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ORIGINAL PAPER

Production of L-tryptophan by enantioselective hydrolysis of D,L-tryptophanamide using a newly isolated bacterium

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Bacterial strain ZJB-09211 capable of amidase production has recently been isolated from soil samples. The strain is able to asymmetrically hydrolyze L-tryptophanamide from D,L-tryptophanamide to produce L-tryptophan in high yield and with excellent stereoselectivity (enantiomeric excess > 99.9 %, and enantiomeric ratio > 200). Strain ZJB-09211 has been identified as *Flavobacterium aquatile* based on the cell morphology analysis, physiological tests, and the 16S rDNA sequence analysis. Optimization of the fermentation medium led to an about six-fold increase in the amidase activity of strain ZJB-09211, which reached 501.5 U L^{-1}. Substrate specifity and stereoselectivity investigations revealed that amidase of *F. aquatile* possessed a broad substrate spectrum and high enantioselectivity.

Keywords: L-tryptophan, *Flavobacterium aquatile*, amidase, enantioselective biocatalysis

Introduction

L-Tryptophan (L-Trp) has a broad range of applications in the pharmaceutical and food industries, and as a precursor in the preparation of other chiral compounds (Azuma et al., 1993; Koçaba et al., 2006; Mateus et al., 2004). Many chemical and biological methods for L-Trp production have been developed. Current biological methods, which include fermentation and enzymatic synthesis, are less economically attractive than the chemical ones (Katsumata & Ikeda, 1993; Ikeda, 2006; Winnicka & Kańska, 2009; Eggers et al., 1988). The fermentation route suffers from low yields; whereas the enzymatic route requires a chiral substrate as the starting material and is hampered by the inhibitory activity of another substrate, indole. However, the chemical methods usually result in the formation of mixtures of L-Trp and D-tryptophan (D-Trp).

Therefore, improved biological methods for L-Trp production involving stereoselective enzymes are being sought for. Amidases possess excellent enantioselectivity and, therefore, have become promising tools for the synthesis of chiral carboxylic acids and their derivatives (Shaw et al., 2003; Martinkova & Mylerova, 2003; Wang et al., 2010). In particular, amino-acid amidases can steroselectively hydrolyze L- or D-amino-acid amide to the corresponding chiral amino acids and ammonia. Because racemic amino-acid amides can be easily synthesized from aldehydes, hydrogen cyanides, and ammonium (Komeda & Asano, 2008; Asano & Yamaguchi, 2005a, 2005b), amidase-catalyzed asymmetrical hydrolysis of amino-acid amides presents a promising method for the production of optically pure amino acids. Although various microbial amidases have been reported, their enzymatic characteristics vary remarkably (Wang et al., 2008). The amidase activity is also significantly affected by the culture conditions, which means that optimization of the culture medium is a crucial requirement (Açıkel et al., 2010).

Here, isolation of a microorganism that can produce L-Trp in high yields and with excellent enantioselectivity through asymmetric hydrolysis of the L-enantiomer present in racemic tryptophanamide

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