Supplementary Information

DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton

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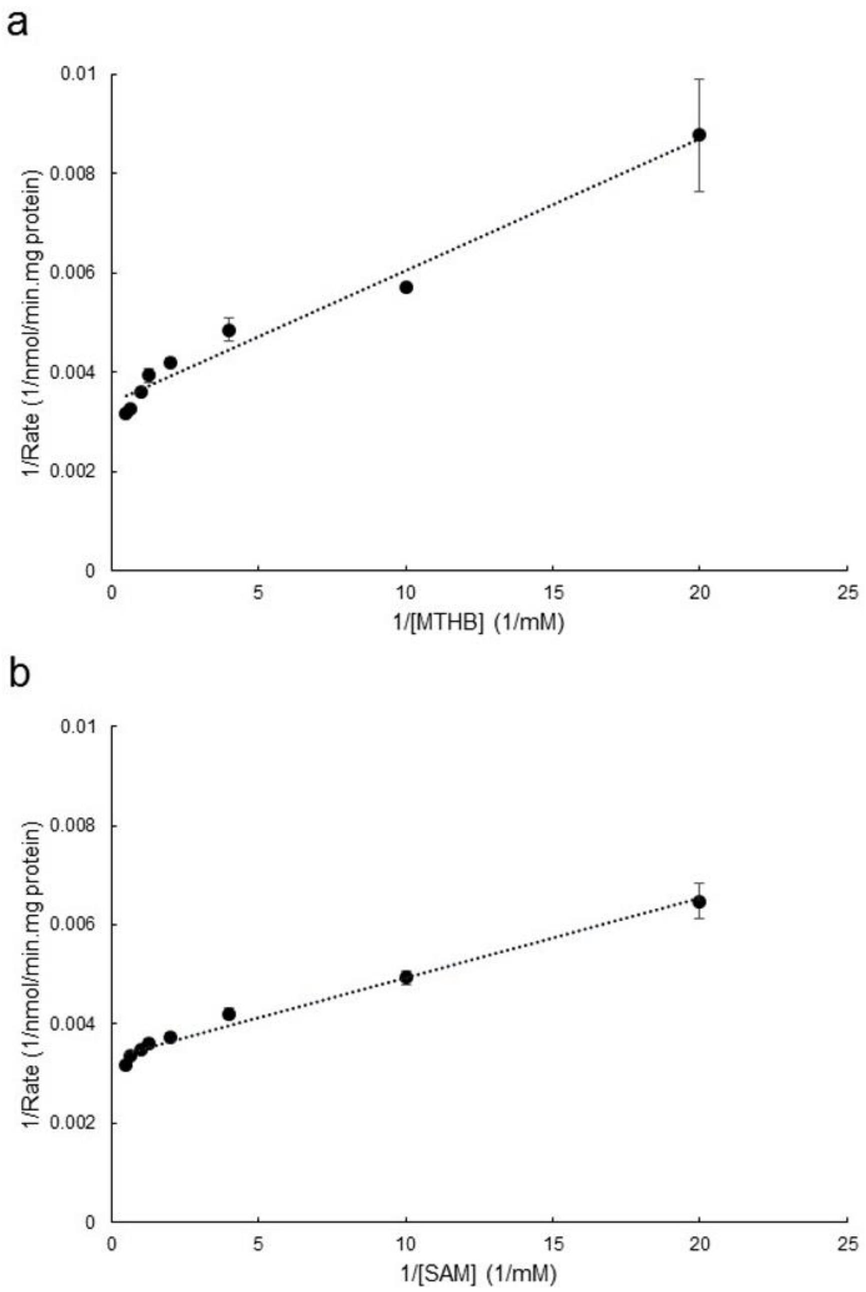
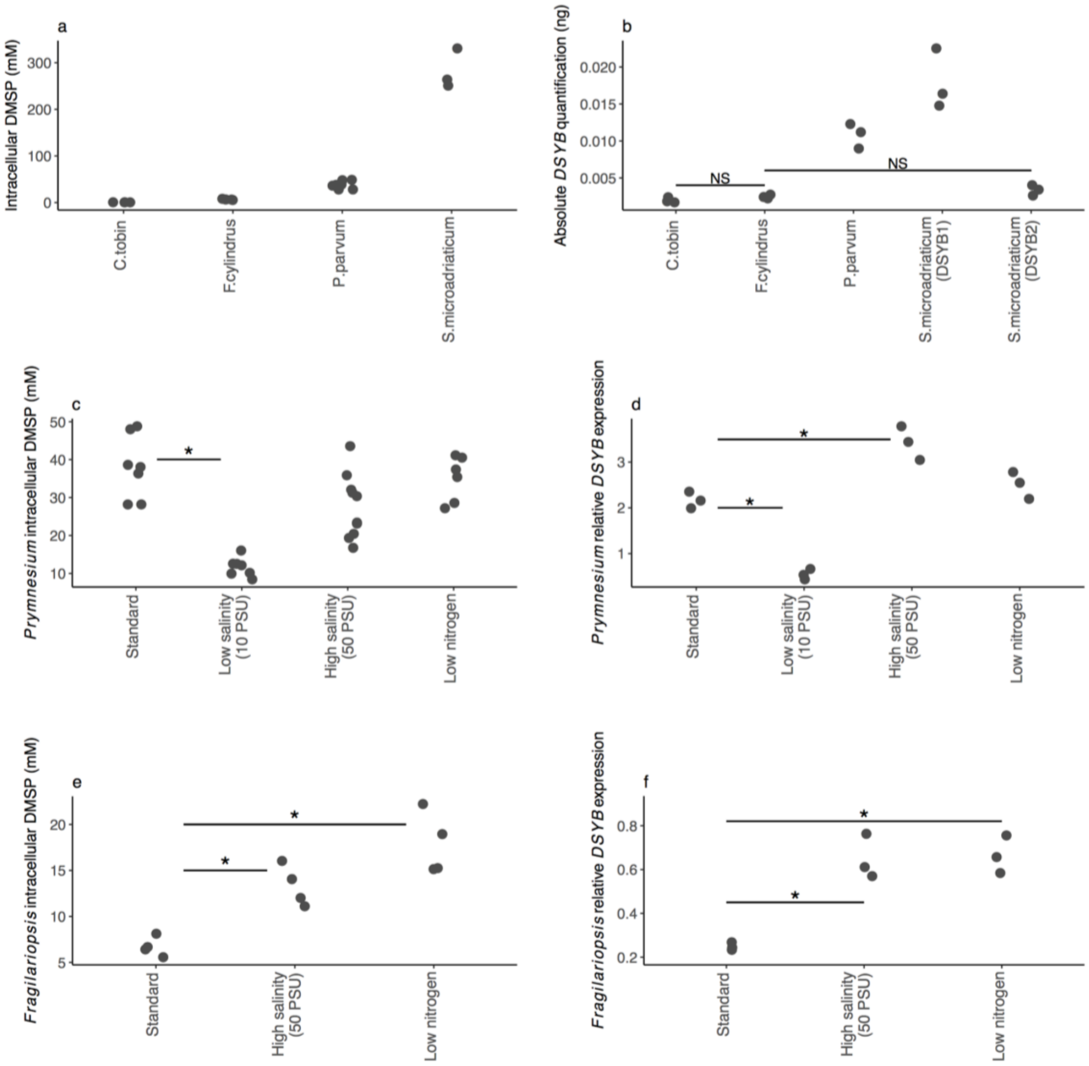
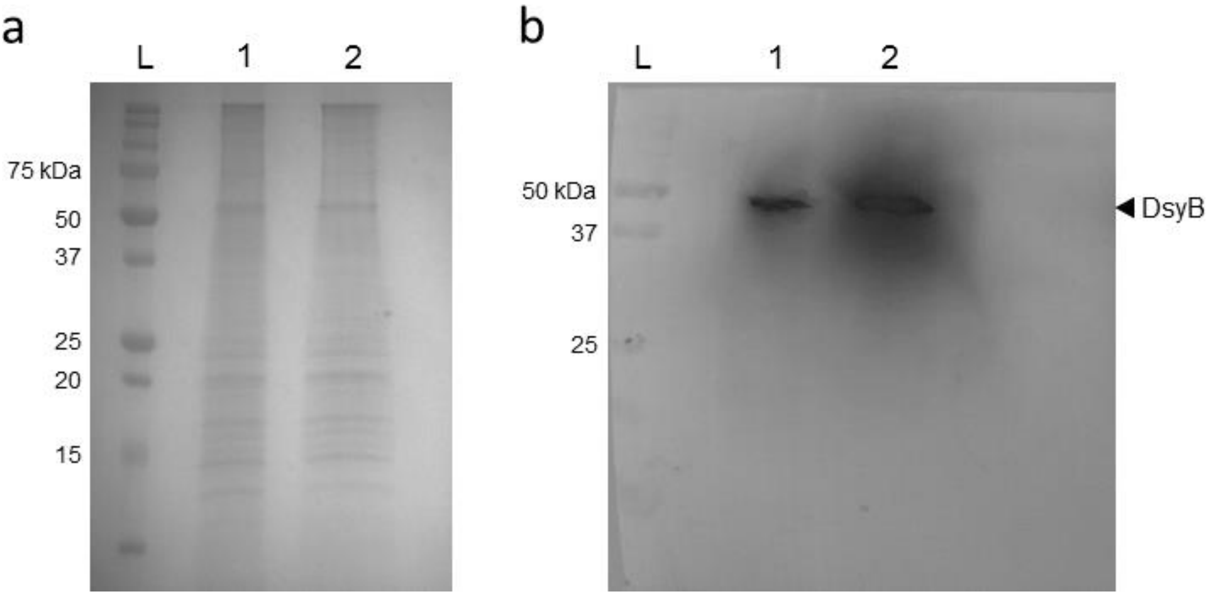


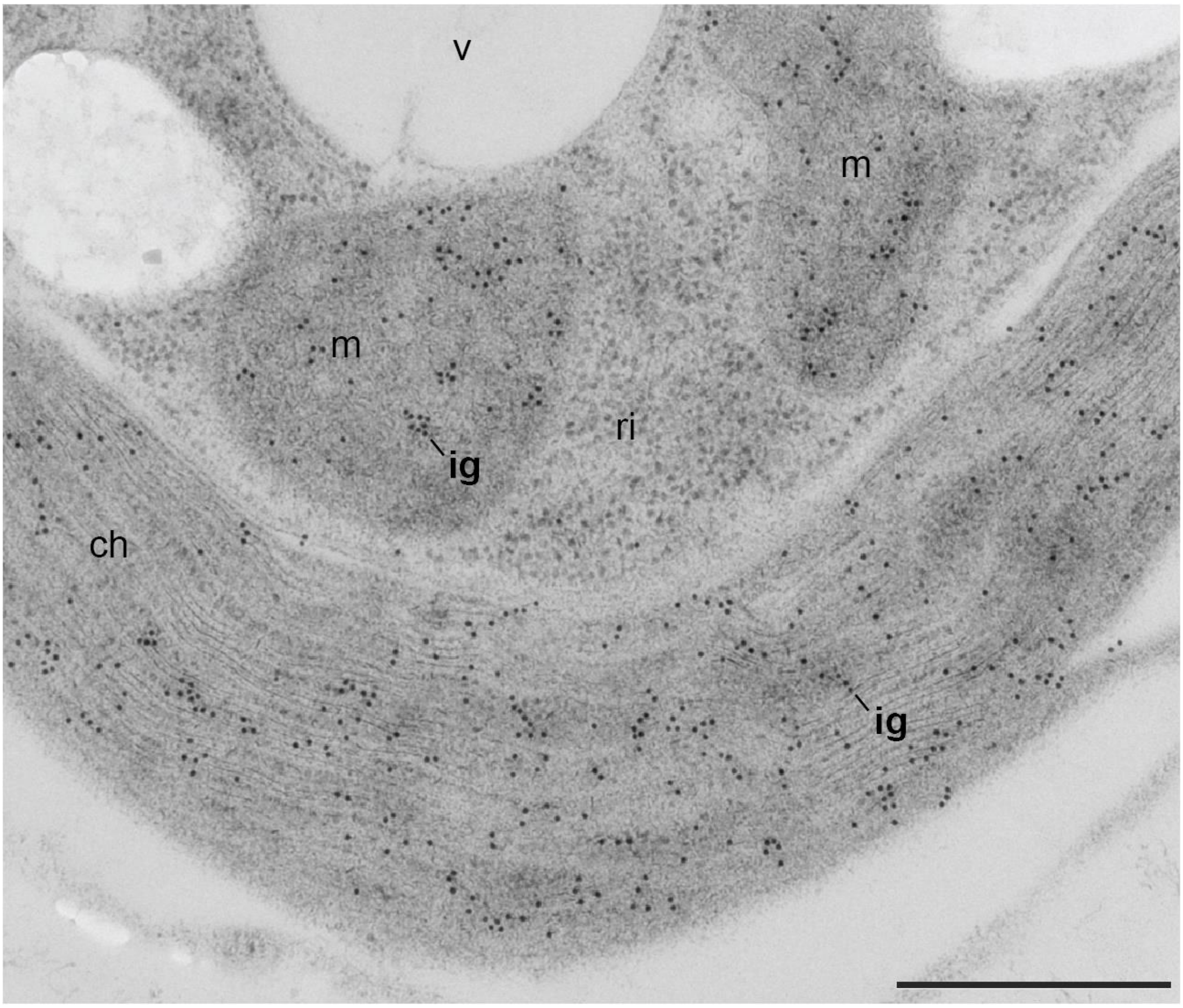
Supplementary Figure 1. DSYB Lineweaver-Burk plots. Results of DSYB enzyme assays with purified protein, with inverse of reaction rate (y axis) plotted against inverse of substrate concentration (x axis), over a range of substrate concentrations, in order to determine enzyme kinetics values for V_{max} and K_M. **a,** for methylthiohydroxybutyrate (MTHB). **b,** for *S*-adenosyl methionine (SAM). Experiments were repeated twice and done using two biological replicates and two technical replicates with error bars of standard deviation shown (n=4).



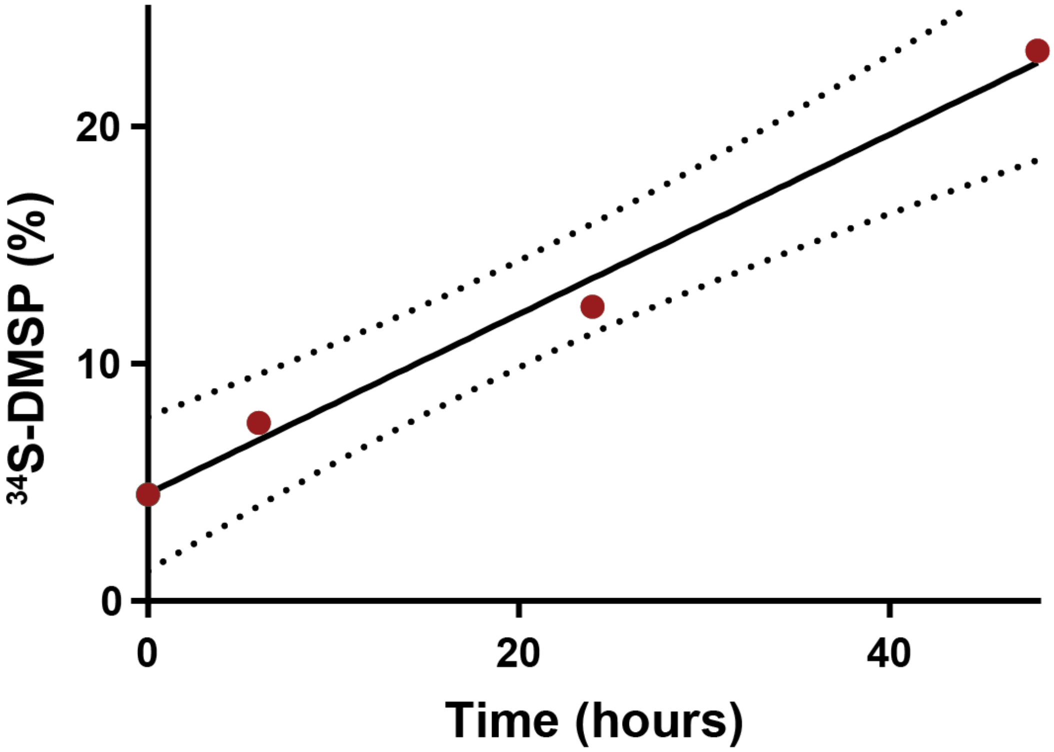
Supplementary Figure 2. DMSP production and *DSYB* expression in different species or growth conditions. a, b, DMSP production and absolute *DSYB* expression in different phytoplankton species grown under standard conditions. **c, d,** DMSP production and relative *DSYB* expression in *Prymnesium parvum* CCAP946/6 grown under different conditions. **e, f,** DMSP production and relative *DSYB* expression in *Fragilariopsis cylindrus* CCMP1102 grown under different conditions. For DMSP production, at least three biological replicates were used for each condition/species. For RT-qPCR, three biological replicates and three technical replicates were used for each gene/condition. All experiments were replicated at least twice. Low nitrogen refers to a concentration of 88.2 µM $NaNO_3$, 10% of standard nitrogen. Sample numbers are n=3 cultures/RT-qPCR reactions. Significance was determined by two-tailed Student's *t*-tests ($P < 0.05$). For visual clarity, only non-significant differences (NS) are indicated for **a** and **b**, while significant differences (*) are indicated for **c-f**. *P* values: **a**, Chry. vs Frag.: 0.0000260; Chry. vs Prym.: 0.00000141; Chry. vs Symb.: 0.0000389; Frag. vs Prym.: 0.00000533; Frag. vs Symb.: 0.0000434; Prym. vs Symb.: 0.000602; **b**, Chry. vs Frag.: 0.126; Chry. vs Prym.: 0.000873; Chry. vs Symb.1: 0.00255; Chry. vs Symb.2: 0.0385; Frag. vs Prym. 0.00104; Frag. vs Symb.1: 0.00284; Frag. vs Symb.2: 0.106; Prym. vs Symb.1: 0.0500; Prym. vs Symb.2: 0.00208; Symb.1 vs Symb.2: 0.00372; **c**, std. vs low sal.: 0.000000440; std. vs high sal.: 0.0664; std. vs low N: 0.455; **d**, std. vs low sal.: 0.000239; std. vs high sal.: 0.00639; std. vs low N: 0.156; **e**, std. vs high sal.: 0.00166; std. vs low N: 0.000752; **f**, std. vs high sal.: 0.00263; std. vs low N: 0.00130.



Supplementary Figure 3. Western blots of DSYB. a, *P. parvum* CCAP946/6 extracts (cells grown in normal (35 PSU) and low (10 PSU) salinity conditions) were run on an SDS-PAGE gel. **b**, Western blot of the SDS gel with antibody to *P. parvum* DSYB using colorimetric detection with NBT/BCIP. Lanes: L, protein standard ladder; 1, *P. parvum* extract (10 PSU); 2, *P. parvum* extract (35 PSU). SDS gel stained with Coomassie blue and Western blot was visualised by colorimetric detection of alkaline phosphatase activity using NBT/BCIP. Experiments were repeated twice on different biological replicates.



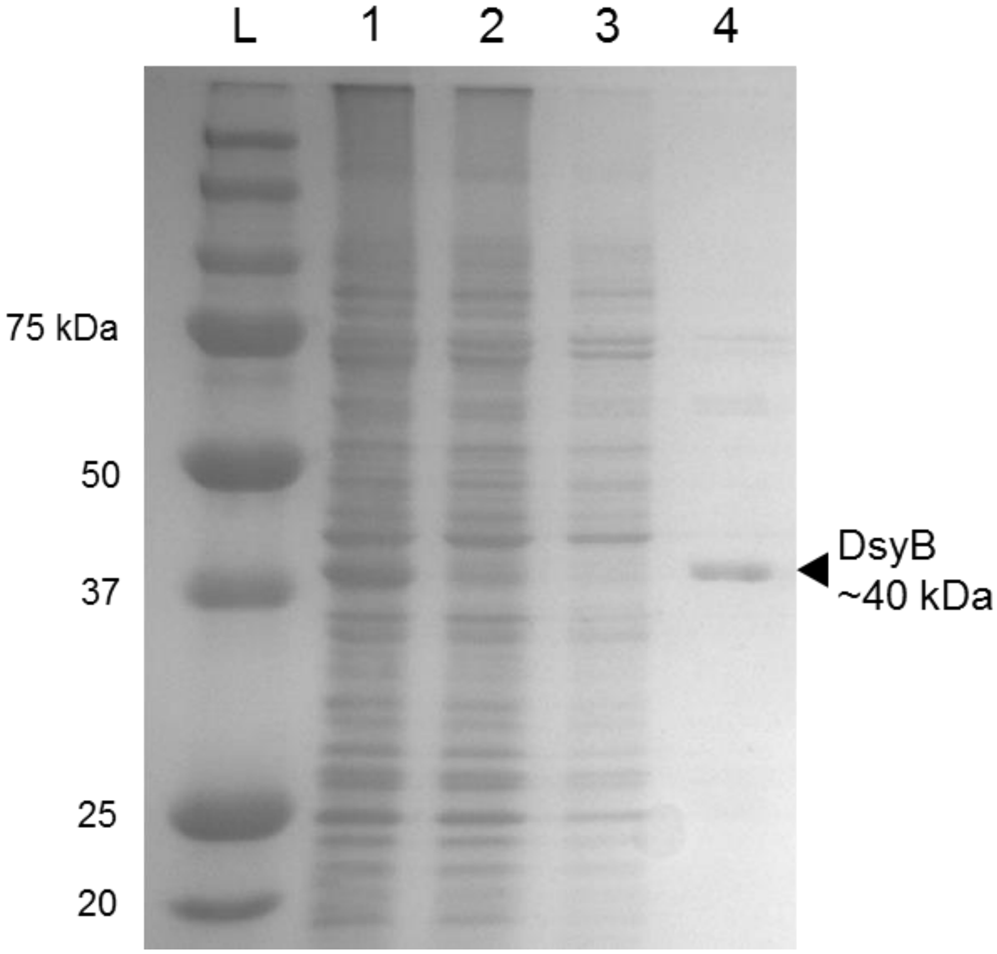
Supplementary Figure 4. Immunogold localisation of DSYB in *Prymnesium parvum* CCAP946/6. Electron micrograph of part of a *P. parvum* cell showing location of DSYB by immunogold labelling. Scale bar is 500 nm. Abbreviations: ch, chloroplast; ig, immunogold; m, mitochondrion; ri, ribosome; V, vacuole. Experiments were repeated twice and two samples (n=2) were used for each experiment.



Supplementary Figure 5. Relative abundance of ^{34}S-DMSP in *Prymnesium parvum* cells following incubation with ^{34}S-labelled sulfate. Each data point represents the average of 3 technical replicates (n=3), standard errors are too small to be visible but are equal to 0.038 at 0 hr; 0.076 after 6 hrs; 0.040 after 24 hrs and 0.159 after 48 hrs. Linear regression is represented as a solid line ($y = 0.379x + 4.512$; $R^2 = 0.989$), with 95% confidence interval as dashed lines.

(figure shown in separate file)

Supplementary Figure 6. Phylogenetic tree of environmental DsyB/DSYB protein sequences. An approximately maximum likelihood phylogenetic tree of DysB/DSYB sequences retrieved from metagenome and metatranscriptome datasets in relation to the ratified sequences (Fig. 1b). Sequences predicted to be bacterial DsyB and eukaryotic DSYB are indicated by curved arrows. Font colour represents the source of the sequences. Based on 1,487 sequences.



Supplementary Figure 7. SDS-PAGE gel showing purification of *C. tobin* DSYB. *C. tobin* DSYB was expressed in *E. coli* and purified using Ni-affinity chromatography, with elutions at different concentrations of imidazole. Lanes: L, protein standard ladder; 1, lysate before purification; 2, eluted fraction at 25 mM imidazole; 3, eluted fraction at 50 mM imidazole; 4, eluted fraction at 100 mM imidazole containing purified DSYB. SDS gel stained with Coomassie blue. Experiments were repeated twice on different biological replicates.

Supplementary Table 1. DMSP production in strains of bacteria/phytoplankton and activity of the corresponding cloned *dsyB*/*DSYB* genes.

Strain/Plasmid	**Intracellular DMSP concentration (mM)***	**Gene cloned**	**DMSHB production from *R. leguminosarum* J391 expressing cloned *DSYB*/*dsyB* from this strain (pmol DMSP mg protein^{-1} min^{-1})***	**DMSHB production from *L. aggregata dsyB*$^{-}$ mutant J571 expressing cloned *DSYB*/*dsyB* from this strain (pmol DMSP mg protein^{-1} min^{-1})***
Labrenzia aggregata LZB033	9.6†	*dsyB*	2.98 ± 0.603	426 ± 11.8
Acropora cervicornis	NT‡	*DSYB*	2.48 ± 0.221	290 ± 40.1
Acropora millepora	NT	non-DSYB methyltransferase (Cluster022229) from Raina et al., 2013[1]	ND§	ND
Alexandrium tamarense ATSP1-B	NT	*DSYB*	3.31 ± 0.223	504 ± 26.4
Chrysochromulina tobin CCMP291	0.611 ± 0.08	*DSYB*	2.39 ± 0.228	407 ± 64.2
Chrysochromulina sp. PCC307	0.196 ± 0.0394	*DSYB*	NT	NT
Fragilariopsis cylindrus CCMP1102	6.71 ± 0.92	*DSYB*	3.12 ± 0.659	311 ± 11.9
		non-DSYB methyltransferase (207357) from Lyon et al., 2011[2]	ND	ND
Lingulodinium polyedrum CCMP1936	NT	*DSYB*	2.49 ± 0.345	364 ± 0.000161
Symbiodinium microadriaticum CCMP2467	282 ± 35.0	*DSYB*	3.41 ± 0.337	363 ± 11.7
Prymnesium parvum CCAP946/6	54.3 ± 5.97	*DSYB*	2.97 ± 0.599	NT
Prymnesium parvum CCAP941/6	20.6 ± 3.05	*DSYB*	NT	NT
Prymnesium parvum CCAP946/1A	53.8 ± 4.58	*DSYB*	NT	NT
Prymnesium parvum CCAP946/1D	35.5 ± 1.50	*DSYB*	NT	NT
Prymnesium parvum CCAP946/1B	48.4 ± 6.29	*DSYB*	NT	NT
Prymnesium patelliferum CCAP946/4	25.3 ± 2.39	*DSYB*	NT	NT
pLMB509 (empty vector control)	-	none	ND	ND

*based on experiments repeated at least twice, mean of three biological replicates (n=3) and standard deviation shown

†concentration for *Labrenzia* from Curson et al.[3]

‡NT, not tested

§ND, not detected

Supplementary Table 2. Summary of iMicrobe transcriptome expression and *DSYB* copy number.

	Dinoflagellates	Haptophytes	Diatoms	Ochrophyta
Number of transcriptomes with DSYB	26(30)*	11(12)	5(30)	3(15)
Average TPM† values for DSYB sequences‡	42.9 ± 28.7	107 ± 49.6	13.8 ± 9.55	6.66 ± 1.18
Transcriptomes that express >1 DSYB copy	23	3	1	0
Range in number of DSYB copies	1 to 12	1 to 2	1 to 5	1

*number in brackets is the total number of transcriptomes of that taxon in iMicrobe

†TPM, transcripts per kilobase million

‡averages based on cumulative TPM values for each species with standard deviation shown and sample numbers were n=26 for dinoflagellates, n=11 for haptophytes, n=5 for diatoms, n=3 for ochrophyta

Supplementary Table 4. *In vitro* assays with purified DSYB protein and heat-denatured *P. parvum* cell lysate and Michaelis-Menten kinetics data

Assay components	Activity (nmol DMSHB mg protein^{-1} min^{-1})*
DSYB	N.D.†
DSYB + SAM	N.D.
DSYB + MTHB	N.D.
DSYB + SAM + MTHB	N.D.
DSYB + *P. parvum* lysate	N.D.
DSYB + *P. parvum* lysate + SAM	7.14 ± 2.26
DSYB + *P. parvum* lysate + MTHB	N.D.
DSYB + *P. parvum* lysate + SAM + MTHB	94.0 ± 2.82
DSYB + *P. parvum* lysate + SAM + Met	N.D.
DSYB + *P. parvum* lysate + SAM + MTOB	N.D.
DSYB + *P. parvum* lysate + SAM + MMPA	N.D.

Substrate	V_{max} (nmol mg protein^{-1} min^{-1}) ‡	K_{cat} (s^{-1})	K_M (μM) ‡	Specific activity, K_{cat}/K_M (M^{-1}s^{-1})
MTHB	294 ± 7.7	0.499	88.2 ± 15	5,664
SAM	303 ± 4.5	0.515	60.1 ± 14	8,497

*background DMS from DMSHB/DMSP present in the *P. parvum* lysate was subtracted from DMS values used for activities, mean of 3 biological replicates (n=3) with standard deviations shown

†N.D., not detected

‡based on experiments that were repeated twice with two biological replicates and two technical replicates (n=4) for each experiment, standard deviations are shown

Supplementary Table 5. Localisation signals predicted from *in silico* sequence analysis of DSYB proteins.

Strain	SignalP 4.1 prediction	ChloroP 1.1 prediction	TargetP 1.1 prediction
Acropora cervicornis	-*	C†	M‡
Alexandrium tamarense ATSP1-B	-	-	M
Chrysochromulina tobin CCMP291	-	-	M
Fragilariopsis cylindrus CCMP1102	-	C	M
Lingulodinium polyedrum CCMP1936	-	C	M
Prymnesium parvum CCAP946/1B	-	-	M
Symbiodinium microadriaticum CCMP2467	-	-	M

*no localisation signal predicted

†C, chloroplast

‡M, mitochondrion

Supplementary Table 7. Metatranscriptome sample information.

Metatranscriptome/ Project	Sample ID	Biome	Location, latitude, longitude	Size fraction	Source
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
GeoMICS	SRR1782575	Marine, seawater, depth 5 m	P1: North Pacific Ocean, 48.575, -125.5	2 μm – 53 μm	NCBI
GeoMICS	SRR1781979	Marine, seawater, depth 5 m	P1: North Pacific Ocean, 48.575, -125.5	2 μm – 53 μm	NCBI
GeoMICS	SRR1782572	Marine, seawater, depth 5 m	P1: North Pacific Ocean, 48.575, -125.5	0.2 μm – 2 μm	NCBI
GeoMICS	SRR1782573	Marine, seawater, depth 5 m	P1: North Pacific Ocean, 48.575, -125.5	0.2 μm – 2 μm	NCBI
GeoMICS	SRR4095707	Marine, seawater, depth 5 m	P6: North Pacific Ocean, 48.743, -127.667	2 μm – 53 μm	NCBI
GeoMICS	SRR4095708	Marine, seawater, depth 5 m	P6: North Pacific Ocean, 48.743, -127.667	2 μm – 53 μm	NCBI
GeoMICS	SRR4095709	Marine, seawater, depth 5 m	P6: North Pacific Ocean, 48.743, -127.667	0.2 μm – 2 μm	NCBI
GeoMICS	SRR4095710	Marine, seawater, depth 5 m	P6: North Pacific Ocean, 48.743, -127.667	0.2 μm – 2 μm	NCBI

Supplementary Table 8. *Tara* Oceans metatranscriptome *dsyB*, *DSYB* and DMSP lyase transcript abundance.

Sample ID	**Total number of sequences**	**Number of transcripts**									
		dsyB	***DSYB***	***dddD***	***dddK***	***dddL***	***dddP***	***dddQ***	***dddY***	***dddW***	***Alma1***
ERR598943	26,541,646	3	2	4	81	1	2,095	279	0	0	1
ERR598945	19,465,217	6	12	60	380	1	636	47	0	0	0
ERR598952	13,686,168	2	5	0	22	1	1,259	156	0	0	0
ERR598953	23,584,453	17	0	1	5	24	3,599	122	6	0	0
ERR598956	24,881,437	49	1	16	1	2	3,227	30	2	0	0
ERR598976	102,893,943	29	38	22	448	12	7,775	829	21	0	0
ERR598977	25,301,577	7	0	1	8	2	1,759	69	0	0	0
ERR598988	24,203,323	136	1	5	12	2	2,491	113	0	0	0
ERR598999	27,948,130	17	1	35	1	11	3,788	77	1	0	0
ERR599004	23,015,118	29	0	33	1	11	2,586	134	0	0	0
ERR599010	19,979,067	14	19	9	8	18	517	56	4	0	0
ERR599011	19,967,844	4	0	6	0	3	1,506	109	1	0	0
ERR599015	27,767,579	26	0	8	3	21	2,771	151	1	0	0
ERR599016	21,315,539	19	2	6	15	4	1,802	106	2	0	0
ERR599022	21,372,057	7	18	11	26	3	1,194	102	15	0	0
ERR599027	19,109,390	6	7	115	540	3	1,710	89	0	0	0
ERR599031	16,274,217	12	0	1	1	1	1,489	10	0	0	0
ERR599035	37,264,126	12	26	179	774	4	2,058	101	0	0	0
ERR599067	19,304,566	2	0	36	0	13	2,975	84	6	0	0
ERR599098	16,420,448	2	3	7	1	3	904	105	1	0	0
ERR599102	50,611,881	5	2	4	68	2	4,773	277	0	0	0
ERR599104	18,052,511	5	24	80	392	1	1,316	9	0	0	0
ERR599109	25,942,802	26	0	2	1	0	2,914	19	0	0	0
ERR599113	14,927,388	29	0	3	24	4	1,630	86	1	0	0
ERR599169	22,146,064	1	0	7	9	2	2,525	325	0	0	0
ERR599176	19,776,131	6	17	27	804	1	1,348	13	0	0	0
Total *Tara* sequences /transcripts	**681,752,622**	**471**	**178**	**678**	**3,625**	**150**	**60,647**	**3,498**	**61**	**0**	**1**
Total transcripts per million sequences		**0.691**	**0.261**	**0.994**	**5.32**	**0.220**	**89.0**	**5.13**	**0.0894**	**0**	**0.000147**

Supplementary Table 10. Strains and plasmids used in this study.

Strain/Plasmid	**Description**	**Reference**
Escherichia coli 803	Strain used for routine transformations	Wood (1966)[4]
Escherichia coli BL21 DE3	Strain used for DSYB expression for protein purification	New England BioLabs
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 J571	*Labrenzia aggregata* LZB033 with mutation in *dsyB* gene	Curson et al. (2017)[3]
Prymnesium parvum CCAP946/6	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions and different environmental conditions	CCAP culture collection
P. parvum CCAP941/1A	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	CCAP culture collection
P. parvum CCAP941/6	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	CCAP culture collection
P. parvum CCAP946/1D	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	CCAP culture collection
P. parvum CCAP946/1B	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	CCAP culture collection
Prymnesium patelliferum CCAP946/4	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	CCAP culture collection
Chrysochromulina sp. PCC307	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions and different environmental conditions	Plymouth culture collection of marine algae
Chrysochromulina tobin CCMP291	Haptophyte used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions and different environmental conditions	Hovde *et al*. (2015)[6]
Symbiodinium microadriaticum CCMP2467	Dinoflagellate used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions	Baumgarten *et al*. (2013)[7]
Fragilariopsis cylindrus CCMP1102	Diatom used for growth experiments to determine intracellular DMSP concentration and *DSYB* expression under standard conditions and different environmental conditions	Mock *et al*. (2017)[8]
pLMB509	Plasmid vector for taurine-inducible expression of cloned genes in *Rhizobium*	Tett et al. (2012)[9]
pRK415	Wide host-range plasmid vector with IPTG-inducible *lac* promoter	Keen et al. (1988)[10]
pRK2013	Helper plasmid used in triparental matings	Figurski and Helinski (1979)[11]
pBIO2258	*Labrenzia aggregata* IAM12614 bacterial *dsyB* gene cloned in pLMB509	Curson et al. (2017)[3]
pBIO2268	*Fragilariopsis cylindrus* CCMP 1102 methyltransferase gene predicted (Lyon *et al*. 2011[2]) to have a role in DMSP biosynthesis (FRACYDRAFT_207357), codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2269	*Acropora millepora* methyltransferase gene predicted (Raina et al. 2013[1]) to have a role in DMSP biosynthesis, codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2270	*Acropora cervicornis DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2271	*Alexandrium tamarense* ATSP1-B *DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2272	*Chrysochromulina tobin* CCMP 291 *DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2273	*Fragilariopsis cylindrus* CCMP 1102 *DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2274	*Lingulodinium polyedrum* CCMP 1936 *DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2275	*Prymnesium parvum* CCAP 946/6 *DSYB* cloned in pRK415	This study
pBIO2276	*Symbiodinium microadriaticum* CCMP 2467 *DSYB,* codon-optimised, synthesised and cloned in pLMB509	This study
pBIO2277	*C. tobin DSYB*, codon-optimised, synthesised and subcloned (from pBIO2272) in pET16b, and used for protein purification	This study

Supplementary Table 11. Oligonucleotide primers used in this study.

Primer name	Sequence (5' to 3')*	Use
PpDSYBp1	GCGGATCCGAAGGAGATATACATCATATGCTGCGCCTCGCCCCTCGC	Cloning of *P. parvum* CCAP 946/6 *DSYB* into pRK415 for pBIO2275
PpDSYBp2	GCGAATTCTTATGGTTTGTAAGCGACGATGAGC	Cloning of *P. parvum* CCAP 946/6 *DSYB* into pRK415 for pBIO2275
qF_ParvDSYB	ACTGCAGCATGTCACTGTCA	RT-qPCR amplification of *P. parvum* CCAP 946/6 *DSYB*
qR_ParvDSYB	CCCCGTGATCATCTCCATGG	RT-qPCR amplification of *P. parvum* CCAP 946/6 *DSYB*
qF_SymbioDSYB1	GAAGGTTGTGACGGGCCATA	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 *DSYB1*
qR_SymbioDSYB1	TCGCCAGGTAATCGAAGACG	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 *DSYB1*
qF_SymbioDSYB2	GTTCTCCTCGAAGCCTGCTT	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 *DSYB2*
qR_SymbioDSYB2	TCATGGTCAACGACAGCCTC	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 *DSYB2*
qF_ChrysDSYB	CACGGCCAAGTACCTTGTCA	RT-qPCR amplification of *C. tobin* CCMP 291 *DSYB*
qR_ChrysDSYB	GACTGTCGACTTGAGTCCCG	RT-qPCR amplification of *C. tobin* CCMP 291 *DSYB*
qF_FragiDSYB	CTATCAGCTGCTGACCCAGG	RT-qPCR amplification of *F. cylindrus* CCMP 1102 *DSYB*
qR_FragiDSYB	AACCATACCTCCCGGTCGTA	RT-qPCR amplification of *F. cylindrus* CCMP 1102 *DSYB*
qF_ParvActin	GCTACTCCTTCACCACCACC	RT-qPCR amplification of *P. parvum* CCAP 946/6 β-actin gene
qR_ParvActin	GCTCGTACGACTTCTCGAGG	RT-qPCR amplification of *P. parvum* CCAP 946/6 β-actin gene
qF_SymbioActin	AAGGCCAATCGTGAACGGAT	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 β-actin gene
qR_SymbioActin	GCACACAATCCCGGTTGTTC	RT-qPCR amplification of *S. microadriaticum* CCMP 2467 β-actin gene
qF_ChrysActin	GGTGTGATGGTGGGTATGGG	RT-qPCR amplification of *C. tobin* CCMP 291 β-actin gene
qR_ChrysActin	CATGTCGTCCCAGTTGGTCA	RT-qPCR amplification of *C. tobin* CCMP 291 β-actin gene
qF_FragiActin	TGGGGATGAAGCACAAGCAA	RT-qPCR amplification of *F. cylindrus* CCMP 1102 β-actin gene
qR_FragiActin	AGGATGAGCTTCAGGGGCTA	RT-qPCR amplification of *F. cylindrus* CCMP 1102 β-actin gene

*restriction sites included in primers for cloning are underlined

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