Dimethylsulphoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process

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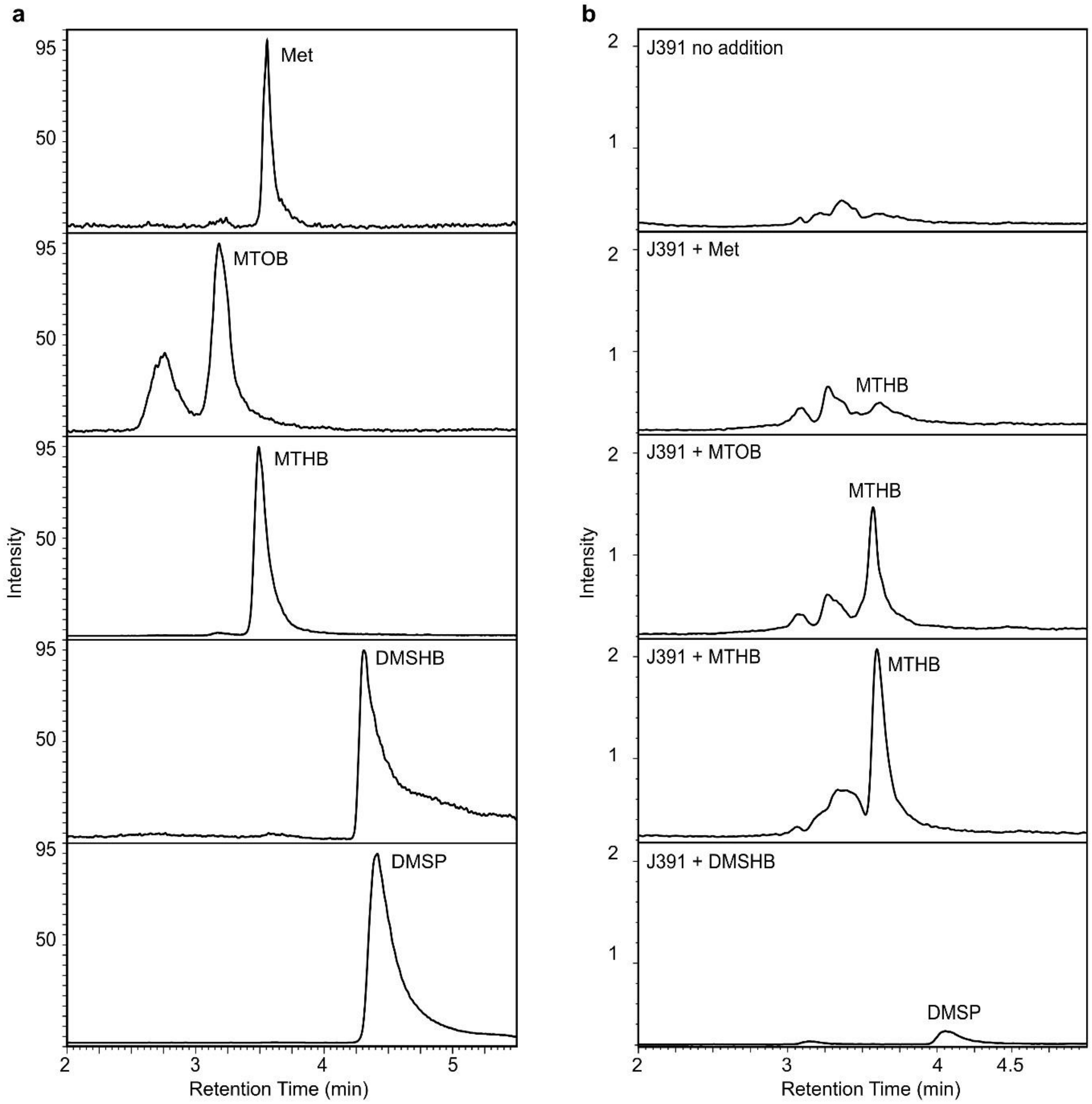
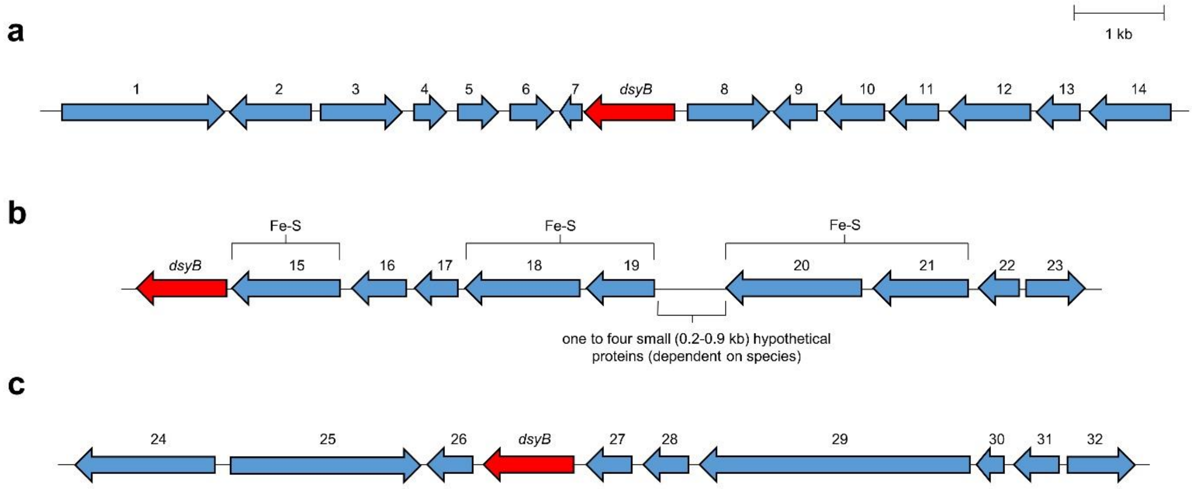
Supplementary Figures



Supplementary Figure 1. LC-MS traces for standards and *Rhizobium leguminosarum* J391. LC-MS chromatograms for **a**, Met (m/z 150), MTOB (m/z 147), MTHB (m/z 149), DMSHB (m/z 165) and DMSP (m/z 135) standards, **b**, MTHB production from *R. leguminosarum* J391 incubated with Met, MTOB or MTHB, and DMSP production from *R. leguminosarum* J391 incubated with DMSHB.



Supplementary Figure 2. Gene maps showing genomic locations of *dsyB* in selected *dsyB*-containing bacteria. a, Gene map for *Labrenzia aggregata* LZB033 and *L. aggregata* IAM12614. **b**, Gene map for *Salipiger mucosus* DSM16094, *Pelagibaca bermudensis* HTCC2601, *Thalassiobius gelatinovorus* DSM5887, *Donghicola xiamenensis* DSM18339, *Antarctobacter heliothermus* DSM11445, *Pseudooceanicola nanhaiensis* DSM18065, *Citreicella* sp. 357, *Citreicella aestuarii* DSM22011, *Sediminimonas qiaohouensis* DSM21189, *Roseivivax halodurans* JCM10272. Genes encoding protein products predicted to be involved in Fe-S cluster assembly are marked. **c**, Gene map for *Rhizobiales* bacterium HL-109. Predicted gene products: 1. tricarboxylate transporter; 2. AraC family transcriptional regulator; 3. nucleotide phosphate sugar epimerase; 4. hypothetical protein; 5. dehydratase; 6. MaoC-like dehydratase; 7. hypothetical protein; 8. agmatinase; 9. acetyltransferase; 10. cob(II)yrinic acid a,c-diamide reductase; 11. adenine phosphoribosyltransferase; 12. *S*-methyladenosine phosphorylase; 13. hypothetical protein; 14. cytochrome C1; 15. cysteine desulfurase; 16. hypothetical protein; 17. hypothetical protein; 18. SufD Fe-S cluster assembly protein; 19. SufC Fe-S cluster assembly ATP-binding protein; 20. SufB iron-regulated ABC transporter membrane component; 21. cysteine desulfurase; 22. BadM/Rrf2 family transcriptional regulator; 23. transposase; 24. serine protease; 25. DNA polymerase; 26. acetyltransferase; 27. peroxiredoxin; 28. peroxiredoxin; 29. uncharacterised membrane protein; 30. hypothetical protein; 31. tyrosine phosphatase; 32. outer membrane immunogenic protein.

Supplementary Tables

Supplementary Table 1. DMSP production by tested strains of bacteria and *Rhizobium leguminosarum* expressing cloned *dsyB* genes.

Strains	**Growth medium***	**DMSP production (pmol µg protein^{-1})**	**Estimated intracellular DMSP concentration (mM)**
Labrenzia aggregata LZB033	MBM (minimal)	8.4 ± 2.2	1.3
	MBM (minimal, 0.5 mM NH_4Cl)	99.8 ± 1.2	9.6
L. aggregata J571 (LZB033 *dsyB*$^-$)	MBM (minimal)	ND†	ND
	MBM (minimal, 0.5 mM NH_4Cl)	ND	ND
L. aggregata IAM12614	MBM (minimal)	3.6 ± 0.2	0.6
	MBM (minimal, 0.5 mM NH_4Cl)	32.9 ± 2.7	5.1
Oceanicola batsensis HTCC2597	YTSS (complete)	40.5 ± 2.0	6.3
Pelagibaca bermudensis HTCC2601	YTSS (complete)	259 ± 52.7	40.6
Sagittula stellata E-37	MBM (minimal)	11.1 ± 0.3	1.7
Sediminimonas qiaohouensis DSM21189	Marine broth 2216 + 3% NaCl (complete)	122 ± 4.6	19.1
Thalassobaculum salexigens DSM19539	Marine broth 2216 (complete)	4.8 ± 1.6	0.8
Amorphus coralli DSM19760	YTSS (complete)	8.2 ± 0.2	1.3
Bacillus mycoides DSM2048‡	Nutrient agar (complete)	ND	ND
Streptomyces varsoviensis DSM40346‡	GYM Streptomyces medium (complete)	ND	ND
Ruegeria pomeroyi DSS-3§	YTSS (complete)	ND	ND
Nesiotobacter exalbescens DSM16456§	Marine broth 2216 (complete)	ND	ND
R. leguminosarum J391	Y (minimal)	ND	ND
R. leguminosarum J391:pBIO2258 (cloned *L. aggregata* IAM12614 *dsyB*)	Y (minimal)	4.2 ± 0.4	0.7
R. leguminosarum J391:pBIO2258	Y (minimal) + 0.5 mM Met	13.9 ± 0.1	2.2
R. leguminosarum J391:pBIO2258	Y (minimal) + 0.5 mM MTOB	14.9 ± 0.1	2.3
R. leguminosarum J391:pBIO2258	Y (minimal) + 0.5 mM MTHB	11.7 ± 0.2	1.8
R. leguminosarum J391:pBIO2260 (cloned *O. batsensis* HTCC2597 *dsyB*)	Y (minimal) + 0.5 mM MTHB	33.2 ± 1.0	5.2
R. leguminosarum J391:pBIO2262 (cloned *A. coralli* DSM19760 *dsyB*)	Y (minimal) + 0.5 mM MTHB	29.1 ± 0.1	4.6
R. leguminosarum J391:pBIO2263 (cloned *P. bermudensis* HTCC2601 *dsyB*)	Y (minimal) + 0.5 mM MTHB	31.8 ± 0.9	5.0
R. leguminosarum J391:pBIO2264 (cloned *S. varsoviensis* DSM40346 *dsyB*)	Y (minimal) + 0.5 mM MTHB	ND	ND

* No added methylated sulphur compounds unless stated otherwise; 10 mM succinate was carbon source for all minimal media; 10 mM NH_4Cl was nitrogen source for all media unless stated otherwise.

† ND, not detected.

‡ Strains with DsyB homologue below cutoff (E value > $1e^{-67}$) for predicted functionality.

§ Strains with no significant DsyB homologue.

Supplementary Table 2. Metagenome information and results of DsyB metagenomics searches.

Metagenome/ Project	Accession number	Biome	Location	Total genes	Database	Number of DsyB sequences*	Number of RecA sequences†	Estimated % of bacteria with DsyB
Tara Oceans	PRJEB7988	Marine	Various	111,530,851	EMBL	120	26396	0.45
Global Ocean Sampling	CAM_PROJ_GOS	Marine	Various	22,607,701	CAMERA	15	5512	0.27
Prairie grassland	4504798.3	Grassland	Iowa, USA	3,096,464	MG-RAST	0	317	0
Rothamsted soil	4453247.3	Grassland	Rothamsted, UK	1,166,789	MG-RAST	0	99	0
Forest soil	4446153.3	Soil	Luquillo, Puerto Rico	689,464	MG-RAST	0	68	0
Waseca farm soil	CAM_PROJ_FarmSoil	Soil	Minnesota, USA	183,297	CAMERA	0	15	0
Metasoil	METASOIL-F1D_FASTA	Soil	Rothamsted, UK	1,003,757	EMBL	0	54	0
Metasoil	METASOIL-F2AI_FASTA	Soil	Rothamsted, UK	1,180,858	EMBL	0	97	0
Metasoil	METASOIL-F3I_FASTA	Soil	Rothamsted, UK	798,555	EMBL	0	62	0
Jutland Miseq	4687209.3	Soil	Jutland, Denmark	255,518	MG-RAST	0	NC‡	0
Rainforest vs. Arctic tundra	4689236.3	Soil	Norway /Malaysia	3,195,645	MG-RAST	0	NC	0
USA Black mountain	4690510.3	Soil	N. Carolina, USA	7,265,095	MG-RAST	0	NC	0

* E value ≤ $1e^{-67}$

† E value ≤ $1e^{-50}$

‡ NC, not calculated as assembled sequences were too short.

Supplementary Table 3. Strains used in this study.

Strain	Description	Reference
Escherichia coli 803	Strain used for routine transformations	Wood (1966)[1]
E. coli BL21	Strain for overexpression of cloned genes in pET vectors	Studier and Moffat (1986)[2]
Rhizobium leguminosarum J391	Streptomycin-resistant derivative of wild type strain 3841 used for library screening and expression of genes cloned in plasmid pLMB509	Young *et al*. (2006)[3]
Labrenzia aggregata LZB033	Wild type strain, isolated from ME3 site	This study
L. aggregata IAM12614	Wild type strain	Uchino *et al*. (1998)[4]
Sagittula stellata E-37	Wild type strain	Gonzalez *et al*. (1997)[5]
Oceanicola batsensis HTCC2597	Wild type strain	Cho and Giovannoni (2004)[6]
Amorphus coralli DSM19760	Wild type strain	Zeevi Ben Yosef *et al*. (2008)[7]
Pelagibaca bermudensis HTCC2601	Wild type strain	Cho and Giovannoni (2006)[8]
Sediminimonas qiaohouensis DSM21189	Wild type strain	Wang *et al*. (2009)[9]
Thalassobaculum salexigens DSM19539	Wild type strain	Urios *et al*. (2010)[10]
Streptomyces varsoviensis DSM40346	Wild type strain	Skerman *et al*. (1980)[11]
Bacillus mycoides DSM2048	Wild type strain	Fahmy *et al*. (1985)[12]
Ruegeria pomeroyi DSS-3	Wild type strain	Gonzalez *et al*. (2003)[13]
Nesiotobacter exalbescens DSM16456	Wild type strain	Donachie *et al*. (2006)[14]
J570	Rifampicin-resistant derivative of *Labrenzia aggregata* LZB033	This study
J571	J570 with mutation in *dsyB*	This study
J572	J570 with mutation in *dddL*	This study

Supplementary Table 4. Plasmids used in this study.

Plasmid	Description	Reference
pLAFR3	Wide host-range cosmid vector, used for library construction	Staskawicz et al. (1987)[15]
pET21a	Plasmid vector for expression of cloned genes in *E. coli*	Merck Millipore
pLMB509	Plasmid vector for taurine-inducible expression of cloned genes in *Rhizobium*	Tett et al. (2012)[16]
pRK415	Wide host-range plasmid vector with IPTG-inducible *lac* promoter	Keen et al. (1988)[17]
pRK2013	Helper plasmid used in triparental matings	Figurski and Helinski (1979)[18]
pBIO1879	Spectinomycin-resistant derivative of pK19*mob* suicide plasmid, used for mutagenesis	Todd et al. (2011)[19]
pBIO2252	pLAFR3 cosmid from *Labrenzia aggregata* IAM12614 library that contains ~30 kb genomic DNA including *dsyB*	This study
pBIO2253	Internal fragment of *L. aggregata* LZB033 *dsyB* cloned in pBIO1879	This study
pBIO2254	Internal fragment of *L. aggregata* LZB033 *dddL* cloned in pBIO1879	This study
pBIO2256	*L. aggregata* LZB033 *dddL* cloned in pET21a(+)	This study
pBIO2258	*L. aggregata* IAM12614 *dsyB* cloned in pLMB509	This study
pBIO2260	*Oceanicola batsensis* HTCC2597 *dsyB* cloned in pLMB509	This study
pBIO2262	*Amorphus coralli* DSM19760 *dsyB* cloned in pLMB509	This study
pBIO2263	*Pelagibaca bermudensis* HTCC2601 *dsyB* cloned in pLMB509	This study
pBIO2264	*Streptomyces varsoviensis* DSM40346 *dsyB* cloned in pLMB509	This study
pBIO2266	*L. aggregata* IAM12614 *dsyB* subcloned from pBIO2258 into pRK415	This study

Supplementary Table 5. Oligonucleotide primers used in this study.

Primer name	Sequence (5’ to 3’)*	Use
LZB02157XbaFOR2	GCACTTCATCTAGATTTGTTTTCACTGC	pBIO2253
LZB02157pK19Eco	CCGGGTTGAATTCGGTGTGCTGAA	pBIO2253
LZBpKLXbaFOR	CGGTTCCTCTAGACCGGTGGCAGCG	pBIO2254
LZBpKLEcoREV	GGCCCAGGCCAGAATTCCCGGCTCC	pBIO2254
labdddLNde	GGAATTCCATATGCTGGAAAAAGACACTTCGG	pBIO2256
labdddLBam	CGGGATCCGCTCATCCCTTCTTGCG	pBIO2256
21095NdeFOR1	GGAGGATAACATATGCCTGTCGCCAGG	pBIO2258
21095BamREV1	GCAGTCTGCGGATCCTTGCCGAAGAACG	pBIO2258
OB06780NdeFOR	GGAGTACCATATGCATCCAGCGACCG	pBIO2260
OB06780EcoREV	CGCCCGAATTCGCAAATGCGTCGC	pBIO2260
AcordsyBNdeFOR	GCGAGATACCAGCCATATGACCCTCC	pBIO2262
AcordsyBSacREV	GCCCCGAGCTCAGGCGCCTCAACCC	pBIO2262
PberdsyNdeFOR	GCGGGAGAGACATATGGGGCCGGTC	pBIO2263
PberdsyBNdeREV	GCGCATATGGGGAATTCCCGTTCCGCG	pBIO2263
SvardsyBNdeFOR	GCCCCACCGGCATATGGACATCGTG	pBIO2264
SvardsyBSacREV2	GATCGAGCTCTGCTGCACCACGTCAAC	pBIO2264
dsyBFOR	CTTGACGCCACAGCATGTTG	qRT-PCR amplification of *dsyB*
dsyBREV	TCCGTCCTTTCACCAGAAAC	qRT-PCR amplification of *dsyB*
dddLFOR	CGCTCCTGAAACGCAGATA	qRT-PCR amplification of *dddL*
dddLREV	GGCAGTATGGCTACGAGAAA	qRT-PCR amplification of *dddL*
recAFOR	CACTGGAAATTGCCGATACG	qRT-PCR amplification of *recA*
recAREV	CACCATGCACTTCGACTTG	qRT-PCR amplification of *recA*
rpoDFOR	ACAAGTTCTCCACCTATGCG	qRT-PCR amplification of *rpoD*
rpoDREV	CGATTTCATGCAGCATCTGG	qRT-PCR amplification of *rpoD*

*Restriction sites included in primers are underlined.

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