Simple and efficient route for the production of terpenes by enzymatic means

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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 in a short organic synthesis from natural farnesol. FPP could be bioconverted in a batch process by the recombinant patchoulol synthase (PTS) to yield a mixture of sesquiterpenoids. Surprisingly, the composition of the oil varied in comparison to the natural essential oil.

Optimizing FPP organic synthesis

For the development of a semi-synthetic approach based on the biotransformation of FPP, large amounts of this high-value substrate are required. The procedure according to Keller et al. [1] was found to be the most convenient method for scale-up. The renewable, plant derived educt farnesol is phosphorylated stepwise in a fast and cost effective reaction at low temperatures. During the reaction, mono-, di-, tri- and polyphosphate derivatives of farnesol are formed (Fig. 2). Purification of the crude reaction mixture was performed using a custom build silica gel 60 (230-400 mesh) column (40 cm x 8 cm). The eluent consisted initially of a mixture 1:1 I-ProH / 25 % NH4OH (6:4) which was subsequently changed to a higher ratio of 1:3 I-ProH to accelerate the elution of phosphate esters. The purification delivered a FPP enriched solution, which contained small concentrations of the mono- and triphosphate esters. The reaction was scaled-up to yield product quantities of approximately 1 g FPP per batch reaction with minor contaminations of the mono- and polyphosphate species.

Cloning and Expression of recombinant terpene synthases

The previously described patchoulol synthase of P. cbin [2, 3, 4] was amplified from a cDNA-library extracted from plant material. The gene was fused to various purification tags and to the fusion protein thioredoxin (Fig. 4). For fast and reliable cloning results, the PCR-based cloning method Gibson assembly [5] was performed. Custom peptide recognition sites for cleavage of the tags and fusion protein could easily attached in a one-step reaction. Constructs were transformed into E. coli BL21(DE3) and derivative strains. DNA-sequencing revealed a variation from the originally published AA-sequence of 4 %. All strains were compared based on their ability to produce soluble and active enzyme. The protein tended to form inclusion bodies. By use of IPTG concentration-dependent induction strains and fusion to thioredoxin, the solubility of the enzyme could be improved significantly. Activity of the enzyme was maintained even without cleavage of the fusion protein.

Discussion and Outlook

By combining a short and reliable organic synthesis step with an one-step enzymatic biotransformation using a recombinant plant sesquiterpene synthase, high value sesquiterpenoids could be successfully produced. This semi-synthetic approach serves as a proof-of-principle for further upscale studies in enzyme reactors and bubble column reactors, focused on possible integration in industrial processes. Various immobilization strategies are currently developed to enable efficient recovery and improve the durability of the biocatalyst. Due to their similar properties but high product promisscuity, terpene synthases are very interesting components in biotechnological fragrance production processes.

Enzymatic production of patchouli essential oil

Biotransformation of the substrate FPP was carried out in batch reactions using crude protein extracts and HISTag-purified PTS enzyme. Reactions were extracted with organic solvents and analyzed by GC-FID and GC-MS. The GC chromatograms of the biotransformation products showed significant variation from native patchouli oil (Fig. 6). According to GC-MS identification, Germacrene A was formed as the main product, (−)-patchoulol 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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