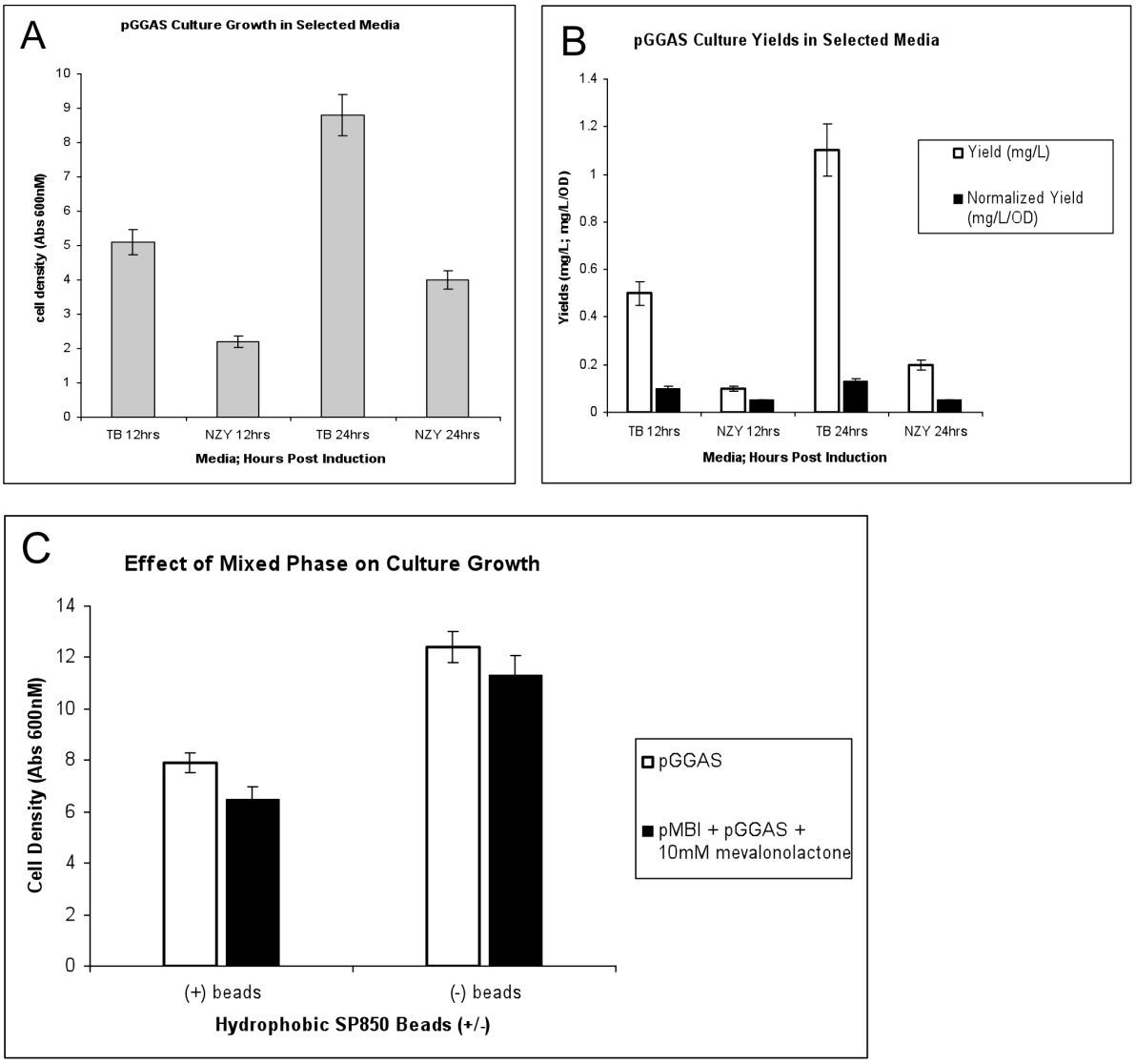
Supplementary Information for:

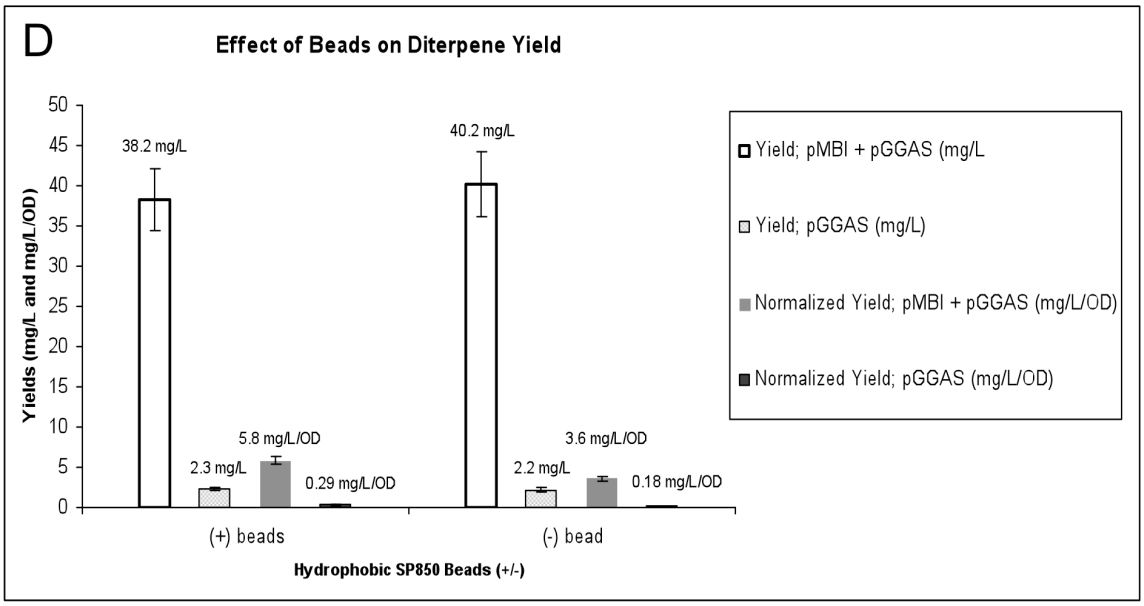
Increasing diterpene yield with a modular metabolic engineering system in *E. coli*: comparison of MEV and MEP isoprenoid precursor pathway engineering

Dana Morrone, Luke Lowry, Mara K. Determan, David M. Hershey, Meimei Xu, Reuben J. Peters*

Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011, USA

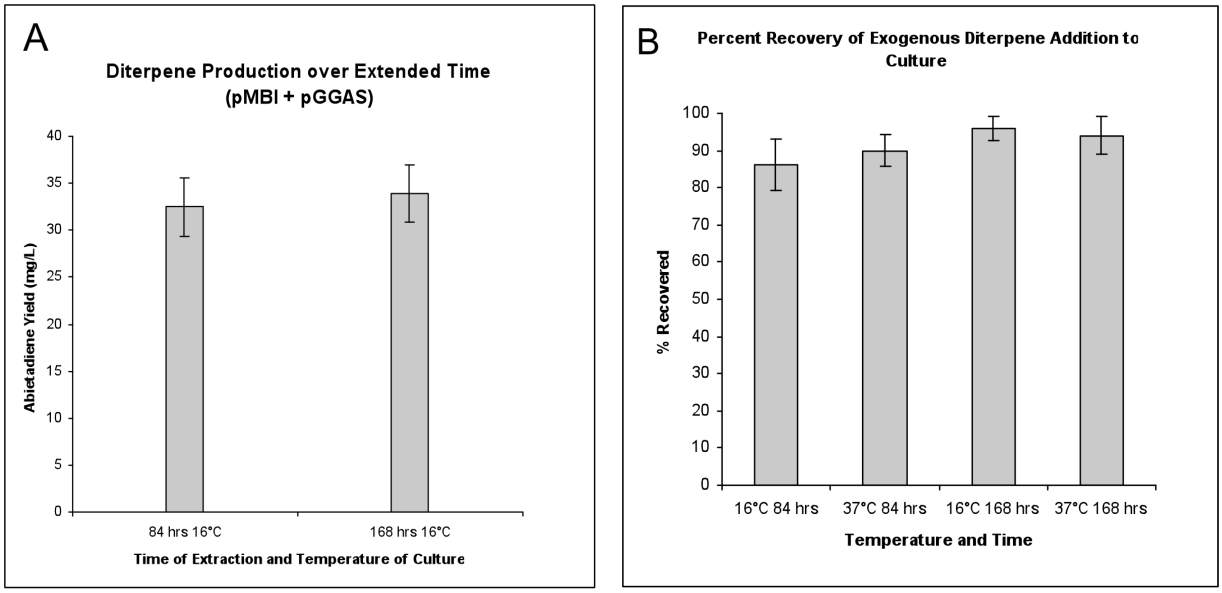
Supplemental Figure 1





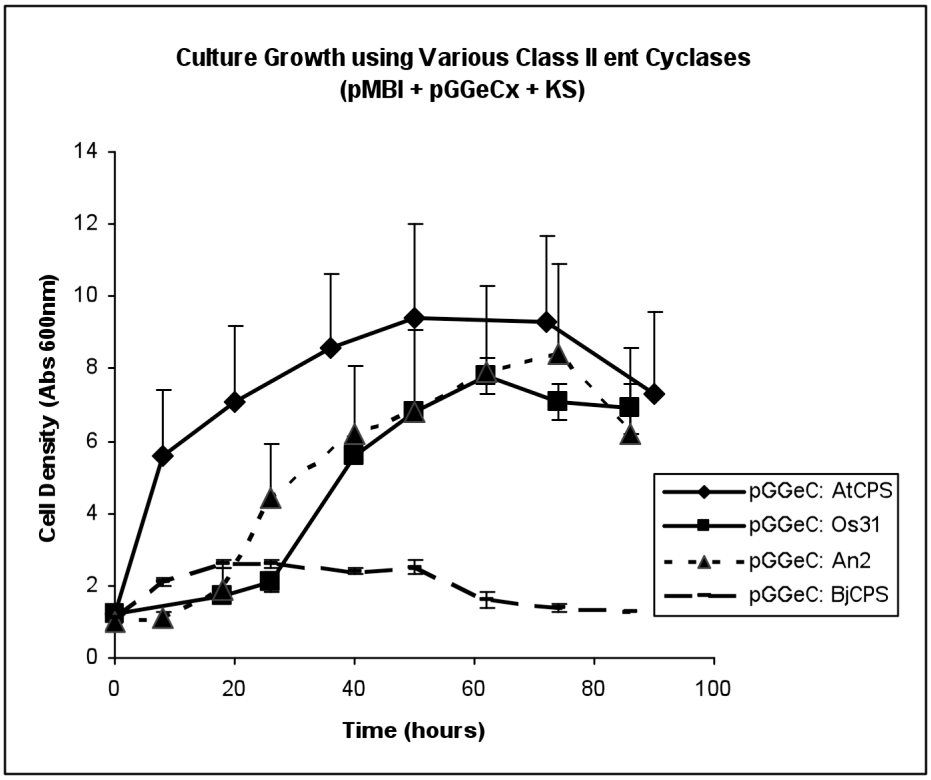
A) Comparison of initial growth for pGGAS containing *E. coli* C41 in TB and NZY media as determined by cell density at 12 and 24 hours post IPTG induction. B) Comparison of abietadiene yields from pGGAS containing *E. coli* C41 in TB and NZY media at 12 and 24 hours. Assayed as per materials and methods. C) Effect of hydrophobic beads on TB culture growth for engineered strains producing abietadiene. Values listed depict maximum cell densities reached during 84 hour fermentation. It was found that the addition of beads [(+) beads], inhibited cell growth in shake flasks. D) Effect of hydrophobic beads on abietadiene yields for the engineered strains. Yields, both net and normalized, are indicated above bars. Mixed phase assays and culturing in TB were performed as per Cyr et al. (2007). All data is from cultures grown in parallel, with error bars depicting the standard deviation of duplicate cultures.

Supplemental Figure 2



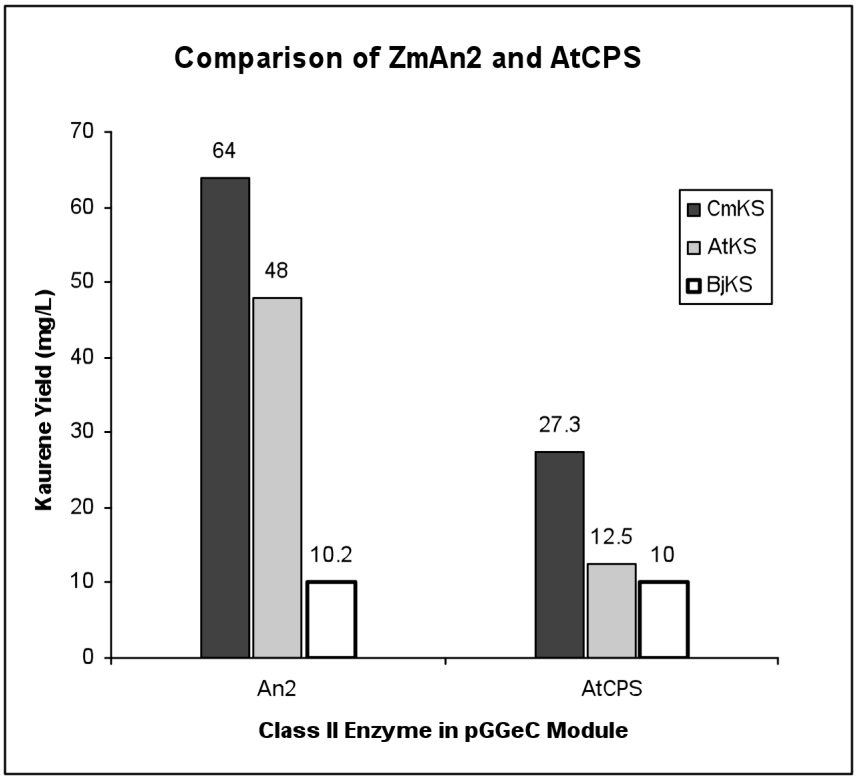
A) Abietadiene production from pMBI + pGGAS (10mM mevalonalactone) up to 168 hours. At 84 hours post induction, the culture was terminated (pH 2) to prevent further abietadiene production. The single culture was assayed by extraction for abietadiene content immediately following termination at 84 hours, and then allowed to shake at 16 degrees C for an additional 84 hours (168 hours total) and assayed again. The lack of change in abietadiene content indicates no significant loss to volatility under growth conditions. Trials performed in duplicate and analyzed as described in the materials and methods section. B) Recovery of 50 µg exogenously added *ent*-kaurene (5 mg/mL in 1:1 DMSO/Methanol) to 50 mL cultures of *E. coli*. These were allowed to incubate at the given times and temperatures before organic extraction. Recovered amounts indicate a significant lack of volatilization and/or product loss under culture conditions. Trials performed in duplicate and assayed as described in the materials and methods section.

Supplemental Figure 3



Assessment of culture growth when utilizing various *ent*-CPP specific class II diterpene cyclases versions of pGG*e*C. While AtCPS, Os31, and ZmAn2 allowed for sufficient growth, BjCPS was repeatedly found to confer poor growth, and subsequently, lower yields. All data is from cultures grown in parallel, with error bars depicting the standard deviation of duplicate cultures.

Supplemental Figure 4



Comparison of *ent*-kaurene production with An2 or AtCPS co-expressed with various KS enzymes (CmKSd40, AtKSd41, and BjKS). An2 or AtCPS were inserted into the pGGeC module and then coupled with pMBI and pDEST-KS. 84 hour expression, performed using 10 mM mevalonolactone, and subsequent analysis were performed as per materials and methods for abietadiene. The two plant class I enzymes (CmKS and AtKS) underwent N-terminal amino acid truncation to construct pseudo-mature enzymes amendable to recombinant expression. All data is from cultures grown in parallel.