Fusion protein and solubility enhancing strategies for heterologous expression of novel plant sesquiterpene synthases

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Introduction

Plant essential oils consist mainly of terpenoids, which are used extensively by the fragrance industry in everyday personal care products and costly perfumes. The extraction process of the relevant plant source material is often laborious, unreliable and cost demanding. Biotechnology enables new approaches to interesting terpene compounds. We over-expressed a plant enzyme catalyzing the synthesis of (+)-zizaene, an interesting and valuable precursor to α-vetivone, in a recombinant E. coli host. As the class of plant sesquiterpene synthases are considered hard-to-express in a soluble form, different solubility enhancing strategies were evaluated in this study. The recombinant enzyme catalyzed the production of (+)-zizaene from farnesyl pyrophosphate.

Expression and purification of the recombinant synthase

Expression experiments using the His-tagged construct (pET16b) yielded no detectable soluble protein production, even during low temperature cultivations. In contrast, both the csPsa-Promoter driven induction as well as fusion to a ubiquitin-modifier moeity resulted in strong and efficient soluble expression of the plant enzyme in E. coli. The relatively low cultivation temperatures (15 °C) needed for csPsa induction slow down the protein translation machinery in the organism, so that proper folding is possible. The SUMO domain is highly soluble in E. coli and was shown previously to enable and enhance the solubility of fusion partners attached to the N-terminus [4]. Purification was performed on a sepharose column decorated with Ni²⁺ (GE Healthcare HiTrap™ IMAC FF 5 ml), using a two elution step method. Both enzymes were successfully purified as shown by SDS-PAGE analysis and western blots of the corresponding fractions.

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. Fusing a modified ubiquitin moiety to the sesquiterpene synthase significantly increased the yield of soluble enzyme while retaining its activity. In addition, use of a csPsa controlled induction at very low temperatures was able to increase expression levels. An IMAC-based Ni²⁺ chromatography step, exploiting a N-terminal His-Tag epitope, produced high amounts of purified (> 90 %) enzyme. In GC-FID analysis of batch bioactivity assays, (+)-zizaene could be identified as the only product resulting from cyclization of FPP. Further studies will be undertaken to characterize kinetic parameters of the novel synthase.

Enzymatic production of (+)-zizaene

Bioconversions of the substrate farnesyl pyrophosphate (FPP) were carried out in ml-scale batch reactions using purified zizaene synthase enzyme (elution fractions). The liquid phase consisting of buffer, enzyme, and substrate were overlaid by isocetane to yield a two-phase system. Optimal reaction conditions were pH 7.0, 1h @ 30 °C. After a short extraction process, the upper organic phase was analyzed by GC-FID and compared to standard sesquiterpene compounds. Both recombinant enzymes were active and produced (+)-zizaene from FPP.

Cloning of protein expression plasmids

The plant Vetrerio zizanoides grows natively in Madagascar and is reknown for its pleasant vetiver oil, consisting mainly of khusimol and α-β-vetivone. Although no terpene cyclase catalyzing the formation of these two components is known to date, a CDNA sequence coding for their precursor (+)-zizaene (GenBank HI931360) was identified earlier [2], but no further data describing the enzyme was published up to date. The sequence was carefully codon optimized, synthesized as two independent double-stranded DNA strings, and cloned into expression vectors using a modified Gibson-assembly [3] approach. Constructs were transformed into E. coli BL21(DE3) competent cells to utilize the T7-Promoter and csPsa-Promoter (cold shock protein A) driven protein expression.

Literature


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