Development of a recombinant *E. coli* whole-cell biocatalyst for selective oxidation of sesquiterpenes

Supervisor / Institute:

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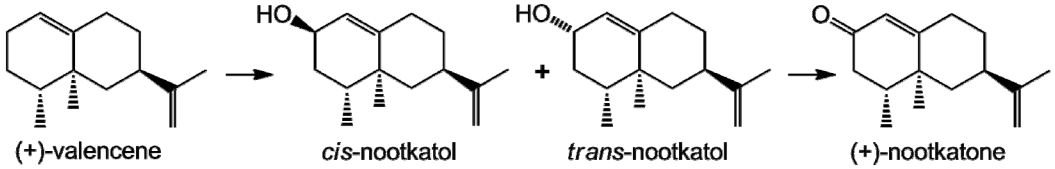
Background:

Sesquiterpenoids are a highly diverse class of natural products that have historically provided a rich source for discovery of biologically active small molecules, such as fragrances, flavourings or pharmaceuticals. These substances are typically produced in low abundance in the host plants, and their isolation consequently suffers from low yields. Furthermore, their chemical syntheses can also be difficult and often involve toxic compounds. For these reasons an attractive alternative strategy is the development of an efficient recombinant microbial whole-cell biocatalyst. A key step here is establishing methods to carry out cytochrome P450-based oxidation chemistry *in vivo*. As a model process selective oxidation of (+)-valencene to (+)-nootkatone with recombinant *E. coli* cells will be developed.



Due to its pleasant grapefruit-like aroma and other interesting sensory characteristics, (+)-nootkatone represents a highly sought-after specialty chemical, with a current market value of ~ 4000 € per kg.

Aims of the project:

The main goal of the project is the development of a recombinant *E. coli* whole-cell biocatalyst for the biooxidation of (+)-valencene to (+)-nootkatone. In our previous work P450 CYP109B1 from *Bacillus subtilis* was implemented and optimised for allylic oxidation of (+)- valencene to a mixture of nootkatol and (+)-nootkatone, with a preference for the alcohol (M. Girhard et al. *Microb. Cell. Fact.* 2009, 8, 36). In the actual project the focus will be on further oxidation of nootkatol to the final product (+)-nootkatone *in vivo* and optimisation of the *E. coli* whole-cell system regarding co-factor regeneration, high productivity and efficient product recovery.

Requirements:

- Master degree in biochemistry, chemistry, biotechnology, or a related discipline.
- Experience in gene cloning and heterologous expression in microbial hosts.