

Supplementary materials

Table S1. Strains and plasmids used in this study.

Strains or plasmids	Descriptions	Source
Strains		
BS1101	MG1655 with RecE and RecF pathways associated genes removed and with endA encoding endonuclease removed	(Bao et al., 2019)
ZMJ01	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411	This study
ZMJ02	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411-2	This study
ZMJ03	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(19)	This study
ZMJ04	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(24)	This study
ZMJ05	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(37)	This study
ZMJ06	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(38)	This study
ZMJ07	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(39)	This study
ZMJ08	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(40)	This study
ZMJ09	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(41)	This study
ZMJ10	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(42)	This study
ZMJ11	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(43)	This study
ZMJ12	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(44)	This study
ZMJ13	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(45)	This study
ZMJ14	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(46)	This study
ZMJ15	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(47)	This study
ZMJ16	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(48)	This study
ZMJ17	BS1101 harboring pAC-6409mva,3933-agGPPS-tri1411(49)	This study
ZMJ18	BS1101 harboring pAC-6409mva,3933-agGPPS-3950(44)	This study
ZMJ19	BS1101 harboring pAC-6409mva,3933-agGPPS-4091(44)	This study
ZMJ20	BS1101 harboring pAC-6409mva,3933-agGPPS-4926(44)	This study
ZMJ21	BS1101 harboring pAC-6409mva,3933-agGPPS-5066(44)	This study
ZMJ22	BS1101 harboring pAC-6409mva,3933-agGPPS-6118(44)	This study
ZMJ23	BS1101 harboring pAC-6409mva,3933-agGPPS-8401(44)	This study
ZMJ24	BS1101 harboring pAC-6409mva,3933-agGPPS-8653(44)	This study
ZMJ25	BS1101 harboring pAC-6409mva,3933-agGPPS-9309(44)	This study
ZMJ26	BS1101 harboring pAC-6409mva,3933-agGPPS-9982(44)	This study
ZMJ27	BS1101 harboring pAC-6409mva,3933-agGPPS-5307(44)	This study
ZMJ28	BS1101 harboring pAC-6409mva,3933-agGPPS-1430(44)	This study
ZMJ29	BS1101 harboring pAC-6409mva,3933-agGPPS-1335(44)	This study
ZMJ30	BS1101 harboring pAC-6409mva,3933-agGPPS-1607(44)	This study
ZMJ31	BS1101 harboring pAC-6409mva,3933-agGPPS-3780(44)	This study
ZMJ32	BS1101 harboring pAC-6409mva,3933-agGPPS-7423(44)	This study
ZMJ33	BL21(DE3) harboring pET21-tri1411	This study
ZMJ34	BL21(DE3) harboring pET21-tri1411(44)	This study
Plasmids		
pAC-6409mva	P _{lacUV5} ::AtoB,HMGS,HMGR;P _{trc} ::MK,PMK,PMD,idi;P15a;Cm ^r	(Bao et al., 2021)
3933-agGPPS-tri1411	P _{trc} ::AgGPPS,1411;ColE1;Amp ^r	This study
3933-agGPPS-tri1411-2	P _{trc} ::AgGPPS,1411-2;ColE1;Amp ^r	This study

3933-agGPPS-tri1411(19)	P _{trc} ::AgGPPS,1411(19);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(24)	P _{trc} ::AgGPPS,1411(24);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(37)	P _{trc} ::AgGPPS,1411(37);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(38)	P _{trc} ::AgGPPS,1411(38);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(39)	P _{trc} ::AgGPPS,1411(39);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(40)	P _{trc} ::AgGPPS,1411(40);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(41)	P _{trc} ::AgGPPS,1411(41);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(42)	P _{trc} ::AgGPPS,1411(42);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(43)	P _{trc} ::AgGPPS,1411(43);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(44)	P _{trc} ::AgGPPS,1411(44);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(45)	P _{trc} ::AgGPPS,1411(45);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(46)	P _{trc} ::AgGPPS,1411(46);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(47)	P _{trc} ::AgGPPS,1411(47);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(48)	P _{trc} ::AgGPPS,1411(48);ColE1;Amp ^r	This study
3933-agGPPS-tri1411(49)	P _{trc} ::AgGPPS,1411(49);ColE1;Amp ^r	This study
3933-agGPPS-3950(44)	P _{trc} ::AgGPPS,3950(44);ColE1;Amp ^r	This study
3933-agGPPS-4091(44)	P _{trc} ::AgGPPS,4091(44);ColE1;Amp ^r	This study
3933-agGPPS-4926(44)	P _{trc} ::AgGPPS,4926(44);ColE1;Amp ^r	This study
3933-agGPPS-5066(44)	P _{trc} ::AgGPPS,5066(44);ColE1;Amp ^r	This study
3933-agGPPS-6118(44)	P _{trc} ::AgGPPS,6118(44);ColE1;Amp ^r	This study
3933-agGPPS-8401(44)	P _{trc} ::AgGPPS,8401(44);ColE1;Amp ^r	This study
3933-agGPPS-8653(44)	P _{trc} ::AgGPPS,8653(44);ColE1;Amp ^r	This study
3933-agGPPS-9309(44)	P _{trc} ::AgGPPS,9309(44);ColE1;Amp ^r	This study
3933-agGPPS-9982(44)	P _{trc} ::AgGPPS,9982(44);ColE1;Amp ^r	This study
3933-agGPPS-5307(44)	P _{trc} ::AgGPPS,5307(44);ColE1;Amp ^r	This study
3933-agGPPS-1430(44)	P _{trc} ::AgGPPS,1430(44);ColE1;Amp ^r	This study
3933-agGPPS-1335(44)	P _{trc} ::AgGPPS,1335(44);ColE1;Amp ^r	This study
3933-agGPPS-1607(44)	P _{trc} ::AgGPPS,1607(44);ColE1;Amp ^r	This study
3933-agGPPS-3780(44)	P _{trc} ::AgGPPS,3708(44);ColE1;Amp ^r	This study
3933-agGPPS-7423(44)	P _{trc} ::AgGPPS,7423(44);ColE1;Amp ^r	This study
pET21-tri1411	P _{T7} ::1411,6xHis;ColE1;Amp ^r	This study
pET21-tri1411(44)	P _{T7} ::1411(44),6xHis;ColE1;Amp ^r	This study

Table S2. Primers used in this study.

Primer name	Primer sequence
GPPS-1411-1411-gibson-R	GCATGCCTGCAGGTCGACTCTAGAG
GPPS-1411-1411-gibson-F	CCGATCAAAATCTCTGTTCCGTAATTCATTAAAGAGGAGAAAGGTACCGGAT
del19-1411-F	CATGCTGTTTAC
del24-1411-F	AACACCTGCTTCCCGTCTTTACCC
del37-1411-F	TCTTTCACCCCGGGTAAATCT
del38-1411-F	TCTAAAGCTGAACCGCCGCT
del39-1411-F	GAGAAAGGTACCGGATCATGAAAGCTGAACCGCCGCTG
	GCTGAACCGCCGCTGAACCA

del40-1411-F	GAACCGCCGCTGAACCACTCTA
del41-1411-F	CCGCCGCTGAACCACTCTAC
del42-1411-F	CCGCTGAACCACTCTACCATCTCTACCAA
del43-1411-F	CTGAACCACTCTACCATCTCTACCAACCA
del44-1411-F	AACCACTCTACCATCTCTACCAACCAG
del45-1411-F	CACTCTACCATCTCTACCAACCAGTCTTCTT
del46-1411-F	TCTACCATCTCTACCAACCAGTCTTCTTC
del47-1411-F	ACCATCTCTACCAACCAGTCTTCTTCTTCTGA
del48-1411-F	ATCTCTACCAACCAGTCTTCTTCTTCTGAC
del49-1411-F	TCTACCAACCAGTCTTCTTCTTCTGAC
del73-1411-F	GACTTCGAATACATCCAGTCTATCCAC
del-1411-R	GTGATGGTGATGGTGATGCATGATCCGGTACCTTTCTCCTCTTTAATGA
del44-8401-F	ATCTCTTCTTCTAACGGTATCATCAACCCG
del44-7423-F	CCGCCGCTGTCTCACTCTTCTA
del44-3708,1335-F	CTGTCTACCGCTGCTGCTGGT
del-7423,8401,3708,1335-R	GTGATGGTGATGGTGATGCATCGGTACCTTTCTCCTCTTTAATGAATTAATTCTGAC
del44-TRS-R	CATGATCCGGTACCTTTCTCCTCTTTAATG
del44-3950-F	GAGAAAGGTACCGGATCATGAACCACTCTACCATCTCTACCAACCA
del44-4091-F	GAGAAAGGTACCGGATCATGCCGCCGCTGAACCACTCTA
del44-5066-F	GAGAAAGGTACCGGATCATGATCTCTTCTTCTAACCGTATCACCAACC
del44-6118-F	GAGAAAGGTACCGGATCATGAACTACCAGCCGCCGATC
del44-8653-F	GAGAAAGGTACCGGATCATGAAAGTTGAATCTCCGCTGCTGAAC
del44-9982,9309-F	GAGAAAGGTACCGGATCATGAACCACTCTCACAACCTGTCTAC
del44-5307-F	GAGAAAGGTACCGGATCATGATCTCTTCTTCTAACGGTATCATCAACC
del44-1430-F	GAGAAAGGTACCGGATCATGATCTCTACCAACCTGATCTCTTCTTCTAACG
del44-4926-F	GAGAAAGGTACCGGATCATGTTCTCTGACCTGAACCCGATCCG
del44-1607-R	CATCGGTACCTTTCTCCTCTTTAATGAATT
del44-1607-F	AGAGGAGAAAGGTACCGATGGGTGGTATGAAACCGGAACCG
PET-del44-1411-F	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTCTAGAGGG
PET-del44-1411-R	AACCACTCTACCATCTCTACCAACCA

Table S3. Tricyclene synthases derived from different species.

Genes used in this study.		
Name	Accession NO.	Species
9309	XP_016539309	<i>Capsicum annuum</i>
9982	PHT29982	<i>Capsicum baccatum</i>
8653	PHT98653	<i>Capsicum chinense</i>
5307	NP_001295307	<i>Lycopersicon esculentum</i>
4091	XP_019234091	<i>Nicotiana attenuata</i>
1411	XP_009791411	<i>Nicotiana sylvestris</i>
1335	XP_009791335	<i>Nicotiana sylvestris</i>
1607	XP_009791607	<i>Nicotiana sylvestris</i>
3708	XP_009773708	<i>Nicotiana sylvestris</i>
7423	XP_009757423	<i>Nicotiana sylvestris</i>

4926	XP_016464926	<i>Nicotiana tabacum</i>
3950	XP_009593950	<i>Nicotiana tomentosiformis</i>
5066	TMX05066	<i>Solanum chilense</i>
8401	XP_015068401	<i>Solanum pennellii</i>
1430	XP_006351730	<i>Solanum tuberosum</i>
6118	XP_015166118	<i>Solanum tuberosum</i>

Table S4. Tricyclene and by-product titers of different TSs

Name	Tricyclene titer (mg/L)	α -Pinene titer (mg/L)	Camphene titer (mg/L)	Sabinene titer (mg/L)	β -Pinene titer (mg/L)	Limonene titer (mg/L)	% Tricyclene of Total Monoterpenes
1411	0.299	0.000	0.066	0.045	0.221	0.179	36.9%
4091(44)	0.135	0.000	0.082	0.017	0.210	0.174	21.8%
4926(44)	0.340	0.052	0.072	0.046	0.261	0.339	30.7%
5066(44)	0.459	0.089	0.380	0.023	0.331	0.339	28.3%
8653(44)	4.329	0.290	0.528	0.329	1.306	1.148	54.6%
9309(44)	0.816	0.062	0.115	0.027	0.315	0.248	51.5%
5307(44)	1.993	0.332	1.884	0.089	1.193	1.427	28.8%
8401(44)	4.196	0.503	2.754	0.177	2.093	2.157	35.3%
3708(44)	0.000	0.556	0.111	0.579	1.339	1.724	0.0%
1411(44)	48.173	2.227	1.306	4.641	12.655	10.265	60.8%

Fig.S1 SDS-PAGE analysis of wild-type 1411 and 1411 (44).

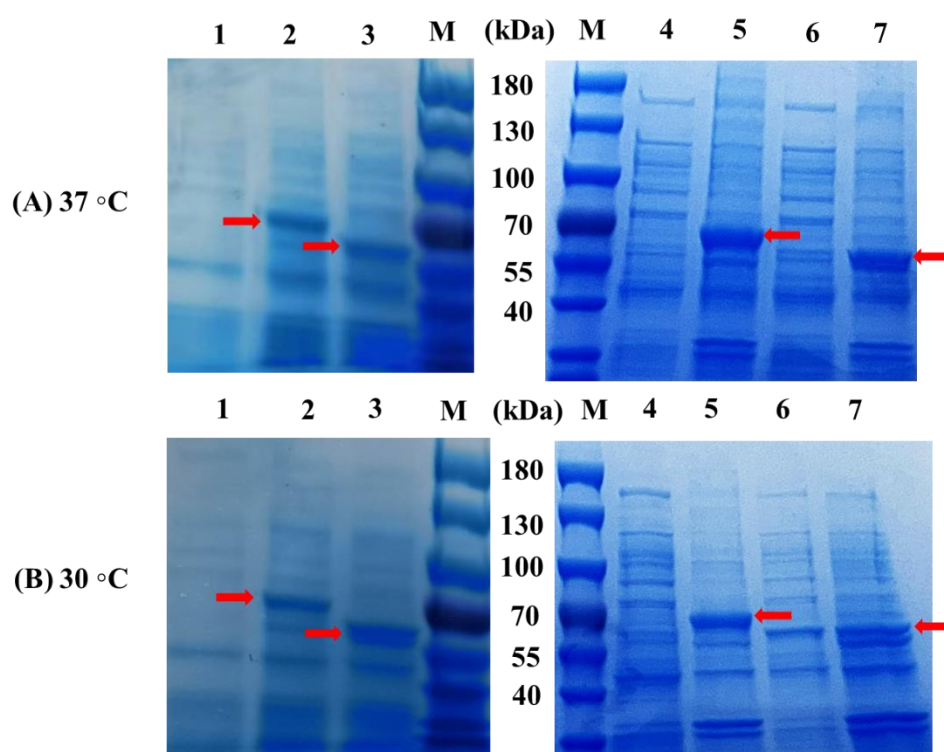


Figure S1. SDS-PAGE analysis of wild-type 1411 and 1411 (44). The induction temperatures were 37 °C (A) and 30 °C (B), respectively. Lane M represented the standard protein molecular weight. Lane 1 was the uninduced culture sample of wild-type 1411. Lane 2 and 3 were the induced culture samples of 1411 and 1411(44), respectively. Lane 4 and 5 were the supernatant and resuspended pellet of cell lysate of 1411, respectively. Lane 6 and 7 were the supernatant and resuspended pellet of cell lysate of 1411(44), respectively. The bands corresponding to wild-type 1411 (71.5 kDa) and 1411(44) (66.9 kDa) were indicated by red arrows.