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Development of a biological chassis for amine synthesis

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PURPOSE OF THE ABSTRACT

Chiral amines are building blocks for various pharmaceuticals and fine chemicals. Biocatalysis using amine dehydrogenases (AmDHs) can enable the direct synthesis of chiral primary amines from prochiral ketones using ammonia as inexpensive amine donor while generating water as sole by-product (Ducrot et al., 2000; Mutti and Knaus, 2021). These AmDHs are NAD(P)H-dependent enzymes that perform a reductive amination, and can be utilized in the form of purified formenzymes, cell-free extracts, or whole-cells (Houwman et al., 2019). The latter are advantageous in terms of increased enzyme stability, low preparation cost, and direct applicability. However, toxicity of substrates, intermediates, and/or products is, among others, a potential drawback when living cells are used. We therefore aimed at improving the biocatalytic potential of E. coli strains expressing oxidoreductase enzymes for sustainable chiral amine production. In this context, we adapted E. coli BL21 (DE3) cells to grow in the presence of high amine concentrations via adaptive directed evolution using the GM3 technology for automated continuous culture (Mutzel and Mutzel, 2000). The evolved strains displayed up to a five-fold increase in amine tolerance compared to the wild-type strain. Co-expression of genes encoding for AmDH and formate dehydrogenase (FDH) activity in the tested strains, enabled the stereoselective bioamination (ee >99%) of a set of prochiral methyl ketones without any exogenous addition of NAD(P)H. In fact, using a resting cell setup, the adapted cells exhibited superior biocatalytic performance contrasted to the non-adapted ones. Notably, with the adapted cells, some of the tested substrates were converted to the corresponding amine with approximately 80% conversion at high substrate loading (i.e., 200 mM). These results were attributed to an increased survivability of the adapted cells compared to the non-adapted cells during the biotransformation reaction. Future work aims at obtaining a chassis of production by continuing to improve the strains and analyzing the adaptation mechanisms. In conclusion, our E. coli resting cell system represents an important advance towards the development of more robust biocatalysis for amine production with excellent chemical and optical purity in a frame of sustainable chemistry.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Whole-cell biocatalysis | Amine toxicity | Adaptive evolution

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