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# Synthesis and characterization of novel astragalin galactosides using β-galactosidase from *Bacillus circulans*

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ABSTRACT

Astragalin (kaempferol-3-*O*-β-D-glucopyranoside, Ast) is a kind of flavonoid known to have anti-oxidant, anti-HIV, anti-allergic, and anti-inflammatory effects. It has low solubility in water. In this study, novel astragalin galactosides (Ast-Gals) were synthesized using β-galactosidase from *Bacillus circulans* and reaction conditions were optimized to increase the conversion yield of astragallin. Purified Ast-Gal1 (11.6% of Ast used, w/w) and Ast-Gal2 (6.7% of Ast used, w/w) were obtained by medium pressure chromatography (MPLC) with silica $C_{18}$ column and open column packed with Sephadex LH-20. The structures of Ast-Gal1 and Ast-Gal2 were identified by nuclear magnetic resonance (NMR) to be kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-galactopyranoside and kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 4)-β-D-galactopyranoside, respectively. The water solubility of Ast, Ast-Gal1, and Ast-Gal2 were 28.2 ± 1.2 mg/L, 38,300 ± 3.5 mg/L, and 38,800 ± 2.8 mg/L, respectively. The $SC_{50}$ value (the concentration required to scavenge 50% of the ABTS·+) of Ast, Ast-Gal1, and Ast-Gal2 were 5.1 ± 1.6 μM, 6.5 ± 0.4 μM, and 4.9 ± 1.1 μM, respectively. The $IC_{50}$ values (the half maximal inhibitory concentration) of Ast, Ast-Gal1, and Ast-Gal2 against angiotensin converting enzyme (ACE) were 171.0 ± 1.2 μM, 186.0 μM, and 139.0 ± 0.2 μM, respectively.

## 1. Introduction

Kaempferol-3-*O*-*β*-D-glucopyranoside (Ast, astragalin) is a flavonoid extracted from leaves of persimmon, mulberry, root of *Astragalus membranaceus* Bunge (Hwang-ki), or green tea [1]. In preclinical studies, Ast has shown numerous pharmacological activities such as anti-oxidant [2], anti-HIV, anti-allergic [3], and anti-inflammatory activities [4]. However, Ast is poorly soluble in water, although it already has one glucosyl unit in the C-3 position of its C ring. Poor water solubility has resulted in its slow absorption, inadequate and variable bioavailability, gastrointestinal mucosal toxicity, and delayed clinical development [5,6]. Transglycosylation catalyzed by enzymes has been used to improve the physicochemical properties (such as water solubility and oxidative stability) of various compounds [7] to enter cells throughout sodium-glucose co-transporter 1 [8]. Until now, only Ast glucosides are alternatives that can overcome this problem. Kim et al. (2012) [9] have reported that Ast glucosides have higher MMP1 inhibition and antioxidant activity [9]. Especially, it has been reported that Ast glucosides, but not Ast, can inhibit fructose transporter (GLUT5) [10].

β-Galactosidases (EC 3.2.1.23) catalyze the hydrolysis and transgalactosylation of β-D-galactopyranosides (such as lactose) [11]. These

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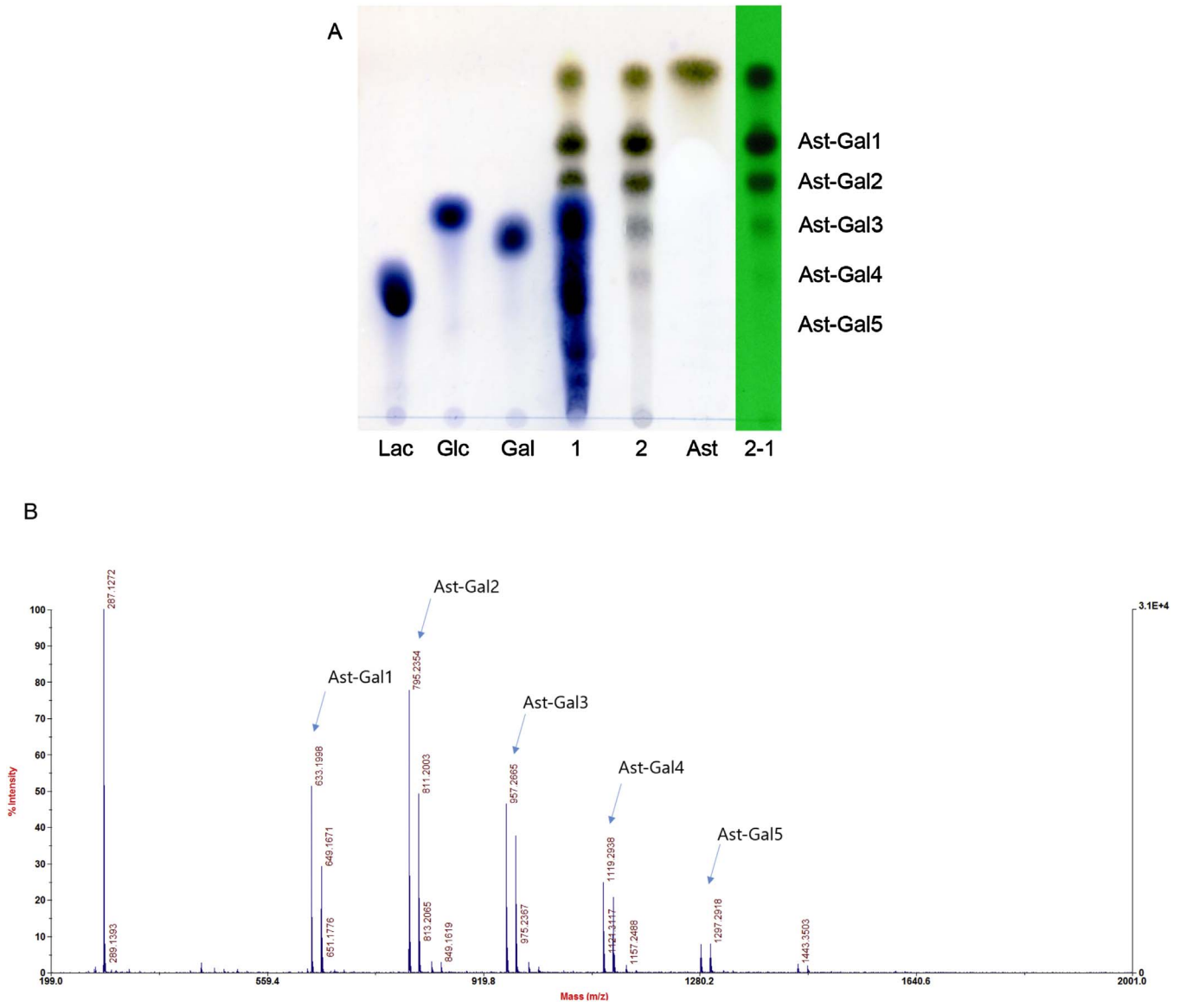


**Fig. 1.** Analysis of β-galactosidase acceptor reaction products using thin layer chromatography (A) and MALDI-TOF-MS (B). (A) Lac: lactose 100 mM; Glc: glucose 100 mM; Gal: galactose 100 mM; lane 1: acceptor reaction products; lane 2: galactosylation products after removing saccharides via MPLC $C_{18}$ column; Ast, astragalin 50 mM; lane 2-1: Ast-Gal detected by 254 nm UV light. (B) MALDI-TOF-MS spectra of Ast-Gals reaction mixture. Ast acceptor reaction mixture containing 3 U/mL β-galactosidase, 300 mM lactose, 50 mM astragalin in 20 mM potassium phosphate buffer (pH 6.0) was reacted at 60 °C for 12 h. One μl of reaction mixtures was spotted onto silica gel 60$F_{254}$TLC plate (Merck, Darmstadt, Germany) and developed in nitromethane: *n*-propyl alcohol: water (2: 5: 1.5, v/v/v) solvent. Ast and Ast-Gals on TLC were visualized under $UV_{254nm}$ or by dipping the TLC plate into a solvent mixture of 0.5 (w/v) N-(1-naphthyl) ethylenediamine dihydrochloride and 5% (w/v) sulfuric acid in methanol followed by heating at 125 °C for 10 min. MALDI-TOF MS.

enzymes are profusely utilized in the dairy industry due to its ability to hydrolyze lactose from milk [12]. In addition, they can produce various sizes of galactooligosaccharides [13–15] as alternative sweetener. Among β-galactosidases, β-galactosidases from *Bacillus circulans* have been a subject of investigation because of their high transgalactosylation activities compared to other β-glactosidases [14–17]. They have four isoforms: β-gal-A (189 kDa), β-gal-B (154 kDa), β-gal-C (134 kDa), and β-gal-D (91 kDa). They contribute to galactooligosaccharides synthesis with different productivities [18]. The transferase activity of β-galactosidase from *Bacillus circulans* has been applied to the synthesis of lactosucrose [19], acetyl-lactosamine [20], and galactosylated derivates [21,22]. This enzyme is an industrial enzyme. It is commercially available at food grade with trade name of Biolacta FN5.

In this study, we used commercially produced β-galactosidase from *Bacillus circulans* for transgalactosylation using lactose as a substrate with Ast as an acceptor. The effects of reaction factors were optimized using response surface method (RSM) program based on the conversion yields of Ast to Ast-Gals products. The structures and biological function of Ast-Gals were determined.

## 2. Materials and methods

### 2.1. Transgalactosylation of astragalin

The commercial β-galactosidase produced from *Bacillus circulans* (Lactazyme-B™, Genofocus, Daejeon, Korea) was used. Ast was provided by Amore Pacific Corporation (Yongin, Korea). Ast was prepared with absolute dimethylsulfoxide (DMSO) as a stock solution of 500 mM. Ast acceptor reaction mixture contained 3 U/mL β-galactosidase, 300 mM lactose, and 50 mM astragalin in 10% (v/v) DMSO (Duksan, Gyeonggi-do, Korea) and 20 mM potassium phosphate buffer (pH 6.0). The reaction was incubated at 60 °C for 12 h. One μl of reaction mixtures was spotted onto silica gel 60$F_{254}$TLC plate (Merck, Darmstadt, Germany) and developed in nitromethane: *n*-propyl alcohol: