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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

Cloning and functional expression of the D-glucoside 3-dehydrogenase complex from *Rhizobium* sp. S10 in *Escherichia coli* and its application for rare sugars production
（*Rhizobium* sp. S10由来D-グルコシド3-デヒドロゲナーゼ遺伝子複合体のクローニングと大腸菌内での組換え酵素生産および希少糖生産への応用）

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Chapter 1: D-Glucoside 3-dehydrogenase (G3DH) producing microorganisms were screened from soil through two-step screening; medium screening and enzyme assay screening. G3DH is an enzyme that regioselectively dehydrogenates glycosides at the C-3 position to the corresponding 3-ketoglycoside. In this study, a novel G3DH with high activity was isolated from a soil microorganism and characterized as a flavin adenine dinucleotide-dependent dehydrogenase. The sequence analysis from newly isolate strain indicated that this microorganism belongs to genus *Rhizobium*, therefore, called *Rhizobium* sp. L35. The molecular weight of G3DH from *Rhizobium* sp. L35 was determined to be 67,000 by SDS-PAGE and 131,000 by size-exclusion chromatography, suggesting that it is a dimeric enzyme. The maximum G3DH activity could be detected at pH 7.5 and 40°C. This enzyme was stable between pH 6.0 and 11.0, and below 40°C (half-life of 3 hr at 40°C and 50 min at 45°C). Finally, it showed broad substrate specificity towards various glycosides, especially β-1,4-linked disaccharides such as cellobiose and lactose.

Chapter 2: The novel isolated *Rhizobium* sp. S10 was identified as D-glucoside 3-dehydrogenase producing microbes. Therefore, the gene encoding for G3DH from *Rhizobium* sp. S10 was cloned and overexpressed in *Escherichia coli* strain JM109 as a soluble enzyme. The yield flavoenzyme contains 1,686 bp of a complete open reading frame and encodes for 561 amino acid residues. The flavin adenine dinucleotide binding region locates in the N-terminal region of G3DH. The recombinant G3DH was purified with specific activity of 38.54 u/mg and the molecular weight was estimated to be 66 kDa by SDS-PAGE. The purified rG3DH showed highest activity at pH 7.0, 40°C toward cellobiose. It can also oxidize a broad range of mono-disaccharides including saccharide derivatives.

Chapter 3: D-Glucoside 3-dehydrogenase from *Rhizobium* sp. L35 and *Rhizobium* sp. S10 showed an outstanding oxidation activity on the C-3 position of various glycosides to their corresponding 3-ketoglycosides. The partial purified G3DH from *Rhizobium* sp. L35 was

utilized to produce D-allose from cellobiose and the final yield was approximately 30%, which was three-time higher than the conventional method. The resting cell reaction by the recombinant *E. coli* harboring pQE60-G3DH expression vector was performed. The glycosides oxidizing activity combined with chemical reaction, could produce D-gulose from lactitol via 3-ketolactitol with roughly 8% yield.