# Characterization of D-Glucoside 3-Dehydrogenase from *Rhizobium* sp. L35 and Its Application for D-Allose Production

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## ABSTRACT

***D-allose is a rare monosaccharide that possesses some interesting features for enhancing the antitumor effects of chemoradiotherapy and treatment of cancer, however, efficient production of D-allose with high percent yield has not yet been reported. In this study, we proposed a utilization of D-glucoside 3-dehydrogenase together with chemical reaction to improve the D-allose production. A D-glucoside 3-dehydrogenase, which regioselectively dehydrogenates glycosides at the C-3 position to the corresponding 3-ketoglycoside, was newly isolated as Rhizobium* sp. *L35 and characterized as a flavin adenine dinucleotide-dependent dehydrogenase. Its molecular weight was determined to be 67,000 by SDS-PAGE and 131,000 by size-exclusion chromatography, suggesting that it is a dimeric enzyme. Its optimum pH and temperature with respect to activity were 7.5 and 40°C, respectively. It was stable between pH 6.0 and 11.0, and below 40°C (half-life of 3 h at 40°C and 50 min at 45°C). The enzyme showed broad substrate specificity towards various glycosides, especially β-1,4-linked disaccharides such as cellobiose and lactose. Finally, D-allose production was performed by a three-step process of enzymatic-dehydrogenation, chemical reduction and acid-hydrolysis, using cellobiose as the starting material. The yield of D-allose was estimated to be 30% from cellobiose. This result indicates that D-allose can be produced by this strategy three-fold higher than the conventional method.***

**Keywords:** D-Glucoside 3-dehydrogenase, 3-Ketoglycoside, Oxidoreductase, Rare sugar, D-Allose

## INTRODUCTION

D-Glucoside 3-dehydrogenase (G3DH; EC 1.1.99.13) was firstly discovered and characterized in *Agrobacterium tumefaciens* (Hayano et al., 1967) and later found in other organisms such as *Flavobacterium saccharophilum* (Takechi et al., 1986; Takeuchi et al., 1988), *Agaricus bisporus* D649 (Morrison et al., 1999), *Halomonas* sp. α-15 (Kojima et al., 1999), and *Stenotrophomonas maltophilia* CCTCC M 204024 (Zhang et al., 2006). G3DH is a flavin adenine dinucleotide-dependent (FAD-dependent) oxidoreductase that catalyzes the dehydrogenation of hydroxyl group at the C-3 position of D-glucose and other aldopyranosides to their corresponding 3-ketoglucoside and 3-ketopyranosides (Maeda et al., 2001). When disaccharides are provided as substrates, G3DH would catalyze from the non-reducing end of the glycosides (Bernaerts, 1963).

A number of enzymes producing 3-ketoglycosides have been reported, including pyranose oxidase (EC 1.1.3.10) from *Phanerochaete chrysosporium* (Giffhorn, 2000) and pyranose dehydrogenase (EC 1.1.99.29) from *A. bisporus* (Volc et al., 1997; Volc et al., 1998), *Macrolepiota rhacodes* (Volc et al., 2001), and *A. xanthodermus* (Kujawa et al., 2007). These oxidoreductases are well known for their application for the sugars bioconversion, and for blood glucose level measurement (Kojima et al., 1999). In addition, the utilization of G3DH from *Halomonas* sp. α-15 for measurement of 1,5-anhydro-D-glucitol, a clinical marker for diabetes, has an advantage over other oxidoreductases because of its regioselectivity property (Tsugawa et al., 1996).

The reaction products, 3-ketoglycosides are attractive for production of commodity chemicals such as polymers and surfactants (Eltz, 1968) and for production of rare sugars. Rare sugars are defined as saccharides and their derivatives occurring only in a small quantity in nature. D-allose, classified as a rare sugar, has been reported several times for its physiological features and has attracted attention as a precursor for other unnatural compounds production (Levin et al., 1995; Kasiganesan et al., 2009) and as an anti-tumor drug (Moyroud et al., 1999; Yamaguchi et al., 2008).