Comparative Biochemical Characterisation of D-Allulose 3-Epimerase Among Different Strains and Its Applications for D-Allulose Production

呂詠懷(5109)

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Outline

一、Introduction

二、D-Allulose 3-epimerase of *Bacillus* sp. origin manifests profuse heat-stability and noteworthy potential of d-fructose epimerization

三、Biochemical characterization, structure-guided mutagenesis, and application of a recombinant D-allulose 3-epimerase from *Christensenellaceae* bacterium for the biocatalytic production of D-allulose

四、Conclusion

Abstract

A novel D-allulose 3-epimerase (DaeB) was successfully cloned and expressed from the plant probiotic *Bacillus* sp. KCTC 13219. This enzyme maintains high activity across a broad pH range of 6.0–11.0 and catalyzes the conversion of d-fructose to D-allulose at temperatures between 35–70 °C, with activity consistently exceeding 50%. DaeB exhibits exceptional stability, demonstrating a half-life of 25 days at 50 °C, alongside high catalytic efficiency (k_{cat} = 367 s^{-1}). Further application in the conversion of high-concentration D-fructose (700 g/L) combined with yeast fermentation simultaneously yielded approximately 200 g/L D-allulose and 214 g/L ethanol. Furthermore, the other DAEase which is characterized from bacteria belonging to the family *Cristenellaceae*. Recombinant CbDAE exhibits optimal activity at pH 7.5 and 55 °C, retaining over 60% activity between 40–70 °C, with catalytic enhancement achievable through Co^{2+} supplementation. At 55 °C, its half-life is 12.4 hours, displaying high affinity for D-fructose but low catalytic efficiency. Structure-guided engineering yielded the optimal double mutant G36N/W112E, exhibiting a 4.21-fold increase in activity and reducing reaction time by 40% for 500 g/L D-fructose. Successful applications were also demonstrated in honey and apple juice. Both DAEases above provides highly efficient biocatalyst with significant potential for industrial applications.

Reference

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