Supplementary information

Microbial Production of γ-Curcumene in Metabolically Engineered *Escherichia coli* Using a Novel γ-Curcumene Synthase from *Parthenium argentatum*

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Table S1. Information of terpene synthases used in the phylogenetic tree

Protein	GenBank accession number	Function	Species	Reference
MrBOS	KJ020282	(–)-α-Bisabolol synthase	*Matricaria recutita*	1
EeBOS	MH048990	(–)-α-Bisabolol synthase	*Eremanthus erythropappus*	2
CcBOS	XP_024994640.1	(–)-α-Bisabolol synthase	*Cynara cardunculus*	3
MrFS	KJ020283	β-Farnesene synthase	*Matricaria recutita*	1
AaFS	AY835398	(*E*)-β-Farnesene synthase	*Artemisia annua*	4
AmBOS	LC106015	(+)-α-Bisabolol synthase	*Artemisia maritima*	5
AkBOS	LC106016	(+)-α-Bisabolol synthase	*Artemisia kurramensis*	5
AaADS	AJ251751	Amorpha-4,11-diene synthase	*Artemisia annua*	6
MrTPS4	KJ020284	Bicyclogermacrene synthase	*Matricaria recutita*	1
AabrBOS1	LC469617	(+)-α-Bisabolol synthase	*Artemisia abrotanum*	7
AabrBOS2	LC469618	(+)-α-Bisabolol synthase	*Artemisia abrotanum*	7
PatTpsA	AY508726	γ-Curcumene synthase	*Pogostemon cablin*	8
LcCedS	MK431785	Cedrol synthase	*Leucosceptrum canum*	9
MpFS	AF024615	(*E*)-β-Farnesene synthase	*Mentha* x *piperita*	10
RelspS	MK775336	Isoprene synthase	*Ralstonia eutropha*	11

Table S2. Primer sequences used in this study

Plasmid	DNA	Name	Sequence
pET28-PaCS	pET28 (backbone)	V-pET28-PaCS-F	ATCCGGCGGCAATCCTCGAGCACCACCACCACCA
		V-pET28-PaCS-R	AGGAAGGTGCTCATGCTGCTGCCCATGGTATATC
	PaCS	I-pET28-PaCS-F	CCATGGGCAGCAGCATGAGCACCTTCCTGGTTAG
		I-pET28-PaCS-R	TGGTGGTGCTCGAGGATTGCCGCCGGATGAACCA
pTSN-PaCS-Mm	pTSN-Bisa-Mm (backbone)	V-pTSN-PaCS-Mm-F	CGGCGGCAATCTAATATTCATTAAAGAGGAGAAA
		V-pTSN-PaCS-Mm-R	AGGAAGGTGCTCATGGTTTAACCTCCTGTGTGAA
	PaCS	I-pTSN-PaCS-Mm-F	CAGGAGGTTAAACCATGAGCACCTTCCTGGTTAG
		I-pTSN-PaCS-Mm-R	CTCTTTAATGAATATTAGATTGCCGCCGGATGAA
pSSN12Didi-PaCS-IspA-Mm	pSSN12Didi-MrBBS-IspA (backbone)	V-pSSN-PaCS-Mm-F	GGCAATCTAATATTCATTAAAGAGGAGAAAGGTACCC
		V-pSSN-PaCS-Mm-R	AGGTGCTCATGGTTTAACCTCCTGTGTG
	PaCS	I-pSSN-PaCS-Mm-F	AGGTTAAACCATGAGCACCTTCCTGGTTAG
		I-pSSN-PaCS-Mm-R	TTAATGAATATTAGATTGCCGCCGGATG

Table S3. Nucleotide sequences of *PaCS* cDNA and *E. coli* codon-optimized *PaCS*

Source	Nucleotide sequence	Reference
PaCS cDNA (red letters: *PaCS* ORF)	CCACCCTTAGAATATTTTGAGATGTCAACCTTTTTAGTTTCAAGTGCTTCC TTTTCTTCATCTATCTTACCATCGGCTGTTGATGACAATTGTGGCACGAAG CAGAATGTTATCCGCAACACTACGAATTTCAATGCTAGCATATGGGGAG ATCAATTTCTTACACCTGATGAGCCAGAAGATCTAGCGATGGAGAAACA AGCTGTTGAAGAGCTCAAAGAAGAAGTAAGAAAAGAGCTAATGATCAAA GCTTCCTCCAACGAACCACTGCAACATATGAAGATGATAGACCTTATTG ATGTAGTCCAACGCCTTGGAATAGCCTATCATTTTGAAGACGAGATCGA GGAAGCCTTGCAACATATCTATGTAACATATGGCGAAAAATGGATCGGT CATAACAACCTACAAAACATTGCTCTTTGGTTCCGACTCCTTCGACAAC AAGGCTTCAACCTTTCATCAGAAATATTCAAAAACCATATGGACGAGGA GGGACGTTTTAAGGAATCCTTTTGTGCTGATGCTCAAGGATTGCTTGCTT TGTATGAAGCATCATATATGAAGGTGGAAGGAGAAAAAGTACTCGATGAT GCCCTCGAGTTCACTAAAACTCACCTTGTAATCATAGCGAAGGATCCTT CTTGTGATTCTTTATTAAGAACCCAAATACAACAAGCACTAAAGCAGCCA CTCCGGAAAAGGTTGCCAAGGCTAGAGGCCGTGCGTTACATACCTATT TACCAACAAGAACCTTCCCACAATGAGGTCTTACTGAAGCTTGCAAAGT CTGATTTTAATGTGCTACAATCAATGCACAAAAAGGAGCTCAGCCAAATT TGCAAGTGGTGGAAGGACTTGGACATGCAAAACAAACTACCTTATGTTC GTGACAGATTGATAGAAGGCTATTTTTGGATACTAGGAGTCTATTTTGAGC CTCAACATTCTCGTACAAGAATGTTCCTAATGAAAACATCCATGTGGTTG ATTGTTTTAGATGACACGTTTGATAACTATGGTACTTATGAAGAGCTCAAG ATCTTTACAGAAGCTGTTGAAAGATGGTCAATGAGTTGTTTGGATACGCTT CCAGAATACATGAAACTAATATATCAAGAACTTTTGAATCATCACCAAGA AATGGAGGAACTAATTGAAAAGGAAGGAAAAGCATACCAGATCCAATAT GTGAAAGAGATGGCAAAAGAGGTTATTCGAAACAACTTAGTGGAAGCTA GATGGTTAAAAGAGGGGTATATGCCAACTCTGGAAGAGTACATGTCTGTT TCAGTGAAGACTTGTACCTACGGCTTGATGATAGCGAGATCTTATGTTGG TAGAGCAGATCATATGGTCACTGAGGATACATTTAAATGGGTCACCACAT ACCCTCCTATTGTTAAAGCTGCATGTTTTATTTTAAGACTTATGGATGATAT TACCACCCACAAGGAGGAACAAGAAAGAGGCCATGTTGCTTCAAGCA TCGAATGCTACCAAAATGAAACCGGAGCATCCGAGGAGGAAGCATGTA AATTCATCTTAAAAAAAATTGAAGATGCATGGAAAGTTATAAACAGAGACT CTCTCCGGCCAACAGAAATCCCATTTCCTCTACTTATGTCGACGATCAA CTTTGCACGTGCGTGTGATGTCATTTATAAAGTTAACGATGGCTACACTC ATGCTGAGGAAGAAATGATAAGTCACATCAAATCGATCTTGGTTCATCCT GCAGCTATTTAAGATGTAAGATCGATTAAATGCATGCGGTTTGTTTGTGTT TACAAAATCGTATAGTTATAATTTATGTGCCATAAGCTTGTACGCATGTCA GCATGTGTGAAAAGAGTATATTAATCTGGCCATGCATGTACGTTGTTCATA TGTGCATGCCATGTTGATCAGCAATAATCCATGTGATGTAAGAGTATGCTT CTTATTTTGTATTCTATTGTACTTGTGCATAGATCG	This study
E. coli codon optimized *PaCS*	ATGAGCACCTTCCTGGTTAGCAGCGCAAGCTTCAGCAGCTCGATTCTC CCGTCAGCAGTCGATGATAATTGTGGTACCAAACAGAATGTCATACGTA ACACAACCAATTTCAATGCAAGCATTTGGGGCGACCAGTTTCTCACTC CCGATGAACCGGAAGATCTGGCTATGGAAAAACAAGCGGTTGAGGAG CTGAAAGAGGAAGTTCGTAAGGAACTGATGATTAAAGCGAGCAGTAAC GAACCGCTGCAGCACATGAAAATGATTGACCTGATCGACGTTGTTCAG CGTCTGGGCATCGCATATCATTTCGAAGACGAAATTGAGGAGGCACTA CAGCACATTTACGTTACCTACGGTGAGAAGTGGATTGGTCATAACAATCT GCAGAACATTGCTCTGTGGTTCCGACTGCTGCGTCAGCAGGGTTTCAA	This study

	CCTGAGCAGCGAAATTTTTAAAAACCACATGGATGAAGAAGGCCGTTTT AAAGAGAGCTTTTGCGCCGATGCACAGGGCCTGCTGGCCCTCTATGA AGCCAGCTACATGAAAGTTGAGGGCGAAAAAGTCCTGGATGACGCGC TGGAATTTACTAAAACCCATCTGGTTATAATAGCGAAGGATCCGAGCTGT GATAGCCTGCTGCGTACCCAGATACAGCAAGCGCTGAAACAGCCGCT GCGTAAGCGCCTGCCGCGTCTGGAAGCTGTTCGTTACATTCCGATCTA TCAACAGGAACCCTCGCATAACGAAGTTCTGCTGAAACTGGCAAAGAG CGATTTTAATGTGCTGCAGAGCATGCATAAAAAAGAACTGAGCCAAATTT GCAAATGGTGGAAAGATCTGGATATGCAAAATAAGCTGCCGTATGTTCG CGATCGTCTGATCGAAGGGTACTTTTGGATTCTGGGAGTTTATTTTGAAC CGCAGCACAGCCGCACCAGGATGTTCCTGATGAAAACTAGCATGTGG CTGATTGTTCTGGATGATACCTTTGACAACTATGGTACCTATGAGGAACT GAAAATTTTTACCGAGGCGGTGGAACGCTGGAGCATGAGCTGCCTGG ATACACTGCCTGAATACATGAAACTGATTTATCAAGAGCTGCTGAATCAC CATCAAGAGATGGAGGAGCTGATTGAGAAGGAGGGTAAGGCATATCAA ATCCAGTATGTAAAAGAAATGGCAAAAGAAGTTATTCGTAACAATCTCGT CGAAGCCCGTTGGCTTAAGGAGGGTTACATGCCGACACTGGAGGAAT ACATGAGCGTTAGCGTTAAAACGTGTACCTATGGACTGATGATCGCCCG TAGCTATGTGGGGCGTGCCGATCACATGGTTACCGAAGACACCTTCAA ATGGGTTACCACCTATCCGCCCATTGTGAAAGCCGCTTGTTTTATTCTG CGGCTGATGGACGATATTACCACCCATAAAGAGGAGCAAGAACGCGG ACATGTTGCCAGCAGCATTGAATGCTATCAGAACGAGACCGGGGCGA GCGAAGAAGAAGCTTGTAAGTTTATCCTGAAAAAGATTGAAGACGCGTG GAAAGTTATCAATCGTGACAGCTTGCGCCCGACCGAAATCCCGTTTCC GCTGCTGATGAGCACCATAAATTTTGCGAGGGCCTGCGACGTTATTTAT AAAGTGAATGATGGTTATACGCATGCTGAAGAAGAAATGATTTCCCATAT CAAGAGCATCCTGGTTCATCCGGCGGCAATCTAA	

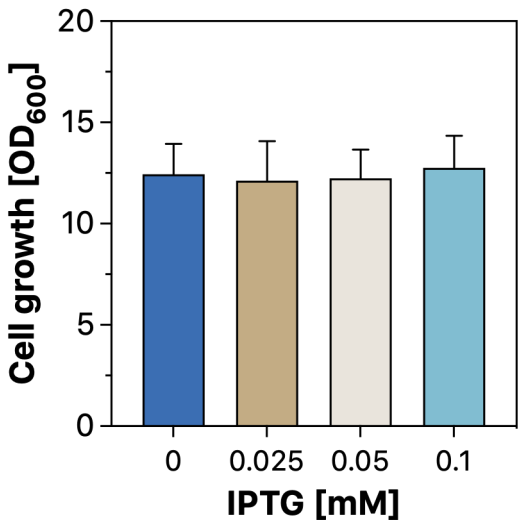

Figure S1. **Cell growth of *E. coli* SBA01 strain producing γ-curcumene in the presence of different concentrations of IPTG.** Different concentrations of IPTG (0–0.1 mM) were added to the *E. coli* SBA01 cells harboring pTSN-PaCS-Mm and pSSN12Didi-PaCS-IspA-Mm. The cells were grown on TB medium at 30°C with 10 g/L glycerol and 20% (*v/v*) *n*-dodecane. Data represent averages from three replicate cultures, and error bars indicate standard deviation.

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