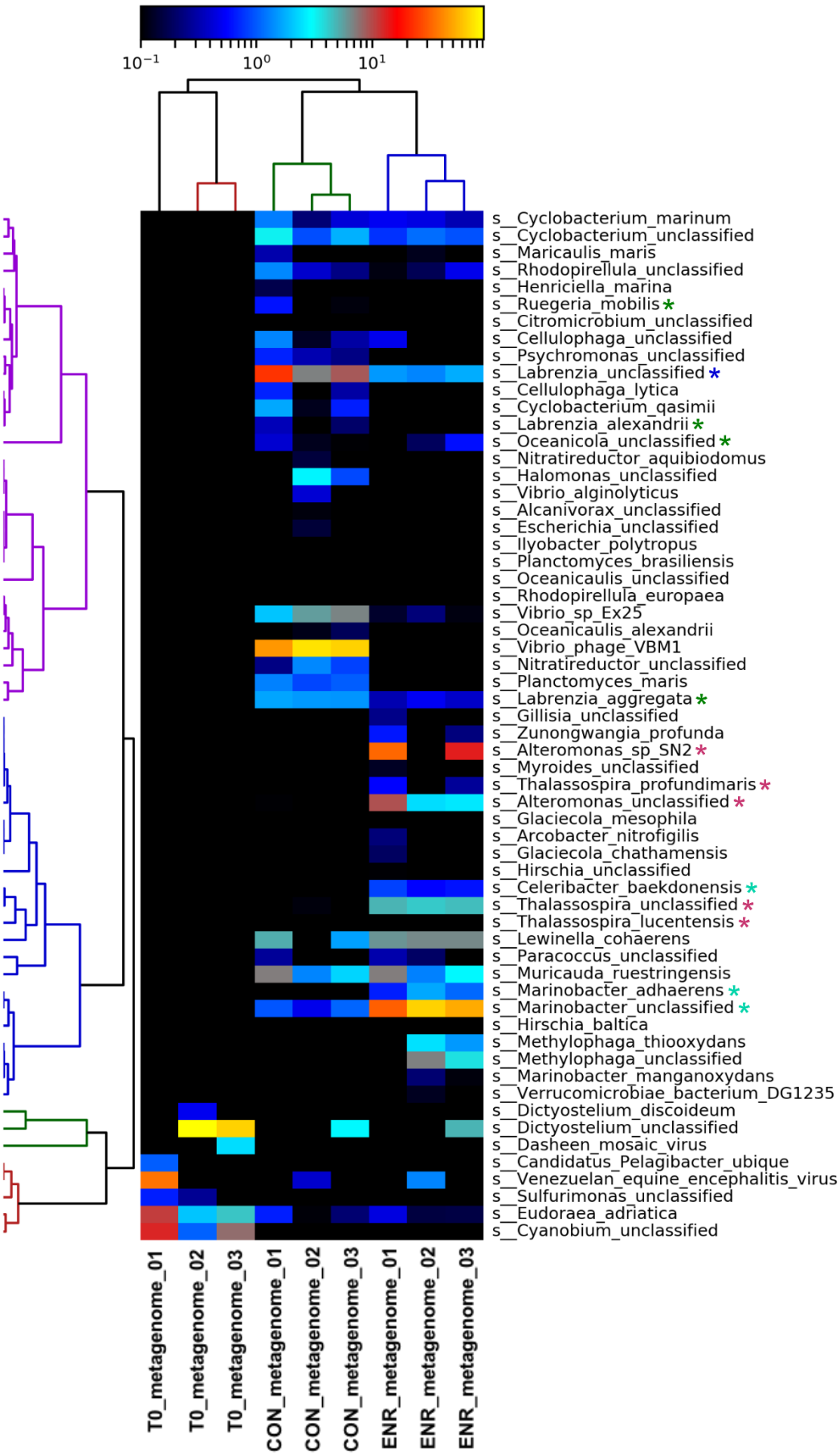
Supplementary Figure 4. Taxonomic profiling of the 16S rRNA gene amplicon sequencing data showing percentage abundance at the class level from natural Stiffkey saltmarsh surface sediment (Time 0) (n=3 biologically independent samples), and from 'Control' (n=4 biologically independent samples), and 'Enriched' (n=3 biologically independent samples), incubation experiments after 14 days. Only classes that are ≥ 0.5 % abundant are represented. (*) indicates those classes known to include DMSP-producing members, (x) indicates classes belonging to Eukaryotes.



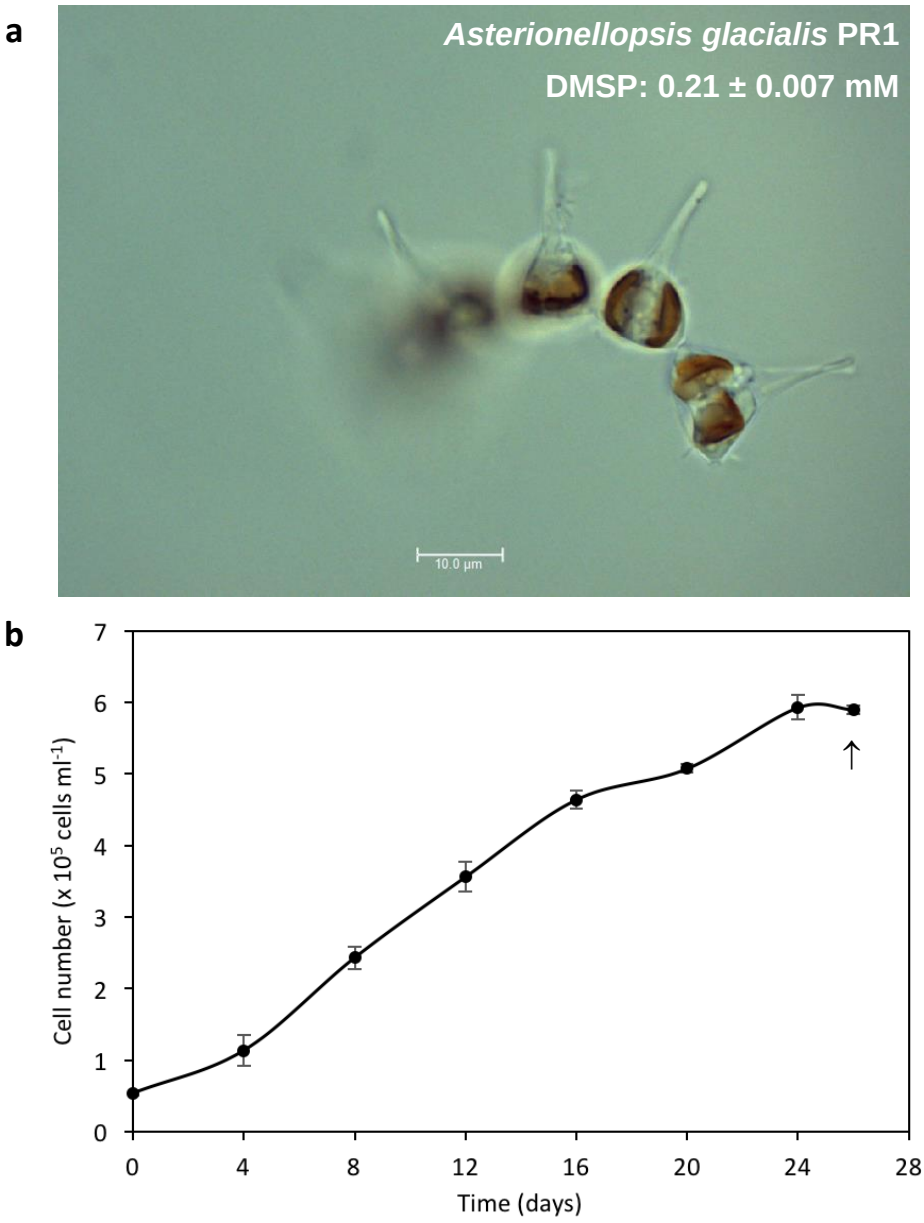
Supplementary Figure 5. Bubble plot depicting the 50 most abundant genera from the 16S rRNA gene amplicon sequencing data from natural Stiffkey saltmarsh surface sediment (T0= Time zero independent replicates 1-3), and from control (CON, replicates 1-4) and enriched (ENR, replicates 1-3) incubation experiments after 14 days. Bubble areas in the panel are proportional to the relative sequence abundances of microbial groups in each sample denoted by 'Relative abundance (%)' (e.g. 0 = 0 - 5 % relative abundance, 5 = 5 - 10 % relative abundance). Bubbles are coloured to indicate those genera that contain *mmtN* (green), *dsyB* (olive), both *mmtN* and *dsyB* (blue), those which contain representatives that produce DMSP with unknown DMSP synthesis genes (orange), and those genera not known to produce DMSP (pink).



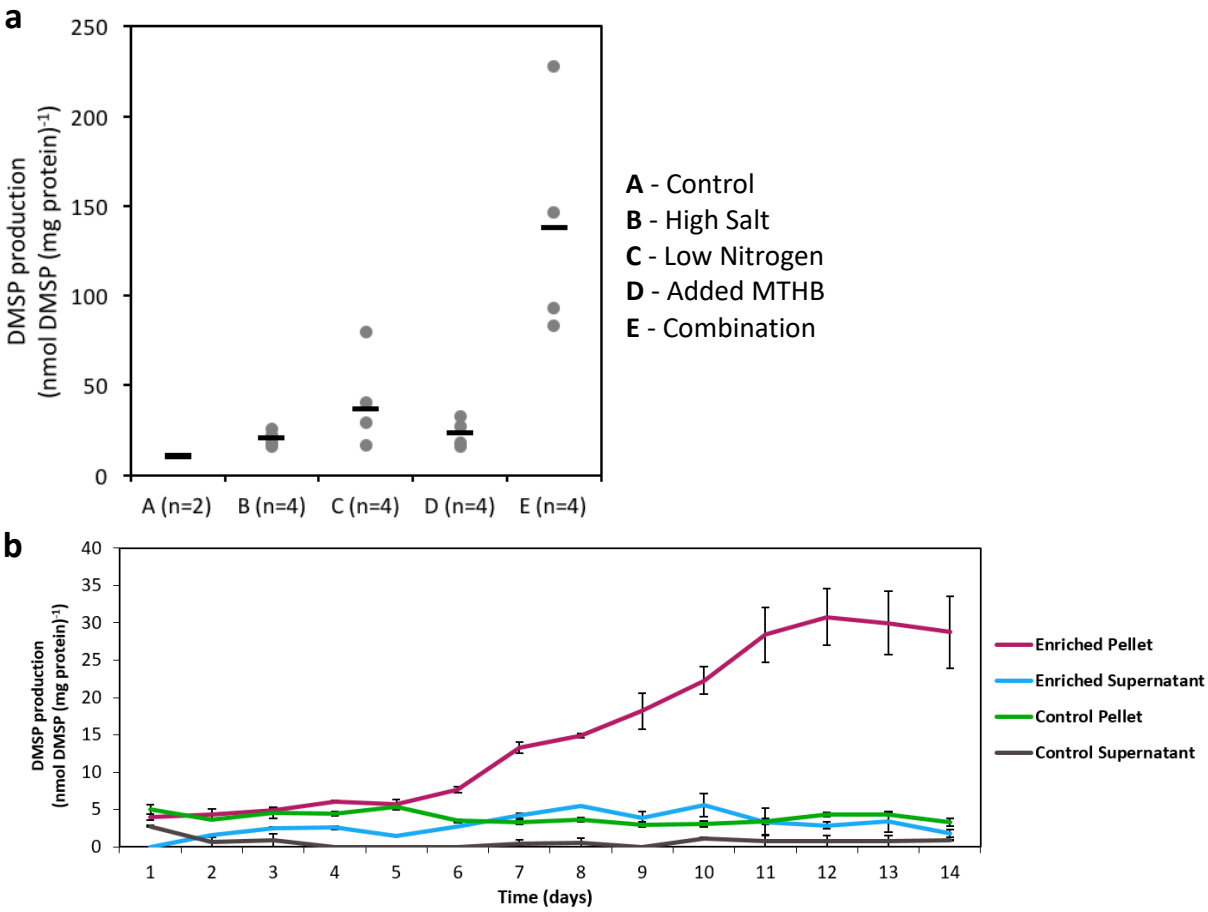
Supplementary Figure 6. Taxonomic profiling of the diversity of the *dsyB* gene amplicon sequencing data showing percentage abundance at the genus level from natural Stiffkey saltmarsh surface sediment (Time 0) (n=2 biologically independent samples), and from 'Control' (n=4 biologically independent samples), and 'Enriched' (n=4 biologically independent samples), incubation experiments after 14 days. Only classes that are ≥ 0.5 % abundant are represented.


10^{-1}
10^{0}
10^{1}
s__Cyclobacterium_marinum
s__Cyclobacterium_unclassified
s__Maricaulis_maris
s__Rhodopirellula_unclassified
s__Henriciella_marina
s__Ruegeria_mobilis *
s__Citromicrobium_unclassified
s__Cellulophaga_unclassified
s__Psychromonas_unclassified
s__Labrenzia_unclassified *
s__Cellulophaga_lytica
s__Cyclobacterium_qasimii
s__Labrenzia_alexandrii *
s__Oceanicola_unclassified *
s__Nitratireductor_aquibiodomus
s__Halomonas_unclassified
s__Vibrio_alginolyticus
s__Alcanivorax_unclassified
s__Escherichia_unclassified
s__Ilyobacter_polytropus
s__Planctomyces_brasiliensis
s__Oceanicaulis_unclassified
s__Rhodopirellula_europaea
s__Vibrio_sp_Ex25
s__Oceanicaulis_alexandrii
s__Vibrio_phage_VBM1
s__Nitratireductor_unclassified
s__Planctomyces_maris
s__Labrenzia_aggregata *
s__Gillisia_unclassified
s__Zunongwangia_profunda
s__Alteromonas_sp_SN2 *
s__Myroides_unclassified
s__Thalassospira_profundimaris *
s__Alteromonas_unclassified *
s__Glaciecola_mesophila
s__Arcobacter_nitrofigilis
s__Glaciecola_chathamensis
s__Hirschia_unclassified
s__Celeribacter_baekdonensis *
s__Thalassospira_unclassified *
s__Thalassospira_lucentensis *
s__Lewinella_cohaerens
s__Paracoccus_unclassified
s__Muricauda_ruestringensis
s__Marinobacter_adhaerens *
s__Marinobacter_unclassified *
s__Hirschia_baltica
s__Methylophaga_thiooxydans
s__Methylophaga_unclassified
s__Marinobacter_manganoxydans
s__Verrucomicrobiae_bacterium_DG1235
s__Dictyostelium_discoideum
s__Dictyostelium_unclassified
s__Dasheen_mosaic_virus
s__Candidatus_Pelagibacter_ubique
s__Venezuelan_equine_encephalitis_virus
s__Sulfurimonas_unclassified
s__Eudoraea_adriatica
s__Cyanobium_unclassified
T0_metagenome_01
T0_metagenome_02
T0_metagenome_03
CON_metagenome_01
CON_metagenome_02
CON_metagenome_03
ENR_metagenome_01
ENR_metagenome_02
ENR_metagenome_03

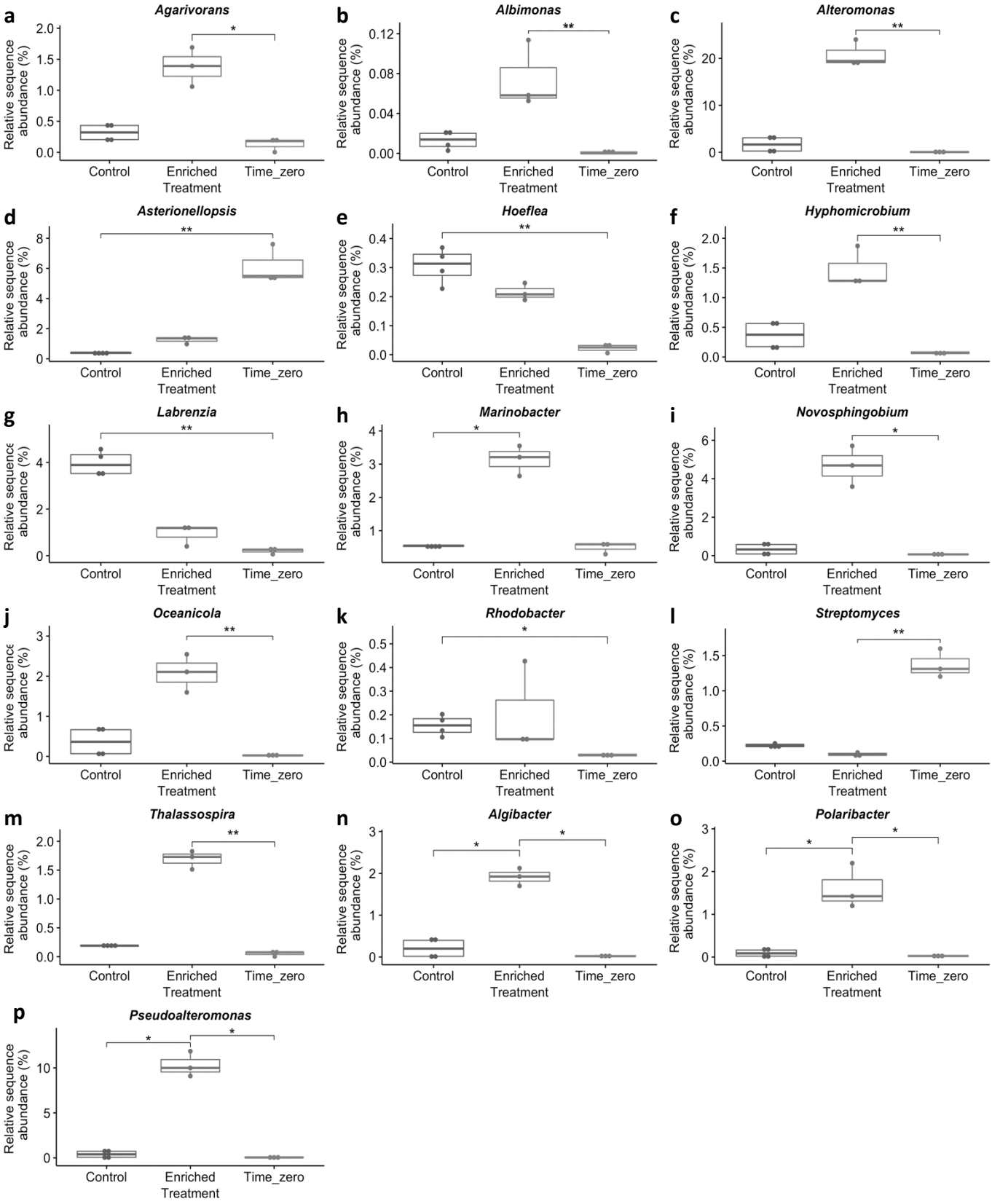
Supplementary Figure 7. Species level phylogenetic analysis of the metagenomes from natural Stiffkey saltmarsh surface sediment (T0_metagenome_01-03), and from control (CON_metagenome_01-03) and enriched (ENR_metagenome_01-03) incubation experiments after 14 days (n=3 pooled samples). Abundance for species is in logarithmic scale reporting the 100 most abundant clades according to the 90^{th} percentile of the abundance of each clade with a custom colour map. Hierarchical clustering is performed with average linkage, using Bray-Curtis distance for clades (the tree on the left) with 1M unique clade-specific marker genes identified from ~17,000 reference bacterial, archaeal, viral and eukaryotic genomes, and using correlation for similarity and relatedness between samples (the tree at the top). All the descendent links below a cluster node are the same color if it is the first node below the cut threshold. The genera/species marked with coloured * indicate those that contain *mmtN* (pink), *dsyB* (green), both *mmtN* and *dsyB* (blue), and those which contain representatives that produce DMSP with unknown DMSP synthesis genes (cyan) (Supplementary Table 4). Note: for DMSP categories, species level correlations take precedent over predictions based at the genus level.



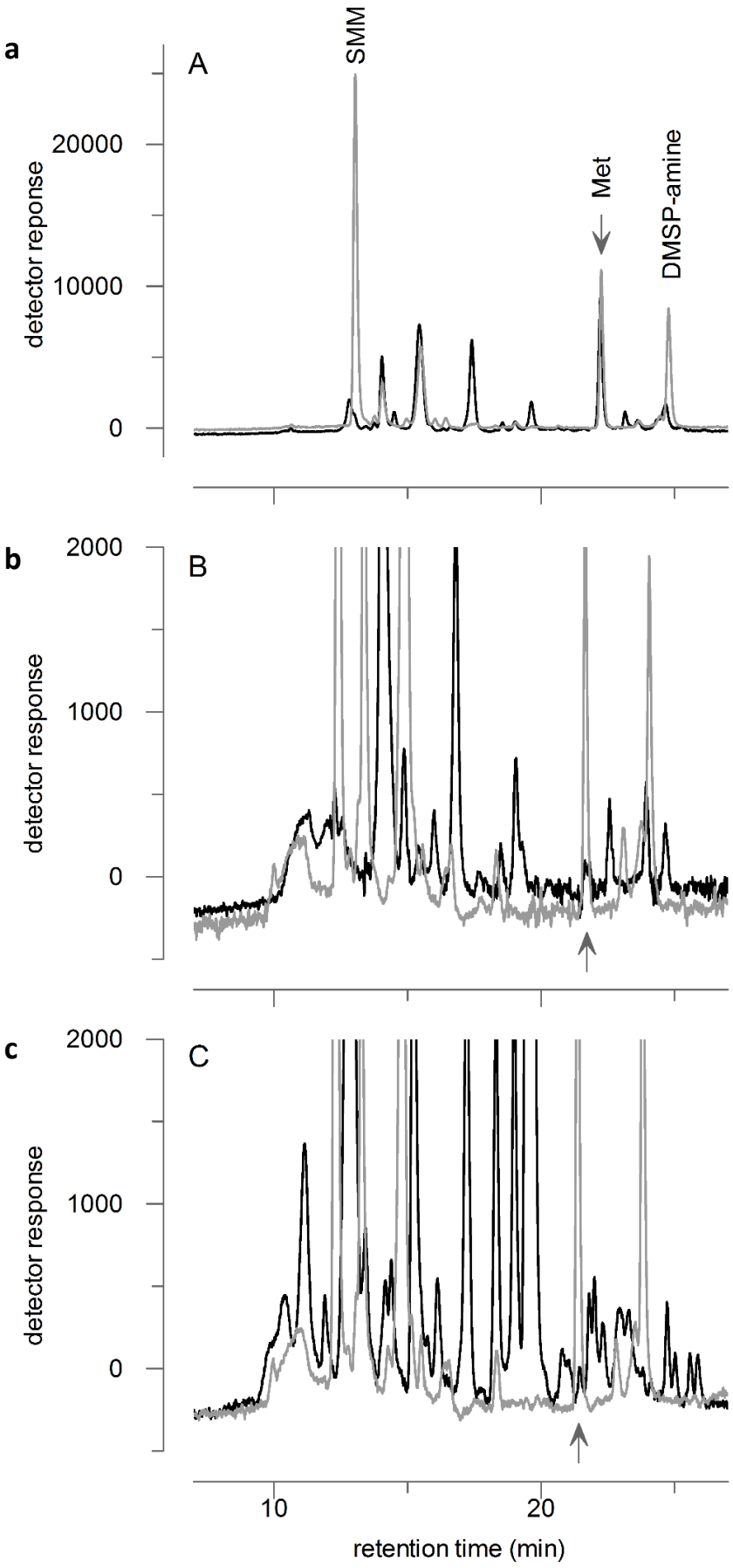
Supplementary Figure 8. (A) Light microscopy image of a colony of *Asterionellopsis glacialis* PR1 isolated from Stiffkey saltmarsh surface sediment. The image was taken by optical microscopy using a Leica DM6000 fitted with a 100x objective. Each of the spikes belongs to a single diatom. The intracellular DMSP concentration is indicated (n=3 biologically independent samples, with the mean value calculated). (B) Growth of *A. glacialis* PR1 over 26 days, ↑ indicates day of sampling for quantification of DMSP. Error bars display standard deviation of triplicate biological replicates from the mean value (n=3 biologically independent samples).



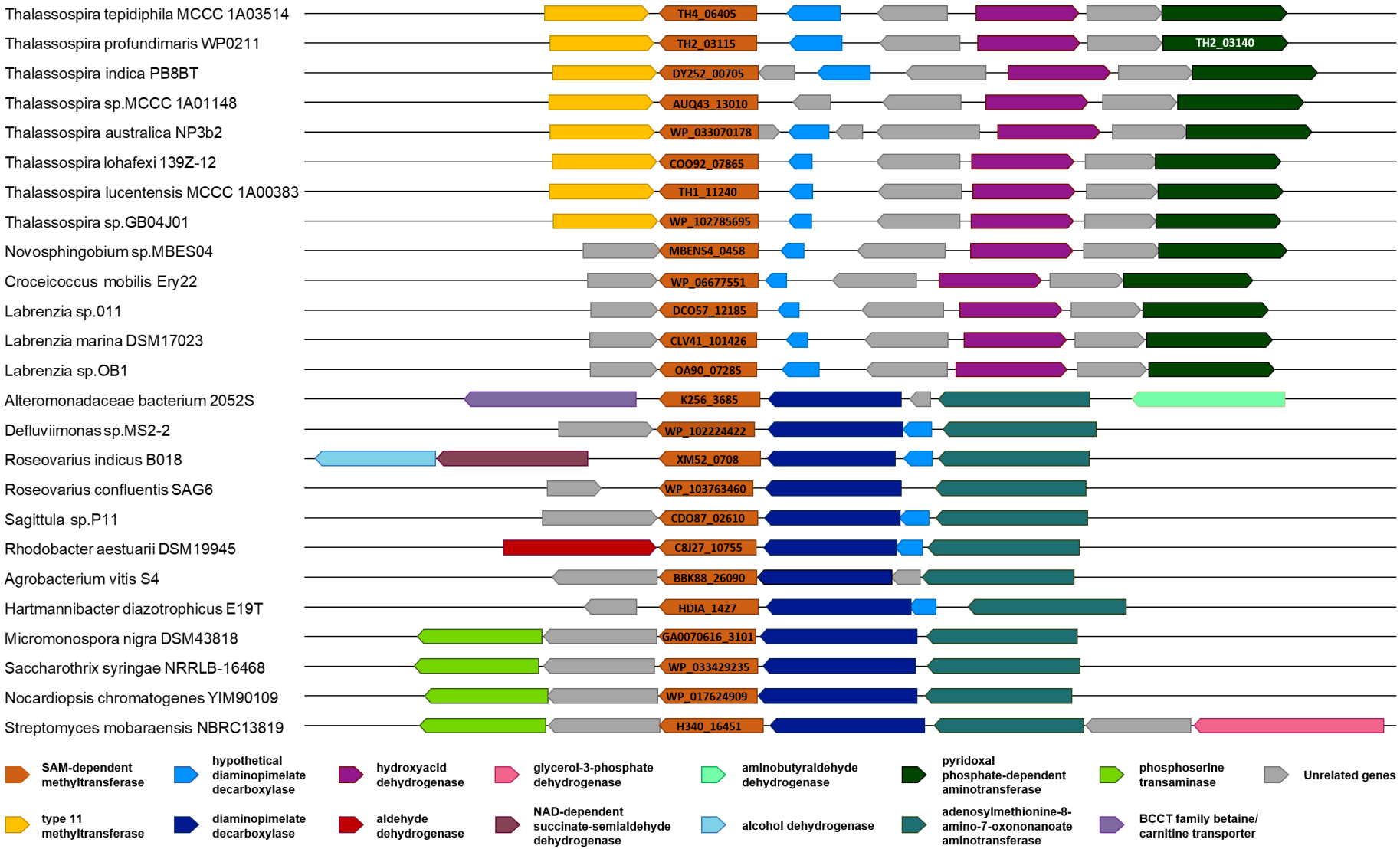
Supplementary Figure 9. Incubation experiments used to develop an enrichment method for DMSP-producing bacteria from Stiffkey saltmarsh surface sediment. (a) DMSP (nmol DMSP (mg protein)$^{-1}$) was monitored in the particulate (after centrifugation) after 7 day incubations of sediment slurry from Stiffkey saltmarsh. Control condition was MBM (35 PSU, 10 mM NH_4Cl), other conditions tested were high salinity, reduced nitrogen levels (0.5 mM NH_4Cl), additional MTHB (0.1 mM) and a combination of all these conditions. (n=2 and 4 biologically independent samples, the black line represents the mean value) (b) A combination media was selected (see Methods) and DMSP was monitored in the supernatant and pellet (following centrifugation) of sediment slurry experimental samples over an incubation period of 14 days. (n=3 biologically independent samples), error bars represent standard deviation from the mean value.


a
Agarivorans
b
Albimonas
c
Alteromonas
d
Asterionellopsis
e
Hoeflea
f
Hyphomicrobium
g
Labrenzia
h
Marinobacter
i
Novosphingobium
j
Oceanicola
k
Rhodobacter
l
Streptomyces
m
Thalassospira
n
Algibacter
o
Polaribacter
p
Pseudoalteromonas
Relative sequence abundance (%)
Control
Enriched
Time_zero
Treatment

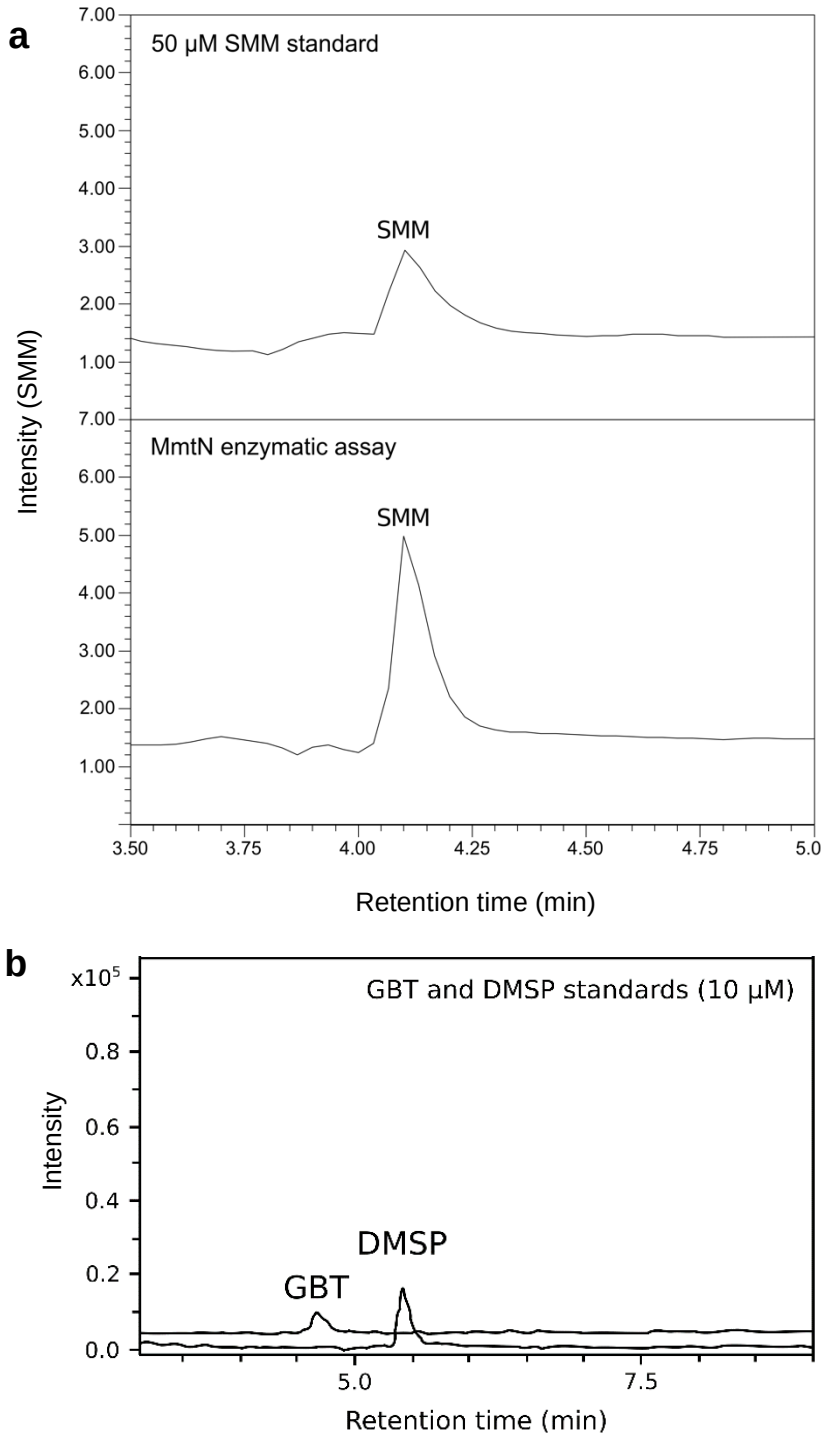
Supplementary Figure 10. Box plots showing relative sequence abundances of genera from the 16S rRNA gene amplicon data from Stiffkey saltmarsh surface sediment (Time zero, n=3 independent replicates), and from control (n=4 independent replicates) and enriched (n=3 independent replicates) incubation experiments after 14 days. Known DMSP-producing genera are shown (a-m) alongside genera not known to make DMSP but whose abundance was significantly higher in the enriched experiments (n-p). Kruskal-Wallis rank sum test generated overall p-values that indicated a significant difference existed at least once, and then pairwise comparisons were made between treatments using Dunn's test with "BH" p-value adjustment (precise p values are in Supplementary Table 6). Significance values are indicated as follows: * = $p \leq 0.05$, ** = $p \leq 0.01$, and *** = $p \leq 0.001$. Median values are indicated by the solid line within each box, and the box extends to upper and lower quartile values. Outlier data points are indicated by closed circles.


a
A
SMM
Met
DMSP-amine
detector reponse
20000
10000
0
b
B
detector response
2000
1000
0
c
C
detector response
2000
1000
0
10
20
retention time (min)

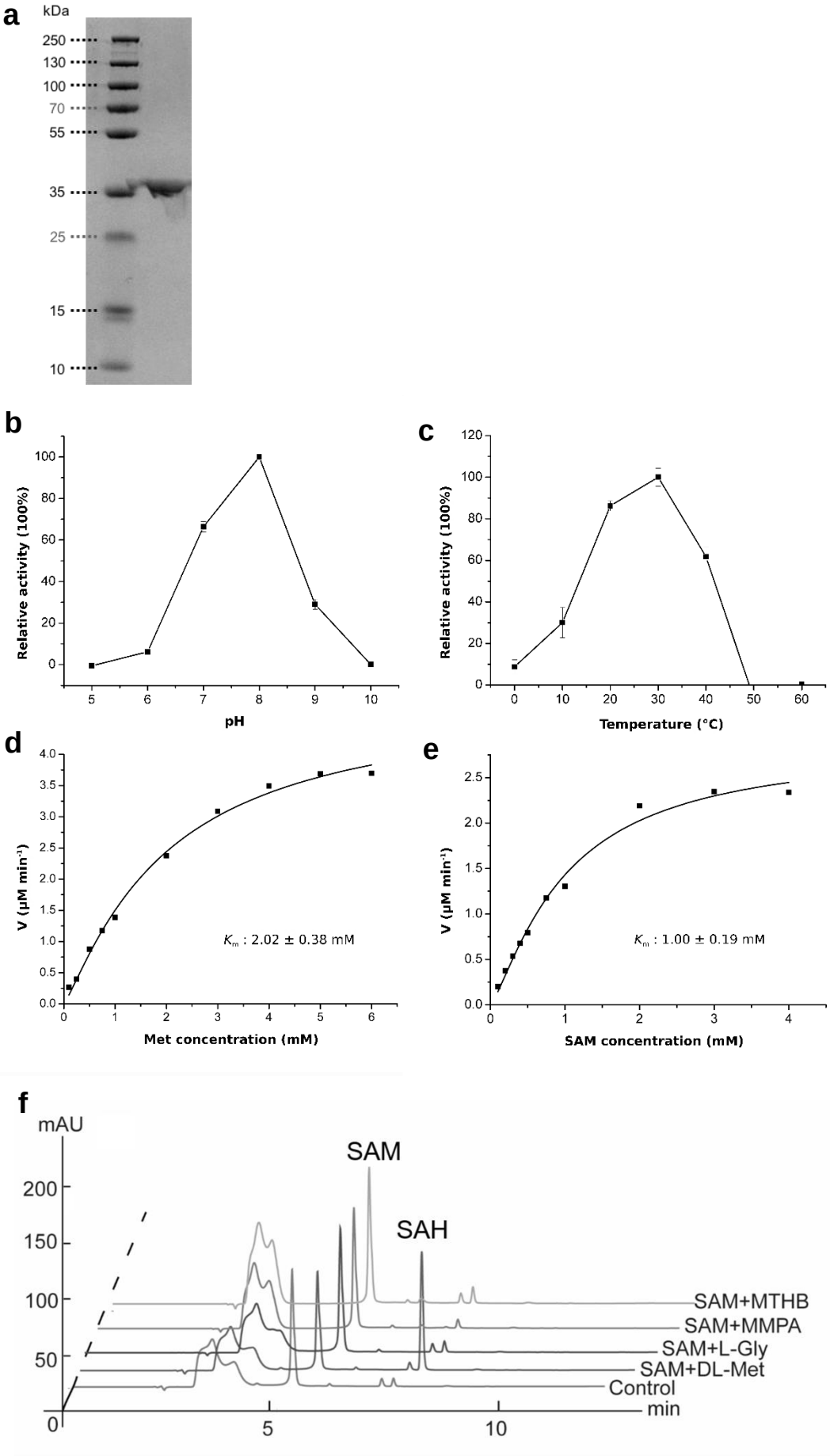
Supplementary Figure 11. Measurement of SMM, Met and DMSP-amine as fluorescent adducts of ortho-pthaldialdehyde, in seawater, sediment and cell extracts. (a) Standards (20 μM) of SMM, Met and DMSP-amine (grey) and 50x diluted *Novosphingobium* sp. BW1 cell extract (black); (b) Standards (5 μM) of SMM, Met and DMSP-amine (grey) and Stiffkey seawater (black); (c) Standards (10 μM) of SMM, Met and DMSP-amine (grey) and 5x diluted Stiffkey oxic sediment. For A, B and C, the peak of methionine is indicated with an arrow. Precise co-elution of Met and DMSP-amine was further confirmed by spiking the *Novosphingobium* sample with 5 μM each of Met and DMSP-amine; co-elution of the Met peak with standard was confirmed for seawater and sediment samples by spiking the samples with 1 μM Met. While the peak of SMM standard co-eluted precisely with a very weak shoulder on a broad, unresolved peak in the *Novosphingobium* extract, the identification was considered inadequate. Experiment was performed once and measurements were performed in triplicate.


Thalassospira tepidiphila MCCC 1A03514
TH4_06405
Thalassospira profundimaris WP0211
TH2_03115
TH2_03140
Thalassospira indica PB8BT
DY252_00705
Thalassospira sp.MCCC 1A01148
AUQ43_13010
Thalassospira australica NP3b2
WP_033070178
Thalassospira lohafexi 139Z-12
COO92_07865
Thalassospira lucentensis MCCC 1A00383
TH1_11240
Thalassospira sp.GB04J01
WP_102785695
Novosphingobium sp.MBES04
MBENS4_0458
Croceicoccus mobilis Ery22
WP_06677551
Labrenzia sp.011
DCO57_12185
Labrenzia marina DSM17023
CLV41_101426
Labrenzia sp.OB1
OA90_07285
Alteromonadaceae bacterium 2052S
K256_3685
Defluviimonas sp.MS2-2
WP_102224422
Roseovarius indicus B018
XM52_0708
Roseovarius confluentis SAG6
WP_103763460
Sagittula sp.P11
CDO87_02610
Rhodobacter aestuarii DSM19945
C8J27_10755
Agrobacterium vitis S4
BBK88_26090
Hartmannibacter diazotrophicus E19T
HDIA_1427
Micromonospora nigra DSM43818
GA0070616_3101
Saccharothrix syringae NRRLB-16468
WP_033429235
Nocardiopsis chromatogenes YIM90109
WP_017624909
Streptomyces mobaraensis NBRC13819
H340_16451
SAM-dependent methyltransferase
hypothetical diaminopimelate decarboxylase
hydroxyacid dehydrogenase
glycerol-3-phosphate dehydrogenase
aminobutyraldehyde dehydrogenase
pyridoxal phosphate-dependent aminotransferase
phosphoserine transaminase
Unrelated genes
type 11 methyltransferase
diaminopimelate decarboxylase
aldehyde dehydrogenase
NAD-dependent succinate-semialdehyde dehydrogenase
alcohol dehydrogenase
adenosylmethionine-8-amino-7-oxononanoate aminotransferase
BCCT family betaine/ carnitine transporter

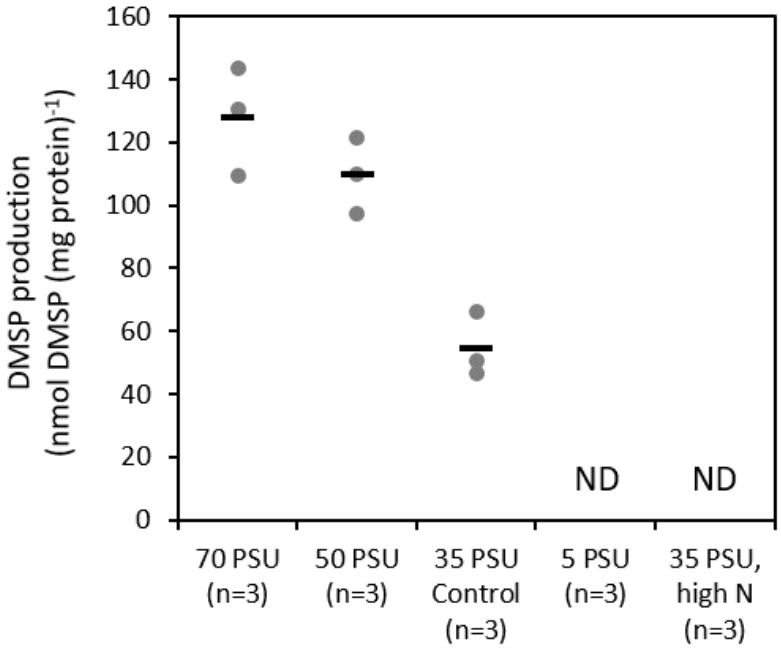
Supplementary Figure 12. Gene maps showing genomic locations of *mmtN* in selected *mmtN*-containing bacteria. The species names of the bacteria are indicated with their strain identifier. Genes are indicated as arrows and are to scale. The *mmtN* gene is shown in orange and belongs to a family of SAM-dependent methyltransferases. The *mmtN* gene locus tag of each species is indicated within the gene. A colour coded key indicates the predicted protein products of the genes immediately surrounding *mmtN*, many of which are predicted to be involved in DMSP synthesis in the respective bacteria. Grey arrows indicate genes that are not currently known/predicted to be involved in DMSP synthesis. All of the coloured genes shown for *Novosphingobium* sp. MBES04 are present in the *Novosphingobium* sp. BW1 cosmids pBIO0438 and pBIO0782. The aminotransferase gene (TH2_03140) knocked out in *T. profundimaris* strain J597 (with a reduced DMSP production phenotype) is indicated.



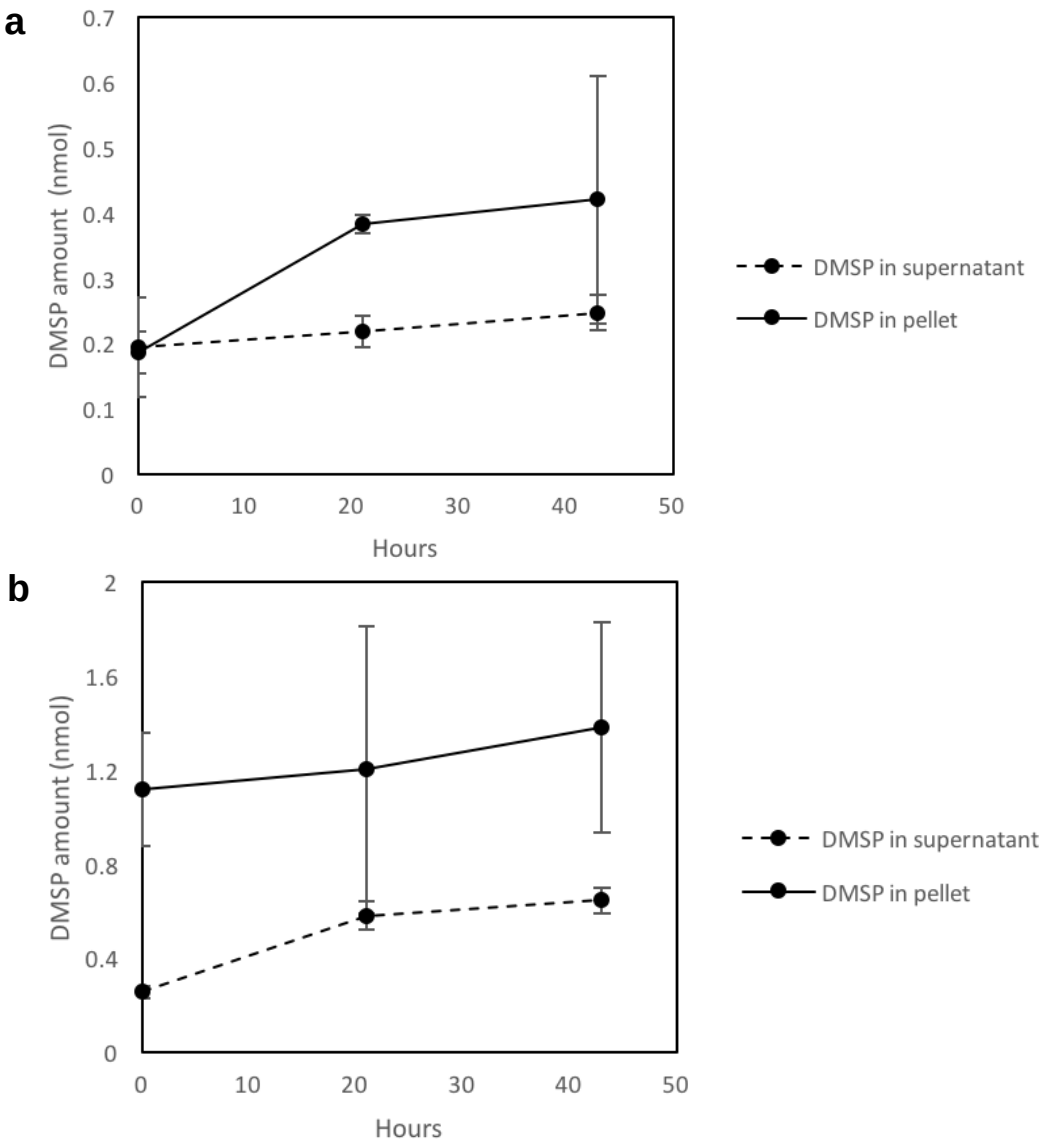
Supplementary Figure 13. (a) LC-MS chromatograms of 50 µM SMM (m/z 165) standard and of the SMM reaction product of purified MmtN protein when incubated with both Met and AdoMet. (b) LC-MS chromatogram showing DMSP (m/z 135) and GBT (m/z 118) 10 µM standards. Experiment was performed once with measurements in triplicate.


a
kDa
250
130
100
70
55
35
25
15
10
b
Relative activity (100%)
pH
c
Relative activity (100%)
Temperature (°C)
d
V (μM min⁻¹)
K_m : 2.02 ± 0.38 mM
Met concentration (mM)
e
V (μM min⁻¹)
K_m : 1.00 ± 0.19 mM
SAM concentration (mM)
f
mAU
SAM
SAH
SAM+MTHB
SAM+MMPA
SAM+L-Gly
SAM+DL-Met
Control
min

Supplementary Figure 14. Characterisation of MmtN. All experiments were repeated three times with similar results. (a) A 1:10 dilution of purified His-tagged MmtN run on a 12 % precast SDS-PAGE gel alongside PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (Sigma). (b-f) Protein characterisation, where relative activity is determined as the production of SAH from SAM. (n=3 parallel experiments, error bars represent standard deviation away from the mean value). An uncropped version of this gel is in Supplementary Fig. 18. (b) The effect of pH on the enzymatic activity of MmtN. Activity at pH 8.0 was defined as 100%. (c) The effect of temperature on the enzymatic activity of MmtN. Activity at 30°C was defined as 100%. (d) A non-linear fit curve for Met methylation by MmtN. Initial rates of SAH generation were determined with 3.34 µM MmtN and 0.1 – 6 mM Met in the reaction buffer. The kinetic constants of MmtN were measured under the optimal pH (8.0) and optimal temperature (30°C). K_M was 2.02 ± 0.38 mM. (e) A non-linear fit curve for SAM demethylation by MmtN. Initial rates of SAH generation were determined with 3.34 µM MmtN and 0.1 – 4 mM SAM in the reaction buffer, also at pH 8 and 30°C. K_M was 1.00 ± 0.19 mM. (f) Detection of the MTHB, MMPA, L-Gly and DL-Met methylation activity of MmtN via the intensity of absorbance of SAM/SAH on HPLC (260 nm), alongside a control of just the MmtN enzyme. The separate traces represent different reaction systems and include different potential substrates.



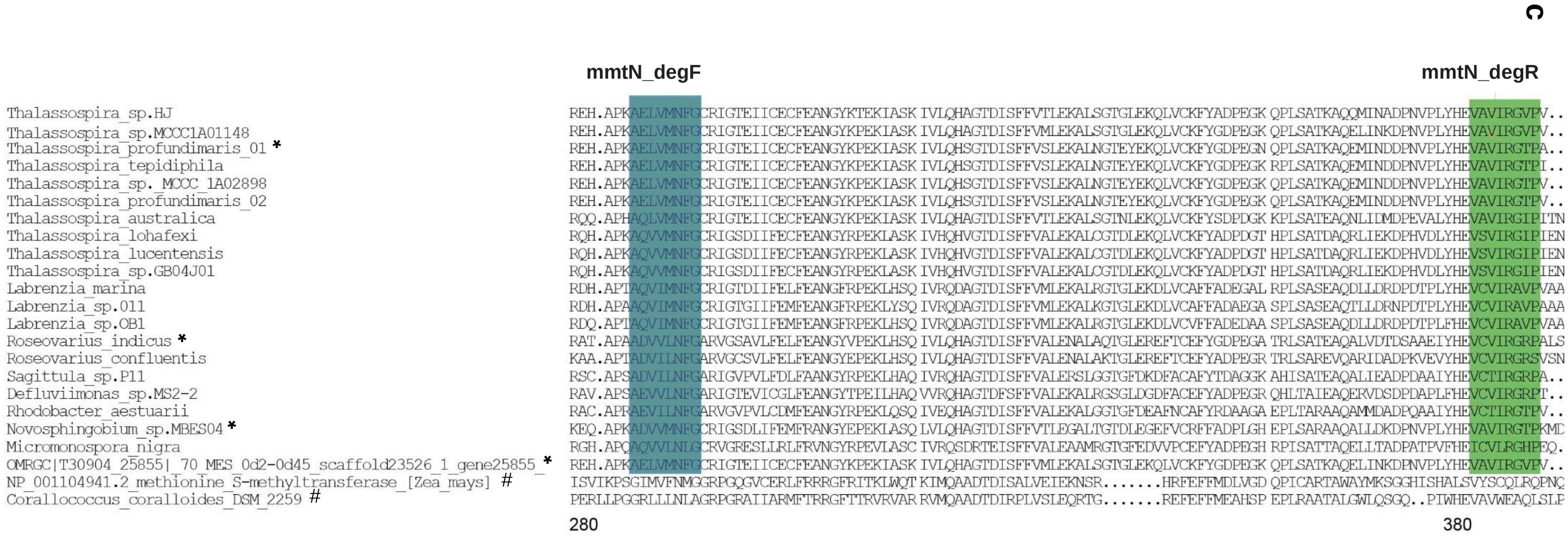
Supplementary Figure 15. The effect of increased (50, 70 PSU) / decreased (5 PSU) salinity or increased nitrogen (10 mM NH_4Cl) on DMSP levels in *T. profundimaris* DSM17430 versus a control condition of 35 PSU, 0.5 mM NH_4Cl. (n=3 biologically independent samples, the black line represents the mean value). ND, not detected.

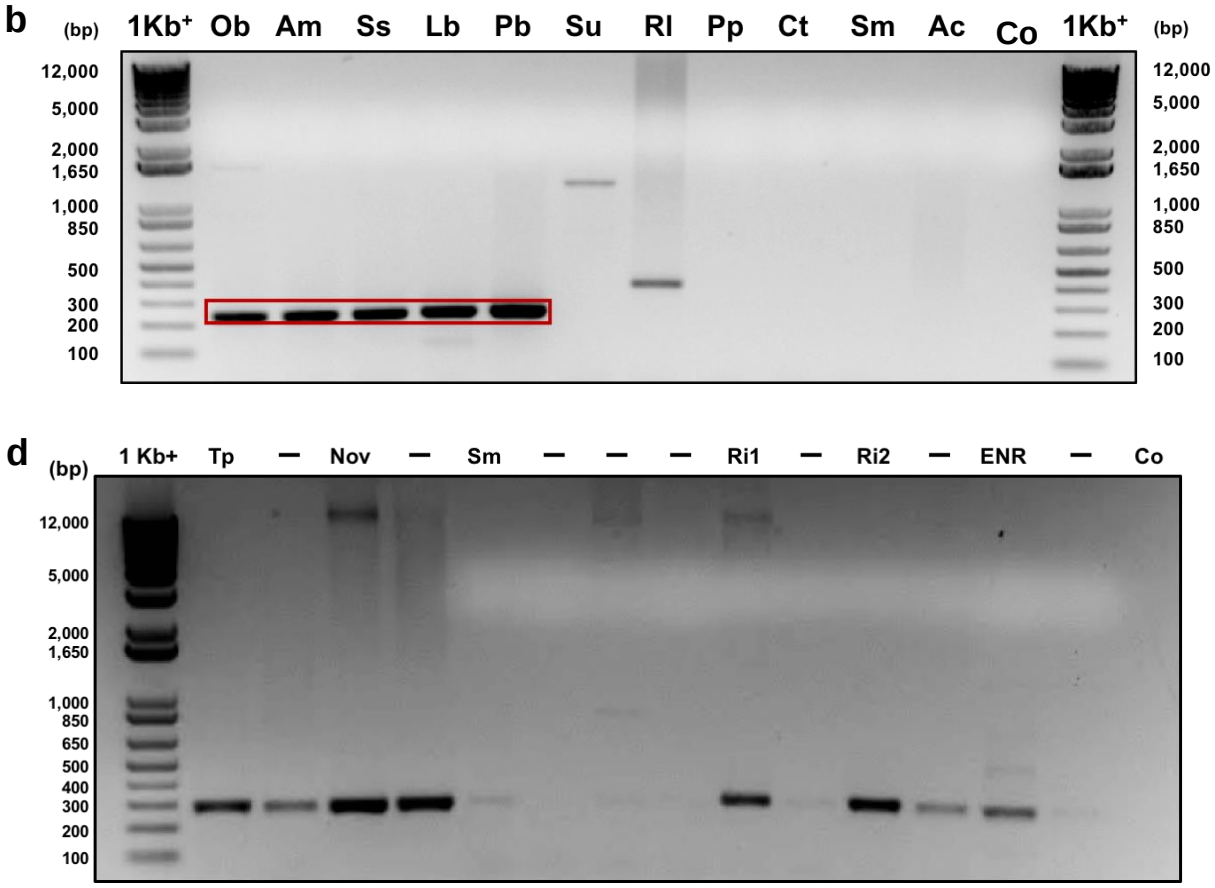


Supplementary Figure 16. DMSP amounts in axenic bacterial cells (pellet) and their cell-free supernatant when incubated in sterilised natural seawater (a) The *dsyB*-containing isolate, *P. bermudensis*, and (b) the *mmtN*-containing isolate *Novosphingobium* sp. BW1. Samples were taken at time zero, after 21 hours and 43 hours (for a-b), and the DMSP determined by alkaline lysis purge and trap GC quantification. Error bars display standard deviation from the mean value (n=3 biologically independent samples). Note that the *L. aggregata dsyB*$^-$ strain imports the DMSP from the seawater after 21 hours incubation (in pellet), whereas all wild type DMSP-producing bacteria show increased intracellular (pellet) and extracellular (supernatant) DMSP levels after 21 and 43 hours incubation.

a


dsyB_deg1F
dsyB_deg2R
Caenispirillum_salinarum_AK4
Thalassobaculum_salexigens_DSM_19539
Rhodobacteraceae_bacterium_PD-2
Roseibacterium_elongatum_DSM_19469_DFL-43
Pseudooceanicola_atlanticus_22II-s11g
Oceanicola_batsensis_HTCC2597 *
Salipiger_mucosus_DSM_16094
Roseivivax_halodurans_JCM_10272
Citreicella_sp._357
Pelagibaca_bermudensis_HTCC2601
Sediminimonas_qiaohouensis_DSM_21189
Oceanicola_sp._HL-35
Oceanicola_sp._S124
Oceanicola_nanhaiensis_DSM_18065
alpha_proteobacterium_BAL199
Nisaea_denitrificans_DSM_18348
Amorphus_coralli_DSM_19760 *
Maritimibacter_alkaliphilus_HTCC2654_
Sagittula_stellata_E-37 *
Stappia_stellulata_DSM_5886
Labrenzia_aggregata_CECT4801 *
Labrenzia_sp._CP4
Labrenzia_alexandrii_DFL-11
Defluviimonas_sp._20V17
Bacillus mycoides DSM2048 #
Streptomyces varsoviensis DSM40346 #
50.....60.....70.....80.....90.....100.....110.....120.....130.....140.

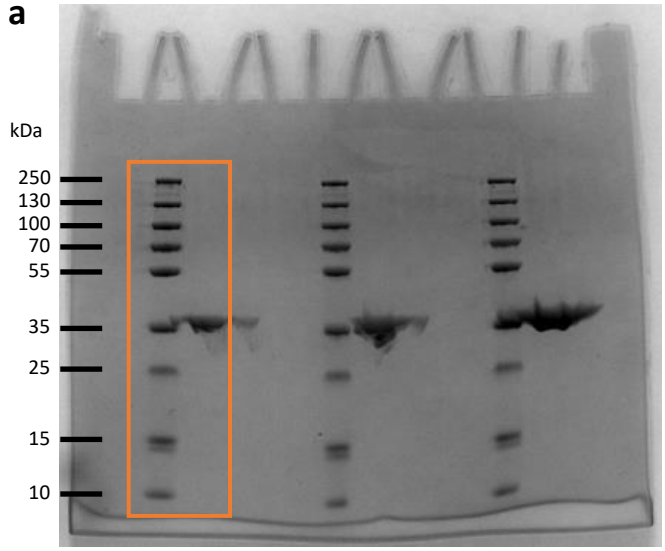

c
mmtN_degF
mmtN_degR
Thalassospira_sp.HJ
Thalassospira_sp.MCCC1A01148
Thalassospira_profundimaris_01 *
Thalassospira_tepidiphila
Thalassospira_sp._MCCC_1A02898
Thalassospira_profundimaris_02
Thalassospira_australica
Thalassospira_lohafexi
Thalassospira_lucentensis
Thalassospira_sp.GB04J01
Labrenzia_marina
Labrenzia_sp.011
Labrenzia_sp.OB1
Roseovarius_indicus *
Roseovarius_confluentis
Sagittula_sp.P11
Defluviimonas_sp.MS2-2
Rhodobacter_aestuarii
Novosphingobium_sp.MBES04 *
Micromonospora_nigra
OMRGC|T30904_25855|_70_MES_0d2-0d45_scaffold23526_1_gene25855_ *
NP_001104941.2_methionine_S-methyltransferase_[Zea_mays] #
Corallococcus_coralloides_DSM_2259 #
280
380



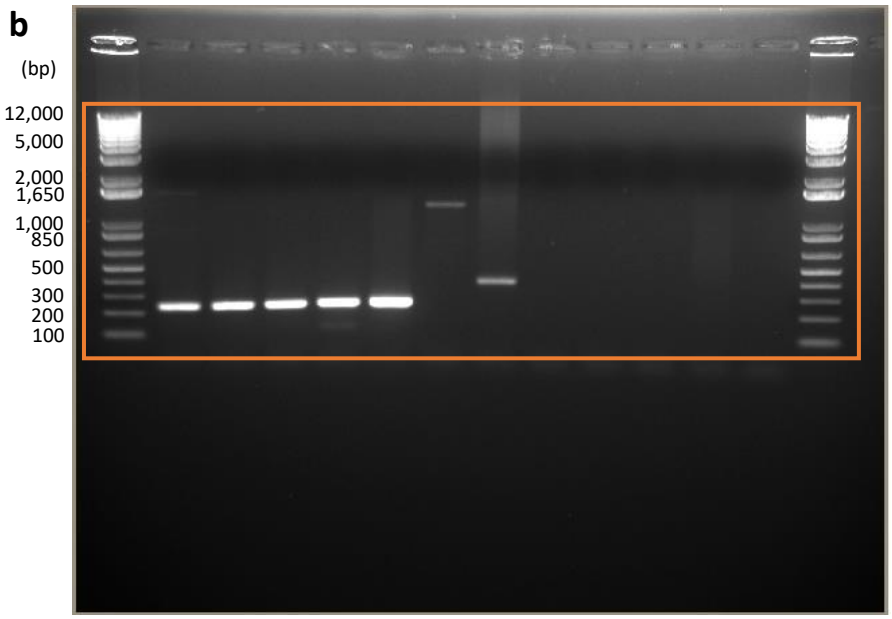
Supplementary Figure 17. (a) Alignment of functional DsyB protein sequences and non-functional DsyB-like sequences (#) (Curson *et al.*[1,6]). The amino acid locations spanning the dsyB_deg1F (teal) and the dsyB_deg2R (orange) primer sequences are indicated, as are the DsyB amino acid locations. Strains used in primer optimisation are indicated by *. (b) The optimised PCR amplification of *dsyB* from genomic DNA using *dsyB* degenerate primers. Positive controls include *O. batsensis* (Ob), *A. coralli* (Am), *S. stellata* E-37 (Ss), *L. aggregata* LZB033 (Lb) and *P. bermudensis* HTCC2597 (Pb); negative controls include *Sulfitobacter* EE36 (Su), *R. leguminosarum* J391 (Rl); negative controls containing the eukaryotic *DSYB* gene include *Prymnesium parvum* (Pp), *Chrysochromulina tobin* (Ct), *Symbiodinium microadriaticum* (Sm) and *Acropora cervicornis* (Ac); and a water control (Co). *dsyB* is indicated by a 246 bp fragment (the red box) in all positive controls. This experiment was

repeated three times with similar results. (c) Alignment of functional MmtN protein sequences and the N-terminus of *Zea mays* MMT, and a bacterial MMT sequence (#). The amino acid locations spanning the mmtN_degF (blue) and the mmtN_degR (green) primer sequences are indicated, as are the MmtN amino acid locations. Strains used in primer optimisation are indicated by *. (d) The optimised PCR amplification of *mmtN* from genomic DNA using *mmtN* degenerate primers. Positive controls include *T. profundimaris* (Tp), *Novosphingobium* sp. BW1 (Nov), *S. mobaraensis* (Sm), *R. indicus* (Ri1+2) and environmental DNA from the enriched sediment (ENR); and a water control (Co). Lanes marked with (–) relate to test PCR cycles not used in this manuscript. *mmtN* is indicated by a 301 bp fragment in all positive controls. This experiment was repeated two times with similar results. Uncropped versions of these gel images are in Supplementary Fig. 18.

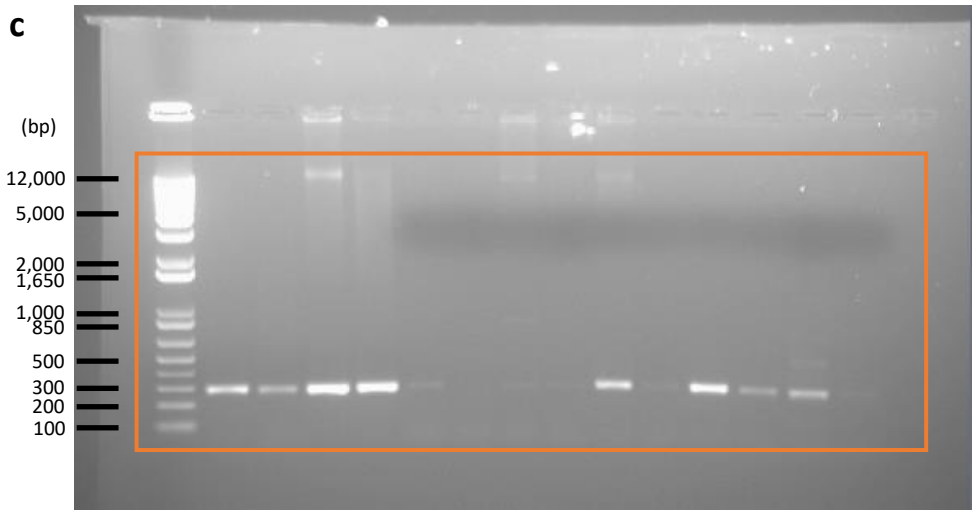
a


kDa
250
130
100
70
55
35
25
15
10

b


(bp)
12,000
5,000
2,000
1,650
1,000
850
500
300
200
100

c


(bp)
12,000
5,000
2,000
1,650
1,000
850
500
300
200
100

Supplementary Figure 18. (a) The full length, unmanipulated 12% SDS-PAGE gel of Supplementary Figure 14a (purified His-tagged MmtN) plus Prestained Protein Ladder. Also the full length agarose gel images of (b) Supplementary Figure 17b (the optimised PCR amplification of *dsyB* from genomic DNA using *dsyB* degenerate primers) plus 1Kb+ ladder and (c) Supplementary Figure 17d (The optimised PCR amplification of *mmtN* from genomic DNA using *mmtN* degenerate primers) plus 1Kb+ ladder. Orange boxes display where the gel images were cropped.

Supplementary Tables:

Supplementary Table 1. (a) Characteristics of sampling sites including DMSP concentration of sediment and overlying seawater at selected sampling sites. (b) DMSP concentrations in Stiffkey surface sediment sampled at other dates.

a. Sampling site (August 2016)	**Location (Lat, Long)**	**Depth**	**nmol DMSP (g^{-1} or ml^{-1})***	**Methionine (μM)***	**Salinity (PSU)**	**pH**	**Temp. (°C)**
Stiffkey sediment	52.964947, 0.925655	Oxic, 0-1 cm	128 ± 13.9	3.9 ± 0.89			
		Anoxic, 1-5 cm	9.8 ± 1.8	NT†			
		Anoxic, 5-10 cm	4.6 ± 0.74	NT			
		Anoxic, 5-15 cm	3.9 ± 0.43	NT			
Stiffkey pond water			0.66 ± 0.10	0.34 ± 0.06	32	7.5	19
Cley sediment	52.957825, 1.046553	Oxic, 0-1 cm	91 ± 15.6	1.0 ± 0.88			
Cley pond water			1.1 ± 0.03	NT	32	7.6	17
Yarmouth sediment	52.614855, 1.715255	Oxic, 0-1 cm	103 ± 30.4	2.7 ± 0.20			
Yarmouth pond water			0.91 ± 0.08	NT	30	7	17
Mariana Trench sediment (4,500 m)	10.409167, 142.35694	Anoxic, 4,500 m	5.0 ± 0.34	NT			
Mariana surface water			6.3×10^{-3} ± 1.6×10^{-3}	NT	34	8.2	29.7

* Mean of three biological replicates (n=3) and standard deviation shown
† NT, not tested

b. Sampling site	**Location (Lat, Long)**	**Date**	**nmol DMSP (g^{-1} or ml^{-1})**
Stiffkey sediment	52.964947, 0.925655	April 2015	61.9 ± 17.3
		January 2016	87.1 ± 8.9
		August 2016	128 ± 13.9
		October 2017	106 ± 26.3
		July 2018	76.4 ± 12.5

* Mean of three biological replicates (n=3) and standard deviation shown

Supplementary Table 2. Environmental parameters of Stiffkey saltmarsh.

O_2 saturation (%)			Nutrient concentration (μM)*					
0 mm	**80 mm**	**160 mm**	**DOC†**	**TDN‡**	**Nitrate**	**Nitrite**	**Ammonia/ ammonium**	**Phosphate**
62	34	29	300 ± 5	40 ± 0.6	0.87 ± 0.03	0.33 ± 0.01	13.5 ± 0.6	1.4 ± 0.01

* Mean of three biological replicates (n=3) and standard deviation shown
† DOC, dissolved organic carbon
‡ TDN, total dissolved nitrogen

Supplementary Table 3. qPCR analysis for *dsyB* and *mmtN* on DNA extracted from the phyllosphere and rhizosphere of *S. anglica* leaves and roots.

dsyB* gene abundance (copies {g plant material^{-1}})		***mmtN* gene abundance (copies {g plant material^{-1}})***	
Phyllosphere	**Rhizosphere**	**Phyllosphere**	**Rhizosphere**
9.4×10^5 $\pm$ 3.0×10^4	3.4×10^6 $\pm$ 4.4×10^5	1.5×10^4 $\pm$ 438	4.0×10^4 $\pm$ 3.1×10^3

*Mean of three replicates (n=3) and standard deviation shown

Supplementary Table 4. Species shown to produce DMSP, including those containing *dsyB, mmtN* or unknown DMSP synthesis genes, as of June 2018.

(Table shown in separate file)

Supplementary Table 5. Analysis of potential DMSP-producing species in 16S rRNA amplicon data from Stiffkey surface sediment. Mean total percentages of genera potentially containing DMSP-producing species (see Supplementary Table 3) ± standard deviation for each treatment are shown, as well as pairwise comparison adjusted *p*-values. Differences between DMSP-producing genera across treatment groups and DMSP gene categories were first assessed for normality using the Shapiro-Wilks test, followed by analysis of variance, and Tukey multiple comparison of means test in the 's20x' statistical package in R.

Gene category	Sediment treatment	Total % of genera potentially containing DMSP-producing species*	Pairwise comparisons†
mmtN	T0	1.9 ± 0.44	T0 vs CONTROL, $p > 0.05$ T0 vs ENRICHED, $p = 0.0000092$
	ENRICHED	28.7 ± 2.2	ENRICHED vs CONTROL, $p = 0.000028$
	CONTROL	7.5 ± 3.4	-
dsyB	T0	2.3 ± 0.57	T0 vs CONTROL, $p = 0.003$ T0 vs ENRICHED, $p = 0.0096$
	ENRICHED	10.5 ± 1.3	ENRICHED vs CONTROL, $p > 0.05$
	CONTROL	11.7 ± 3.4	-
Unknown DMSP-producing isolates	T0	6.6 ± 1.1	T0 vs CONTROL, $p = 0.000016$ T0 vs ENRICHED, $p = 0.008$
	ENRICHED	4.4 ± 0.28	ENRICHED vs CONTROL, $p = 0.0004$
	CONTROL	0.9 ± 0.10	-
Total DMSP-producing strains	T0	9.1 ± 0.67	T0 vs CONTROL, $p > 0.05$ T0 vs ENRICHED, $p = 0.000029$
	ENRICHED	42.2 ± 0.88	ENRICHED vs CONTROL, $p = 0.000067$
	CONTROL	15.0 ± 5.5	-

*Mean of three biological replicates (n=3) and standard deviation shown
†Pairwise comparison adjusted *p*-values.

Supplementary Table 6. Evaluation of differences in genus level microorganism communities within Stiffkey saltmarsh surface sediment treatment groups for (a) known DMSP producers, and (b) microorganisms with unknown DMSP production. (Time zero, n=3 independent replicates), control (n=4 independent replicates) and enriched (n=3 independent replicates) treatment groups were compared. A Kruskal-Wallis rank sum test generated overall p-values, indicating at least one significant difference across the treatment groups. Pairwise comparisons were made using the non-parametric, two-sided Dunn's test with "BH" p-value correction for multiple pairwise comparisons.

	Pairwise comparisons from Dunn's test with "BH" *p*-value correction		
Genus	**Control - Enriched**	**Control – T0**	**Enriched – T0**
a. DMSP Producers			
Albimonas	NS*	NS	0.008
Alteromonas	NS	NS	0.007
Asterionellopsis	NS	0.007	NS
Hoeflea	NS	0.010	NS
Labrenzia	NS	0.007	NS
Marinobacter	0.026	NS	NS
Novosphingobium	NS	NS	0.016
Oceanicola	NS	NS	0.007
Rhodobacter	NS	0.026	NS
Streptomyces	NS	NS	0.007
Thalassospira	NS	NS	0.007
b. Potential DMSP Producers			
Agarivorans	NS	NS	0.016
Algibacter	0.043	NS	0.027
Hyphomicrobium	NS	NS	0.007
Polaribacter	0.038	NS	0.032
Pseudoalteromonas	0.050	NS	0.023

*NS = not significant, $p>0.05$

Supplementary Table 7. Metagenome information and results of *mmtN/dsyB* metagenomic searches in OM-RGC and Stiffkey metagenomes. Bacterial sequences were normalised to gene length and the shortest read length**.** Estimated percentages were calculated using *recA* abundance.

(Table shown in separate file)

Supplementary Table 8. DMSP production in selected strains of bacteria and activity of the corresponding cloned *mmtN* genes.

(Table shown in separate file)

Supplementary Table 9. Analysis of the 16S rRNA, *dsyB* and *mmtN* genes amplified by qPCR from DNA extracted from various surface sediment and water samples.

Sample	16S DNA (copies g^{-1} or ml^{-1})*	*dsyB* DNA (copies g^{-1} or ml^{-1})*	*mmtN* DNA (copies g^{-1} or ml^{-1})*	Total percentage of DMSP producers (%)	Number of potential DMSP-producing organisms in the sediment/water (cells g^{-1} or ml^{-1})	Average number of potential DMSP-producing organisms in the coastal sediment (cells g^{-1})
Stiffkey sediment	$7.9 \times 10^9 \pm 2.2 \times 10^9$	$1.8 \times 10^7 \pm 1.7 \times 10^6$	$1.1 \times 10^6 \pm 2.2 \times 10^5$	0.24%	1.9×10^7	
Yarmouth sediment	$2.1 \times 10^{10} \pm 5.8 \times 10^9$	$4.9 \times 10^7 \pm 3.1 \times 10^7$	$1.2 \times 10^6 \pm 1.6 \times 10^5$	0.24%	5.1×10^7	
Cley sediment	$1.3 \times 10^{10} \pm 3.5 \times 10^9$	$1.2 \times 10^7 \pm 2.6 \times 10^6$	$4.0 \times 10^5 \pm 8.4 \times 10^4$	0.10%	1.3×10^7	**Sediment**†: $2.7 \times 10^7 \pm 1.7 \times 10^7$
Mariana trench sediment	$1.3 \times 10^7 \pm 3.5 \times 10^6$	$4.5 \times 10^5 \pm 7.1 \times 10^4$	$1.1 \times 10^4 \pm 135$	3.49%	4.4×10^5	
Stiffkey pond water	$5.9 \times 10^3 \pm 2.1 \times 10^3$	115 ± 47.6	5.0 ± 1.25	0.57%	33.5	
Coastal seawater	$3.4 \times 10^5 \pm 9.5 \times 10^4$	$1.1 \times 10^3 \pm 393$	49.2 ± 12.9	0.34%	1.2×10^3	

* Mean of three replicates (n=3) and standard deviation shown
† calculated from values for Stiffkey, Yarmouth and Cley coastal sediment

Supplementary Table 10. Cell counts of bacterial copy numbers in Stiffkey sediment samples.

	Cell count (dilution factor 1:10000)					Average cell count ml^{-1}*	Average cell count g^{-1}*
Replicate 1	153	243	169	228	246	$1.0 \times 10^{10} \pm 1.9 \times 10^{9}$	$2.0 \times 10^{10} \pm 3.7 \times 10^{9}$
Replicate 2	163	229	189	214	263	$1.0 \times 10^{10} \pm 1.7 \times 10^{9}$	$2.0 \times 10^{10} \pm 3.2 \times 10^{9}$
Replicate 3	184	214	197	227	223	$1.0 \times 10^{10} \pm 7.9 \times 10^{8}$	$2.0 \times 10^{10} \pm 1.5 \times 10^{9}$
						Average:	**$2.0 \times 10^{10} \pm 3.0 \times 10^{9}$**

* Mean of five replicates (n=5) and standard deviation shown

Supplementary Table 11. Metatranscriptome sample information

Metatranscriptome/ Project	Sample ID	Biome	Location, latitude, longitude	Size Fraction	Database
Tara Oceans	ERR598943	Marine, seawater, depth 5 m	South Pacific Ocean, -5.2529, -85.1545	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598945	Marine, seawater, depth 5 m	Southern Ocean, -60.2287, -60.6476	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598952	Marine, seawater, depth 30 m	North Pacific Ocean, 2.0299, -84.5546	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598953	Marine, seawater, depth 177 m	South Pacific Ocean, -12.9794, -96.0232	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598956	Marine, seawater, depth 150 m	South Pacific Ocean, -8.9109, -140.2845	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598976	Marine, seawater, depth 5 m	North Atlantic Ocean, 36.1715, -29.0230	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598977	Marine, seawater, depth 60 m	Arabian Sea, 14.5536, 70.0128	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR598988	Marine, seawater, depth 120 m	South Pacific Ocean, -9.0714, -140.5973	0.22 μm – 3 μm	EBI
Tara Oceans	ERR598999	Marine, seawater, depth 600 m	South Pacific Ocean, 8.9729, -139.2393	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599004	Marine, seawater, depth 450 m	North Pacific Ocean, 6.3599, -103.0598	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599010	Marine, seawater, depth 5 m	South Atlantic Ocean, -20.9354, -35.1803	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599011	Marine, seawater, depth 5 m	Arabian Sea, 14.6059, 69.9776	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599015	Marine, seawater, depth 375 m	North Pacific Ocean, 14.2025, -116.6433	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599016	Marine, seawater, depth 75 m	Indian Ocean, -16.9534, 53.9601	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599022	Marine, seawater, depth 5 m	South Atlantic Ocean, -30.1367, -43.2899	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599027	Marine, seawater, depth 40 m	South Atlantic Ocean, -47.2007, -57.9446	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599031	Marine, seawater, depth 600 m	Arabian Sea, 20.8457, 63.5851	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599035	Marine, seawater, depth 5 m	South Atlantic Ocean, -47.1863, -58.2902	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599067	Marine, seawater, depth 380 m	North Pacific Ocean, 2.0649, -84.5546	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599098	Marine, seawater, depth 5 m	Indian Ocean, -6.9570, 53.9801	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599102	Marine, seawater, depth 5 m	Arabian Sea, 19.0393, 64.4913	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599104	Marine, seawater, depth 90 m	Southern Ocean, -62.2231, -49.2139	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599109	Marine, seawater, depth 340 m	Arabian Sea, 19.0351, 64.5638	0.22 μm – 1.6 μm	EBI
Tara Oceans	ERR599113	Marine, seawater, depth 50 m	South Pacific Ocean, -12.9723, -96.0122	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599169	Marine, seawater, depth 5 m	South Pacific Ocean, -13.0023, -95.9759	0.22 μm – 3 μm	EBI
Tara Oceans	ERR599176	Marine, seawater, depth 5 m	Southern Ocean, 62.0385, -49.5290	0.22 μm – 3 μm	EBI

Supplementary Table 12. *Tara* Oceans metatranscriptome *mmtN* transcript abundance, alongside *dsyB, DSYB* and DMSP lyases calculated in Curson *et al* (2018)[1].

(Table shown in separate file)

Supplementary Table 13. Seawater incubation experiment comparing DMSP production by wild type *L. aggregata* and the *dsyB*$^-$ mutant strain.

Time point	DMSP production (nmol)				
	Seawater	Wild type strain		*dsyB*$^-$ strain	
		Cell-free supernatant	Cell pellet	Cell-free supernatant	Cell pellet
0 h	0.07 ± 0.001	0.06 ± 0.002	0.07 ± 0.004	0.06 ± 0.000	0
21 h	0.06 ± 0.000	0.08 ± 0.005	0.13 ± 0.020	0	0.04 ± 0.04

* Mean of three replicates (n=3) shown with standard deviation

Supplementary Table 14. Species and accession numbers of the sequences used to create HMM profiles for bioinformatics analysis for *mmtN* (June 2018).

MmtN-containing species (April 2017)	Accession Number
Novosphingobium sp. MBES04	WP_052321947
Croceicoccus mobilis	WP_066775518
Thalassospira sp. HJ	WP_044830103
Thalassospira sp. MCCC_1A01148	WP_062957385
Thalassospira indica	WP_064788038
Thalassospira tepidiphila MCCC_1A03514	WP_064780488
Thalassospira australica	WP_033070178
Thalassospira lucentensis	WP_022734010
Thalassospira sp. MCCC_1A02898	WP_063085993
Thalassospira profundimaris sp. DSM17430	WP_008888945
Labrenzia sp. OB1	WP_068409229
Roseovarius indicus 01	WP_064261696
Roseovarius indicus 02	WP_057814729
Roseovarius indicus 03	KRS18724
Rhodobacter aestuarii	WP_076485456
Saccharothrix syringae	WP_033429235
Micromonospora nigra	WP_091090849
Agrobacterium vitis	WP_071204336
Nocardiopsis chromatogenes	WP_017624909
Streptomyces mobaraensis NBRC_13819	EME99407

Supplementary Table 15. Strains used in this study.

Strain	Description	Reference
Escherichia coli 803	Strain used for routine transformations	Wood (1966)[2]
E. coli BL21	Strain for overexpression of cloned genes in pET vectors	Studier and Moffat (1986)[3]
E. coli JM101	Strain for expression of *lacZ* gene in blue-white screen	Yanisch-Perron et al. (1985)[4]
Rhizobium leguminosarum J391	Streptomycin-resistant derivative of wild type strain 3841 used for library screening and expression of genes cloned in plasmid pLMB509 or pRK415	Young et al. (2006)[5]
Labrenzia aggregata LZB033	Wild type strain, isolated from ME3 site, *dsyB*+	Curson et al. (2017)[6]
Sagittula stellata E-37	Wild type strain, *dsyB*+ control for degenerate primer optimisation	Gonzalez et al. (1997)[7]
Oceanicola batsensis DSM21189	Wild type strain, *dsyB*+ control for degenerate primer optimisation	Cho and Giovannoni (2004)[8]
Amorphus coralli DSM19760	Wild type strain, *dsyB*+ control for degenerate primer optimisation	Zeevi Ben Yosef et al. (2008)[9]
Pelagibaca bermudensis HTCC2597	Wild type strain, *dsyB*+ control for degenerate primer optimisation	Cho and Giovannoni (2006)[10]
Sulfitobacter sp. EE-36	Wild type strain, *dsyB*- control for degenerate primer optimisation	Gonzalez et al. (1996)[11]
Novosphingobium sp. BW1	Wild type strain, isolated from Stiffkey saltmarsh	This study
Stappia sp. BW2	Wild type strain, isolated from Stiffkey saltmarsh	This study
Pseudooceanicola sp. BW3	Wild type strain, isolated from Stiffkey saltmarsh	This study
Labrenzia sp. BW4	Wild type strain, isolated from Stiffkey saltmarsh	This study
Rhodobacterales bacterium sp. BW5	Wild type strain, isolated from Stiffkey saltmarsh	This study
Marinobacter sp. BW6	Wild type strain, isolated from Stiffkey saltmarsh	This study
Alteromonas sp. BW7	Wild type strain, isolated from Stiffkey saltmarsh	This study
Rhodobacter sp. BW8	Wild type strain, isolated from Stiffkey saltmarsh	This study
Oceanicola sp. BW9	Wild type strain, isolated from Stiffkey saltmarsh	This study
Asterionellopsis glacialis PR1	Diatom isolated from Stiffkey saltmarsh	This study
Thalassospira profundimaris DSM17430 (J594)	Wild type strain used to demonstrate DMSP production by *mmtN* expression	DSMZ Culture Collection
Roseovarius indicus B108 DSM26383	Wild type strain used to demonstrate DMSP production by *mmtN* expression	DSMZ Culture Collection
Nocardiopsis chromatogenes DSM44844	Wild type strain used to demonstrate DMSP production by *mmtN* expression	DSMZ Culture Collection
Streptomyces mobaraensis DSM40847	Wild type strain used to demonstrate DMSP production by *mmtN* expression	DSMZ Culture Collection
T. profundimaris J595	Rifampicin-resistant derivative of *T. profundimaris* DSM17340	This study
T. profundimaris J596	*T. profundimaris* J595 with mutation in *mmtN* (TH2_03115)	This study
T. profundimaris J597	*T. profundimaris* J595 with mutation in aminotransferase (TH2_03140) linked to *mmtN*	This study
Pseudobacteriovorax antillogorgiicola DSM103413	Wild type strain, contains homologue of plant-like MMT enzyme	DSMZ Culture Collection
Corallococcus coralloides DSM2259	Wild type strain, contains homologue of plant-like MMT enzyme	DSMZ Culture Collection
Stigmatella aurantiaca DSM17044	Wild type strain, contains homologue of plant-like MMT enzyme	DSMZ Culture Collection
Myxococcus fulvus DSM16525	Wild type strain, contains homologue of plant-like MMT enzyme	DSMZ Culture Collection

Supplementary Table 16. Plasmids used in this study.

Plasmid	Description	Reference
pLAFR3	Wide host-range cosmid vector, used for library construction	Staskawicz et al. (1987)[12]
pET21a	Plasmid vector for expression of cloned genes in *E. coli*	Merck Millipore
pRK2013	Helper plasmid used in triparental matings	Figurski and Helinski (1979)[13]
pBIO1879	pK19mob suicide vector derivative used to create knockout mutants in *T. profundimaris*	Todd et al. (2011)[14]
pLMB509	Expression vector used to express *mmtN* in *T. profundimaris* mutant	Tett et al. (2012)[15]
pBIO2275	*Prymnesium parvum* CCAP946/6 *DSYB* cloned in pRK415, control for degenerate primer optimisation	Curson et al. (2018)
pBIO2276	*Symbiodinium microadriaticum* CCMP2467 *DSYB*, codon-optimised, synthesised and cloned in pLMB509, control for degenerate primer optimisation	Curson et al. (2018)
pBIO2272	*Chrysochromulina tobin* CCMP291 *DSYB*, codon-optimised, synthesised and cloned in pLMB509, control for degenerate primer optimisation	Curson et al. (2018)
pBIO2270	*Acropora cervicornis DSYB*, codon-optimised, synthesised and cloned in pLMB509, control for degenerate primer optimisation	Curson et al. (2018)
pBIO2279	pLAFR3 cosmid from a *Novosphingobium* sp. BW1 library that contains ~21 kb genomic DNA including *mmtN*	This study
pBIO2280	pLAFR3 cosmid from a *Novosphingobium* sp. BW1 library that contains ~30 kb genomic DNA including *mmtN*	This study
pBIO2281	*Novosphingobium* sp. BW1 *mmtN* cloned in pET21a(+)	This study
pBIO2282	*Novosphingobium* sp. BW1 *mmtN* cloned into pLMB509	This study
pBIO2283	*T. profundimaris* DSM17340 *mmtN* cloned in pET21a(+)	This study
pBIO2284	*R. indicus mmtN* cloned in pET21a(+)	This study
pBIO2285	*N. chromatogenes mmtN* cloned in pET21a(+)	This study
pBIO2286	*S. mobaraensis mmtN* cloned in pET21a(+)	This study
pBIO2287	*T. profundimaris mmtN* internal fragment cloned in pBIO1879 for knockout mutagenesis	This study
pBIO2278	*T. profundimaris* aminotransferase gene (TH2_03140) internal fragment cloned in pBIO1879 for knockout mutagenesis	This study

Supplementary Table 17. Oligonucleotide primers used in this study.

Primer name	Sequence (5' to 3')*	Use
8F	AGAGTTTGATCCTGGCTCAG	Forward primer used to amplify the 16S rRNA gene for identification from *A. glacialis*
27F	AGAGTTTGATCCTGGCTCAG	Forward primer used to amplify the 16S rRNA gene for identification from bacteria
1492R	GGTTACCTTGTTACGACTT	Reverse primer used to amplify the 16S rRNA gene for identification from bacteria and *A. glacialis*
Eub_338F	ACTCCTACGGGAGGCAGCAG	Forward primer used to amplify the 16S rRNA gene for RT-qPCR
Eub_518R	ATTACCGCGGCTGCTGG	Reverse primer used to amplify the 16S rRNA gene for RT-qPCR
Euk_A	AACCTGGTTGATCCTGCCAGT	Forward primer used to amplify the 18S rRNA gene for identification from *A. glacialis*
Euk_B	TGATCCTTCTGCAGGTTCACCTAC	Reverse primer used to amplify the 18S rRNA gene for identification from *A. glacialis*[16]
M13 uni (-43)	AGGGTTTTCCCAGTCACGACGTT	Universal forward primer used to amplify inserts in pLAFR3
M13 rev (-29)	CAGGAAACAGCTATGACC	Universal reverse primer used to amplify inserts in pLAFR3
dsyB_deg1F	CATGGGSTCSAAGGCSCTKTT	Degenerate primer for amplification of *dsyB* in PCR and RT-qPCR
dsyB_deg2R	GCAGRTARTCGCCGAAATCGTA	Degenerate primer for amplification of *dsyB* in PCR and RT-qPCR
mmtN_degF	CCGAGGTGGTCATGAAYTTYGG	Degenerate primer for amplification of *mmtN* in PCR
mmtN_degR	GGATCACGCACACYTCRTGRTA	Degenerate primer for amplification of *mmtN* in PCR
NOmmtN_NdeI-F	CGGATCCCATATGTCTGACGCAGATGACTCC	Cloning of *Novosphingobium* sp. BW1 *mmtN* into pET21a for pBIO21N1
NOmmtN_EcoRI-R	GGAATTCACTCTACCTTGGGGATACC	Cloning of *Novosphingobium* sp. BW1 *mmtN* into pET21a for pBIO21N1
TPmmtN_NdeI-F	CGGATCCCATATGCAACATGCTTTAGAAGAGAGC	Cloning of *T. profundimaris mmtN* into pET21a for pBIO21T2
TPmmtN_EcoRI-R	CGAATTCTTAGGCCGGTGTGCCGCGAATGAC	Cloning of *T. profundimaris mmtN* into pET21a for pBIO21T2
RImmtN_NdeI-F	CGAATTCCATATGACCGATTTCAAAACGCCCG	Cloning of *R. indicus mmtN* into pET21a for pBIO21R3
RImmtN_EcoRI-R	CCCGGATCCTCAACGATTGGACGGATCGGTTTCC	Cloning of *R. indicus mmtN* into pET21a for pBIO21R3
NCmmtN_NdeI-F	CGGATCCCATATGCCGTCCGAGCACACGATG	Cloning of *N. chromatogenes mmtN* into pET21a for pBIO21N4
NCmmtN_EcoRI-R	CGAATTCATCGCCGGTCCTCCTCGTCGG	Cloning of *N. chromatogenes mmtN* into pET21a for pBIO21N4
SMmmtN_NdeI-F	CGGATCCCATATGAGAACAGAGACCGGACCGCC	Cloning of *S. mobaraensis mmtN* into pET21a for pBIO21S5
SMmmtN_EcoRI-R	CGAATTCTACGTGGCGGGTGTGCCCCTGAC	Cloning of *S. mobaraensis mmtN* into pET21a for pBIO21S5
TPSCO_BamHI_F	CGGGATCCGTCGCCTTTATCTTGCAAAG	Cloning of internal gene fragment for generating a single crossover *mmtN* knockout in *T. profundimaris* strain J596
TPSCO_EcoRI_R	CGGAATTCCGTTCCGGAATGTTGCAG	Cloning of internal gene fragment for generating a single crossover *mmtN* knockout in *T. profundimaris* strain J596
03140pKXbaFOR	TGACTCTAGACCCTGATCGACGCCTGCG	Cloning of internal gene fragment for generating a single crossover TH2_03140 aminotransferase knockout in *T. profundimaris* strain J597
03140pKPstREV	TGACCTGCAGCCGGTTGCACCGACATCG	Cloning of internal gene fragment for generating a single crossover TH2_03140 aminotransferase knockout in *T. profundimaris* strain J597

* Restriction sites included in primers underlined

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