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Combining enzyme engineering with bioprocess development for a sustainable metaraminol synthesis

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PURPOSE OF THE ABSTRACT

Chiral amino alcohols are subject of interest in the pharmaceutical and fine chemical industries as they play an important role as active pharmaceutical ingredients or precursor molecules for drugs. In recent years, our working group has focused on metaraminol as showcase for the enzymatic production of chiral amino alcohols. An enzymatic two-step reaction was successfully established, starting with the carboligation of 3-hydroxy benzaldehyde and pyruvate towards the intermediate (R)-3-OH-phenylacetylcarbinol ((R)-3-OH-PAC) catalyzed by the pyruvate decarboxylase of Acetobacter pasteurianus (ApPDC). This step is followed by the reductive amination of (R)-3-OH-PAC towards metaraminol using L-alanine as amino donor catalyzed by the amine transaminase of Chromobacterium violaceum (Cv2025) [1,2]. By coupling the enzymatic cascade with an in-situ liquid-liquid extraction, 69 % metaraminol were yielded after three extraction steps [2]. However, to make the process economically feasible, higher product yields and catalyst reuse are envisaged. However, these goals are challenging due to stability issues. Within the scope of our new project MetaProcess, these challenges are targeted by molecular dynamics simulations elucidating the weak points of the applied enzymes. Additionally, the operational stability of the catalysts is to be enhanced by the application of rational design strategies.

Besides enzyme engineering, biocatalyst costs can be minimized by optimizing the production process of the catalysts themselves. As whole cell catalysts are applied as a cheap catalyst formulation, high cell densities and simultaneously high specific activities of the heterologous produced enzymes inside the cells are targeted. After optimization of the fed-batch cultivation, first approaches with the amine transaminase of Bacillus megaterium showed a specific activity of 0.81 ± 0.03 U/mg dry cell weight and a cell density of 223 ± 14 g/L wet cell weight. The optimized cultivation protocol led to an overall 45-fold increase of activity compared to shake flask experiments. In the future, this fed-batch protocol can be used to produce the engineered enzymes for the metaraminol synthesis. By combining both strategies, enzyme engineering and bioprocess engineering, the enzymatic production process of chiral amino alcohols can be designed in an economically feasible manner representing a green alternative to classical chemical production routes.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

enzyme engineering | bioprocess engineering | enzymatic cascade | chiral amino alcohols

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