Towards the biocatalytic production of α-humulene: Recombinant expression and characterization of α-humulene synthase from Z. zerumbet Smith

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Introduction

Despite their extensive application in industrial sector as flavors and fragrances in food as well as in perfumery and cosmetics products, many terpenes also have valuable bioactive properties. The sesquiterpene α-humulene possesses anti-inflammatory activities [4]. Although sesquiterpene α-humulene possesses anti-inflammatory activities, its content in food as well as in perfumery and industrial sector as flavors and fragrances is limited.

Expression system & optimization of recombinant α-humulene synthase production

A parametric study was conducted to optimize the induction conditions in the production of recombinant α-humulene synthase in shake-flask cultures. After induction with IPTG, cultivation temperature was lowered from 37 °C to 20 °C to increase protein solubility. Densitometric analysis of cell extracts showed the highest yield of soluble HUM is obtained when inducing expression with 0.15 mM IPTG at an OD600 of 1.0 rel. AU. The ratio of soluble to insoluble protein was determined to be 40:60.

Bioactivity assay and enzyme characterization

For characterization experiments the histagged α-humulene synthase was purified from cell extracts by immobilized metal ion chromatography (IMAC) using NTAligands decorated with Co2+-ions. The isoelectric point was determined by 2D gel electrophoresis. To assess the product spectrum of the recombinant HUM, bioactivity assays with the substrate farnesyl diphosphate (FPP) were performed in a mi-scale two-phase system with an aqueous reaction solution consisting of activity buffer, enzyme and substrate, and an organic solvent overlay of iso-octane. After product extraction to the organic phase the samples were analyzed by GC-FID. Chromatograms showed two product peaks (Fig. 3). By comparison to commercially available standards the major product could be identified as α-humulene (94.5 %) and the minor product of the recombinant enzyme as β-caryophyllene (5.5 %).

Characterization of enzyme activity in dependence of temperature and pH was carried out by analyzing reaction rates at various temperatures and pH (Fig. 4). The recombinant HUM showed optimal activity at 38 °C and pH 7.5. Kinetic properties were measured using a discontinuous kinetic assay with above determined optimal reaction conditions.

Conclusion

We have successfully expressed, purified and biochemically characterized the sesquiterpene synthase α-humulene synthase from shampoo ginger (Z. zerumbet Smith) for the biocatalytic production of α-humulene. This is the first time to report key enzymatic properties of α-humulene synthase. Since the synthesis of macrocyclic sesquiterpenes are chemically challenging, bioconversion strategies can provide an efficient route for large scale production. By combining synthetic chemistry (for terpene precursor synthesis) and biotechnology (for recombinant terpene synthase production) high value terpenes can be produced, offering an alternative to approaches based on pathway engineered microorganisms.

References


Acknowledgement

This work is funded by the European Development Fund (EFRE): Innovation Network „Refinement of plant resources“ (ZW 8-80130940).