The Terpene Mini Path: or how to identify and characterize new terpene synthases?

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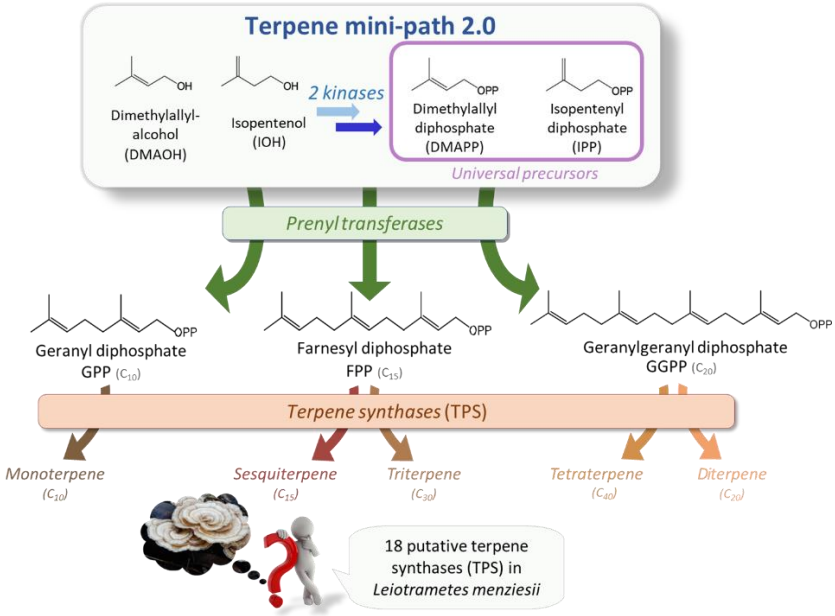
Terpenes are very widespread molecules in the world. These natural compounds have a wide range of interests in the flavour industry, insecticides, food and medicine. Although a hundred thousand structures have been solved to date, the world of terpenes leaves a huge field of possibilities to be discovered. Access to terpenes by natural means remains a challenge. Thanks to the terpene mini-pathway (TMP) (see poster Leydet et al.), we offer a very efficient biotechnological alternative for their access. In only two enzymatic steps we access the universal precursors of all terpenes (IPP and DMAPP) [1]. Then we implemented this simplified pathway with i) a prenyl transferase (FPPS, GPPS, GGPPS) to convert the IPP and DMAPP into C10, C15 or C20 linear diphosphates and ii) a terpene synthase which catalyze the cyclization of these linear molecules into terpenes [2]. Fungi are prolific producers of sesquiterpene compounds with relevant activities. Thanks to a bioinformatic tool recently developed for an accurate identification of fungal sesquiterpene synthases, 18 putative genes have been annotated in the genome of the Polyporales *Leiotrametes menziesii* [3]. All of these genes have been cloned and overexpressed in order to test *in vitro* each purified enzyme with the implemented TMP. The characterization of their products by GC/MS in combination with NMR and by biological activities is in progress. Therefore, our biosynthetic pathway offers an easy method to synthesize different terpenes, to discover new terpene synthases, and to determine their substrate promiscuity.



[1] J. Couillaud, J. Rico, A. Rubini, T. Hamrouni, E. Courvoisier-Dezord, JL. Petit, A. Mariage, E. Darii, K. Duquesne, V. De Berardinis and G. Iacazion. *ACS Omega* **2019**, 4, 7838-7849.

[2] J. Couillaud, K. Duquesne and G. Iacazio. *Chembiochem* **2022**, 23(24), e202100642

[3] H. Hage, J. Couillaud, A. Salamov, M. Loussouarn-Yvon, F. Durbesson, E. Ormeño, S. Grisel, K. Duquesne, R. Vincentelli, I. Grigoriev, G. Iacazio, MN. Rosso. *Microbial Genomics* **2023**, 9(4), 000990.