

CONTROLLING ENANTIOSELECTIVITY OF LIMONENE SYNTHASES BY EXPLORING NATURAL DIVERSITY COMBINED TO MOLECULAR ENGINEERING

Clement P.M. Scipion, CNRS@CREATE, 1 Create Way, #08-01 Create Tower, Singapore
clement.scipion@cnrsatcreate.sg

Shreyash Pramod Borkar, IISER Kolkata & SIFBI (A*STAR), 31 Biopolis Way, Singapore

Jérémy Esque, Toulouse Biotechnology Institute, TBI, Université de Toulouse, CNRS, INRAE, France

Sophie Bozonnet, Toulouse Biotechnology Institute, TBI, Université de Toulouse, CNRS, INRAE, France

Magali Remaud-Siméon, Toulouse Biotechnology Institute, TBI, Université de Toulouse, CNRS, INRAE, France

Xue Bo, SynCTI, NUS, Singapore

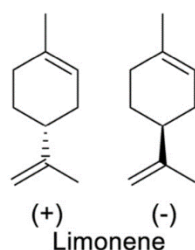
Wen Shan Yew, SynCTI, NUS, Singapore

Chen Xixian, SIFBI (A*STAR), 31 Biopolis Way, Singapore

Isabelle André, Toulouse Biotechnology Institute, TBI, Université de Toulouse, CNRS, INRAE, France

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Terpenes are natural products with more than 80,000 compounds. Predominantly produced by plants, they are key players in a number of functions such as signaling hormones, chemical defense against infection, disease resistance, cell growth modulation, non-exhaustively. They also have important roles in industry as solvents, fragrance, and flavorings, as well as have pharmaceutical applications. Their generic formula is $(C_5H_8)_n$ and most terpenes are derived from three precursors: monoterpenes (C_{10}) from geranyl diphosphate (GPP), sesquiterpenes (C_{15}) from farnesyl diphosphate (FPP) and diterpenes (C_{20}) from geranylgeranyl diphosphate (GGPP).



Here, we are interested in the cyclic monoterpene limonene, which is present in nature as two enantiomers (Figure 1). Its biosynthesis consists of the conversion of the acyclic GPP via the formation of a geranyl cation and ionization/cyclization to an α -terpinyl cation, followed by deprotonation. This synthesis is driven by the enzyme limonene synthase (LS). Limonene is the major component in the oil of citrus fruit peels Its (+)-isomer participates in the fragrance of oranges, and is routinely used as a flavouring agent. It also has solvent applications for cleaning purposes, and has also been reported to have anti-cancer properties. Today, more than 90% of commercial limonene oil comes from citrus extraction.

Figure 1 – Limonene enantiomers

We propose to engineer limonene synthases to improve the production of limonene in *E. coli*. For this, we have first searched the Pfam database for terpene synthase domain, selected reviewed LS-annotated enzymes, and clustered them on the basis of their sequence identity percentage to select in total 8 enantioselective LS. Based on literature, we also developed a stable plasmid system and production protocol for easy screening of LS homologues and their mutants. Using this system, the selected 8 homologues were assessed for limonene production (Figure 2). With the use of GC-MS chiral column, we have confirmed the enantioselectivity of each LS. Using a structure-based engineering strategy, we are now aiming at identifying mutations enabling to switch between (+) and (-) limonene production. In order to improve titer of the system, we developed a high-throughput method, utilizing our plasmid system, automated robotics for cloning and culture, and high-throughput MS for analysis. The work will contribute to the development of the improved biological production of limonene enantiomers from renewable carbon source.

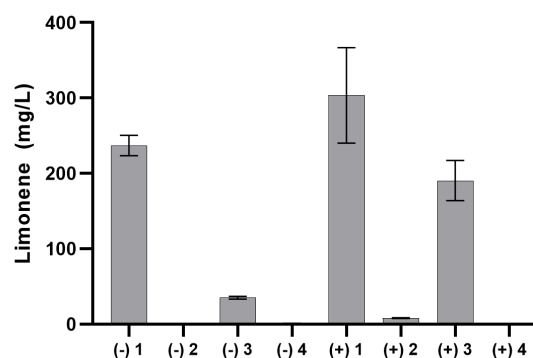


Figure 2 – Screening of different limonene synthases, for the production of (+) or (-) limonene.

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