

Supporting Information

Cloning and characterization of Pfl_1841, a 2-methylenebornane synthase in *Pseudomonas fluorescens* PfO-1

Wayne K. W. Chou¹, Haruo Ikeda² and David E. Cane^{1*}.

¹Department of Chemistry, Box H, Brown University, Providence, Rhode Island 02912-9108 USA. ²Laboratory of Microbial Engineering, Kitasato Institute for Life Sciences, Kitasato University, 1-15-1 Kitasato, Sagamihara, Kanagawa 252-0373, Japan.

*Address correspondence to: David_Cane@brown.edu

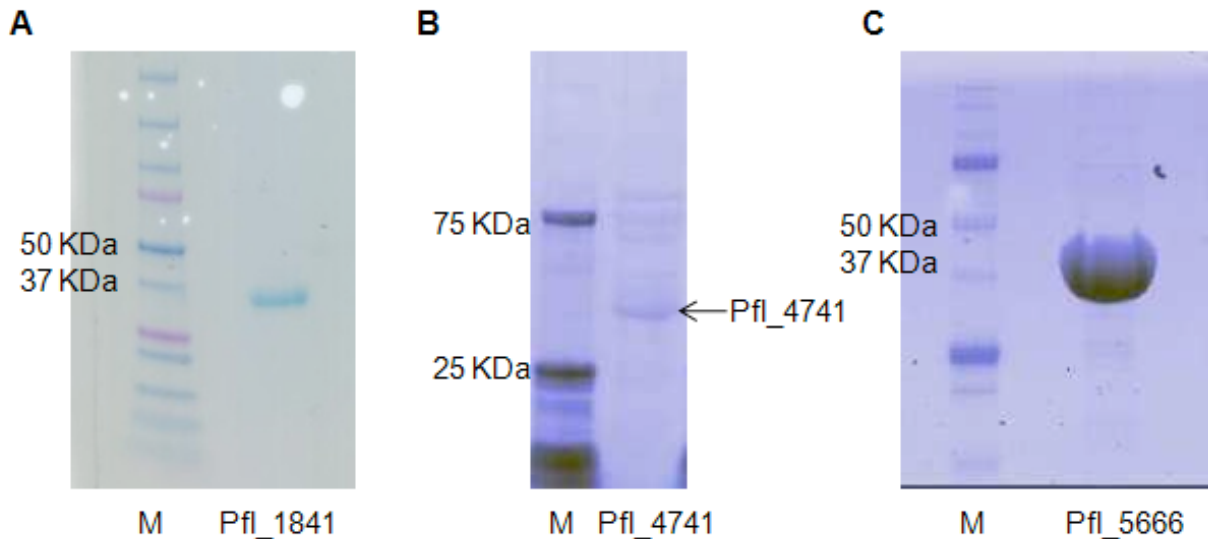


Figure S1. SDS PAGE gels of (A) purified recombinant Pfl_1841, (B), partially purified recombinant Pfl_4741, and (C) purified recombinant Pfl_5666 proteins. Lane M denotes protein MW standards.

```

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
-----MFLP 4
MPDSGTLGTPPPEQGPTPTTLPDVPAPVIPSASVTSAAASDFLAALHPPV 50

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
SLDSGSAGKLTGLAATILSDMVGAPPAELTRLTGPTLRLNGPTGLGTAAL 54
TVFDPAPPPPPAPAAGNFPDVTGDSVLQRIILRGPTGPGTTLAPAVRYG 100

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
MNQSSSARTPRSATAPFIVRAVRCPPPTRIDEALGQEVNERLMEWIS-NI 49
-----MLPPPTDDGVPELFCPGPVRDDPALGETVNDGIVEWAG-QV 40
RISPPSAPAAPPAPDRGRPELFCPGPVRDDPALGEEVNDRVVEWAE-QV 103
RQPGPEAPASAPPAAGRAVPGLYHHPVPEPDPVRVEEVSRRIKRWAEDV 150
..... : . * . : * . : . * : :

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
GIFAGKEE-KIRASDFGRYAMLCHADTNNPDRLLLVACFAALFAVDDHY 98
GIYPGQLD-RLRAYNFGRLIMLTHPATNDPDRLLAAAKCVVAEWATDDYV 89
GIYPGRLD-RLRAGNFGRLIMLAHPATSDPDRLLAATKCVVAEWAADDYM 152
QLYPEEWEGQDFGFSVGRYMGCHPDAPTVDHMLLATRLMVAENAVDDCY 200
: . . : : . . * * : * . : * * : : . * * * *

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
CDDQSLGGRPETVAESLSFALTAIDPVYLPSPFDKELLKQMCDPVIRGL 148
VDEVSLGADPAVVGSRSLAKLHAVVDPARLPARYAPQLDAYRRDEPIATAF 139
VDEVSLGADPTVVGSRSLAKLHVIVDPARLPARYAPQLDQYRREEPIATAF 202
CEDH--GGSPVGLGRRLLLAHTAIDHFHSTAEYTPTWQASLAADAPRRAY 248
: : * . * : . * . : * : : : : : : . .

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
LAYMKRVAQFCTPSQVARVRQITIAMFVTMAAEGPWRLYGTQPTVAEYLA 198
RSAMEHLARYTTVAQLGRFQHQQLGILFLAWNQEADWHVNGRTPPVWEYLV 189
RSAMQHLSRYVSVPLARFQHQMSILFVAWNQEADWHVNRRTPPVWEYLV 252
DSAMGYFVRAATPSQSDRYRHDMARLHLGYLEAGAWAQTGHVPEVWEYLA 298
: * . : : . * * : : : : * . * * * * *

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
SRQVNSFWPCLVLIDLIGGYEVANTYSRDPDIHHVTALASLATTLVNDLY 248
QRHLNLFPPMVLVDVAVAGYELSPDEFDPVRRRAYTTAALANVLLNDIH 239
QRHLNSYLPPMILVDVAVAGYELSPDEFHPLVRRRAFTAGLAAVLLNDIY 302
MRQFNNFRPCPTITDVTGGYELPADLHARPDQRVIALAGNATTIVNDLY 348
* . * : * : * * * : : . * : : : : * . * : : * * :

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
SAYKEHLNETGDFKLPYLLAARHNCSLQEAIDLAAIDHAVMEEYERLHA 298
SGTCESD---TDFNLPRVISIEEGCSLRDAVTRTVEIHNELMHAFVADAA 286
SGAQESD---TDFNLPRVIAAEHNCALEEAITRTVEIHNELMHEFVADAV 349
SYTKELNSPGRHLNLPVVAEREQLCERDAYLKAVEVHNELQHSFEAAAA 398
* * : : * * : : . . . * : : * : : .

Q3KF72|Q3KF72_PSEPF
MOL|M.olivasterospora_KY11048
ZP_04609212.1|Micromonospora_s
Q9F1Y6|MIBS_STRCO
TLMKGTSPVLRRLYLTGLSTWIGGNLEWHKHSARYHI----- 335
TSLSL-IGSPNLRFLADIWAWLGGREWHATTSTRYHGEATTRS- 328
TSLSL-TGSPMLRRFLADTWAWLGGREWHATTARYHKQQGADPA 392
DLAEACPLPPVLRFLRGVAWVDGNHDFHRTNTYRYSLPDFW-- 440
* * : * * . : * * . * * : :

```

Figure S2. Clustal 2.1 multiple sequence alignment of *Pseudomonas fluorescens* PfO-1 Pfl01_1841 (Uniprot ID Q3KF72) with 2-MB and 2-MIB synthases (EBI Clustal W Server, <http://www.ebi.ac.uk/Tools/clustalw2/index.html>). MOL, *Micromonospora olivasterospora* KY11048, draft sequence (2-MB synthase); ZP_04509212, *Micromonospora* sp. ATCC39149 (putative 2-MB synthase); Q9F1Y6, *S. coelicolor* A3(2) (SCO7700, MIB synthase).

```

Q3K6X6|Q3K6X6_PSEPF          MKSTSVSAKASLFSPHGLSGSLLRRGVLRLQLEQLKQQLLIVIEDGERHVF 50
Q3K4A2|Q3K4A2_PSEPF          -----MLAQLPFPALQNLQLPLRLRLWDGHEFNL 28
MGPPT|M.olivasterospora_KY1104 -----
ZP_04609213.1|Micromonospora_s -----
Q9F1Y5|GPPMT_STRCO           -----

Q3K6X6|Q3K6X6_PSEPF          GTPGSALVGEIQVLDPAVWGLVAGNGSIGAGEAFIHGYWRSPLDTAVVRV 100
Q3K4A2|Q3K4A2_PSEPF          G---PTPSVTIVVKDPLMVSQ LTHPSLDALGA AFVEGKLELEGSISEVIR 75
MGPPT|M.olivasterospora_KY1104 -----MQTSTIGKPVLRTPYQRSVAAYWNTNQNDPVLNK 34
ZP_04609213.1|Micromonospora_s -----MTVSTTPTAGSPVLR TQYQKSVAAYWNTNQDPVNL R 37
Q9F1Y5|GPPMT_STRCO           -----MTTETTTATATAKIPAPATPYQEDIARYWN-NEARPVNL R 39
                                     : . .

Q3K6X6|Q3K6X6_PSEPF          FVSNLEVL DAMEGGLARLRPLVRGLHWNLRNTRKGSQKNIAAHYDLGND 150
Q3K4A2|Q3K4A2_PSEPF          VCDELSSALLDEDDDSQFVR-----TLHDKETDAKAI SYHYDLSNA 116
MGPPT|M.olivasterospora_KY1104 LG-----EIDGIYHHHYGVGDY 51
ZP_04609213.1|Micromonospora_s LG-----EIDGLYHHHYGIGNY 54
Q9F1Y5|GPPMT_STRCO           LG-----DVDGLYHHHYGIGPV 56
                                     . **.:.

Q3K6X6|Q3K6X6_PSEPF          LFEQFLDPTMMYSAAQFLSPDSDL EQAQLNKLERICQK LALKPGDHLL EI 200
Q3K4A2|Q3K4A2_PSEPF          FYQLWL DSDMAYSCAYFETGSESL EQAQAKFRHLCKRKLRLQPGDYLLDV 166
MGPPT|M.olivasterospora_KY1104 DPAVL DGPKE TR- DERVIAELHRLETAQANVLLDHLG--DIAPTDRLLDA 98
ZP_04609213.1|Micromonospora_s DPSVL AGPEQTR- DERIIAELHRLETAQADMLLSYL G--EIGPSDRLLDA 101
Q9F1Y5|GPPMT_STRCO           DRAALGDPEHSEYEKKVIAELHRLESAQA EFLMDHLG--QAGPDDTLVDA 104
                                     . . : . * * * * : * * * : :

Q3K6X6|Q3K6X6_PSEPF          GTGWGSMALYAAQHYGCKVTTTLSKEQFAFTAARIERLGLQDV TLLLK 250
Q3K4A2|Q3K4A2_PSEPF          GCGWGGLARYAAREFGAKVFGITLSKEQLALARERVKAEGL EDQIELQLL 216
MGPPT|M.olivasterospora_KY1104 GSGRGGTSLMAHQRF GCHVDGVSISEYQVGFANEQA AVHGVADQVAFHFR 148
ZP_04609213.1|Micromonospora_s GSGRGGT SFMANQRFGCQVDGVSISEYQVDFANRQATDRRVADQVRFHFR 151
Q9F1Y5|GPPMT_STRCO           GCGRGGSMVMMAHRRFGRVGVTL SAAQADFGNRRARELRIDHDHVRSRVC 154
* * * . * : : * * * : : : * : .

Q3K6X6|Q3K6X6_PSEPF          DYRDLT--GQYDKLVS IEMIEAVGHRFLPTYFKQCAQLLKS NGLMLLQAI 298
Q3K4A2|Q3K4A2_PSEPF          DYRDL PQDGRFDKVVSVGMFEHVGHANLAEYCKT LFGAVKEGGLVMNHGI 266
MGPPT|M.olivasterospora_KY1104 NMLDTG--FDNGRYQAVW TNETTMYVDLFELYGEFARLLPFGGRYVCITG 196
ZP_04609213.1|Micromonospora_s NMLDTG--FEAGSRQAVW TNETTMYVDLFELYAEFARLLPFGGRYVCITG 199
Q9F1Y5|GPPMT_STRCO           NMLDTP--FDKGAVTASW NNESTMYVDLHDLFSEHSRFLKVGGRYV TITG 202
: * . : * . : * : . * :

Q3K6X6|Q3K6X6_PSEPF          TIREQRYEQARRGV-DFIQRYIFPGGALPCVQKMLEIVGRD TDMNLLHME 347
Q3K4A2|Q3K4A2_PSEPF          TAKHTDGRPVGRGAGDFIEKYVFPNGELPHLAMISAEIS -EAGLEIVDVE 315
MGPPT|M.olivasterospora_KY1104 CANDVTGG-RSKAVSRIDEHYTCN-----IHRRSQYFAAL AANRLAPIN 239
ZP_04609213.1|Micromonospora_s CYN DVTGG-RSRAVSQIDEHYTCN-----IHPRSA YFKAL AANRFVPIH 242
Q9F1Y5|GPPMT_STRCO           CWNPRYQ-PSKWVSQINAHFECN-----IHSRREYLRAMADNR LVPHT 245
. . : . : : . : . :

Q3K6X6|Q3K6X6_PSEPF          DFGLHYARTLRLWHENFRHAHGRLTELG YDDYFLRLW EFLCYCEGGFLE 397
Q3K4A2|Q3K4A2_PSEPF          SRLRLHYARTLDHWSERLEDNLEAAAKL-VPDQALRIWR LYLACAYAFAR 364
MGPPT|M.olivasterospora_KY1104 VVDLT-AATIPYWELRAKSSVATGIEE-----AFLTAYREGSFHY 278
ZP_04609213.1|Micromonospora_s VIDLT-SATIPYWELRAQSSVATGIED-----PFLTAYREGSFHY 281
Q9F1Y5|GPPMT_STRCO           IVDLT-PDTLPYWELRATSSSLVTGIEK-----AFIESYR DGSFQY 284
. * . * : * . : : . *

Q3K6X6|Q3K6X6_PSEPF          RTIGTAQLVLAKPAAMTEPLLGRFDA----- 423
Q3K4A2|Q3K4A2_PSEPF          GWINLHQILAVKAHADGSHEL PWTRDDIYNP 395
MGPPT|M.olivasterospora_KY1104 LLIVADRI----- 286
ZP_04609213.1|Micromonospora_s LLIAADRV----- 289
Q9F1Y5|GPPMT_STRCO           VLIAADRV----- 292
* : :

```

Figure S3. Clustal 2.1 multiple sequence alignment of *Pseudomonas fluorescens* PfO-1 Pfl01_4741 and Pfl01_5666 proteins (Uniprot ID Q3K6X6 and Q3K4A2, respectively) with 2-MGPP synthase (EBI Clustal W Server, <http://www.ebi.ac.uk/Tools/clustalw2/index.html>). MGPPT, *Micromonospora olivasterospora* KY11048, draft sequence; ZP_04609213, *Micromonospora* sp. ATCC39149; Q9F1Y5, *S. coelicolor* A3(2).

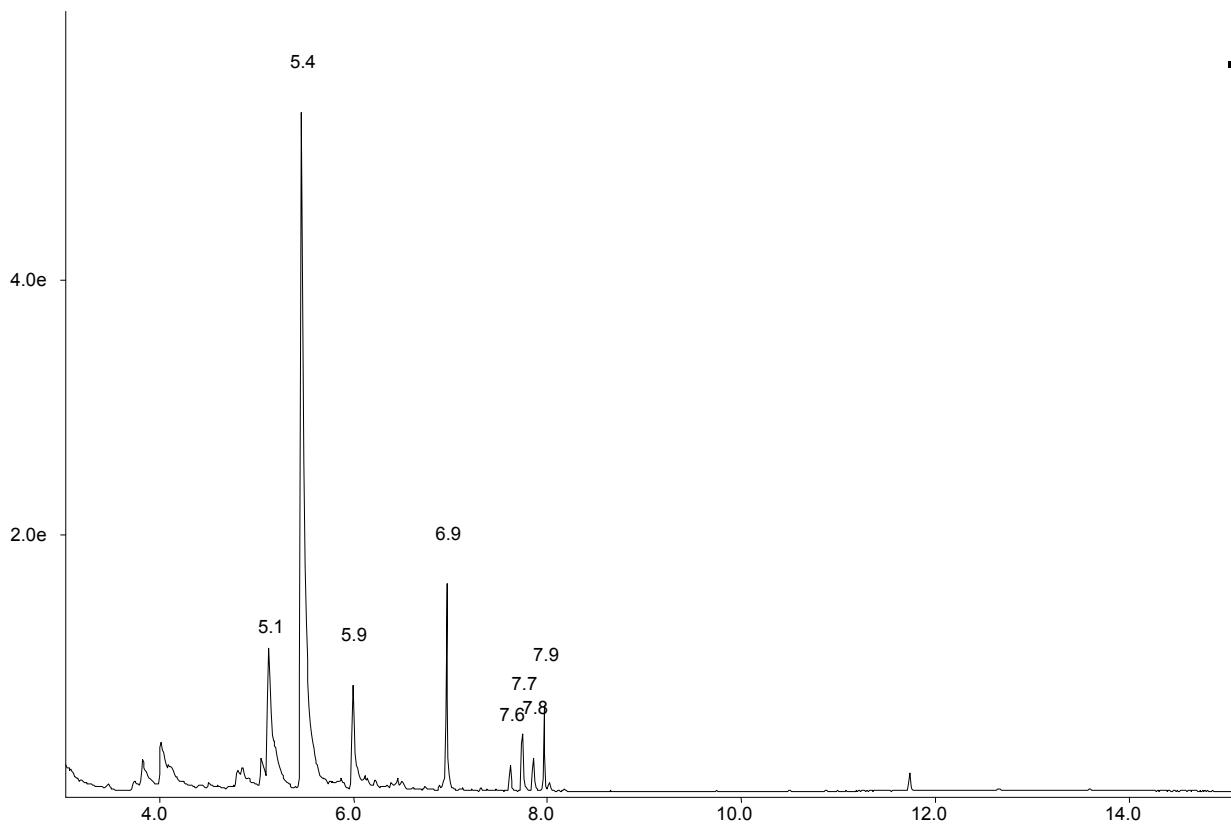


Figure S4. GC-MS trace of pentane extracts from the incubation of 2-MGPP with Pfl_1841.

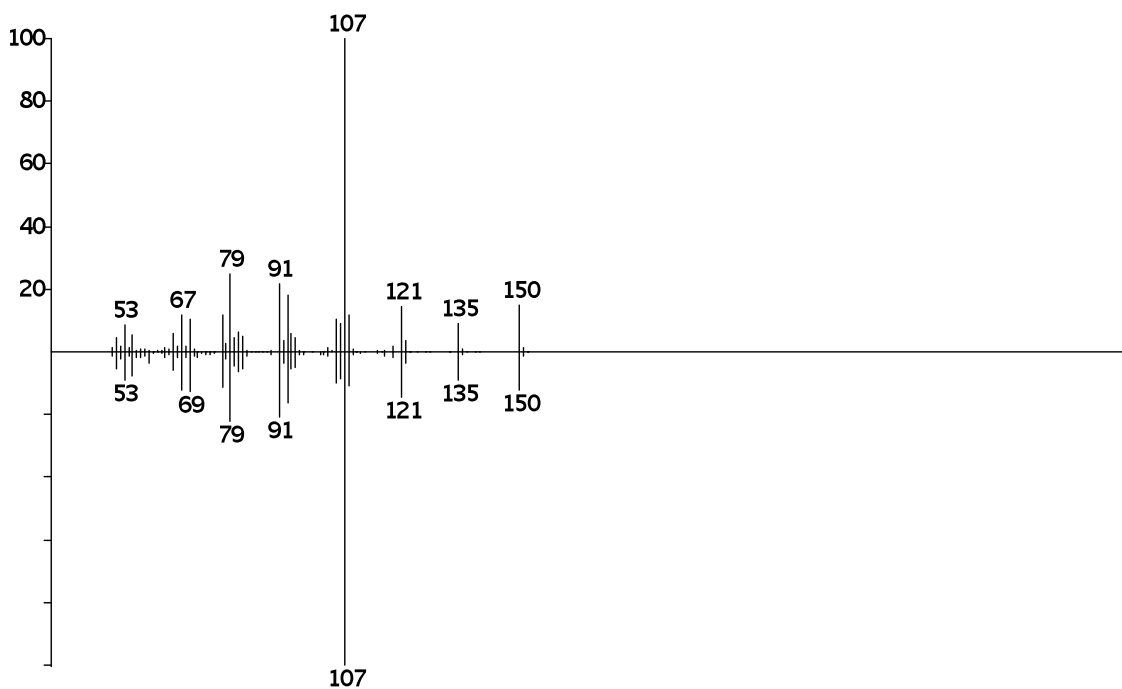


Figure S5. Mass spectrum of 1-methylcamphene produced from the incubation of 2-MGPP with Pfl_1841 (peak at 5.14 min, lower mass spectrum) and from the acid catalyzed rearrangement of 2-MIB (upper mass spectrum).

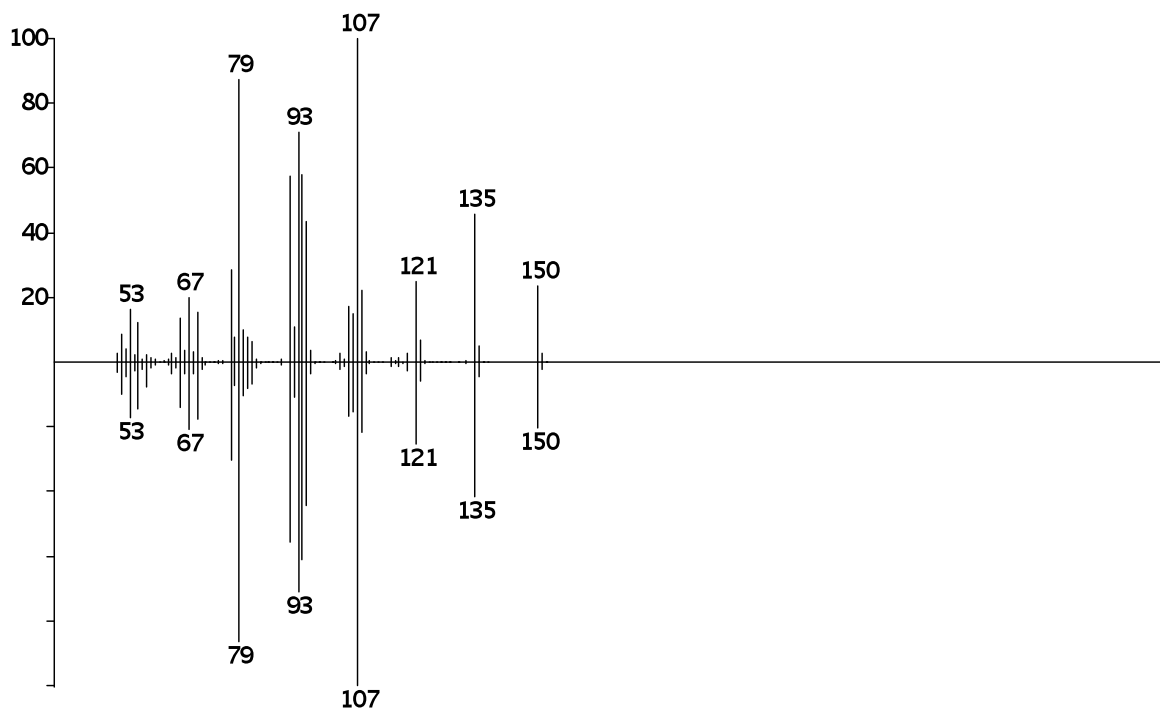


Figure S6. Mass spectrum of 2-methylenebornane produced from the incubation of 2-MGPP with Pfl_1841 (peak at 5.47 min, lower mass spectrum) and from the acid-catalyzed rearrangement of 2-MIB (upper mass spectrum).

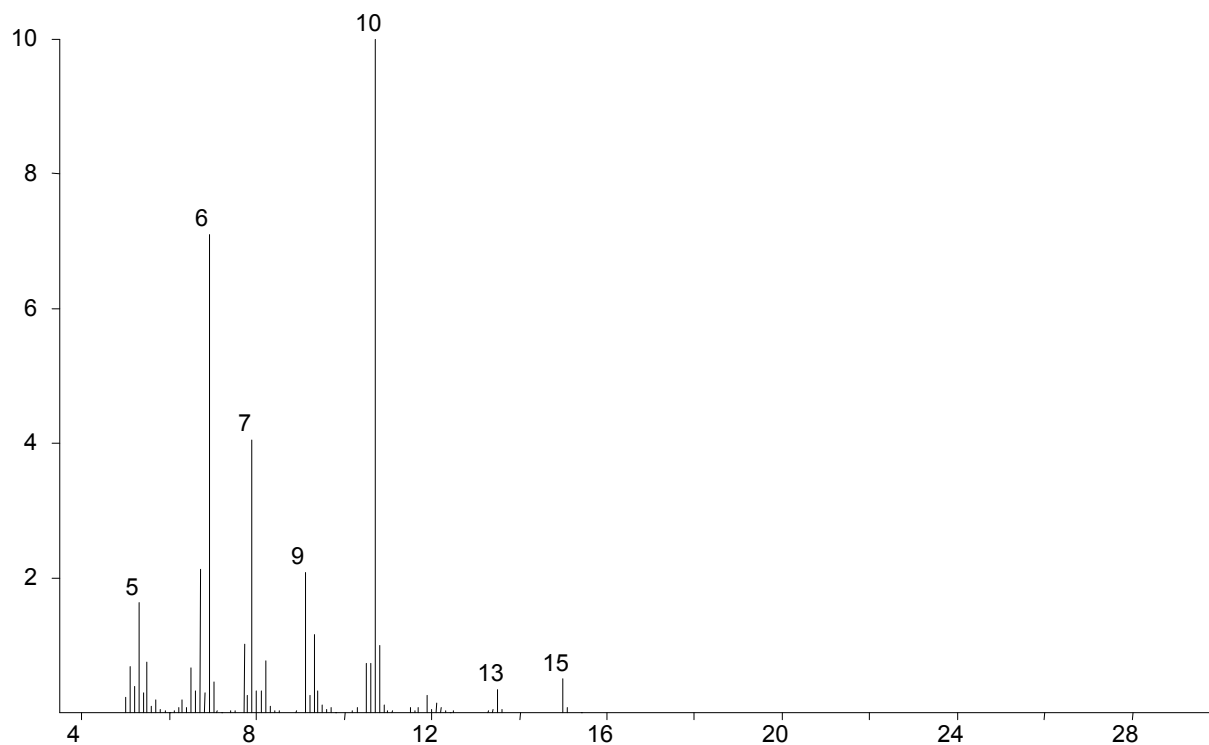


Figure S7. Mass spectrum of unknown peak at 5.99 min produced from the incubation of 2-MGPP with Pfl_1841.

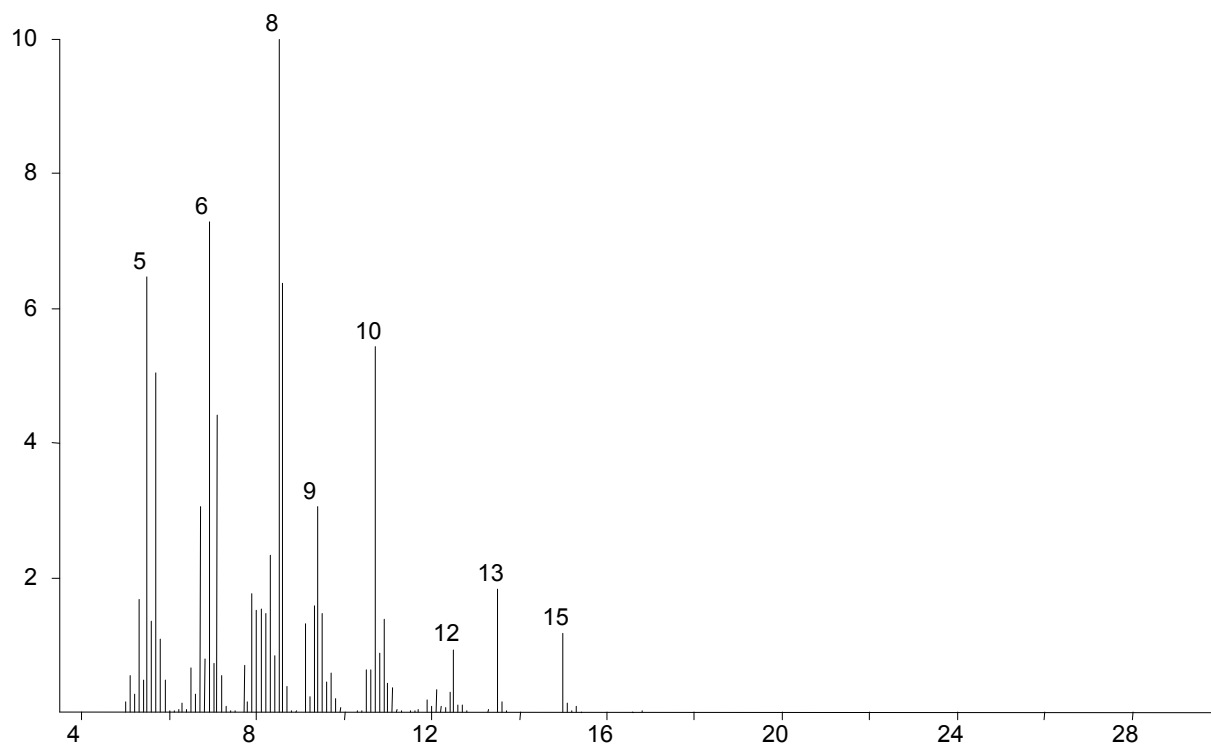


Figure S8. Mass spectrum of unknown peak at 6.96 min produced from the incubation of 2-MGPP with Pfl_1841.

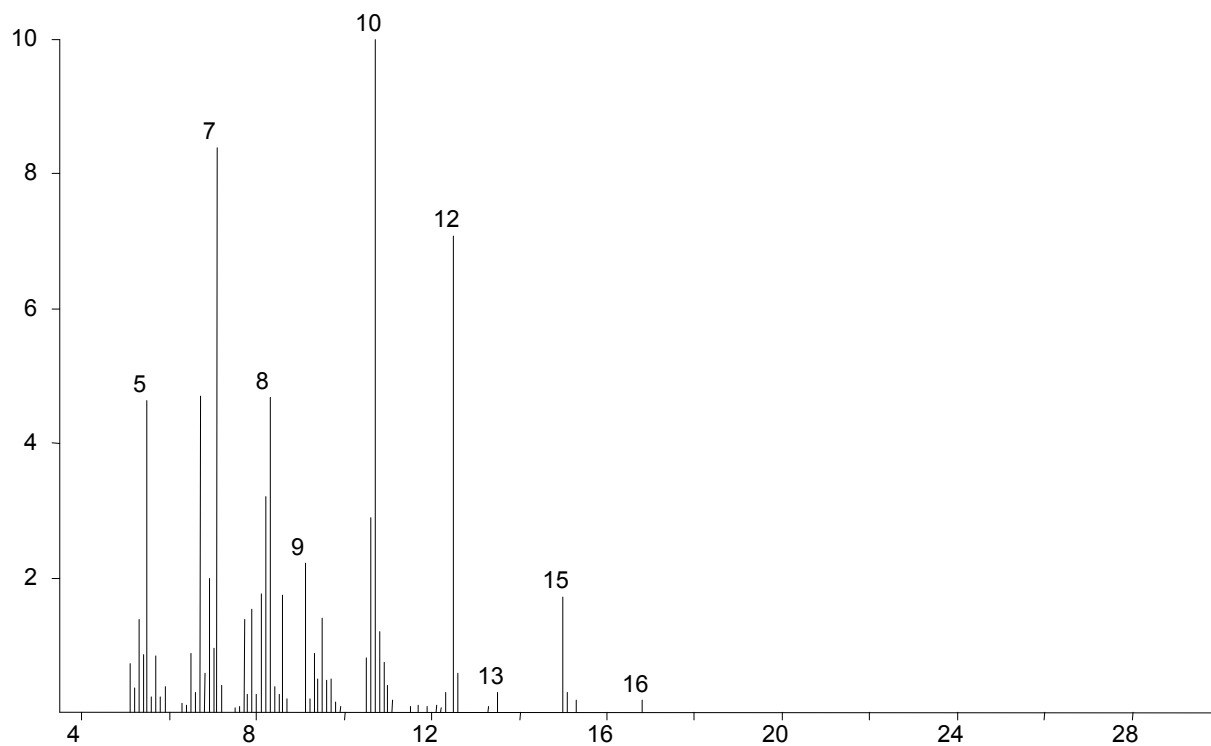


Figure S9. Mass spectrum of unknown peak at 7.01 min produced from the incubation of 2-MGPP with Pfl_1841.

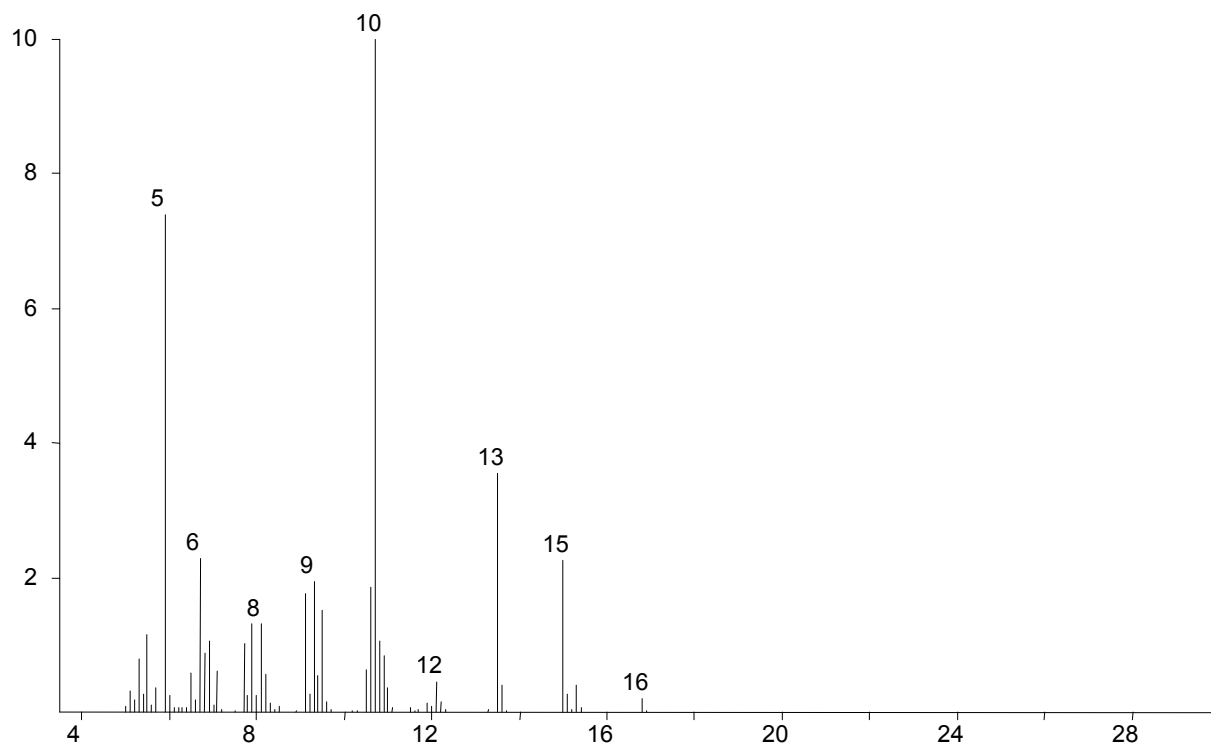


Figure S10. Mass spectrum of unknown peak at 7.75 min produced from the incubation of 2-MGPP with Pfl_1841.

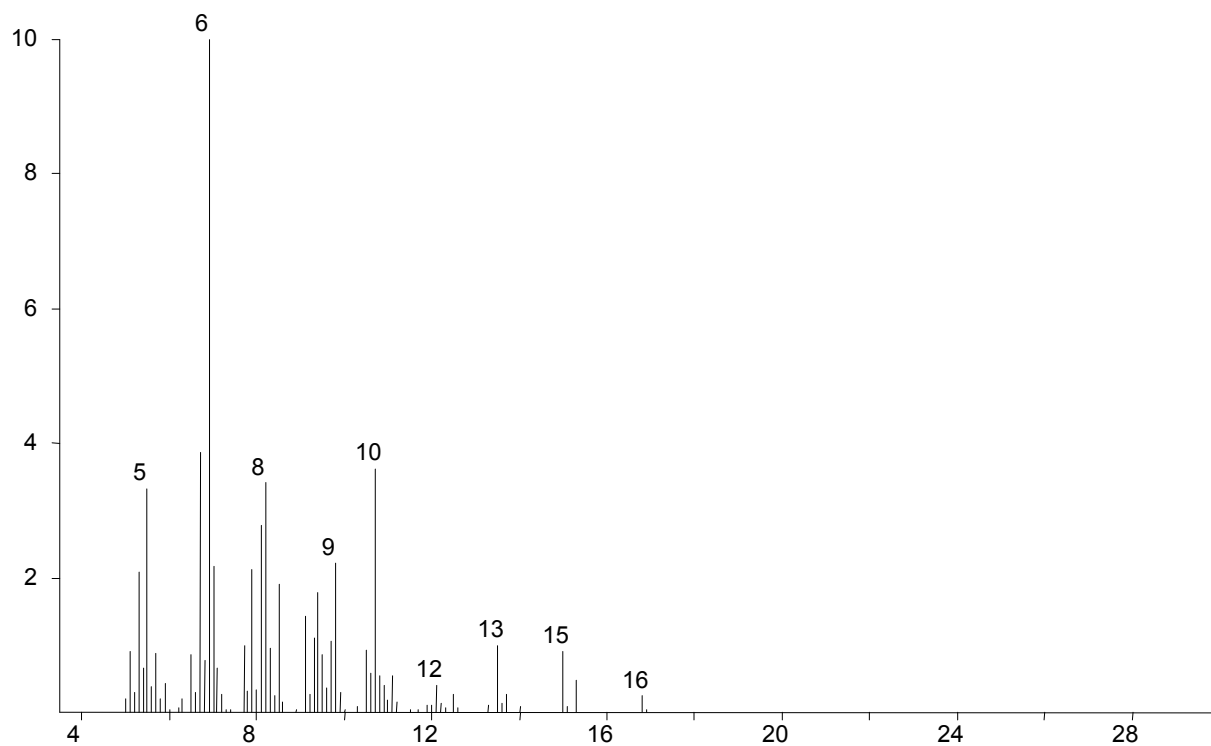


Figure S11. Mass spectrum of unknown peak at 7.86 min produced from the incubation of 2-MGPP with Pfl_1841.

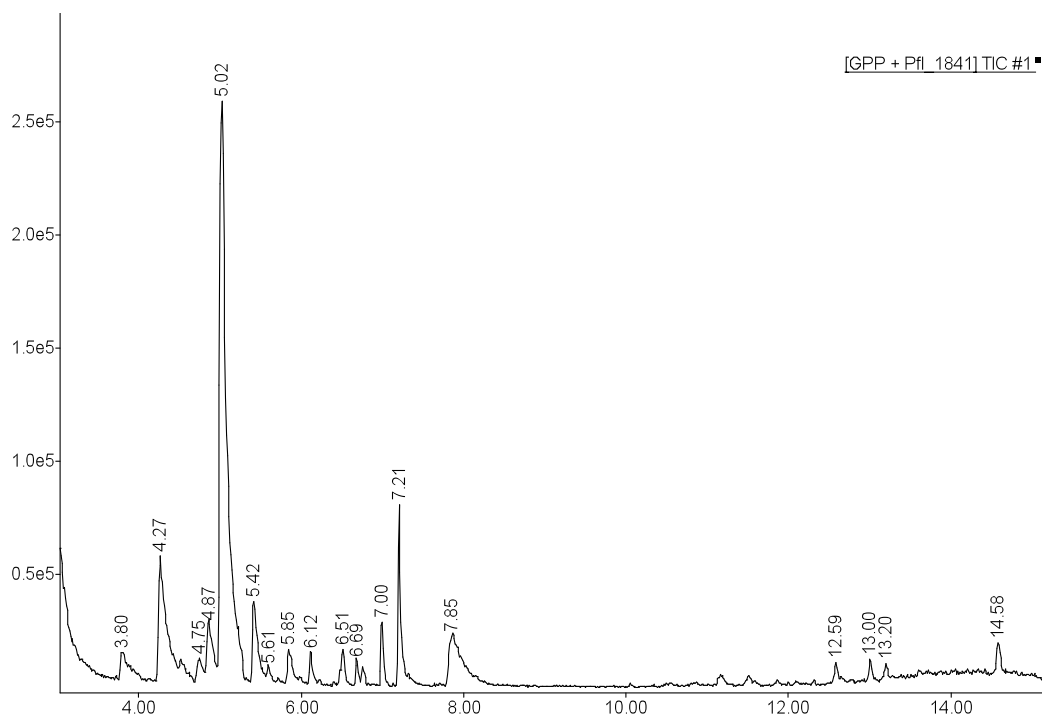


Figure S12. Pentane extracts from the incubation of Pfl_1841 with GPP. Identified peaks are tricyclene (4.75 min.), α -pinene (4.87 min.), α -fenchene (5.02 min.), α -phellandrene (5.61 min.), β -phellandrene (5.85 min.), γ -terpinene (6.12 min.), terpinen-4-ol (7.21 min.) and geraniol (7.85 min.).

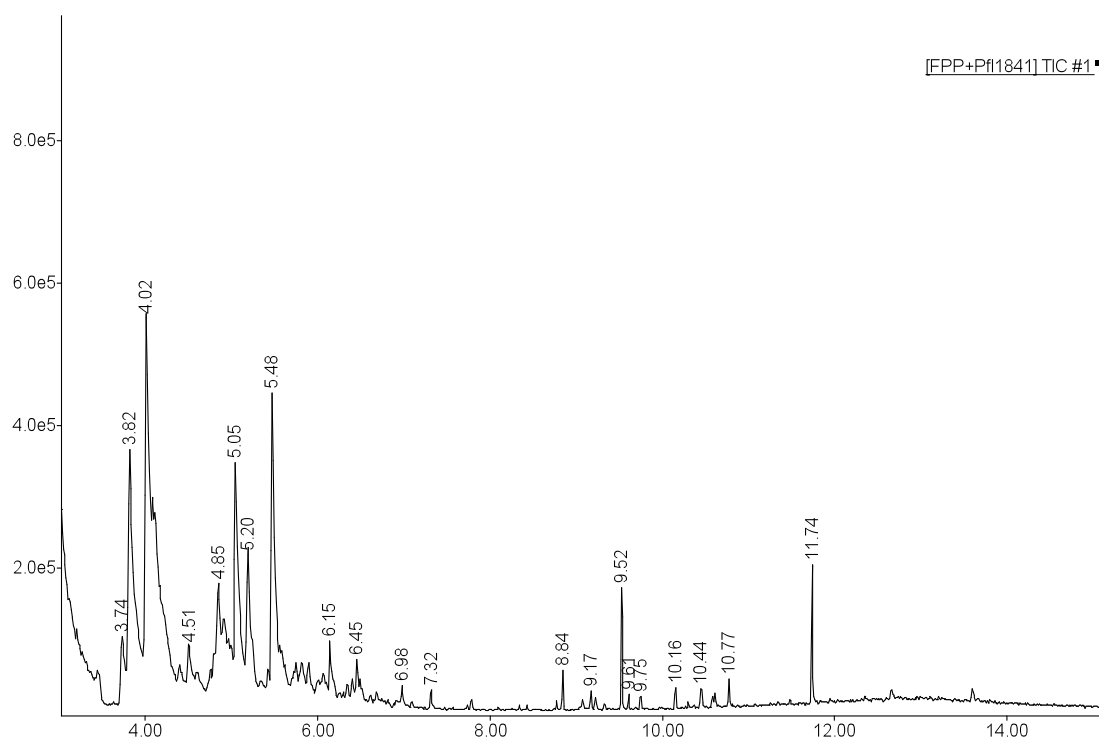


Figure S13. GCMS trace of pentane extracts from the incubation of Pfl_1841 and FPP. Identified sesquiterpene peaks are *E*- β -farnesene (8.84 min.), *E,E*- α -farnesene (9.17 min.), *E*-nerolidol (9.52 min.) and *E,E*-farnesol (10.44 min.).

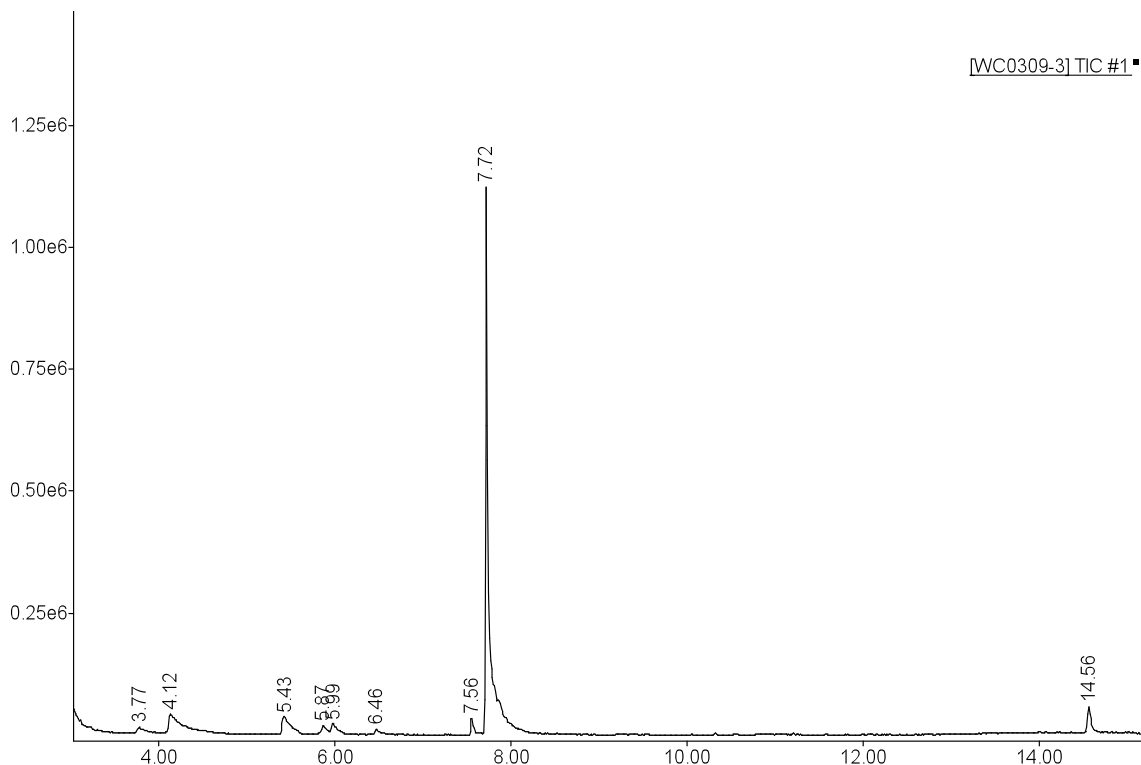


Figure S14. GCMS trace of pentane extracts from the incubation of SAM and GPP with Pfl_4741 followed by treatment with phosphatase/apyrase. The peak at 7.72 minutes corresponds to geraniol.

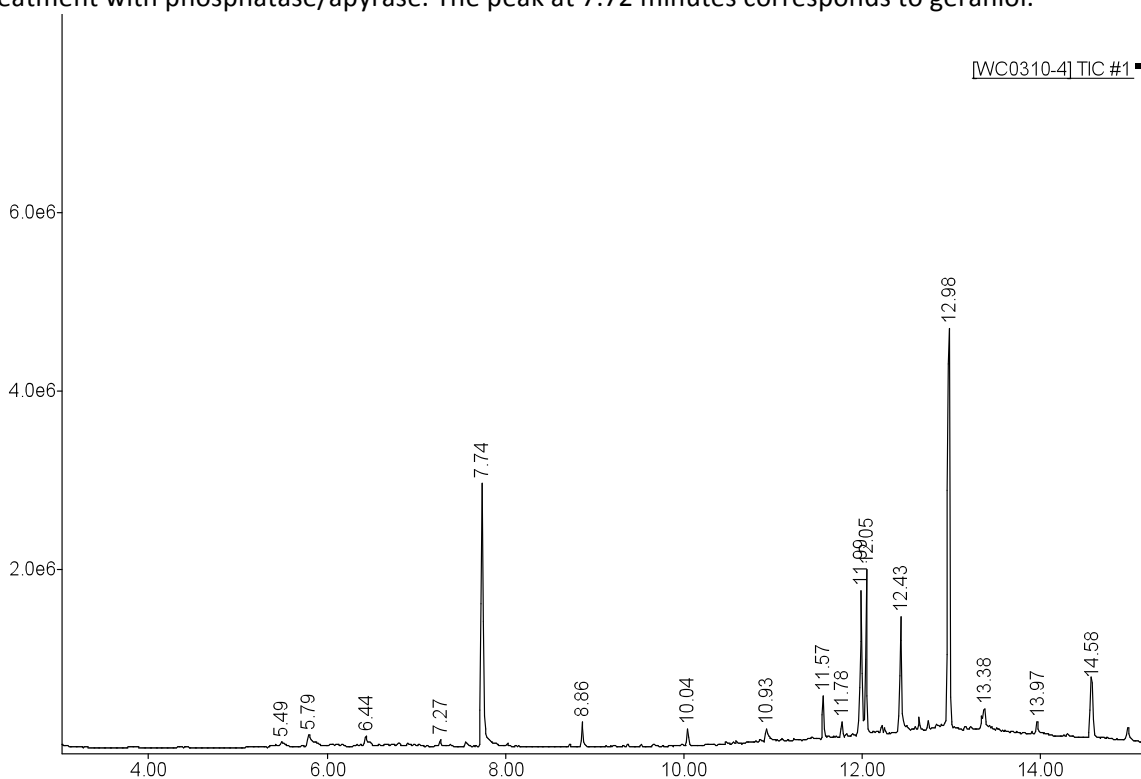


Figure S15. GCMS trace of pentane extracts from the incubation of SAM and GPP with Pfl_5666 followed by treatment with phosphatase/apyrase. The peak at 7.74 minutes corresponds to geraniol.

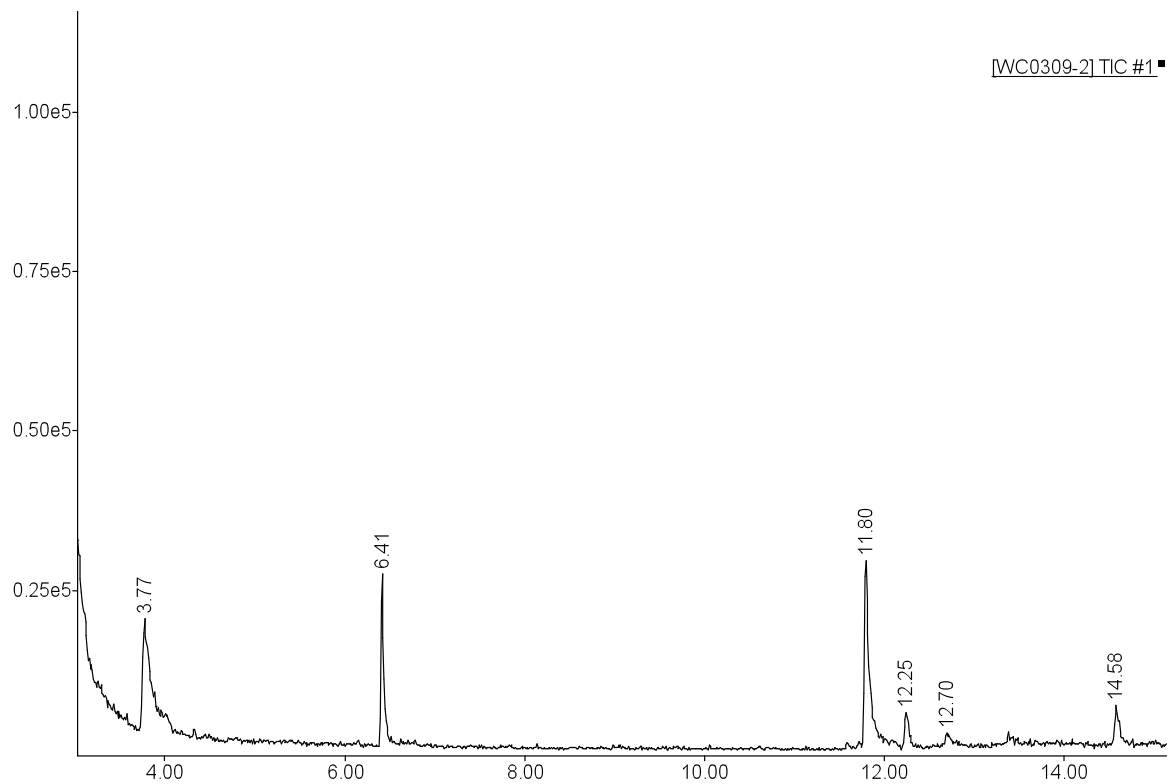


Figure S16. GCMS trace of pentane extracts from *Pseudomonas fluorescens* PFO-1 grown on SFM agar plates for 4 days. None of the GC-MS traces from the seven days of growth revealed terpene products and all were similar to this representative trace.'

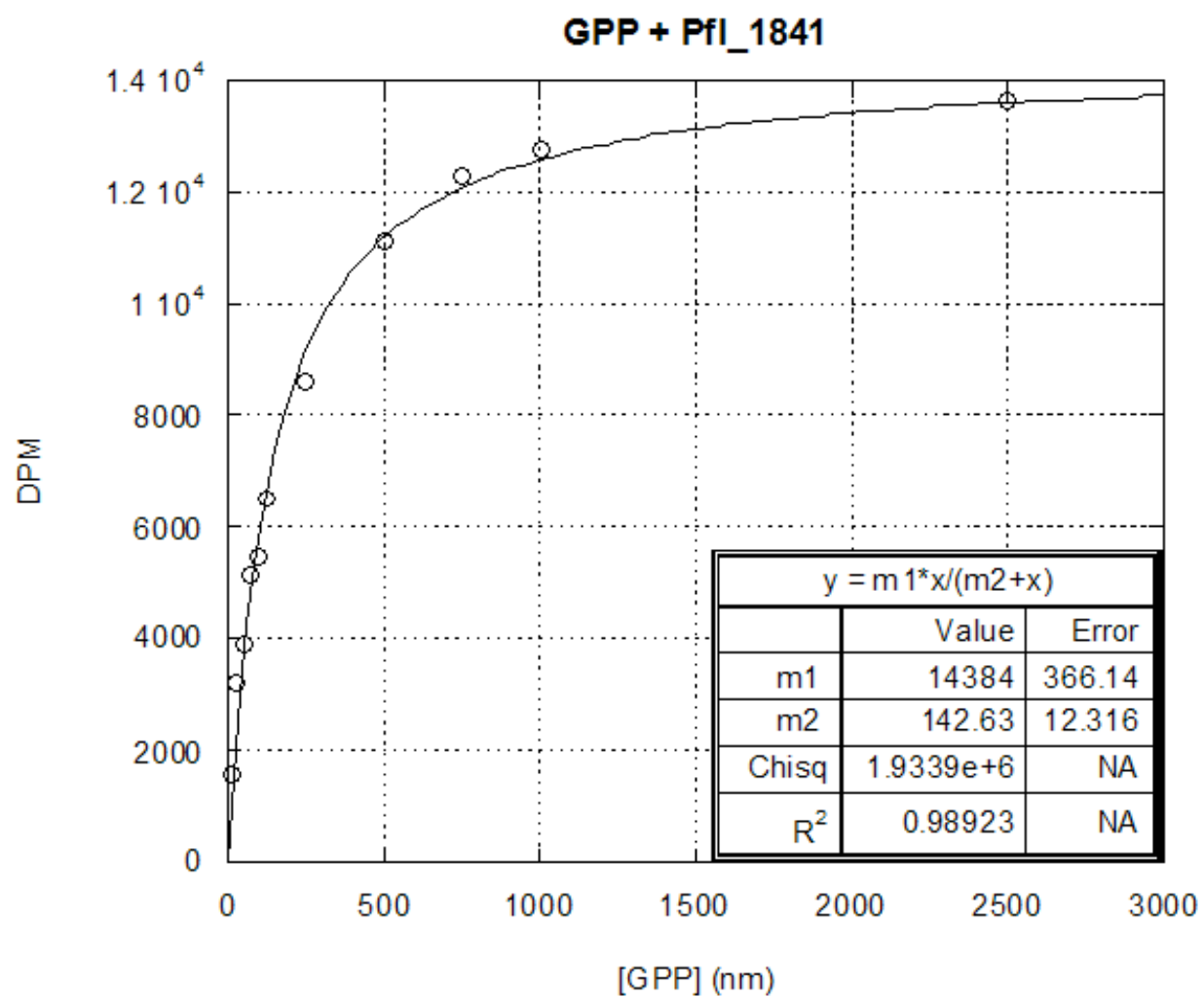


Figure S17. Michaelis-Menten plot of the reaction velocity for the Pfl_1841-catalyzed reaction as a function of the concentration of GPP.

2-methyllimonene/1

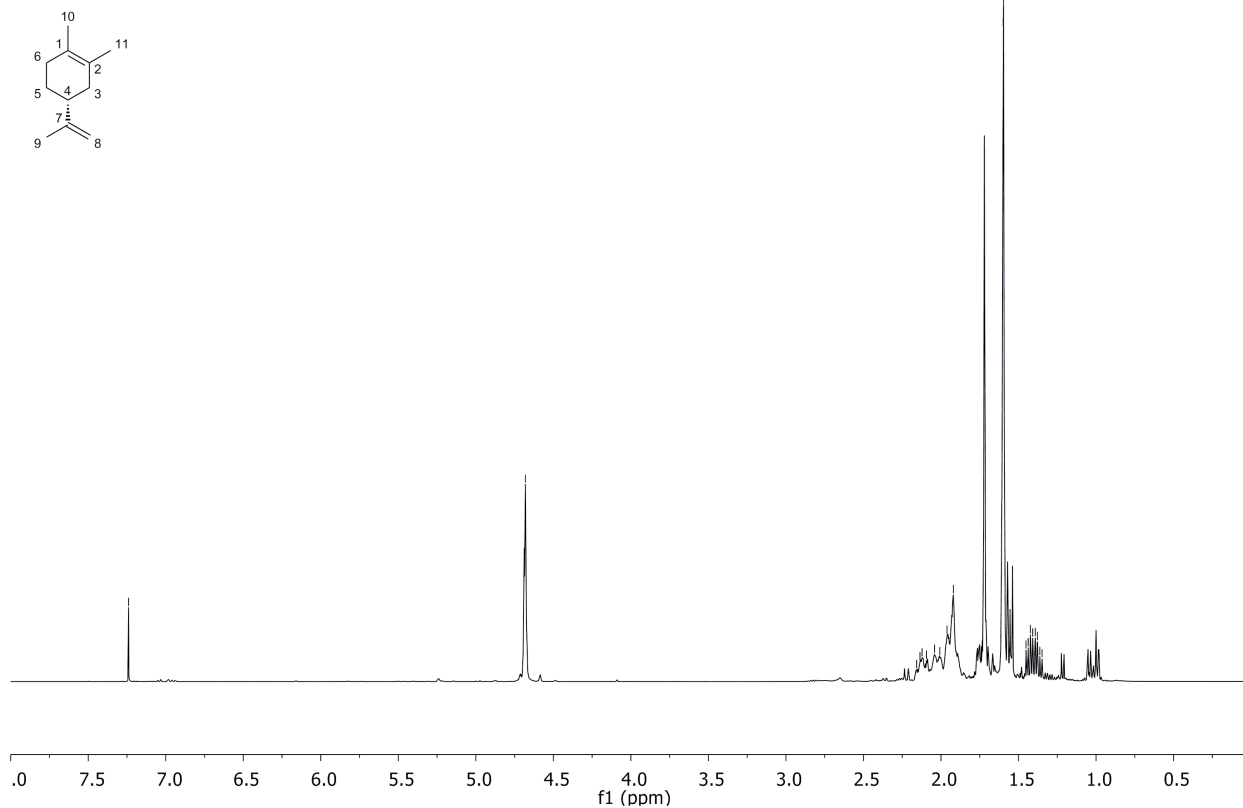
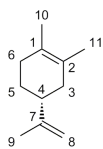


Figure S18 ¹H NMR (400 MHz, CDCl₃) of 2-methyllimonene.

2-methyllimonene/2



— 150.36

— 125.28
— 125.10

— 108.24

— 41.98
— 37.18
— 32.25
— 28.24

— 20.80
— 19.13
— 18.84

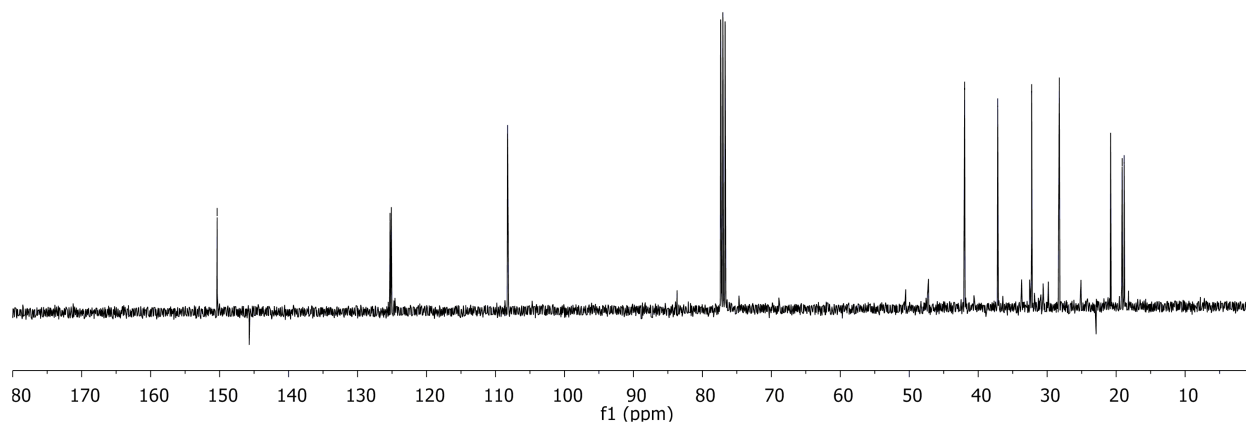


Figure S19 ¹³C NMR (100 MHz, CDCl₃) of 2-methyllimonene.