

Title: Terpene factory

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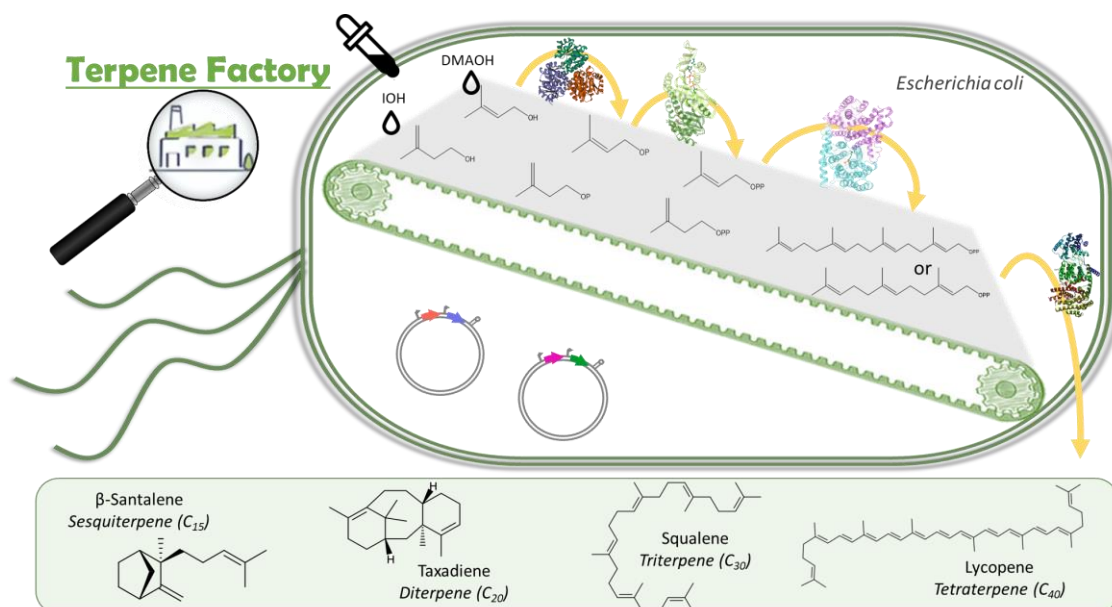
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Terpenoid are one of the most represented families of natural molecules on Earth. To date, more than 100,000 compounds [1] whose structural, biological (antibiotic, anticancer, anti-inflammatory, etc.) and physicochemical (cleaner, flavor, dye, etc.) properties hold the attention of the scientific community [2-3]. However, their access is limited because of the low available quantity by extraction from natural sources; an often expensive and laborious chemical synthesis; and long biosynthetic pathways. We have developed a new *in vitro* production pathway[4]: two enzymes making it possible to obtain diphosphates (DMAPP and IPP the universal precursors of terpenes), a prenyl transferase and finally the enzyme allowing cyclization. This synthesis is carried out from two alcohols with five carbon atoms in order to give different terpene compounds.

By combining bioinformatics, biochemical and molecular biology approaches, we have developed this approach *in vivo*, by joining together two plasmids comprising the four genes necessary for this terpene pathway. Playing on the gene position, but also on the different regulatory elements (plasmid copy number, operonic organization,...), the quantity of terpenes obtained can vary: it is a question of optimizing the experimental conditions, *from genetic construction to bioconversion*.

As a proof of concept we chose a fungal sesquiterpene synthase that produces d-cadinol (an anti-microbial, anti-fungal and anti-inflammatory compound)[5].

In the future the objective is to apply these approaches to all terpenoids: monoterpenes, triterpenes, diterpenes and teraterpenes. We propose a new biosynthetic tool to not only optimize and facilitate access to terpenes, but also to explore the biodiversity and characterize new terpene synthases.



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[2] Bohlmann J, Keeling Cl. *Terpenoid biomaterials*. Plant J. (2008) 54(4):656-69.

[3] Paramasivan K, Mutturi S. *Progress in Terpene Synthesis Strategies through Engineering of Saccharomyces cerevisiae*. Crit. Rev. Biotechnol. (2017) 37(8):974-989.

[4] Couillaud J, Rico J, Rubini A, Hamrouni T, Courvoisier-Dezord E, Petit JL, Mariage A, Darii E, Duquesne K, de Berardinis V, Iacazio G. *Simplified in Vitro and in Vivo Bioaccess to Prenylated Compounds*. ACS Omega. (2019) 4(4), 7838–7849.

[5] Ringel M, Dimos N, Himpich S, Haack M, Huber C, Eisenreich W, Schenk G, Loll B and Brück T. *Biotechnological potential and initial characterization of two novel sesquiterpene synthases from Basidiomycota Coniophora puteana for heterologous production of δ -cadinol*. Microbial Cell Factories (2022) 21 (1):64.