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Building terpene production platforms in yeast.

Introduction:

Plants and microbes commonly make terpenes and terpenoids in small amounts and as complex mixtures, and their chemical synthesis is often costly and inefficient. Hence, there are many efforts to create robust and efficient biological production platforms for this interesting class of molecules. In this study, author's effort was directed towards building a yeast production platform using an unbiased genetic selection approach. They have used metabolic engineering to change the endogenous pathways along with the heterologous expression of exogenous active enzymes that resulted in high production of terpenoid compounds. Many of the target compounds are normally difficult to produce by organic chemical synthesis and metabolic engineering takes advantage of the specificity of enzyme-mediated biosynthesis to generate stereo-chemically pure products from cheap raw materials.

Paper:

Building terpene production platforms in yeast.

Zhuang X and Chappell J. Biotechnol Bioeng. 2015 Sep;112(9):1854-64.

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Abstract:

Yeast strain BY4741 was subjected to EMS mutagenesis, followed by selection for growth in the presence of nystatin, squalestatin, and exogenous cholesterol. This unbiased screen selected for mutant yeast lines having a dispensable mevalonate pathway and containing uncharacterized SUE (sterol uptake enhancement) mutations supporting aerobic uptake of exogenous sterol. These mutants were next screened for high level accumulation of farnesol (FOH), an indicator for high level accumulation of the key intermediate FPP, farnesyl diphosphate. To further improve the FPP pool in these mutants, insertional mutations into the ERG9 gene (coding for squalene synthase) were introduced into those lines capable of accumulating ≥50 mg farnesol/L. This generated another series of lines that accumulated farnesol levels over 70 mg/L in small-scale shake cultures. To evaluate the utility of these lines as a general production platform for specific terpenes, select SUE/erg9 lines were transformed with a vector harboring the Hyoscyamus muticus premnaspirodiene synthase (HPS) gene encoding for a sesquiterpene synthase. The new yeast line ZX178-08 accumulated the highest level of premnaspirodiene, up to 116 mg/L, with FOH levels of 23.6 mg/L. In comparison, the parental line BY4741 accumulated 10 times less premnaspirodiene, 10.94 mg/L, with no farnesol detectable. Co-expression of the HPS gene with an amino-terminal truncated, catalytic form of the hamster HMGR gene, tHMGR, increased premnaspirodiene accumulation to 170.23 ± 30.44 mg/L, almost a 50% increase. Further utility of this yeast line was demonstrated for triterpene production. When engineered for the production of a non-native triterpene, Zx178-08 accumulated upwards of 60 mg/L of botryococcene. To engineer more native triterpene accumulation, additional insertion mutants into the ERG1 gene (coding for squalene epoxidase) were evaluated. Insertion of a simple selection marker followed by over-expression of a heterologous squalene synthase gene resulted in greater than 85 mg/L of squalene. However, when the ERG1 insertional mutant included chromosomal insertion of a truncated, heterologous HMGR gene, squalene production was more than tripled to 270 mg/L.