Supplementary methods 1

Reagents and equipment. All enzymes and chemicals were purchased from New England Biolabs and Sigma-Aldrich Co, respectively, unless otherwise stated. An HP6890 gas chromatograph equipped with a 5973 mass selective detector (Hewlett Packard), a CyclosilB capillary column (30 m × 250 μm i.d. × 0.25 μm thickness, Agilent Technologies), DB5-MS capillary column (30 m × 250 μm i.d. × 0.25 μm thickness, Agilent Technologies), and a Combi PAL auto sample-injector (LEAP Technologies) were used for sesquiterpene analysis. An LS6500 multi-purpose scintillation counter (Beckman coulter) was used for enzyme kinetics.

Synthesis of γ-humulene synthase gene. The γ-humulene synthase gene used herein was designed to maintain the native primary sequence but with optimized codon usage for *E. coli* (Supplementary Material 2); the gene was synthesized from oligonucleotides (Supplementary Table 10) using standard methods[1]. The synthetic gene was digested with NcoI/XbaI, and cloned into pTrc99A to form pTrcHUM. The vector was transformed into *E. coli* DH10B, and the transformants were screened for sesquiterpene production. Several functional clones were found and sequenced. For high-level production of the target enzyme, the gene encoding γ-humulene synthase was cut from pTrcHUM with NcoI/HindIII and cloned into pET29 (Strategene) to form pETHUM.

Saturation and site directed mutagenesis of γ-humulene synthase by overlap

PCR. Both saturation and site directed mutagenesis were carried out using overlap PCR[2] (see Supplementary Table 8 for the primer sequences). DNA fragments encoding the N and C termini were amplified by PCR: 94°C for 30 sec, 50°C for 30 sec, and 68°C for 2 min, repeated 30 times. The reaction mixture contained *Pfu* buffer, 2 mM dNTP, 0.5 µM forward and reverse primers, 2.5 U *Pfu* turbo (Strategene), and 50 ng pTrcHUM in 100 µl. Amplified DNA was gel purified using a kit (Qiagen). Overlap PCR was carried out using the protocols described above. The fully amplified fragment was digested with NcoI/XbaI and cloned into pTrc99A.

Protein expression and purification. Wild type γ-humulene synthase and its variants were cloned into pET29 and transformed into BL21(DE3). Each transformant was inoculated into LB medium (5 ml) containing 50 µg/ml kanamycin (Km) and was grown overnight at 30°C. An aliquot (2 ml) of this seed culture was inoculated into fresh terrific both (TB) medium containing Km (500 ml), and the culture was grown at 30°C. When the culture reached OD_{600nm} of 0.6-0.8, 0.05 mM of IPTG was added, and it was grown at 20°C for 16 hours. Cells were harvested by centrifugation a**t 6,000 ×g** for 15 min. The pellet was suspended in 10-15 ml of BugBuster (Novagen) containing 20 U DNaseI and bacterial protease inhibitor, and was incubated for two hours at 4°C. The solution was then centrifuged at 20,000 ×g for 30 min, and was filtered through a 0.45-µm filter. S-tag™ Thrombin purification kit (Novagen) was used for the purification following the protocol recommended

by Novagen. All purifications were done at half scale. The eluted protein solution was dialyzed twice (PIERCE, MW 3,000 Da) against 1 L of buffer containing 10 mM Tes (pH 7.0), 10 mM $MgCl_2$, 1 mM DTT and 5% glycerol overnight. The protein concentration was measured by Bradford assay. We obtained approximately 3 ml of 25-500 µg/ml of protein solution with about 95% purity (confirmed by SDS-PAGE gel).

Enzyme kinetics. The kinetics studies of γ-humulene synthase and its variants were carried out following the slightly modified method previously reported by Little et. al.[3]. Kinetics for each enzyme was measured in a 40 µl reaction containing 0.05-0.5 µM enzyme, in buffer described in previous section and overlaid with dodecane. The concentration of FPP was varied from 0.229 to 58.6 µM with a fixed ratio of [^{3}H]FPP. Seven to nine different concentrations of FPP were used for each enzyme. As for *E*-β-farnesene synthase, the concentration of FPP was varied from 0.014 to 58.6 µM with a fixed ratio of [^{3}H]FPP. Twelve (12) different concentrations of FPP were used. Each concentration was done in triplicate. The reaction mixture was incubated for 20 minutes at 31°C. To stop the reaction, 40 µL of a solution containing 4 M NaOH and 1 M EDTA was added and mixed. To extract sesquiterpene products, the reaction mixture was vortexed for 1 min, and 400 µL of dodecane was taken from the solution and mixed with 10 mL of scintillation fluid. Radioactivity was measured by scintillation counting. k_{cat}, K_m and k_{cat}/K_m were calculated using Enzyme Kinetics!Pro (ChemSW).

References

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