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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Studies on a novel alditol oxidase produced by *Penicillium* sp. KU-1 isolated from soil
（土壌から単離した*Penicillium* sp. KU-1株が生産する新規アルジトール酸化酵素に関する研究）

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Rare sugars are defined as monosaccharides and their derivatives existing in nature in limited quantities. Recent studies have shown that certain rare sugars have pharmacologically beneficial functions and may be used as low-calorie sweeteners, agrochemicals, and pharmaceuticals. While these desirable properties have been identified, only a limited number of rare sugars have been intensively studied. Most rare sugars have not been studied in depth, since processes for their mass-production have not been established. The conventional enzymatic production strategy for rare sugars is based on isomerization or epimerization reactions. However, in the production of rare sugars by using this strategy, efficient mass-production has not been achieved in most cases, due to the product often has a lower yield due to chemical equilibrium. To overcome this problem, this study focused on alditol oxidases that catalyze the irreversible oxidation of alditols to produce the corresponding aldoses with an expected conversion ratio of 100%. In the process of screening for microorganisms that produce alditol oxidase, *Penicillium* sp. KU-1 was isolated from soil.

Chapter 1: Culture condition of *Penicillium* sp. KU-1 for the production of alditol oxidase

The culture conditions of *Penicillium* sp. KU-1 for the production of alditol oxidase (AldOx) were examined in the solid-state fermentation (SSF) and the submerged fermentation (SmF). In SSF, AldOx activity obtained from wheat-bran culture was about 8-hold higher than that of rice-bran culture. While, AldOx was not produced extracellularly at all under any tested conditions in SmF, so the enzyme was suggested to be specifically produced enzymes only in SSF.

Chapter 2: Purification and characterization of alditol oxidase

The apparent molecular weight of the purified enzyme was estimated to be 143 kDa which is composed of two same subunits. The enzyme showed broad substrate specificity and acted on 19 kinds of alditols. Among them, the highest activity showed toward to erythritol and the highest catalytic efficiency was also observed with erythritol ($k$cat/Km: 355 $M^{-1}s^{-1}$).

Chapter 3: Gene cloning and expression of alditol oxidase in *Escherichia coli* and *Pichia pastoris*

The recombinant AldOx expressed in *E. coli* BL21(DE3) and Rosetta-gami B (DE3) strains were obtained as insoluble inclusion bodies. The formation of inclusion bodies was not improved by using a pCold TF vector suitable for expression of solubilized

proteins. For expression in *P. pastoris*, no detectable AldOx activity was not found in the extracellular fraction for 6 days. In the heterologous expression system, this enzyme could not be obtained in the active form.

Chapter 4: Immobilization of alditol oxidase and its application in production of rare sugars

The anion exchange resin TOYOPEAL DEAE-650M was found to be the most suitable carrier for immobilization of AldOx. The immobilized AldOx could be prepared with an activity yield of about 60%. In addition, efficient production of L-gulose, a rare sugar, was achieved by continuous reaction using the immobilized AldOx repeatedly. The produced L-gulose was identified by ^{13}C NMR analysis and measurement of the specific optical rotation.

Based on the findings in this study, AldOx from *Penicillium* sp. KU-1 has potential applications in the production of various aldoses including rare sugars. In particular, the establishment of an efficient and cost-effective production method of L-gulose will greatly contribute to the pharmaceutical industry. Moreover, the application of AldOx to the production of rare sugars will greatly contribute to further research on rare sugars.