Supporting information

Biosynthesis of 2-methylisoborneol in cyanobacteria

Giglio, S.[1], Chou, W.K.W.[2], Ikeda, H.[3],Cane D.E.[2], Monis, P.T[1].

[1]Australian Water Quality Centre, South Australian Water Corporation, 250 Victoria Square, Adelaide, SA 5008.

[2]Brown University, Department of Chemistry, Box H, Providence, Rhode Island 02912-9108.

[3]Kitasato Institute of Life Sciences, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan.

To whom correspondence should be addressed. Email: steven.giglio@sawater.com.au

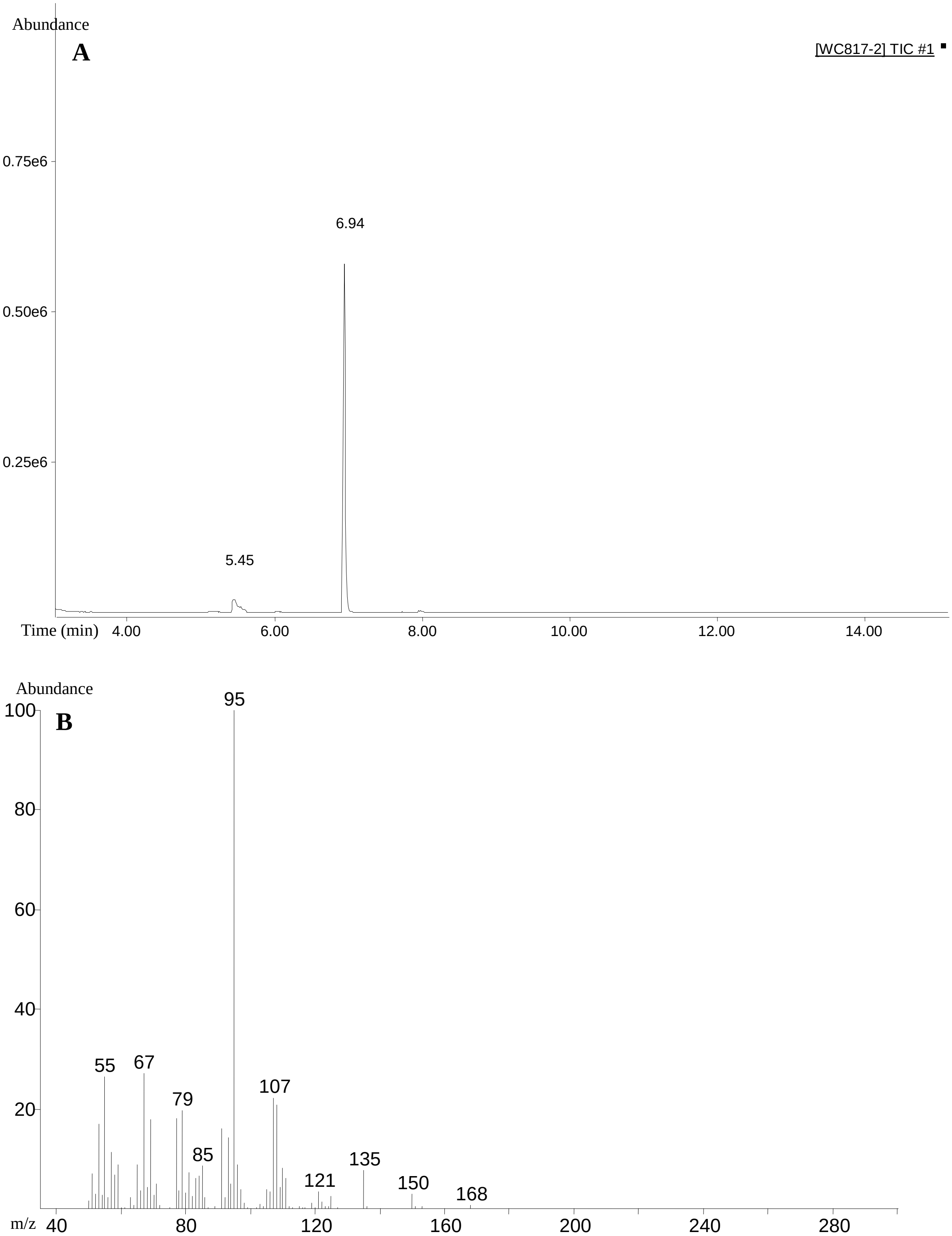

Figure S1. (A) GC-MS TIC of pentane extract from the incubation of 2-Me-GPP (**2**) (60 μM) with MIBS. (B) Mass spectrum of MIB (**3**) produced from MIBS incubation.

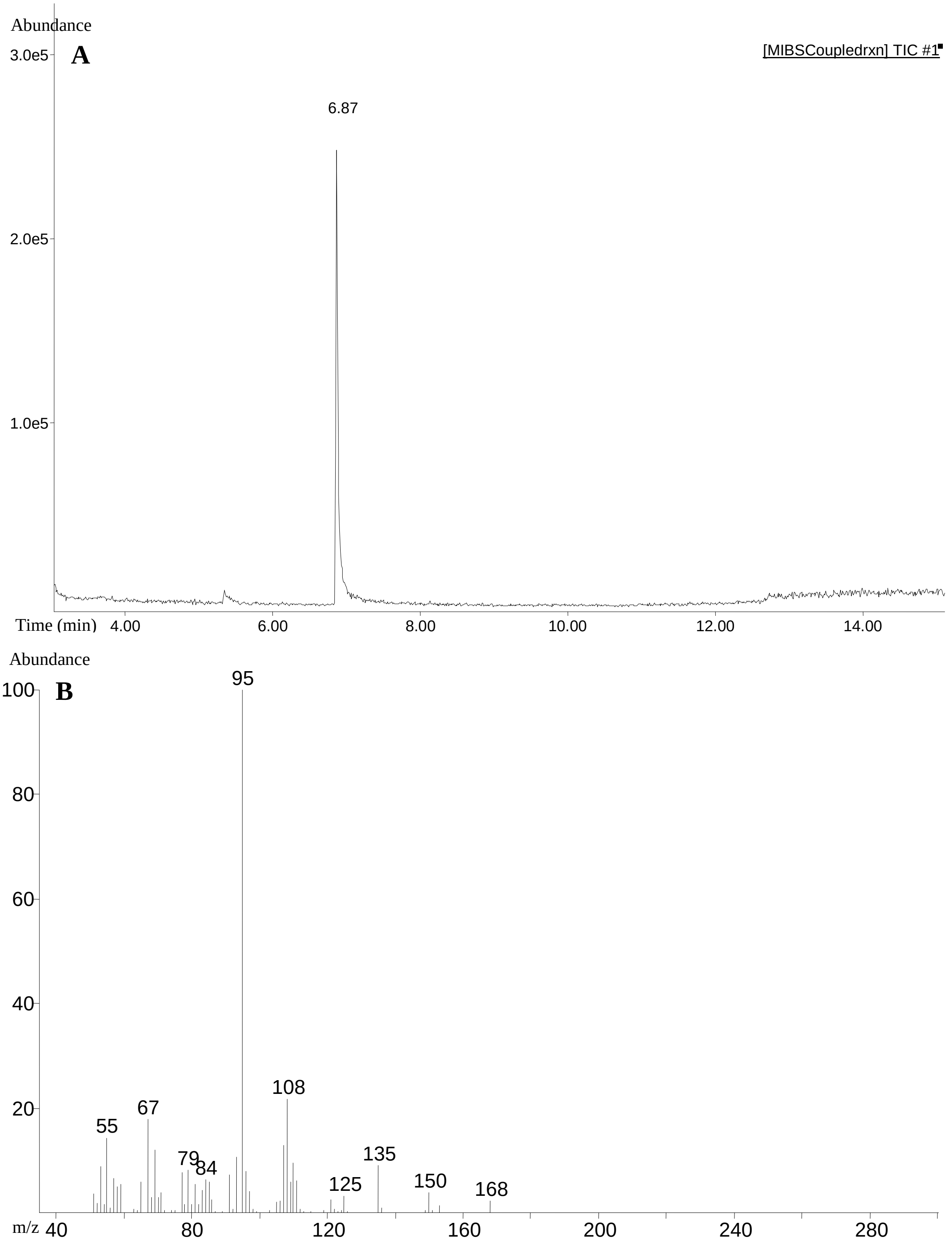

Figure S2. (A) GC-MS TIC of pentane extract from the coupled incubation of GPP (**1**) (60 μM) and SAM (120 μM) with GPPMT and MIBS. (B) Mass spectrum of MIB (**3**) produced from coupled GPPMT and MIBS incubation.

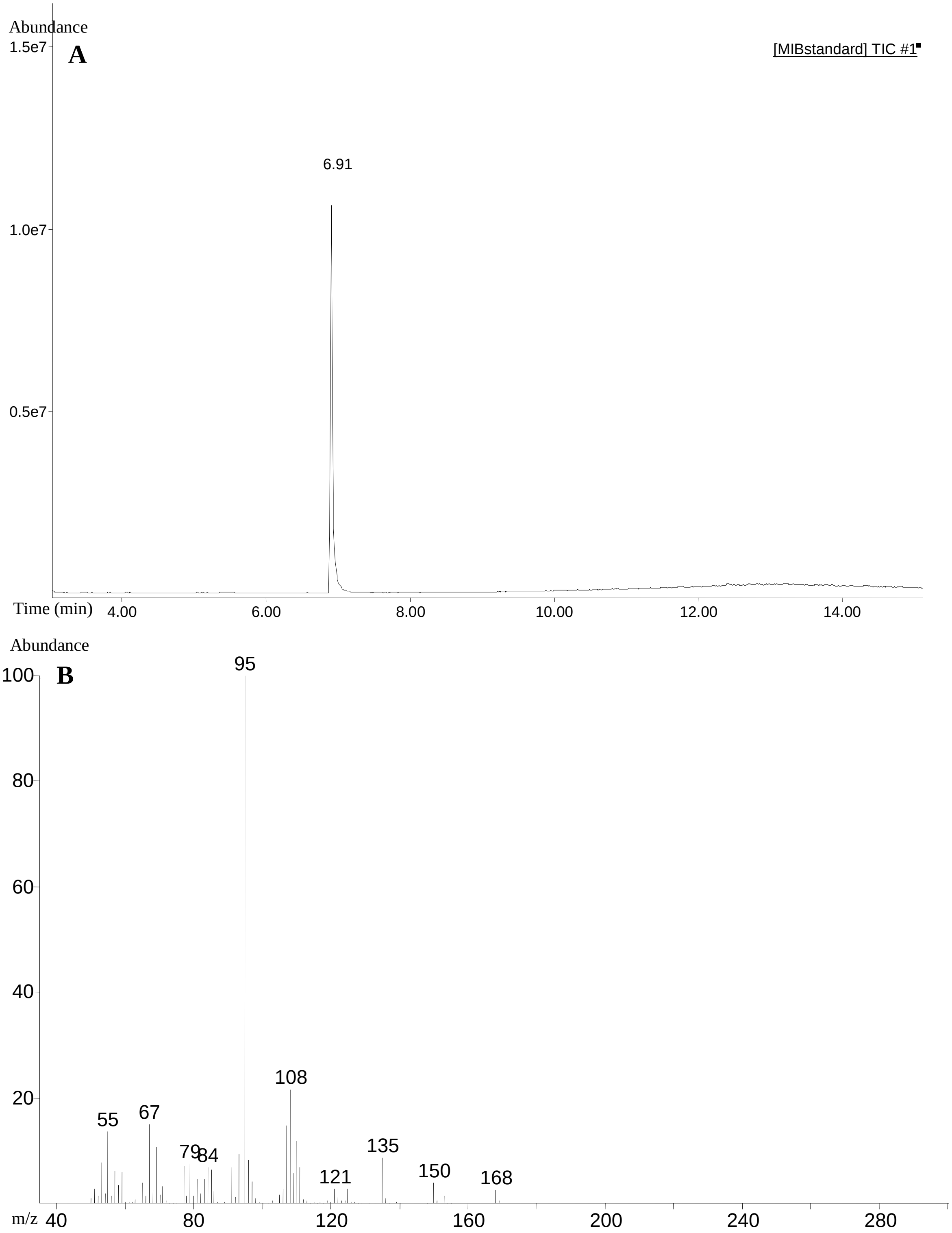

Figure S3. (A) GC-MS TIC of MIB (**3**) standard. (B) Mass spectrum of MIB (**3**) standard.

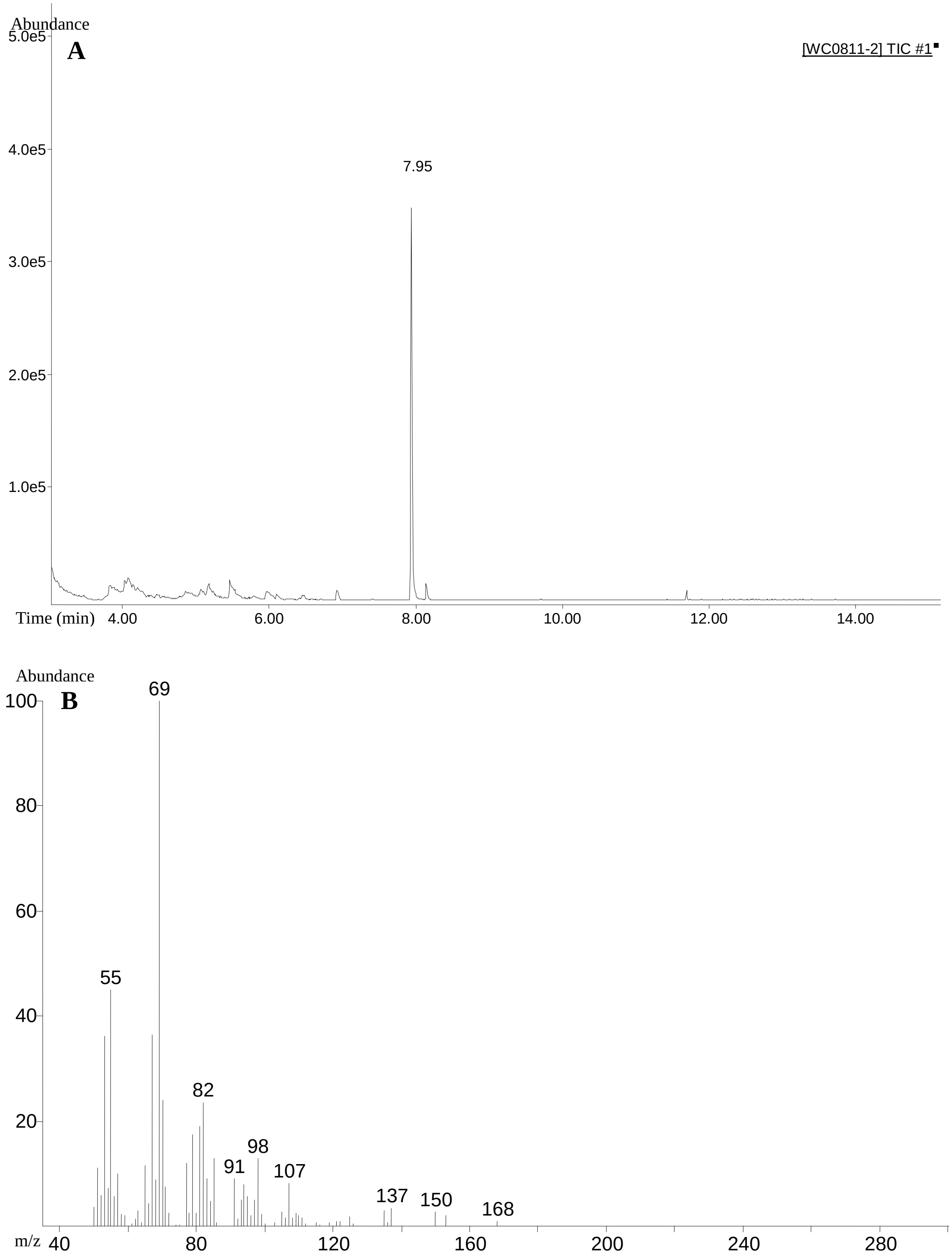

Figure S4. (A) GC-MS TIC of pentane extract from the incubation of GPP (**1**) (60 μM) and SAM (120 μM) with GPPMT (10 μM), followed by incubation with phosphatases. (B) Mass spectrum of E-2-methylgeraniol (**4**) produced from GPPMT and phosphatase incubation.

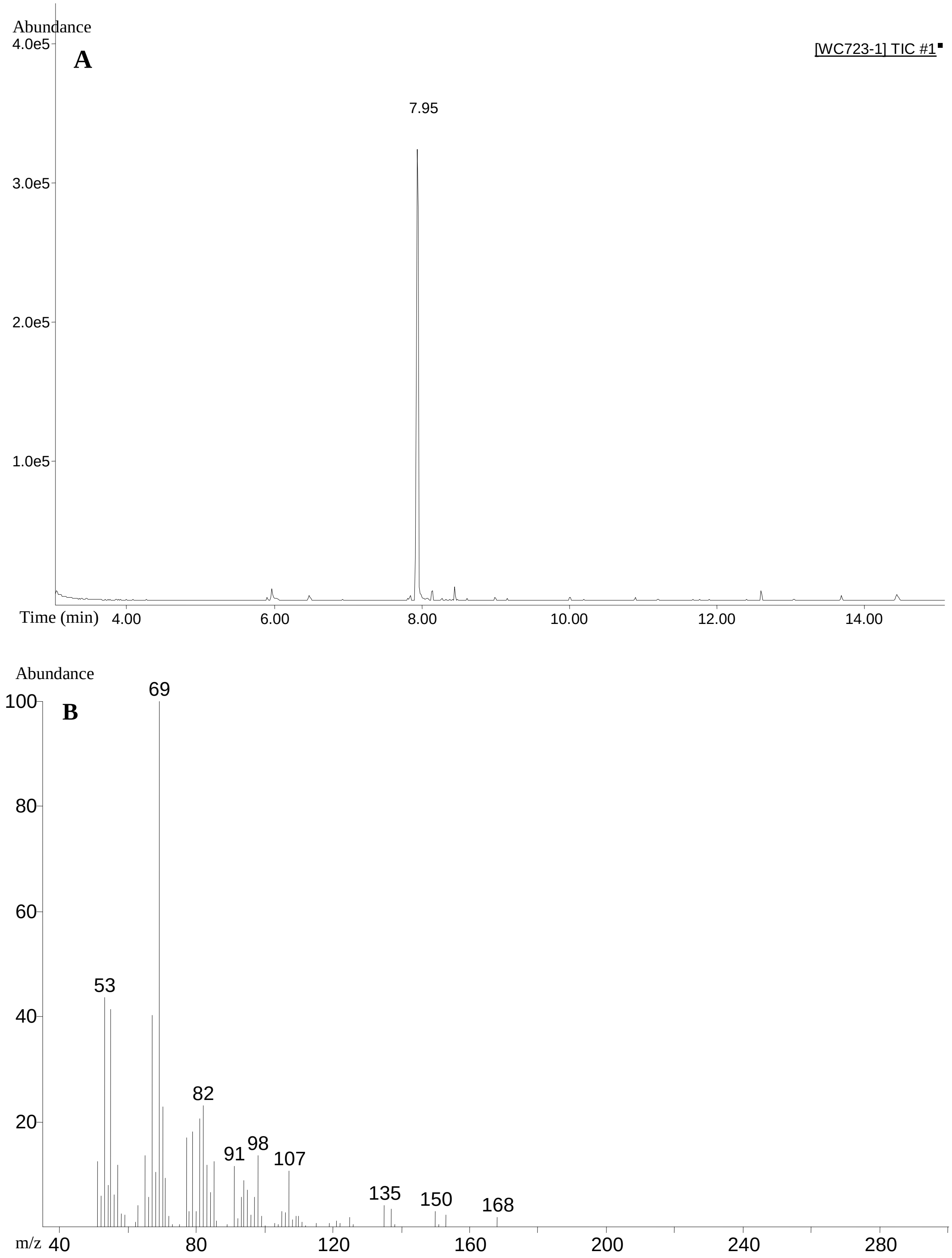

Figure S5. (A) GC-MS TIC of synthetic E-2-methylgeraniol (**4**) standard. (B) Mass spectrum of synthetic E-2-methylgeraniol (**4**) standard.

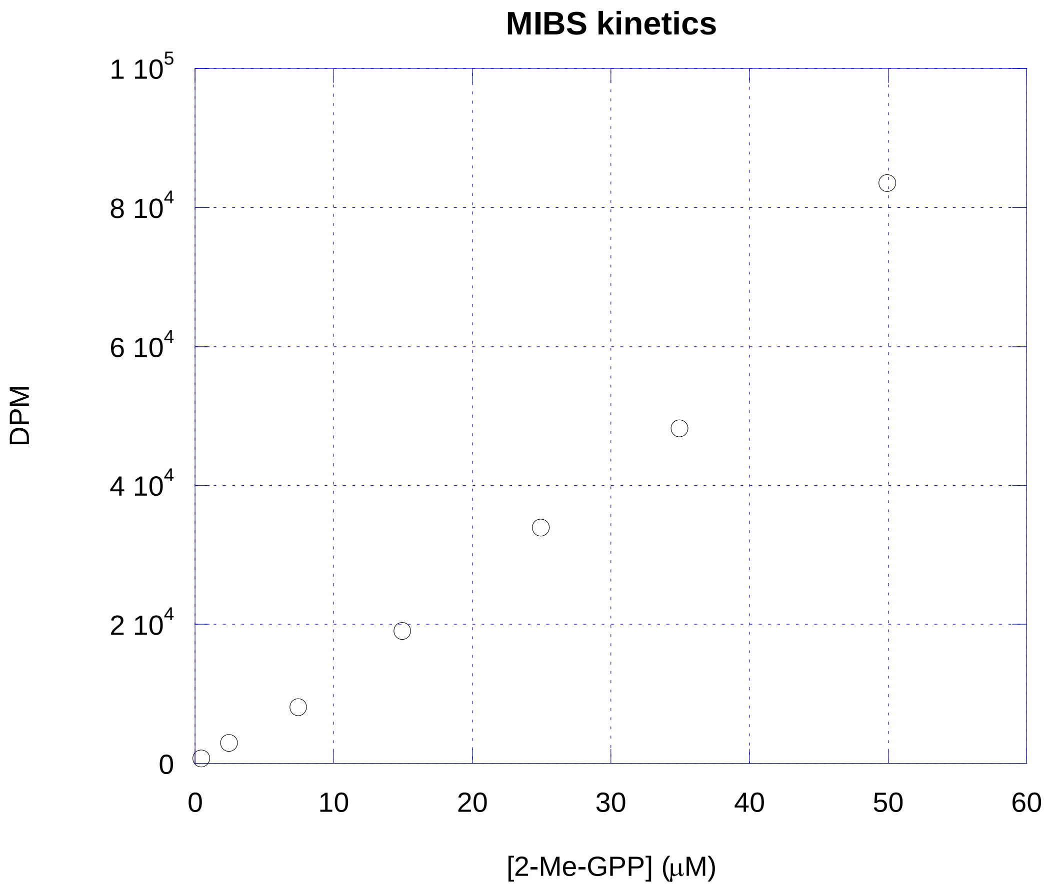

Figure S6. Plot of the reaction velocity for the MIBS-catalyzed formation of MIB (**3**) as a function of the concentration of 2-Me-GPP (**2**) at sub-saturating concentrations of **2**.

Table S1. PCR primers and combination PCRs used throughout this study

PCR primer combinations	Sequence (5’ – 3’)	Assay target	Expected product size
GPPF1 **MIBR1**	GCGCAGTGAATATCAG TATGCCATTCAAATGC	GPPMT and MIBS gene order	2057 bp
MIBF1 **GPPR1**	TGGATAATACGAGTACCAC GTCAGCAACGATGAGC	MIBS and GPPMT gene order	2078 bp
MIBF2 **MIBR2**	ATGAAAGATACCAACTTGGATAATA TTAGGCTAGTGATTGTGAATCTGGC	MIBS	1194 bp
GPPF2 **GPPR2**	GCGCAGTGAATATC TTACCGAATGATGCGGTCAGCAACG	GPPMT	832 bp
PSANROS1F **PSANDDR1**	ATGGCGGTTCTGGTCG CTGTCGTCAGCATAGTAGTCATCAG	Rossmann fold on GPPMT and DDxxx(D/E) Mg^{2+} binding domain on MIBS	1118 bp
PSANROS1F **PSANNSER1**	ATGGCGGTTCTGGTCG GGCAACCGAAAGGAGA	Rossmann fold on GPPMT and NSE triad Mg^{2+} binding domain on MIBS	1555 bp
PSAN562F **PSAN1140R**	AACGATGTTACGGGAGG ATCAAACCCGCATTCC	Spanning region between GPPMT and MIBS	704 bp

Table S2. Sequence comparison of *P. limnetica* (Castaic Lake, GenBank Accession HQ630883) MIBS with bacterial orthologs

Organism name	**GenBank Accesion Number**	**aa**	**Identity (%)**	**Positive matches (%)**
***Streptomyces* sp. AA4**	ZP_05479642	370	58	70
***Catenulispora acidiphila* DSM 44928**	YP_003115314	393	53	68
***Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111**	ZP_04336204	442	41	56
***Micromonospora* sp. ATCC 39149**	ZP_04609212	392	35	49
***Stackebrandtia nassauensis* DSM 44728**	YP_003510780	373	37	49

Table S3. Sequence comparison of *P. limnetica* (Castaic Lake, GenBank Accession HQ630882) GPPMT with bacterial orthologs

Organism name	GenBank Accesion Number	aa	Identity (%)	Positive matches (%)
***Micromonospora* sp. ATCC 39149**	ZP_04609213	289	73	84
***Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111**	ZP_04336205	286	72	84
***Saccharopolyspora erythraea* NRRL 2338**	YP_001105918	442	67	78
***Streptomyces* sp. AA4**	ZP_05479643	283	67	78
***Stackebrandtia nassauensis* DSM 44728**	YP_003510779	282	64	80
***Catenulispora acidiphila* DSM 44928**	YP_003115315	282	64	80

Table S4. Sequence comparison of *P. limnetica* (Castaic Lake, GenBank Accession HQ630884) nucleotide binding protein with bacterial orthologs

Organism name	**GenBank Accesion Number**	**aa**	**Identity (%)**	**Positive matches (%)**
Streptomyces avermitilis	NP_824183	468	60	78
***Saccharopolyspora erythraea* NRRL 2338**	YP_001106174	466	60	76
***Saccharopolyspora erythraea* NRRL 2338**	YP_001107099	466	60	77
***Frankia alni* ACN14a**	YP_716635	475	60	75
***Streptomyces coelicolor* A3(2**	NP_629395	468	58	76
***Frankia* sp. CcI3**	YP_483305	467	60	77
Streptomyces griseus	BAB20506	470	58	75

Table S5. Sequence comparison of *O. limosa* and *P. limnetica* (Lake Biwa) MIB synthases (GenBank Accession numbers HQ630885 and HQ630887 respectively) with closest related bacterial orthologs

Organism name	GenBank Accesion Number	aa	Identity (%)	Positive matches (%)
***Streptomyces* sp. AA4**	ZP_05479642	370	61	71
***Catenulispora acidiphila* DSM 44928**	YP_003115314	393	54	68
***Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111**	ZP_04336204	442	42	57
***Micromonospora* sp. ATCC 39149**	ZP_04609212	392	36	53
***Stackebrandtia nassauensis* DSM 44728**	YP_003510780	373	37	50

Table S6. Sequence comparison of of *O. limosa* and *P. limnetica* (Lake Biwa) GPPMTs (GenBank Accession numbers HQ630886 and HQ630888 respectively) with closest related bacterial orthologs.

Organism name	**Genebank Accesion Number**	**aa**	**Identity (%)**	**Positive matches (%)**
***Micromonospora* sp. ATCC 39149**	ZP_04609213	289	74	84
***Nocardiopsis dassonvillei* subsp. *dassonvillei* DSM 43111**	ZP_04336205	286	72	84
***Stackebrandtia nassauensis* DSM 44728**	YP_003510779	282	68	81
***Streptomyces* sp. AA4**	ZP_05479643	283	66	77
***Saccharopolyspora erythraea* NRRL 2338**	YP_001105918	442	65	76
***Catenulispora acidiphila* DSM 44928**	YP_003115315	282	62	77

Table S7. Sequence comparison of *P. limnetica* MIBS (Castaic Lake, GenBank Accession HQ630883) with MIB synthases cloned in this study, and closest bacterial ortholog.

Organism name	**Genebank Accesion Number**	**aa**	**Identity (%)**	**Positive matches (%)**
***Streptomyces* sp. AA4**	ZP_05479642	370	60	71
P. limnetica (Lake Biwa)	HQ630887	379	97	98
O. limosa	HQ630885	359	94	95

Table S8. Sequence comparison *P. limnetica* GPPMT (Castaic Lake, GenBank Accession HQ630882) with GPPMTs cloned in this study, and closest bacterial ortholog.

Organism name	**Genebank Accesion Number**	**aa**	**Identity (%)**	**Positive matches (%)**
***Micromonospora* sp. ATCC 39149**	ZP_04609213	289	74	84
P. limnetica (Lake Biwa)	HQ630888	257	96	99
O. limosa	HQ630886	257	96	99