SPATIAL ORGANISATION OF ENZYMES IN THE BIOSYNTHETIC LIMONENE PRODUCTION PATHWAY IN *ESHERICHIA COLI*

Tiffany CHAU, Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Singapore 138 669; CNRS@CREATE, 1 Create Way, #08-01 Create Tower, Singapore 138 602
tiffany_chau@sifbi.a-star.edu.sg
Thomas LAUTIER, CNRS@CREATE, 1 Create Way, #08-01 Create Tower, Singapore 138 602
POH Chueh Loo, NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI), Centre for Life Sciences, National University of Singapore, Singapore 117 456
Simon ZHANG Congqiang, Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Singapore 138 669
Cédric Y. MONTANIER, TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France
Claire DUMON, TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France

Key Words: Limonene, Spatial organization, Mevalonate pathway, *Escherichia coli*

The monoterpene, limonene has a wide range of applications, including its use in food, cosmetics, pharmaceuticals, or chemistry. Limonene is naturally produced in plants. However, extraction from plants is unreliable and unsustainable. On the other hand, chemical synthesis of limonene is costly and harmful to the environment. To achieve sustainable goal while meeting the growing demand for limonene, we are developing a synthetic biology approach.

The mevalonate pathway (MVA) was engineered into *Escherichia coli* to produce limonene from glucose[1]. The final two steps of the pathway, the condensation of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) to geranyl diphosphate (GPP) and the cyclisation of GPP to limonene[2], are thought to be rate-limiting, as production of monoterpenes has remained lower compared the production of diterpenes, sesquiterpenes, and tetraterpenes[1].

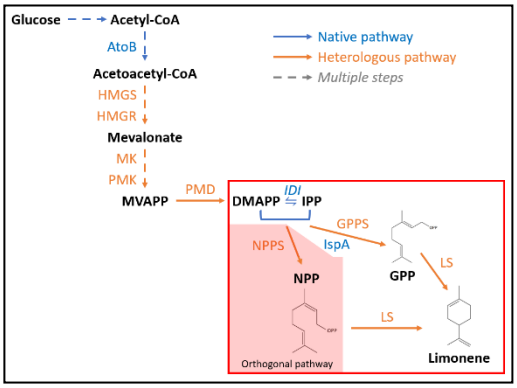

Figure 1: Engineered pathway introduced into E. coli to produce limonene.

Inspired by examples from natural enzymes such as tryptophan synthase, carbamoyl phosphate synthase[3], and the six enzymes involved in the purine biosynthetic pathway in eukaryotes[4], the spatial organization of enzymes is hypothesized to improve pathway flux. In this work, we experiment with translational and post-translational enzyme fusions in an attempt to increase limonene flux by enzyme colocalization.

We aim to increase limonene production using both translational and post-translational methods of assembling enzymes. We also experiment with different enzyme homologues, including utilizing an orthogonal pathway, by the condensation of DMAPP and IPP to neryl diphosphate (NPP) and the subsequent cyclisation of NPP to limonene.

We show that a translational enzyme fusion results in better limonene production compared to free enzymes, as previously reported[5], albeit in *E. coli*. Further work with post-translational assembly will be carried out to investigate if post-translational assembly of these enzyme homologues similarly increases limonene production.

Acknowledgements: This work is funded by the EcoCTs project with the support of the National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme.

---

[1] Alonso-Gutierrez et al., 2013, Metabolic Engineering, 19, pp.33-41.
[2] Sun et al., 2022, Engineering in Life Sciences, 22 (5), pp.407-416.
[3] Miles et al., 1999, Journal of Biological Chemistry, 274 (18), pp.12193-12196.
[4] An et al., 2008, Science, 320 (5872), pp.103-106.
[5] Hu et al., 2020, Journal of Industrial Microbiology & Biotechnology, 47 (6-7), pp.511-523.