

**Novel dimethylsulfoniopropionate biosynthesis enzymes in diverse
marine bacteria, cyanobacteria and abundant algae**

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25 Supplementary Tables

26 **Supplementary Table 1: DMSP cycling gene analysis of the *G. sunshinyii* YC6258 genome using BLASTp against the functional gene list**
 27 **in Supplementary Table 11.**

<i>G. sunshinyii</i> gene ID	Closest homologues	Closest homologues organism	homologue accession ID	E value	Identity
WP_044616278.1	DmdC	<i>Pseudomonas aeruginosa</i> PAO1	WP_003114720.1	0	63.13%
WP_044618173.1	DddT	<i>Pseudomonas</i> sp. J465	ACY01993.1	1.58E-112	40.16%

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30 **Supplementary Table 2: List of proteins studied here with their accession numbers and host organism.**

Organism and enzyme	Accession Number	Protein length	Identity to ^{Gs} DsyGD (%)	Coverage to ^{Gs} DsyGD (%)	E-value
<i>G. sunshinyii</i> YC6258 DsyGD	WP_044616208.1	494	100	100	0
<i>Symploca</i> sp. SIO3E6 DsyGD	NES17792.1	499	46.04	96	6e-153
<i>Oscillatoria</i> sp. SIO1A7 DsyGD	NER39123.1	490	46.60	97	3e-151
<i>Zarconia navalis</i> LEGE 11467 DsyG	WP_264320056.1	276	49.81	54	8e-85
<i>Fragilariopsis cylindrus</i> CCMP1102 DSYE	OEU16654.1	306	38.27	55	2e-56
<i>Planctomycetales bacterium</i> DsyG-like	MCA9139260.1	278	38.85	55	3e-60
<i>Porphyra umbilicalis</i> DsyGD-like	OSX77567.1	458	38.57	55	1e-55
<i>Nitzschia inconspicua</i> DSYE	KAG7362955.1	305	39.43	55	1e-57
	MMETSP ID	Protein length	Identity to ^{Fc} DSYE	Coverage to ^{Fc} DSYE	E-value
<i>Norrisiella sphaerica</i> BC52 DSYE	MMETSP0113_16486 m.23051	284	49.65	92	2e-106
<i>Pelagococcus subviridis</i> CCMP1429 DSYE	MMETSP0882_30218 m.93541	307	61.46	97	6e-136
<i>Bigelowiella longifila</i> CCMP242 DSYE	MMETSP1359_22057 m.30888	304	47.3	95	1e-104
<i>Tetraselmis striata</i> LANL1001 DSYE	MMETSP0803_2078 m.7237	340	52.54	96	7e-110
<i>Pavlova</i> sp. CCMP459 DSYE	MMETSP1381_18054 m.44509	309	67.79	97	8e-158
<i>Ostreococcus prasinus</i> BCC99000 DSYE	MMETSP0933_5616 m.17802	301	56.75	94	1e-114
<i>Exanthemachrysis gayraliae</i> RCC1523 DSYE	MMETSP1464_12967 m.43959	338	68.79	97	9e-160
<i>Chroomonas mesostigmatica</i> CCMP1168 DSYE	MMETSP0047_26906 m.66305	347	50.87	94	2E-108
	MMETSP ID	Protein length	Identity to DsyD	Coverage to DsyD	E-value

<i>Prymnesium parvum</i> Texoma1 DsyD-like	MMETSP0008_14016 m.24446	173	~29.81	~46	4e-09
<i>Alexandrium monilatum</i> CCMP3105 DsyD-like	MMETSP0093_31591 m.94061	259	~27.78	~90	2e-15

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32 **Supplementary Table 5: DMSP production by *Pelagophyta* algae assayed via GC**

Organism	phylum	class	DSYE clade	DMSP production
<i>Chrysocystis fragilis</i> RCC 6172	<i>Ochrophyta</i>	<i>Pelagophyceae</i>	Clade C	86.17 ± 0.28 mM
<i>Aureococcus anophagefferens</i> RCC 4094	<i>Ochrophyta</i>	<i>Pelagophyceae</i>	Clade C	39.25± 2.51 mM
<i>Pelagococcus subviridis</i> RCC 4422	<i>Ochrophyta</i>	<i>Pelagophyceae</i>	Clade C	51.93± 0.90 mM
<i>Chrysoreinhardia</i> sp. RCC 2956	<i>Ochrophyta</i>	<i>Pelagophyceae</i>	Clade C	233.81± 32.1 mM
<i>Pelagomonas calceolata</i> RCC 100	<i>Ochrophyta</i>	<i>Pelagophyceae</i>	Clade C	13.79± 0.46 mM
<i>Ostreococcus tauri</i> *	<i>Chlorophyta</i>	<i>Prasinophyceae</i>	Clade B	0.34 ±0.0032 nmol DMSP/ug protein

33 * *Ostreococcus tauri* DMSP production was normalized by protein concentration.

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35 **Supplementary Table 8: Strains used in this study.**

strain	Description	reference
<i>Gynuella sunshinyii</i> YC6258	Wild type strain	[1]
<i>Escherichia coli</i> 803	Strain used for routine transformations	[2]
<i>E. coli</i> BL21	Strain for overexpression of cloned genes in pET vectors	[3]
<i>E. coli</i> MC4100	Control strain for osmosensitivity test	Horizon Discovery Biosciences Limited
<i>E. coli</i> FF4169	Strain deficient in trehalose production for osmosensitivity test	Horizon Discovery Biosciences Limited
<i>Rhizobium leguminosarum</i> J391	Streptomycin-resistant derivative of wild type strain 3841 used for library screening	[4]
<i>Labrenzia aggregata</i> <i>dsyB</i> ⁻ mutation	<i>Labrenzia aggregata</i> LZB033 with a deletion mutation in <i>dsyB</i> used for methyltransferase domain functional assay. Stain is rifampicin-resistant.	[5]
<i>Ruegeria pomeroyi</i> DSS-3	Rifampicin-resistant wild type strain used for decarboxylase domain functional assay	[6]
<i>Zarconia navalis</i> LEGE 11467	Cyanobacterium with DsyG	This study

36 **Supplementary Table 9: Plasmids used in this study.**

Plasmid	Description	reference
pLAFR3	Wide host-range cosmid vector, used for library construction, tetracycline-resistant	Staskawicz et al. (1987) ^[7]
pLMB509	Plasmid vector for expression of cloned genes in <i>L. aggregata</i> <i>dsyB</i> ⁻ mutation and <i>R. pomeroyi</i> DSS-3, gentamicin-resistant	[8]
pRK2013	Helper plasmid used in triparental mating, kanamycin-resistant	Figurski and Helinski ^[9]
pUCm-T	Plasmid vector used for cloning <i>dsyGD</i> cloned in <i>E. coli</i> FF4169, ampicillin - resistant	Sangon Biotech
pET-16b	Plasmid vector for expression of cloned genes in <i>E. coli</i> BL21(DE3), ampicillin-resistant	Merck Millipore
pET-22b	Plasmid vector for protein purification of cloned genes in <i>E. coli</i> BL21(DE3), ampicillin-resistant	Merck Millipore
pBIO2208	pLAFR3 cosmid from <i>G. Sunshinyii</i> library that contains ~30 kb genomic DNA including <i>dsyGD</i> , tetracycline-resistant	This study

38 **Supplementary Table 10: Oligonucleotide primers used in this study.**

Primer name	Sequence (5' to 3')*	Use
GS_Nde_FOR	GGGAATTCCATATGAAACAAGTCAG	PCR amplification of <i>dsyGD</i>
GS_BamH_REV	GCGGATCCTGAGTCCGTCC	PCR amplification of <i>dsyGD</i>
MT_Gs_Nde_FoR	GGGAATTCCATATGAAACAGGTTTCTTAC	PCR amplification of methyltransferase domain
MT_Gs_Bam_Rev	CGGGATCCCTAAACGAAAGAACCCCA	PCR amplification of methyltransferase domain
DB_Gs_Nde_FoR	GGGAATTCCATATGAACAAATCTACCCGTC	PCR amplification of decarboxylase domain
DB_Gs_Bam_Rev	CGGGATCCCTAAGAGTCGGTACCGG	PCR amplification of decarboxylase domain
RT_Gs_Nde_FoR	GGGAATTCCATATGAAAAAGATCTT	PCR amplification of reductase domain
RT_Gs_Bam_Rev	CGGGATCCCTAGGAGAACTTTGTGAC	PCR amplification of reductase domain
pLMB_PB_FoR	GGAATTCCATATGCAGAGCGAATT	PCR amplification of <i>dsyG</i> from <i>Planctomycetales bacterium</i> and cloning into pLMB509
pLMB_PB_Rev	CCGGAATTCGAATGCTCCCC	PCR amplification of <i>dsyG</i> from <i>Planctomycetales bacterium</i> and cloning into pLMB509
pLMB_PU_FoR	GGAATTCCATATGACCGGCACC	PCR amplification of <i>dsyGD</i> from <i>Porphyra umbilicalis</i> and cloning into pLMB509
pLMB_PU_Rev	CCGGAATTCACCGGTTGCTG	PCR amplification of <i>dsyGD</i> from <i>Porphyra umbilicalis</i> and cloning into pLMB509
pLMB_LEGE_FoR	GGAATTCCATATGAGCGGCTTCG	PCR amplification of <i>dsyG</i> from <i>Z. navalis</i> LEGE 11467 and cloning into pLMB509
pLMB_LEGE_Rev	CCGGAATTCACGGGTGTAGAAG	PCR amplification of <i>dsyG</i> from <i>Z. navalis</i> LEGE 11467 and cloning into pLMB509

pLMB_1A7_FoR	GGAATTCC <u>CATATG</u> CTATCTGAAAACCA	PCR amplification of <i>dsyG</i> from <i>Oscillatoria</i> sp.SIO1A7and cloning into pLMB509
pLMB_1A7_Rev	CCG <u>GAAATTCT</u> TAAAAATTACAGGACTTAC	PCR amplification of <i>dsyG</i> from <i>Oscillatoria</i> sp.SIO1A7 and cloning into pLMB509
pLMB_3E6_FoR	GGGAATTCC <u>CATATG</u> CTGCGCA	PCR amplification of <i>dsyG</i> from <i>Symploca</i> sp. SIO3E6 and cloning into pLMB509
pLMB_3E6_Rev	CCG <u>GAAATTCT</u> CAGATGCAAACCTTTC	PCR amplification of <i>dsyG</i> from <i>Symploca</i> sp. SIO3E6 and cloning into pLMB509
pLMB_NS_FoR	GGAATTCC <u>CATATG</u> AGCGAAGTTGGTAG	PCR amplification of <i>DSYE</i> from <i>Norrsiella sphaerica</i> BC52 and cloning into pLMB509
pLMB_NS_Rev	CG <u>GAAATTC</u> ATTCTGATCATTGACTGC	PCR amplification of <i>DSYE</i> from <i>Norrsiella sphaerica</i> BC52 and cloning into pLMB509
pLMB_PS_FoR	GGAATTCC <u>CATATG</u> GCCGCAAATA	PCR amplification of <i>DSYE</i> from <i>Pelagococcus subviridis</i> CCMP1429 and cloning into pLMB509
pLMB_PS_Rev	CCG <u>GAAATTC</u> ATACAGCGGACC	PCR amplification of <i>DSYE</i> from <i>Pelagococcus subviridis</i> CCMP1429 and cloning into pLMB509
pLMB_FC_FoR	GGAATTCC <u>CATATG</u> GCCCCGC	PCR amplification of <i>DSYE</i> from <i>Fragilariopsis cylindrus</i> CCMP1102 and cloning into pLMB509
pLMB_FC_Rev	CCG <u>GAAATTC</u> ATAAATATTACCTTCGGT	PCR amplification of <i>DSYE</i> from <i>Fragilariopsis cylindrus</i> CCMP1102 and cloning into pLMB509
pLMB_NI_FoR	GGAATTCC <u>CATATG</u> AGCCCGCTG	PCR amplification of <i>DSYE</i> from <i>Nitzschia inconspicua</i> and cloning into pLMB509
pLMB_NI_Rev	CCG <u>GAAATTC</u> GTAGATATCACCTTCAA	PCR amplification of <i>DSYE</i> from <i>Nitzschia inconspicua</i> and cloning into pLMB509

pLMB_PP_FoR	GGAATTCCATATGCATGGTGCACA	PCR amplification of <i>dsyD</i> from <i>Prymnesium parvum</i> Texoma1 and cloning into pLMB509
pLMB_PP_Rev	CCGGAATTCACCACTCGGTTCA	PCR amplification of <i>dsyD</i> from <i>Prymnesium parvum</i> Texoma1 and cloning into pLMB509
pLMB_AM_FoR	GGAATTCCATATGGCCCGTAGTCG	PCR amplification of <i>dsyD</i> from <i>Alexandrium monilatum</i> CCMP3105 and cloning into pLMB509
pLMB_AM_Rev	CCGGAATTCACACAGCGGAAC	PCR amplification of <i>dsyD</i> from <i>Alexandrium monilatum</i> CCMP3105 and cloning into pLMB509
GS_pro_F	ATGAAATCTCTTCAATACGTTTCAGAATCT	PCR amplification of <i>dsyGD</i> and promotor from <i>G. sunshinyii</i> YC6258 and cloning into pUCm-T vector
GS_pro_R	TGCTGACCGTGATGTTCAACCAC	PCR amplification of <i>dsyGD</i> and promotor from <i>G. sunshinyii</i> YC6258 and cloning into pUCm-T vector
q_GS_ <i>dsyG</i> _FOR	AAAATCTCGCCGAAGTGGG	RT-qPCR amplification of <i>dsyG</i> from <i>G. sunshinyii</i> YC6258
q_GS_ <i>dsyG</i> _REV	CGCTTTGGGATGATCTACC	RT-qPCR amplification of <i>dsyG</i> from <i>G. sunshinyii</i> YC6258
q_GS_ <i>rpoD</i> _FOR	TGCTCAACAACCCTGCCTAC	RT-qPCR amplification of <i>rpoD</i> from <i>G. sunshinyii</i> YC6258
q_GS_ <i>rpoD</i> _REV	CGATGGAAATGACCAGACGC	RT-qPCR amplification of <i>rpoD</i> from <i>G. sunshinyii</i> YC6258
q_GS_ <i>recA</i> _FOR	GGCTCCCCTGAAACCACTA	RT-qPCR amplification of <i>recA</i> from <i>G. sunshinyii</i> YC6258
q_GS_ <i>recA</i> _REV	AAAACCTCACCCAAGCGATA	RT-qPCR amplification of <i>recA</i> from <i>G. sunshinyii</i> YC6258
M13 uni (-43)	AGGGTTTTCCAGTCACGACGTT	Universal forward primer used to sequence insert ends in pLAFR3
M13 rev (-29)	CAGGAAACAGCTATGACC	Universal reverse primer used to sequence insert ends in pLAFR3
q_Zn_ <i>revA</i> _FOR	TGGCGGTAGACTTAGTGGTGGTAG	RT-qPCR amplification of <i>recA</i> from <i>Z. navalis</i> LEGE 11467

q_Zn_recA_REV	GTTGCCGTTAGACGATGCCGATT	RT-qPCR amplification of <i>recA</i> from <i>Z. navalis</i> LEGE 11467
q_Zn_GADPH_FOR	TGGACGCATCGGACGCAACT	RT-qPCR amplification of <i>GADPH</i> from <i>Z. navalis</i> LEGE 11467
q_Zn_GADPH_REV	TCGGTCACGAACACGCCTGT	RT-qPCR amplification of <i>GADPH</i> from <i>Z. navalis</i> LEGE 11467
q_Zn_dsyG_FOR	ATCAGCAGAAAGCGGCAGAACT	RT-qPCR amplification of <i>dsyG</i> from <i>Z. navalis</i> LEGE 11467
q_Zn_dsyG_REV	CTGACACCTTCACCGGCCATAA	RT-qPCR amplification of <i>dsyG</i> from <i>Z. navalis</i> LEGE 11467

39 *Restriction sites included in primers are underlined

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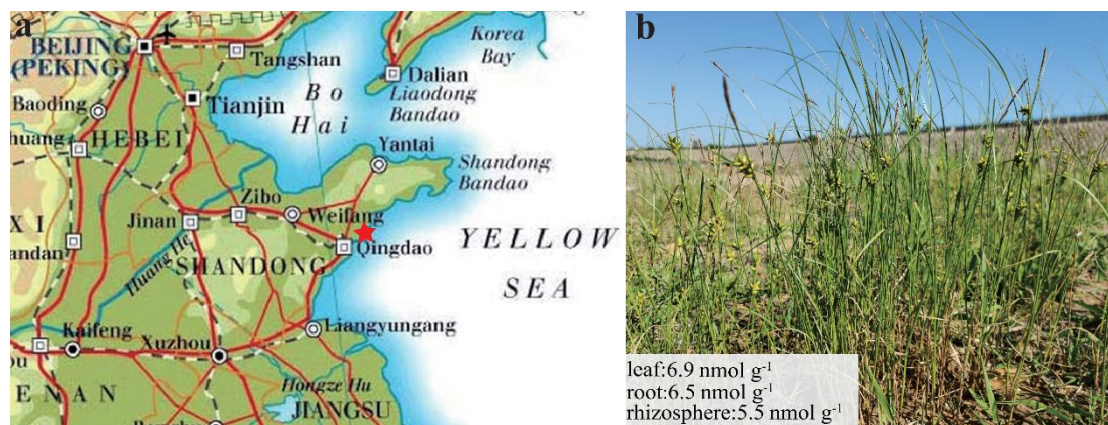
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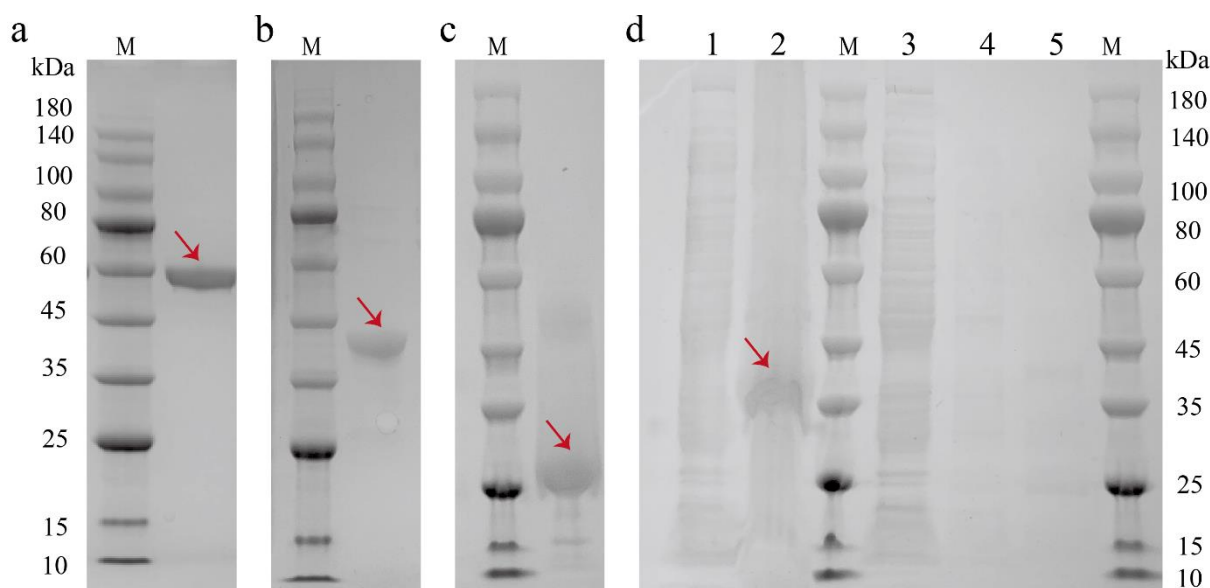
Supplementary Table 11: Accession numbers of previously ratified enzymes.

Protein	Ratified strains	Accession number	Reference
DSYB	<i>Prymnesium parvum</i> CCAP946/6	NA	[10]
	<i>Chrysochromulina tobin</i> CCMP291	KOO32714	
	<i>Lingulodinium polyedrum</i> CCMP1936	NA	
	<i>Alexandrium tamarense</i> ATSP1-B	NA	
	<i>Acropora cervicornis</i>	NA	
	<i>Fragilariopsis cylindrus</i> CCMP1102	OEU17621	
TpMMT	<i>Symbiodinium microadriaticum</i> CCMP2467	OLQ07620	[11]
	<i>Thalassiosira pseudonana</i> CCMP1335	Tp23128	
DsyB	<i>Labrenzia aggregata</i> IAM 12614	EAV42226	[5]
	<i>Pseudooceanicola batsensis</i> HTCC2597	EAQ04968	
	<i>Pelagibaca bermudensis</i> HTCC2601	EAU45958	
	<i>Amorphus coralli</i> DSM 19760	WP_018697905	
MmtN	<i>Thalassospira profundimaris</i> PB8B	OAZ15224	[12]
	<i>Novosphingobium</i> sp. MBES04	GAM03459	
	<i>Roseovarius indicus</i> B108	KRS18724	
	<i>Nocardiopsis chromatogenes</i> YIM 90109	WP_017624909	
	<i>Streptomyces mobaraensis</i> DSM 40847	EME99407	
DmdA	<i>Ruegeria pomeroyi</i> DSS-3	AAV95190	[13]
	<i>Candidatus Pelagibacter ubique</i> HTCC1062	WP_011281570	
	<i>Dinoroseobacter shibae</i> DFL 12	WP_012178987	[14]
	<i>marine gammaproteobacterium</i> HTCC2080	WP_007233625	
	<i>Candidatus Pelagibacter</i> sp. HTCC7211	WP_008546106	[15]
DddD	<i>Candidatus Puniceispirillum marinum</i>	WP_013044947	
	IMCC1322		
	<i>Marinomonas</i> sp. MWYL1	ABR72937	[16]
	<i>Oceanimonas doudoroffii</i>	AEQ39135	
	<i>Psychrobacter</i> sp. J466	ACY02894	[18]
	<i>Halomonas</i> sp. HTNK1	ACV84065	
	<i>Sinorhizobium fredii</i> NGR234	AAQ87407	[16]
DddL	<i>Burkholderia ambifaria</i> AMMD	WP_011659284	
	<i>Pseudomonas</i> sp. J465	ACY01992	[19]
	<i>Sulfitobacter</i> sp. EE-36	ADK55772	[20]
	<i>Rhodobacter sphaeroides</i> 2.4.1	YP_351475	
DddP	<i>Labrenzia aggregata</i> LZB033	KP639184	[5]
	<i>Ahrensia marina</i> LZD062	KP639183	
	<i>Roseovarius nubinhibens</i> ISM	EAP77700	[22]
	<i>Ruegeria pomeroyi</i> DSS-3	WP_044029245	
	<i>Phaeobacter inhibens</i> DSM 17395	AFO91571	[23]
	<i>Oceanimonas doudoroffii</i> DSM 7028	AEQ39091	
	<i>Oceanimonas doudoroffii</i> DSM 7028	AEQ39103	[17]
DddQ	<i>Aspergillus oryzae</i> RIB40	BAE62778	
	<i>Fusarium graminearum</i> PH-1	XP_389272	[22]
	<i>Ruegeria pomeroyi</i> DSS-3	WP_011047333	[6]
	<i>Roseovarius nubinhibens</i> ISM	EAP76002	
DddW	<i>Roseovarius nubinhibens</i> ISM	EAP76001	[6]
	<i>Ruegeria lacuscaerulensis</i> ITI1157	WP_005978225	
	GOS_2632696	ECW91654	[24]
	GOS_7860946	EBP74803	
	GOS_2469775	ECX82089	
DddY	<i>Ruegeria pomeroyi</i> DSS-3	AAV93771	[25]
DddX	<i>Alcaligenes faecalis</i> M3A	ADT64689	[26]
	<i>Desulfovibrio acrylicus</i>	SHJ73420	
	<i>Acinetobacter bereziniae</i>	ENV21217	[27]
	<i>Ferrimonas kyonanensis</i> DSM 18153	WP_028114584	
	<i>Shewanella putrefaciens</i> CN-32	ABP77243	[28]
DddK	<i>Candidatus Pelagibacter ubique</i> HTCC1062	AAZ21215	[31]
	<i>Candidatus Pelagibacter ubique</i> HTCC9022	WP_028037226	
	<i>alphaproteobacterium</i> HIMB5	AFS47241.1	
DddX	<i>Marinobacterium jannaschii</i>	WP_084332639.1	[32]
	<i>Pelagicola</i> sp. LXJ1103	WP_109384856.1	
	<i>Psychrobacter</i> sp. P11G5	WP_068035783.1	
	<i>Sporosarcina</i> sp. P33	WP_081242855.1	
Alma1	<i>Emiliania huxleyi</i> CCMP1516	XP_005784450	[33]

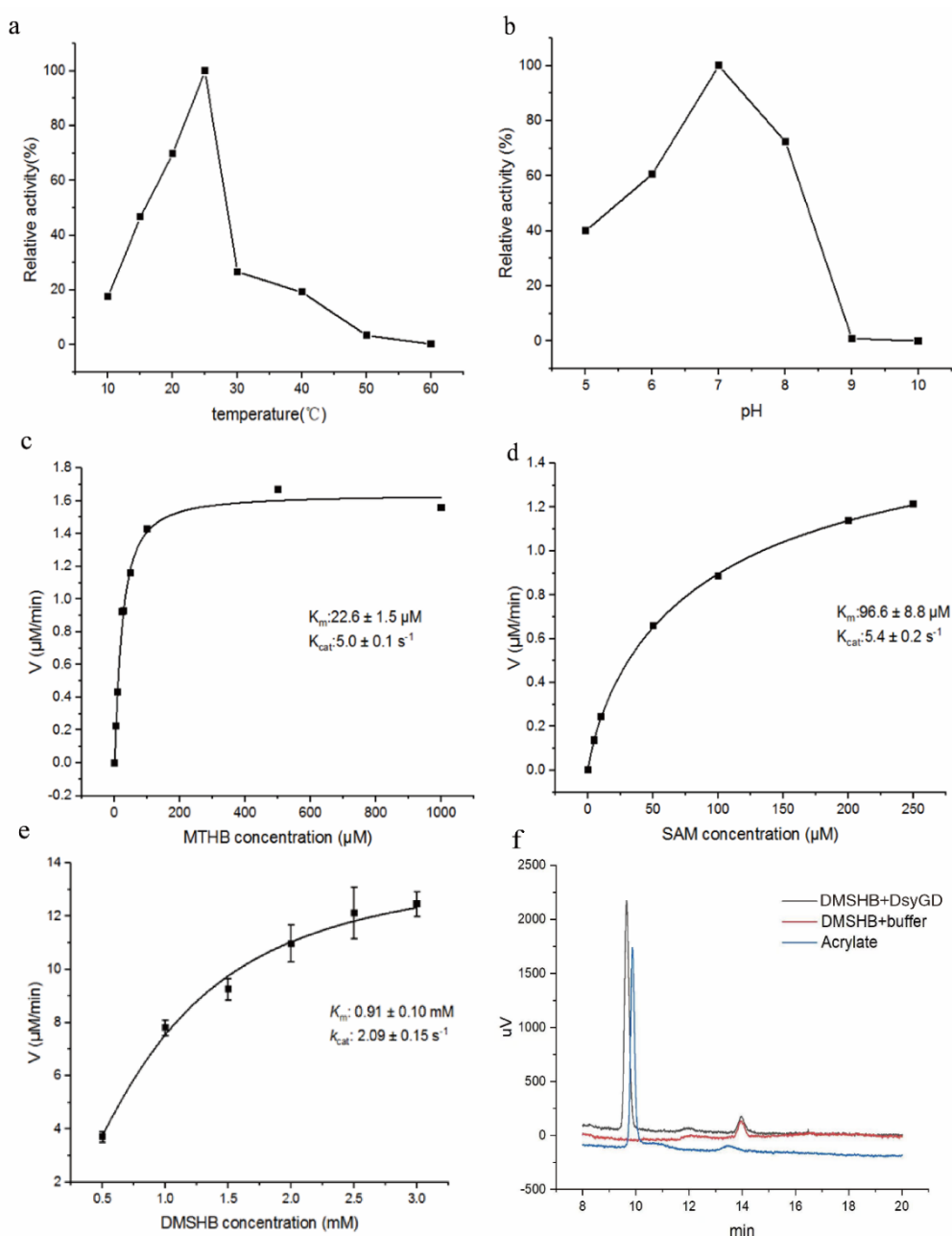
	<i>Emiliana huxleyi</i> CCMP1516	XP_005763983	
MddA	<i>Mycobacterium tuberculosis</i> H37Rv <i>Bradyrhizobium diazoefficiens</i> USDA 110 <i>Bradyrhizobium diazoefficiens</i> USDA 110 <i>Pseudomonas</i> sp. GM41 <i>Pseudomonas deceptionensis</i> <i>Sulfurovum</i> sp. NBC37-1	WP_003416945.1 WP_011084036.1 WP_011088485.1 WP_008148420.1 WP_048359798.1 WP_011980608.1	[34]
DmoA	<i>Hyphomicrobium sulfonivorans</i> 6AK1_A	E9JFX9.1	[35]
MTO	<i>Hyphomicrobium</i> sp. <i>Methylophaga thiooxydans</i> <i>Ruegeria pomeroyi</i>	ATJ26742.1 WP_008290534.1 WP_011242048.1	[36] [37]
DdhA	<i>Rhodovulum sulfidophilum</i>	WP_075783016.1	[38]
Tmm	<i>Methylocella silvestris</i> BL2 <i>Ruegeria pomeroyi</i> DSS-3 <i>Roseovarius</i> sp. 217	ACK52489.1 AAV94838.1 EAO26624.1	[39]
DMSOR	<i>Rhodobacter capsulatus</i> 1DMR_A <i>Escherichia coli</i> <i>Rhodobacter sphaeroides</i>	See the sequence in Song et al., 2020 WP_097479356.1 AAB94874.1	[40] [41]
DmdB	<i>Ruegeria pomeroyi</i> <i>Ruegeria pomeroyi</i> <i>Candidatus Pelagibacter ubique</i>	WP_011047771.1 WP_011046428.1 WP_011281571.1	[42]
DmdC	<i>Ruegeria pomeroyi</i> <i>Burkholderia thailandensis</i> E264 <i>Ruegeria pomeroyi</i> DSS-3 <i>Pseudomonas aeruginosa</i> PAO1 <i>Pseudomonas aeruginosa</i> PAO1 <i>Burkholderia thailandensis</i> E264 <i>Ruegeria lacuscaerulensis</i> ITI-1157	WP_011049476.1 WP_009892931.1 WP_011048615.1 WP_003114720.1 WP_003114561.1 WP_009889880.1 EEX08676.1	[42]
DmdD	<i>Ruegeria pomeroyi</i>	Q5LLW6.1	[42, 43]
AcuH	<i>Ruegeria lacuscaerulensis</i> ITI-1157 <i>Ruegeria pomeroyi</i> DSS-3	EEX08788.1 AAV93475.1	[44, 45]
AcuN	<i>Halomonas</i> sp. HTNK1 <i>Alcaligenes faecalis</i>	ACV84068.1 WP_123051137.1	[18, 30]
DddT	<i>Pseudomonas</i> sp. J465 <i>Psychrobacter</i> sp. J466 <i>Halomonas</i> sp. HTNK1 <i>Marinomonas</i> sp. (strain MWYL1)	ACY01993.1 ACY02893.1 ACV84066.1	[18, 19, 46]



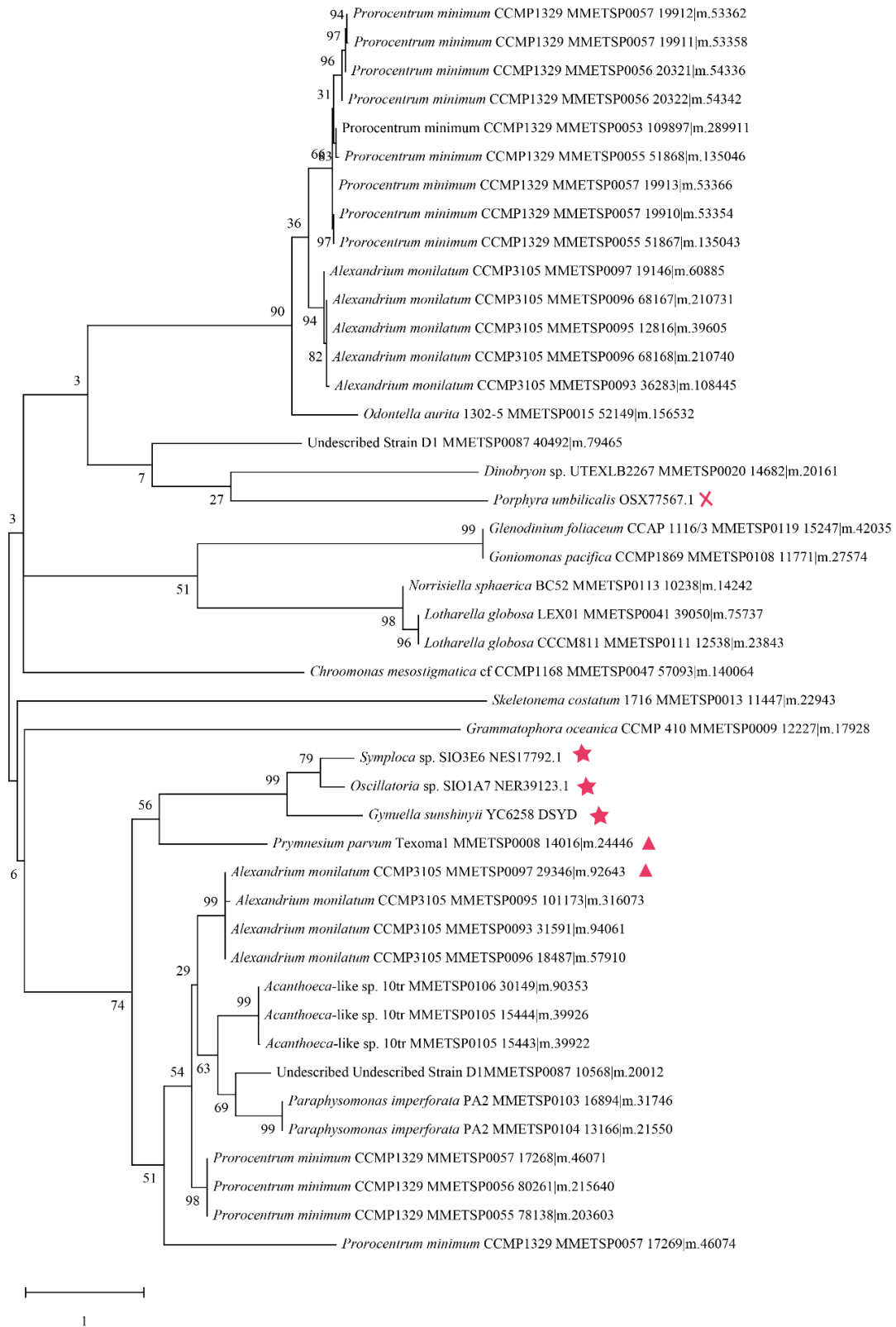
Supplementary Figure 1: *Carex scabrifolia* sampling location in China and associated DMSP levels. **a**, Naturally growing *C. scabrifolia* plants were sampled in biological triplicates near Qingdao, Shandong Province China (120.7°E, 36.5°N), labeled as (★). **b**, The DMSP concentration within *C. scabrifolia* leaves, root and rhizosphere soil samples were calculated and normalized to fresh weight.



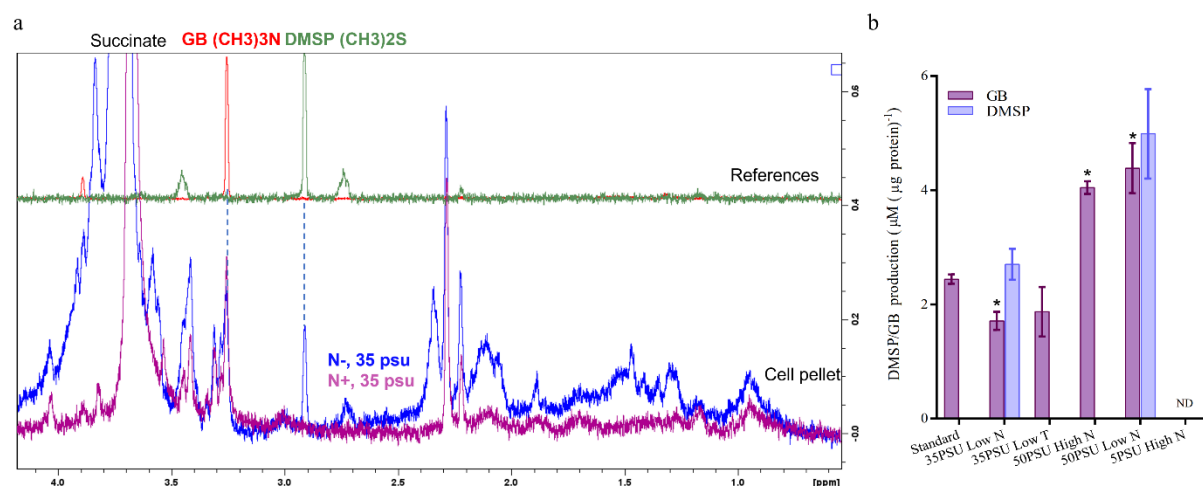
Supplementary Figure 2: Purified *Gynuella sunshinyii* YC6258 DsyGD, DsyG, DsyD and a putative reductase. The purified recombinant His-tagged **a**, DsyGD (56.0 kDa); **b**, candidate reductase protein (35.8 kDa); **c**, DsyD (24.4 kDa); **d**, DsyG (31.7kDa) run on a 12 % precast SDS-PAGE gel. Lanes: M, Prestained Protein Ladder, 10 to 180 kDa (Transgen). For **d**, lane1, lysate before purification; 2, cell fractions precipitate; 3, eluate after loading cell lysate; 4, eluate of washing buffer; 5, eluted fraction by imidazole.



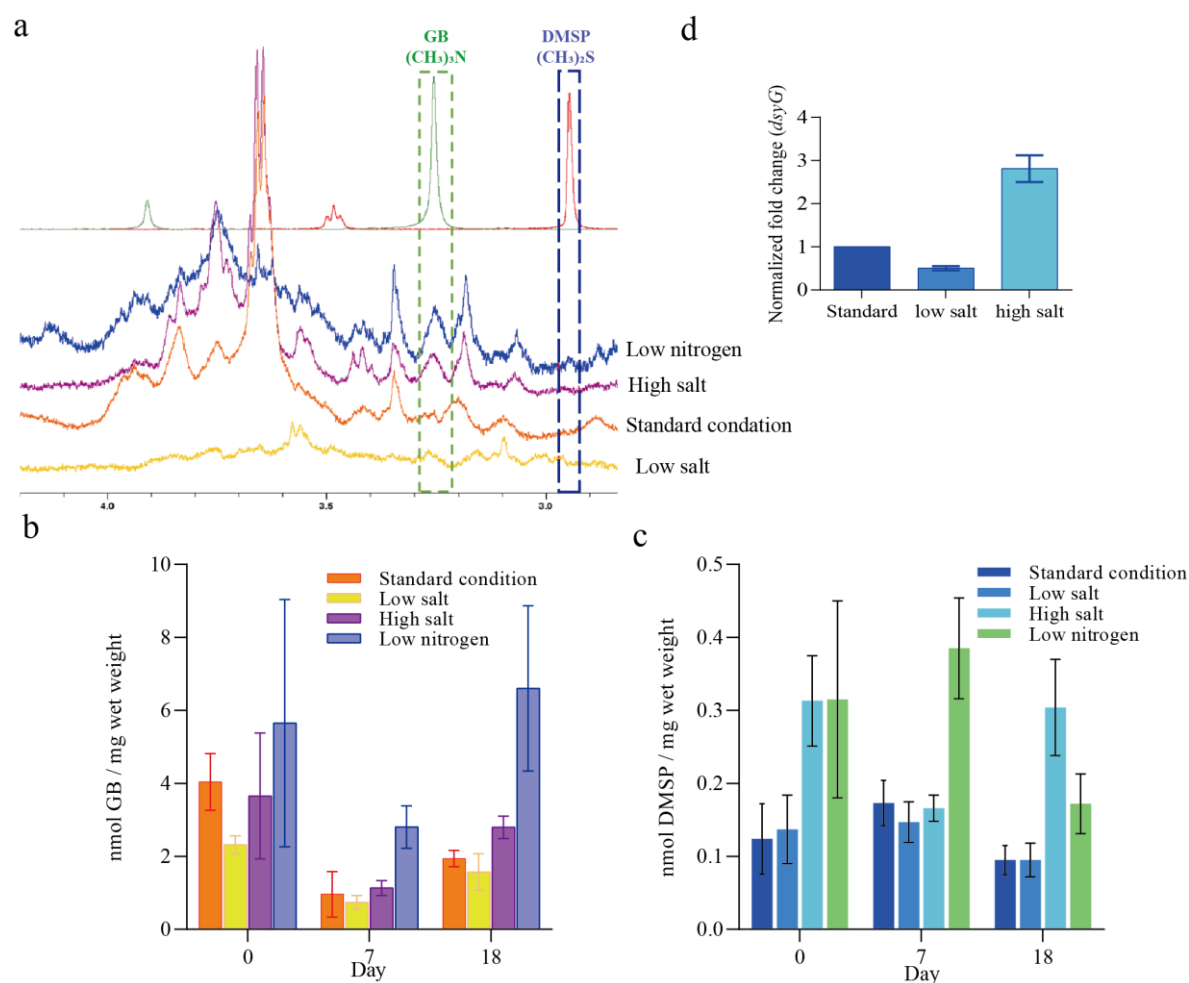
Supplementary Figure 3: Characterization of recombinant DsyGD: (a-d) Protein characterization, where activity is determined as the production of SAH from SAM (Data are presented as the mean $\pm$ standard deviations (SD) of $n = 3$ independent experiments. **a**, Effect of temperature on MSM enzymatic activity of DsyGD. **b**, Effect of pH on MSM enzymatic activity of DsyGD. **c**, Nonlinear fit curves of DsyGD MSM activity with varying SAM concentration. **d**, Nonlinear fit curves of DsyGD activity with varying MTHB concentration. **e**, Nonlinear fit curves of DsyGD DDC activity with varying DMSHB concentration. The kinetic parameters were determined at pH 7.0 and 25°C. **f**, Detection of acrylate produced by alkaline lysis of DMSP formed from the DsyGD-dependent decarboxylation of DMSHB (black line). The acrylate standard is indicated by a blue line. Incubation of DMSHB in buffer controls yielded no acrylate (red line).



Supplementary Figure 4: Maximum likelihood phylogenetic tree of the DsyD domain sequences from the MMETSP database. The tree was constructed in Mega X with proteins obtained from a HMM search using functional DsyD domains from DsyGD proteins. Synthesized DsyD sequences were labeled as (▲). Note, the functional DsyD is the C-terminal domain of the *G. sunshinyii* DsyGD.

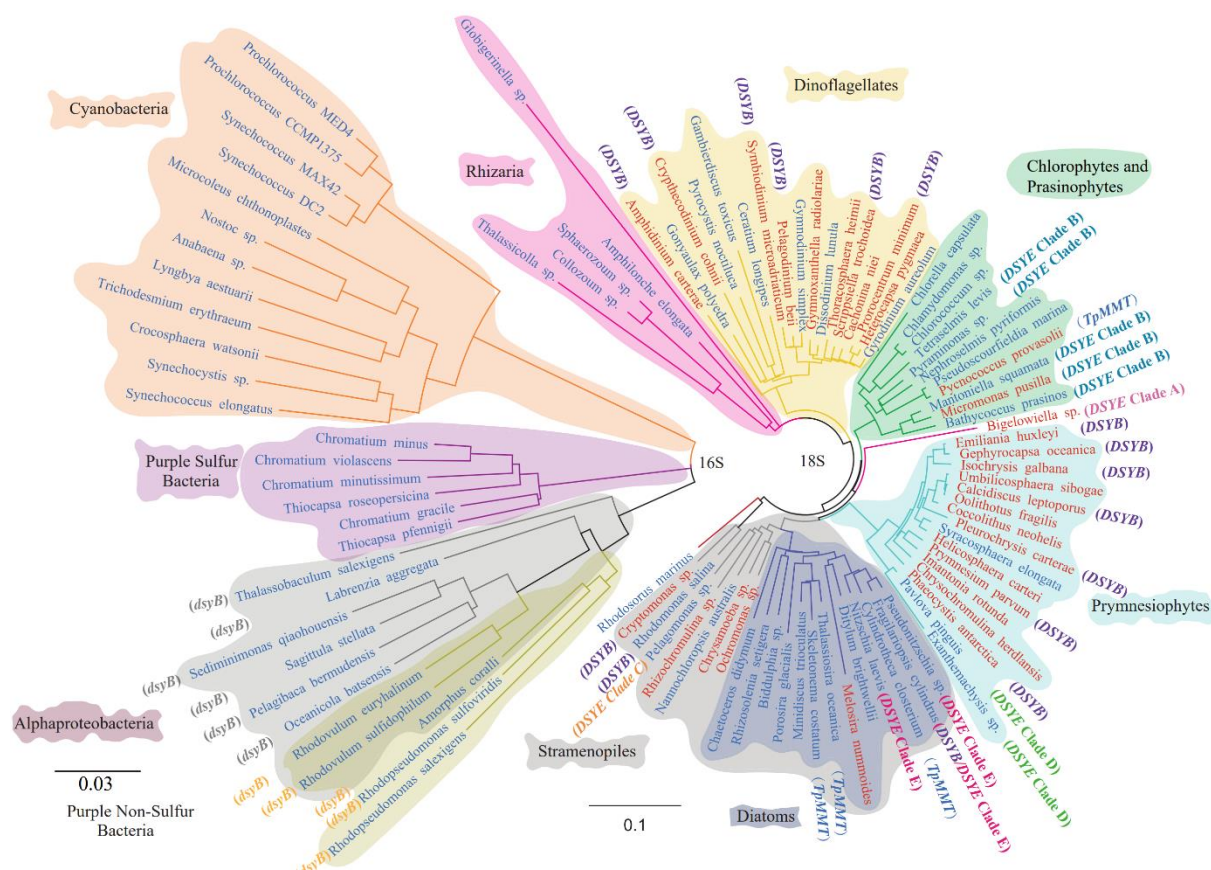


Supplementary Figure 5: Quantification of DMSP and glycine betaine (GB) in *G. sunshinyii* YC6258 by NMR. **a.** Metabolite analysis of *G. sunshinyii* YC6258 in low nitrogen and high nitrogen conditions, with the standard spectra for GB (red spectrum) and DMSP (green spectrum) overlaid. **b.** DMSP and GB production by *G. sunshinyii* YC6258 (n=3). The DMSP peak was only observed in cells grown under low nitrogen conditions (both 35 PSU and 50 PSU), while GB was present in all tested conditions. Note, no DMSP or GB was detected in *G. sunshinyii* grown at 5 PSU. ND, not detected.

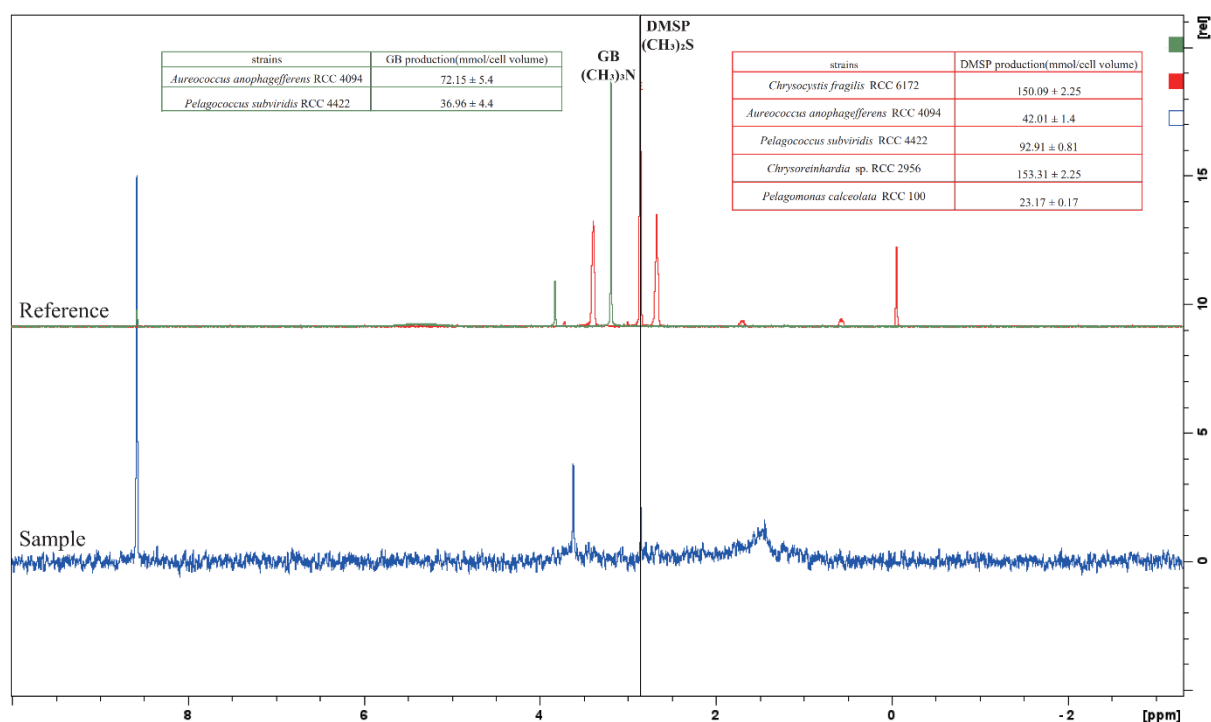


Supplementary Figure 6: Quantification of DMSP and glycine betaine (GB) production in *Zarcinia navalis* LEGE 11467 by NMR. **a.** An example of the spectra

of *Z. navalis* LEGE 11467 in the 4 different conditions after 18 days of growth, with the standard spectra for GB (green spectrum) and DMSP (red spectrum) overlaid. A DMSP peak is only observed in the low nitrogen conditions (blue spectrum). GB was present in all the tested conditions. **b**, GB production detected by NMR under different stress condition on day 0, day 7 and day 18. **c**, DMSP production detected by GC under different stress condition on day 0, day 7 and day 18. Standard condition: 25 PSU tropical marine salt in BG-11 with 17.65 mM nitrate, Low salt: 5 PSU tropical marine salt in BG-11 with 17.65 mM nitrate, High salt: 50 PSU tropical marine salt in BG-11 with 17.65 mM nitrate, Low nitrate: 25 PSU tropical marine salt in BG-11 with 0.5 mM nitrate. **d**, Zn_{dysG} transcription from cultures grown under different conditions. Gene expression was normalized by *recA* and *GADPH*.



Supplementary Figure 7: 16S/18S rRNA gene phylogeny of low and high DMSP producers and the presence of DMSP synthesis genes (modified from McParland, 2018^[47]). Phylogenetic tree generated using Geneious version R11 (<http://www.geneious.com>, Kearse et al. 2012) and 16S and 18S rRNA gene sequences. The prokaryotic producers are grouped by functional groups, while the eukaryotic producers are grouped by the major eukaryotic supergroups. Blue text for species names represents low DMSP producers (intracellular DMSP < 50 mM) and red text for species names represents high DMSP producers (intracellular DMSP > 50 mM). DSYE clades are labeled with different colours. Where known, the presence of *dsyGD*, *dsyB*, *DSYB*, *mmtN* and *TpMMT* and *DSYE* are indicated.



Supplementary Figure 9: Quantification of DMSP and glycine betaine (GB) in *Pelagophyte* algae. The concentration of the DMSP and GB (where detected) was established using pyrazine as internal standard (see Methods). The blue spectrum is for *Chrysoreinhardtia* sp. RCC 2956, with the standard spectra for DMSP (red spectrum) and GB (green spectrum) overlaid. GB was only detected in *Aureococcus anophagefferens* RCC 4094 and *Pelagomonas calceolata* RCC 100 samples (table at left), DMSP was detected in all 5 *Pelagophyte* (table at right).

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