Introduction

Terpene Production

The production of plant essential oils and their derivatives represents an over 9 billion dollar industry when considering just their applications in the food and fragrance industries.1 A staggering 23 million kilograms of citrus oil alone are produced worldwide each year. Up until only a couple decades ago, the production of essential oils was done exclusively by chemical extraction from plant material. However, the sudden emergence of synthetic biology as a versatile and efficient tool has the potential to transform this immense industry, the products of which nearly everyone will come in contact with on a daily basis.

Future Research

Gas Chromatography Mass Spectrometry is in progress to confirm the production of each terpene for all of the genes successfully extracted and integrated so far. We plan on preforming this assay both in our E. coli and yeast models.

By harnessing existing biosynthetic pathways and introducing enzymes taken from plants into more malleable model systems, it will be possible to significantly improve on current methods of the active components of essential oils, most notably the terpenoids. While most plants express terpenes in the range of parts per million and thus require very large scale operations to be commercially viable, early forays into the biological production of terpenes have proven that it is possible to achieve yields well over 100-fold.2 We selected a total of nine different terpenes to produce (see Table), each of which has practical viability, early forays into the biological production of terpenes have proven that it is possible to achieve yields well over 100-fold.2 We selected a total of nine different terpenes to produce (see Table), each of which has practical.

Design

As a shuttle vector, pvU14006 is capable of expression in both E. coli and S. cerevisiae. For cloning in bacteria, it has a prokaryotic origin of replication taken out of pUC19. Two regions of base pair homology with the S. cerevisiae genome allow it to efficiently integrate into the yeast genome. There is a multiple cloning site with a range of different restriction enzymes to make the plasmid compatible with almost all of the most commonly used restriction enzymes, including those used in RFC10 compatible biobricks. A Gal1 inducible promoter is upstream of where the protein coding gene would be inserted. Lastly, the Strep epitope tag was added to each synthase gene for purification and identification.

Our initial project used plant genomic DNA as the template for PCR extraction of the synthase genes. Plants were grown at the Vanderbilt greenhouse, lyophilized and genomic extracted, then run in PCR reactions. Later, we transitioned to a cDNA based protocol for gene isolation in order to avoid potential complications from intron sequences. RNA was extracted from plant samples and converted to cDNA with reverse transcriptase. PCR was run to isolate the genes and the extracted genes were processed by site-directed mutagenesis and overlap-extension PCR to make each gene compatible with standard biobrick assembly. Once each gene was extracted, processed, and integrated into our specially designed vector, they would be integrated into our microbial factories for synthesis. To confirm the synthesis was transcribed, the Strep tag could be used in western blotting. Terpene production would be confirmed by gas-chromatography mass spectrometry.

Project

To make our project an opportunity for symbio education, we organized university undergraduates into “teams” that each worked on production of one terpene. Each group planted and grew the host plant species, genomic extracted DNA, and ran PCR isolations. After troubleshooting over the summer, we successfully isolated every terpene synthase. After remarking the large degree of introns in our isolated genes, we moved on to cDNA templates. Almost all plant hosts successfully had their RNA extracted and their mRNA converted to cDNA. PCR isolation of the synthases successfully isolated several terpene synthases, which were gel extracted for further processing. Although mutagenesis is still ongoing in order to make the parts biobrick compatible, two synthases (sabinene and santalene) were ligated into our vector and transformed into yeast and E. coli. The vector was assembled by PCR isolation of gene cassettes.

Background

Metabolic Engineering Terpene Production

Terpene biosynthesis in plants is part of larger pathways that metabolize isoprenoid intermediates. Genes encoding for enzymes known as synthases catalyze the terminal step in these pathways, from a precursor (commonly farnesyl pyrophosphate (FPP) or geranyl pyrophosphate (GPP)) to the final terpene product. As it happens, two well established and genetically manipulable model organisms, the bacterium Escherichia coli and baker’s yeast, Saccharomyces cerevisiae, produce moderate amounts of GPP and FPP as part of their endogenous non-mevalonate pathway (MEP) and mevalonate pathway (MEV) respectively.3 Although, yeast is the better of the two since a Eukaryote it has a more efficient MEV pathway.

All that is required for either of these organisms to begin producing terpenes is to introduce that single synthase gene. Upon the introduction of the synthase gene, the appropriate terpene will be produced as well as several secondary products including stereoisomers as well as other minor terpenes.

Results

Table 1. Expression of terpene synthases in yeast using S. cerevisiae and E. coli expression systems

References/Acknowledgments

VANDERBILT UNIVERSITY


Figure 1. Homologous Recombination in Yeast

Figure 2. Reverse Transcription PCR

Figure 3. Synthase gene extraction from plant genome