Optimizing terpene synthase expression and bioconversion of FPP as a model system for production of high value sesquiterpenes

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Introduction

Plant essential oils, consisting mainly of terpenoids, are used extensively by the fragrance industry in everyday personal care products and costly perfumes. Extraction processes and steam distillation of the relevant plant source material is often laborious, unreliable and cost demanding. We hereby present one possible alternative route for the production of sesquiterpenoids. The terpene cyclase patchouli synthase (PTS) was chosen as model enzyme. By over-expressing the plant protein in a recombinant E.coli host, large amounts of soluble and active enzyme were acquired. Solubility of the enzyme was further increased by the use of a thioredoxin protein fusion tag. FPP could be bioconverted in a batch process by the recombinant patchouli synthase (PTS) to yield a mixture of sesquiterpenoids. Surprisingly, the composition of the oil varied in comparison to the natural essential oil.

Cloning and Expression of recombinant terpene synthases

The previously described patchouli synthase of P. cibin [1, 2, 3] was amplified from a cDNA-library extracted from plant material. For fast and reliable cloning results, the PCR-based cloning method Gibson assembly [4] was performed. Custom peptide recognition sites for cleavage of the tags and fusion protein could easily be attached in a one-step reaction. Constructs were transformed in E.coli BL21(DE3) and derivative strains. DNA-sequencing revealed a variation from the originally published AA-sequence of 4% (Fig. 4). This variation is probably due to natural sequence variation between cultivars of the exotic plant and shows once again the sequence variability of terpene synthases. All strains were due to natural sequence variation between cultivars of the exotic plant and showed high variability. The fusion protein previously reported to enhance solubility of certain proteins expressed in E.coli [5], increased solubility compared to constructs without fusion protein (Fig. 5). The construct contains an intramolecular polyHis affinity tag. Cleavage of the fusion protein is possible via a TEV (tobacco etch virus)-peptidase site (Fig. 3).

Discussion and Outlook

This study shows, how important the choice of strain and fusion tag strategy is when expressing eukaryotic genes in a prokaryotic host. Thioredoxin-fusion increased the yield of the soluble enzyme while retaining its activity. An IMAC-based Co²⁺ chromatography step yields high amounts of pure enzyme, even though the affinity sequence is located intramolecular. Conversion of FPP to patchouli alcohol and other constituents found in the native patchouli oil was detected by GC-FID and GC-MS. Product composition varied from native patchouli oil, due to several AA mutations. This serves as a proof-of-principle for further upscale-studies with other sesquiterpene synthases in enzyme reactors and enables the development of a bioprocess strategy for these versatile molecules.

Purification of thioredoxin-fused patchouli synthase

Both Ni²⁺ and Co²⁺ ions were evaluated for affinity binding in an IMAC-based purification step. The ions were immobilized on Sartobind® IDA 75 membrane adsorbers (effective membrane area of 75 cm²). After binding of the recombinant enzyme, SDS-PAGE analysis of flow-through fractions during washing of the membrane showed no detectable unbound protein-of-interest. The binding was very specific, although the polyHis is located intramolecular (Fig. 6). The affinity tag might be located in a loop structure on the outer surface of the native protein. Thus it is very accessible for the complexing cations. Protein fractions were eluted with 500 mM imidazole (in 0.1 M sodium acetate, 0.5 M NaCl pH 4.5). Co²⁺ showed a more specific binding in comparison to Ni²⁺. For buffer exchange and protein concentration after purification, Sartorius Vivaspin® Ultrafiltration units with a MWCO of 10 kDa were used.

Enzymatic production of patchouli essential oil

Bioconversion of the substrate FPP was carried out in batch reactions using crude protein extracts and purified PTS enzyme. Reactions were extracted with organic solvents and analyzed by GC-FID and GC-MS. The GC chromatograms of the bioconversion products showed significant variation from native patchouli oil (Fig. 7). According to GC-MS identification, Germacrene A was formed as the main product, (−)-patchouli being one of the major components. This proves how small amino acid variations of terpene synthases can influence the product spectrum dramatically.

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Literature