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Novel thermostable aminotransferase from Streptomyces sp.: discovery and functional characterization

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PURPOSE OF THE ABSTRACT

Aminotransferases (ATAs, E.C. 2.6.1.x) are pyridoxal-5'-phosphate (PLP) dependent enzymes which catalyze the formation of primary chiral amines by transferring an amino group from an amine donor to a prochiral ketone (acceptor), thus generating a new stereogenic centre at the end of the reaction. ATAs are of great industrial interest for the production of enantiomerically pure chiral amines.

In this study, a gene encoding for a putative transaminase was discovered in Streptomyces sp. BV333 employing a combined identification technique, which included functional screening and genome mining. The identified gene shared sequence similarities with thermostable ATA sequences, including those from Thermomicrobium roseii (43% identity) [1] and from hot spring metagenomes (B3-ATA, 41% identity) [2]. The corresponding enzyme (Sbv333-ATA) was successfully produced in Escherichia coli co-expressing GroES/GroEL chaperons.

Interestingly, it was found that Sbv333-ATA was extremely thermostable, with a melting temperature (Tm) that was only marginally lower (85 °C) than those of the most thermostable transaminases previously reported (87–88 °C) [2]. Moreover, Sbv333-ATA displayed a broad substrate specificity, as far as the amino acceptor spectrum is concerned, and a remarkable activity in the transamination of β-ketoesters, which are rarely accepted by known ATAs [3].

Recently, new studies have been carried out to evaluate enzyme stability in the presence of organic (co)solvents. Sbv333-ATA proved to be stable in the presence of up to 20% (v/v) of the water-miscible-co-solvents methanol, ethanol, acetonitrile, dimethylsulfoxide, and in biphasic systems with petroleum ether, toluene and ethyl acetate as organic phase. Furthermore, new amine donors were evaluated as possible substrates for this ATA.

This enzyme was also crystallized, and the high-resolution structures of both the native form and the complex with the inhibitor gabaculine were determined.

Mutagenesis studies are being conducted to broaden the substrate scope of Sbv333-ATA.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

transaminases | enzyme discovery | enzyme engineering | biocatalysis

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