

Communication

## Biotransformation of Ginsenoside Rf to Rh<sub>1</sub> by Recombinant $\beta$ -Glucosidase

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**Abstract:** An *Aspergillus niger* strain was isolated from the soil around ginseng fruit. *In vitro* enzyme assays showed that this strain had the ability to transform total ginsenosides (TGS) into several new products. In a further biochemical study, a  $\beta$ -glucosidase gene isolated from this strain, *bglI*, was expressed in *Saccharomyces cerevisiae*. His-tagged BGL1 protein (~170 kD) showed the ability to transform ginsenoside Rf into Rh<sub>1</sub>.

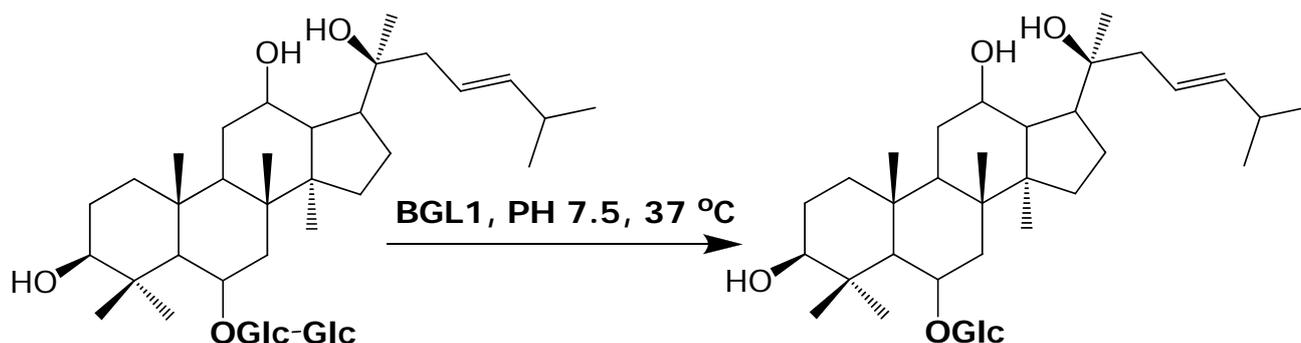
**Keywords:** *Aspergillus niger*;  $\beta$ -glucosidase; ginsenoside Rf; ginsenoside Rh<sub>1</sub>

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## Introduction

The root of *Panax ginseng* C.A. MEYER is frequently used in China as a traditional medicine [1]. Ginsenosides, as the major components of ginseng, have been reported to show various biological activities, *eg.* anti-tumor, anti-inflammatory, immune-modulatory and anti-aging effects [2-5]. Among the 30 previously reported ginsenosides, Rg<sub>3</sub>, compound K (CK) and Rh<sub>1</sub> showed highly cytotoxicity against tumor cells [6-9]. CK was proven to be produced by intestinal microorganisms after oral administration of Rg<sub>3</sub>, and then further esterified to sustain it longer in the body [10-11]. During our continued work on bioactive ginsenosides, an *Aspergillus niger* strain was isolated from the soil around ginseng fruit. *In vitro* enzyme assays showed that this strain had the ability to transform total ginsenosides (TGS) into several new products [12]. In the subsequent biochemical study, a  $\beta$ -glucosidase gene isolated from this strain, *bgl1*, showed the ability to transform ginsenoside Rf to Rh<sub>1</sub> (Figure 1).

**Figure 1.** Scheme of biotransformation from ginsenoside Rf to Rh<sub>1</sub> catalyzed by BGL1.

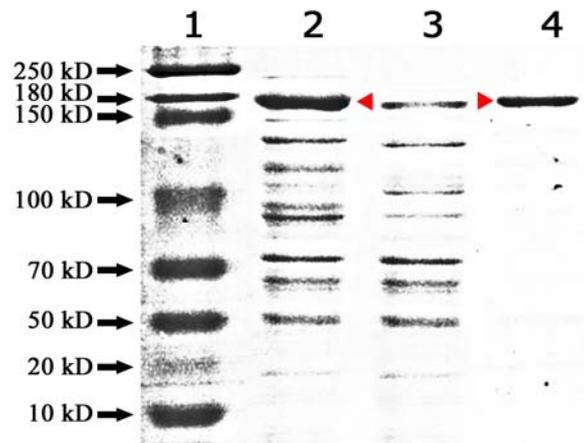


## Results and Discussion

### *Expression and purification of BGL1 in Saccharomyces cerevisiae*

In the present study, the *bgl1* gene isolated from an *Aspergillus niger* strain, which encodes a glucosidase, was cloned into the yeast shuttle vector pRS423 and introduced into *Saccharomyces cerevisiae* (MGY70). SDS-PAGE analysis showed strong expression of a ~170 kDa his-tagged BGL1 protein at 37 °C (Figure 2, lane 2). The empty pRS423 vector was used as control (Figure 2, lane 3). Soluble recombinant protein purified from cultures grown at 37°C by his-tagging yielded a single distinct band after SDS-PAGE (Figure 2, lane 4). After dialysis, the purified recombinant BGL1 was quantified at 0.9  $\mu\text{g } \mu\text{L}^{-1}$  (total of 5.1 mg from 6 g bacteria cell pellet).

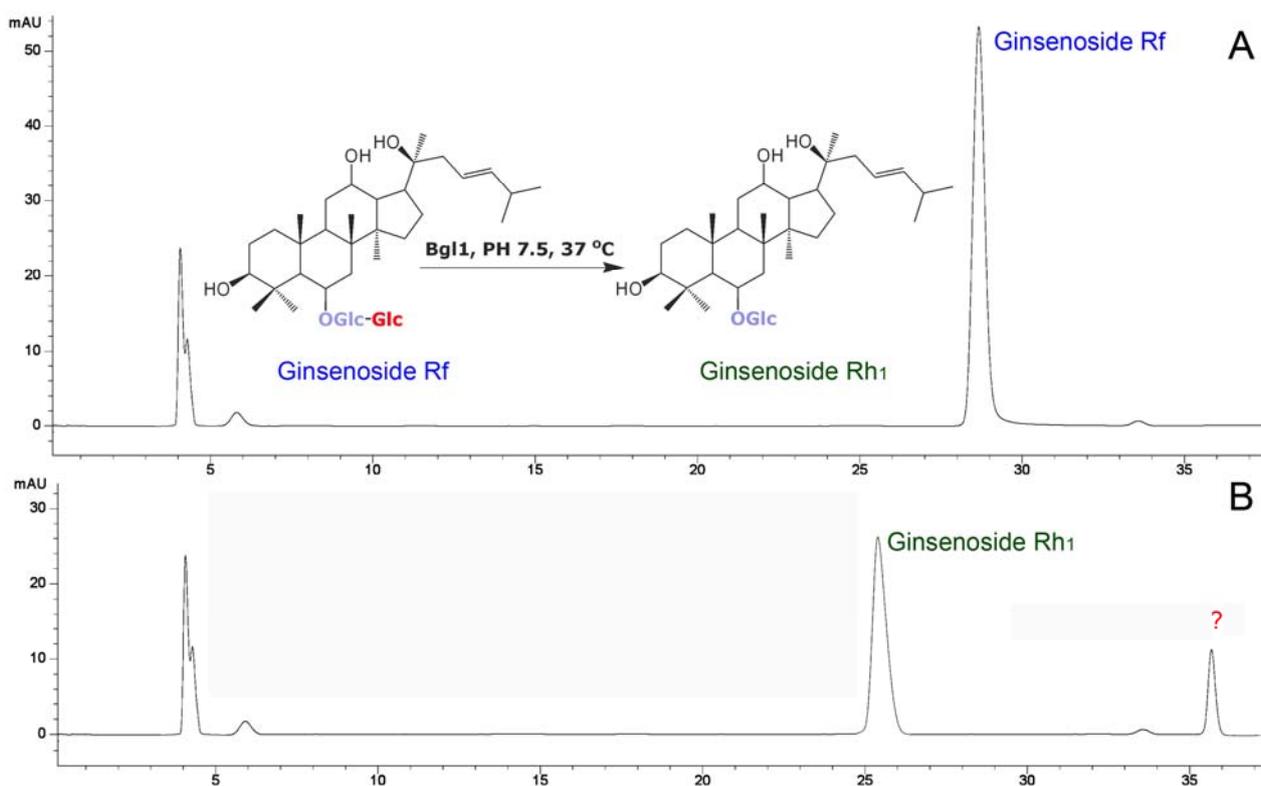
**Figure 2.** SDS-PAGE gel of expression and purification of BGL1. Lane 1: Protein marker, 2: Over-expression of BGL1; 3: Empty control; 4: Purified protein.



### *In vitro* biotransformation of ginsenoside Rf to Rh<sub>1</sub>

Purified BGL1 protein was tested for biotransformation activities with ginsenoside Rf, DM<sub>1</sub>, PM<sub>1</sub>, SM<sub>1</sub> [11] and compound K. The recombinant protein didn't show any glucosidase activities towards compound K, ginsenosides DM<sub>1</sub>, PM<sub>1</sub> and SM<sub>1</sub> but did show the ability to transform ginsenoside Rf into new products, one of which has been identified as ginsenoside Rh<sub>1</sub> by comparison of the retention time with the authentic compound and further confirmed by LC-MS analysis (Figures 3A and B).

**Figure 3.** HPLC trace of enzyme assay. A: Trace of substrate ginsenoside Rf; B: Trace of enzyme assay of ginsenoside Rf reacted with BGL1.



## Conclusions

Ginsenoside Rh<sub>1</sub> had been reported as a bioactive compound with various pharmacological effects [6-9], but the amount in the ginseng was relative minor. In the present study, a recombinant *Aspergillus niger* BGL1 protein showed the ability to transform ginsenoside Rf to Rh<sub>1</sub>, increasing the availability of this compound and hence its potential as a drug.

## Experimental

### General

HPLC runs were carried out on a Zorbax C<sub>18</sub> column (150 x 25 mm, Phenomenex, Torrance, CA, USA) on an Agilent 1100 instrument and UV absorption data ( $\lambda_{203}$ ) were analyzed with Agilent Chemstation Ver 8.01. All solvents used in this study were HPLC grade, purchased from the Chinese Chemical Group, Beijing, P.R. China. *S. cerevisiae* MGY70 was used as host strain and the yeast shuttle vector pRS423 was used for the construct.

### Cloning and Expression of pRS-BGL1 in *S. cerevisiae*

Total RNA of overnight cultured *Aspergillus niger* was extracted using an RNAeasy mini kit, (Qiagen, USA). The full-length BGL1 cDNA was cloned using specific primers designed from the mRNA sequence deposited in GeneBank (Accession No. XM.001398779): sense primer 5'-GC CTCGAG ATGAGGTTCACCTTCGATCGA-3' and antisense primer 5'- GC GAATTC TTAGTGAACAGTAGGCAGAG-3', with underlined nucleotides representing restriction sites included for *XhoI* and *EcoRI*. The PCR product was purified by a Mini-PCR purification kit (Invitrogen, USA), ligated into a pRS423 expression vector, sequenced and then introduced into *Saccharomyces cerevisiae*, selecting for growth on yeast nitrogen base (YNB) minimal medium (Difco) lacking histidine as appropriate.

### Purification of recombinant BGL1 protein

All steps were carried out at 4 °C. His-tagged BGL1 protein was purified from the soluble fraction using a His-Bind purification kit (Novagen) following the manufacture's protocol. Briefly, cells were freeze-thawed 3 times in binding buffer (500 mM NaCl, 20 mM Tris-HCl and 20 mM imidazole, PH 7.9). The suspension was incubated with lysozyme on ice for 30 min, and sonicated, the supernatant was collected by centrifugation at 14,000 g for 20 min and applied to pre-equilibrated His-Bind resin. Bound resin was washed three times with wash buffer (500 mM NaCl, 20 mM Tris-HCl and 60 mM imidazole, PH 7.9), then his-tagged protein was elute twice with three bed volumes of elution buffer (500 mM NaCl, 20 mM Tris-HCl and 1 M imidazole, PH 7.9), dialyzed three times against 1×PBS (140 mM NaCl, 2.7 mM KCl, 1.4 mM KH<sub>2</sub>PO<sub>4</sub> and 8 mM Na<sub>2</sub>HPO<sub>4</sub>, PH 7.4) to remove imidazole and examined by SDS-PAGE on 13% denaturing gel.

### Enzyme Activity

To test the potential of BGL1 to catalyze the biotransformation of ginsenosides, *in vitro* enzyme assay conditions were altered to include incubation at 37 °C for 12 h in 1 mL total volume containing 850 µL Tris-HCl buffer (100 mM, PH 7.0), 50µl purified BGL1 protein (0.9 µg µL<sup>-1</sup>), 100 µL ginsenoside (1 µg µL<sup>-1</sup>). Tested ginsenosides included ginsenosides Rf, DM<sub>1</sub>, PM<sub>1</sub>, SM<sub>1</sub> and compound K. The reaction mixture with ginsenosides was centrifuged and subjected to HPLC for analysis (20 µL injection), at ambient temperature, a linear gradient of 5% to 65% Acetonitrile (containing 0.05% formic acid), (v/v) (flow rate of 1.0 mL/min), and monitored by PDA at A<sub>203</sub>.

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*Sample Availability:* Samples are available from the authors.

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