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Expanding the amine substrate scope of C-N lyases by homologue discovery

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PURPOSE OF THE ABSTRACT

C-N lyases are enzymes that naturally catalyze the cleavage of carbon-nitrogen bonds to yield amines and α,β-unsaturated mono- or dicarboxylic acids. Their ability to reverse this reaction towards C-N bond formation can be a powerful tool for the synthesis of optically pure (un)natural amino acids, which are important synthetic precursors for pharmaceuticals and food additives [1].

The enzyme ethylenediamine-N,N'-disuccinic acid lyase (EDDS lyase), from Chelativorans sp. BNC1, naturally catalyzes a reversible two-step sequential addition of ethylenediamine to two molecules of fumarate, providing (S,S)-EDDS as the final product [2]. This enzyme shows a broad substrate scope, enabling the asymmetric addition of ammonia, various mono- and diamines [2], homo- and hetero cycloalkyl amines [3], arylamines [4,5], arylhydrazines [4], and arylalkylamines [6] to fumarate yielding the corresponding N-functionalized aspartic acids. By means of a one-pot, two-step chemoenzymatic approach, complex heterocycles like pyrazolidinones, dihydrobenzoxazinones and dihydroquinoxalinones could be obtained with high optical purities [4,5]. Furthermore, EDDS lyase can accept a wide variety of (non)natural amino acids with terminal amino groups, supporting the chemoenzymatic synthesis of the fungal natural products aspergillomarasmine A and various related aminocarboxylic acids [7]. Lastly, an engineered variant of EDDS lyase was applied for the enantioselective synthesis of several N-substituted aspartic acids, including precursors to the important dipeptide sweeteners neotame and advantame [8].

The very broad amine substrate scope makes EDDS lyase a promising template for homologue discovery to identify biocatalysts that can convert new unnatural substrates. Here, we used genome mining strategies to select various EDDS lyase homologues from bacteria and explored their biocatalytic applicability. The preliminary results show that some of the homologues are highly promiscuous and can utilize new substrates, expanding the range of C-N bond formation reactions that can be accessed.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

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