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Biorefinery of Renewable Limonene and Oleic Acid by Designing Multi-Enzymatic Routes

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PURPOSE OF THE ABSTRACT

Introduction

Based on the design-build-test (DBT) concept of synthetic biology, the natural biosynthesis pathway of l-menthol from limonene in plant was artificially reconstructed in microbial chassis of Escherichia coli by recruiting microbial enzymes to replace some difficult-to-express plant genes encoding membrane proteins. The biosynthesis of isopulegone removes the last technical hurdle for de novo microbial synthesis and large-scale biomanufacturing of l-menthol, one of the most important flavor chemicals with an annual demand of 30,000 metric tons.

Green synthesis of eco-friendly chemicals or material monomers from renewable feedstocks is of great significance to alleviate the problems of global warming and white pollutions. We designed and constructed a library of non-natural components/devices for biorefinery of oleic acid into bifunctional chemicals of mid-chain length (C8-C10), such as 8-hydroxyoctanoic acid, 9-aminononanoic acid and 1,10-decanedioic acid. A Baeyer-Villiger monooxygenase with unconventional regio-selectivity was designed, and 6 flexible modules of Plug-and-Play type were constructed for easy assembly into different routes to 7 structurally diverse bifunctional chemicals.

Results and Discussion

A microbial cell factory was designed and built in E. coli for synthesis of (+)-cis-isopulegone from (−)-limone, removing the last obstacle to (−)-menthol de novo biosynthesis. The production of (+)-cis-isopulegone, up to 281 mg L−1, was improved by 36 times compared with that of the initial strain.

A biocatalytic cascade process was designed for the normalization of n-nonanoic acid (NA) into 9-hydroxynonanoic acid (9-HNA) which might be further transformed into other C9 bifunctional chemicals, namely 1,9-nonanedioic acid (1,9-NDA) and 9-aminononanoic acid (9-ANA). More importantly, the cascaded biocatalysis system was operated by using different engineered E. coli consortia composed of the desired cell modules. This represents the first examples for valorizing the by-product (n-nonanoic acid) originated from oleic acid biorefinery, improving the carbon atom economy from 50% to 100%.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIBLIOGRAPHY