

# Whole-Cell Biocatalysis for Producing Ginsenoside Rd from Rb1 Using *Lactobacillus rhamnosus* GG

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Ginsenosides are the major active ingredients in ginseng used for human therapeutic plant medicines. One of the most well-known probiotic bacteria among the various strains on the functional food market is *Lactobacillus rhamnosus* GG. Biocatalytic methods using probiotic enzymes for producing deglycosylated ginsenosides such as Rd have a growing significance in the functional food industry. The addition of 2% cellobiose (w/v) to glucose-free de Man-Rogosa-Sharpe broths notably induced  $\beta$ -glucosidase production from *L. rhamnosus* GG. Enzyme production and activity were optimized at a pH, temperature, and cellobiose concentration of 6.0, 40°C, and 2% (w/v), respectively. Under these controlled conditions,  $\beta$ -glucosidase production in *L. rhamnosus* GG was enhanced by 25-fold. Additionally, whole-cell homogenates showed the highest  $\beta$ -glucosidase activity when compared with disrupted cell suspensions; the cell disruption step significantly decreased the  $\beta$ -glucosidase activity. Based on the optimized enzyme conditions, whole-cell *L. rhamnosus* GG was successfully used to convert ginsenoside Rb1 into Rd.

**Keywords:** Whole-cell bioconversion, biocatalysis, *Lactobacillus rhamnosus*, ginsenoside,  $\beta$ -glucosidase

## Introduction

Ginsenosides are primary active components in ginseng roots used for traditional human therapeutic herbal medicines in Asia. Recently, the deglycosylation of ginsenosides before oral ingestion has been a topic of increased interest by the nutraceutical communities [39, 40]. These aglycones are regarded as more biologically potent than glycoside forms of ginsenoside [13, 30]. Ginsenoside aglycones enter the blood stream more effectively than ginsenoside glycosides and show higher nutraceutical activities when consumed by humans [53].

Among various deglycosylated ginsenosides, ginsenoside Rd (Rd), derived from ginsenosides Rb1, Rb2, and Rb3, has been considered as the primary ginseng quality indicator on the market [30]. Multiple researchers have reported the beneficial effects of Rd for humans (*i.e.*, management of mitochondrial dysfunction [58], anti-obesity [25], wound-

healing [27], and immunosuppressive effects [54]). Unlike using chemical (*i.e.*, acid or alkaline) catalysis, the deglycosylation process using bacterial enzymes prevents epimerization, hydration, and hydroxylation [6, 7, 42].

There are two major approaches to produce microbial enzymes for biocatalytic reactions. One common method is using microorganisms (*i.e.*, *E. coli*, *Flavobacterium*) that are genetically modified for high expression of foreign genes and mass production of microbial enzymes [19, 34]. However, using enzymes produced from genetically modified (GM) microorganisms has practical limitations when applied directly to the food industry. Without a doubt, *E. coli* is one of the most well-known, inedible microorganisms for consumers. *Flavobacterium* is also known to contaminate fresh foods and water [5]. Many food consumers have shown a substantial resistance to GM organisms, with recent reports showing that about 70% of people have a negative response to GM organisms used in their food and

about 90% of respondents feel that food products containing GM organisms should be labeled on the package [12, 26]. Because of these issues, the addition of GM and/or *E. coli* ingredients has become a major challenge for industry manufacturers in the world [32, 33].

To address this challenge, an alternative method was developed that utilizes probiotic bacteria (*i.e.*, cell-free extracts, intercellular enzymes, whole-cell enzymes). Probiotic bacteria are capable of naturally producing the microbial enzyme; thus, this method is generally recognized as safe (GRAS). Since 1998, the US Food and Drug Administration has provided information for GRAS bacteria types (such as *Bifidobacterium* spp. and *Lactobacillus* spp.) [11]. All GRAS microorganisms produce different enzymes with different activities. Therefore, cells and enzymes should be screened to characterize enzyme properties before the treatment of GRAS bacteria for the bioconversion of glycosides. Among the various GRAS bacteria, *Lactobacillus rhamnosus* GG (LGG) has been widely used in the nutraceutical and dairy industry because of its resistance to acidic environments [9], amenability to food processing [8], and various beneficial effects on humans [15, 22]. However, little research has been published on the industrial applications of LGG to the biocatalysis of ginsenosides and/or other phytochemicals.

In our previous work, we reported the enhanced cell recovery and/or enzyme activities for biocatalysis of glycosides using various cultural conditions with modified de Man-Rogosa-Sharpe (MRS) broths [17, 29–31, 56]. In this

study, we report the optimal microbial media composition for production of  $\beta$ -glucosidase from LGG. Then, we characterize the best experimental conditions to produce  $\beta$ -glucosidase for further application of ginsenoside deglycosylation.

## Materials and Methods

### Materials

*Panax ginseng* was purchased from a local grocery market in Seoul, Korea. Ginsenoside standards Rb2, Rc, and Rd were purchased from BTGin (Korea); Rh1 and Rh2 were from LKT laboratories (USA); and F2 was from Wako Pure Chemical Industries (Japan). Ginseng extracts and Compound K were produced and isolated using the methods of Kim *et al.* [24]. Chiroinositol and pinitol were kindly donated from Amicogen (Korea). L-Sorbose, L-arabinose, L-rhamnose, lactose, raffinose, myoinositol, mannitol, fructose, glucose, maltose, galactose, sucrose, xylose, cellobiose, ribose, sucralose, mannose, formic acid, and maleic acid were purchased from Samchun Pure Chemical Co., Ltd (Korea). Sucralose, myoinositol, phosphoric acid, lactic acid, glucuronic acid, ascorbic acid, propionic acid, acetic acid, phytic acid, and citric acid were purchased from Sigma (USA). Yeast extract, proteose peptone, and beef extract were purchased from Becton Dickinson (BD) (USA). HPLC-grade acetonitrile, methanol, and water were purchased from J. T. Baker (USA).

### Cell Culture Condition

Fifty-one kinds of modified MRS broths containing various

**Table 1.** Fifty-two kinds of modified MRS media tested in this experiment.

Glucose-free MRS + 4% (w/v) of nutrients							
1	L-Sorbose	6	Myoinositol	11	Glucose	16	2-Deo-glu <sup>a</sup>
2	L-Arabinose	7	Chiroinositol	12	Maltose	17	Cellobiose
3	L-Rhamnose	8	Pinitol	13	Galactose	18	Ribose
4	Lactose	9	Mannitol	14	Sucrose	19	Sucralose
5	Raffinose	10	Fructose	15	Xylose	20	Mannose
Commercial MRS <sup>b</sup> + 2% (w/v) of nutrients							
21	L-Sorbose	29	Mannitol	37	Cellobiose	45	Ascorbic acid
22	L-Arabinose	30	Fructose	38	Ribose	46	Propionic acid
23	L-Rhamnose	31	Glucose	39	Sucralose	47	Acetic acid
24	Lactose	32	Maltose	40	Mannose	48	Phytic acid
25	Raffinose	33	Galactose	41	Met- $\alpha$ -D-glucoside <sup>c</sup>	49	Citric acid
26	Myoinositol	34	Sucrose	42	Phosphoric acid	50	Formic acid
27	Chiroinositol	35	Xylose	43	Lactic acid	51	Maleic acid
28	Pinitol	36	2-Deo-glu	44	Glucuronic acid	52	Control (MRS)

<sup>a</sup>2-Deo-glu denotes 2-deoxy-D-glucose.

<sup>b</sup>Commercially available MRS broth containing 2% (w/v) of glucose as a basal carbon source.

<sup>c</sup>Met- $\alpha$ -D-glucoside denotes methyl- $\alpha$ -D-glucoside.

dietary sugars, sugar alcohols, and organic acids were prepared according to our previous method (Table 1) [29–31]. Briefly, commercially available MRS medium (Criterion, USA) was used for a control group. Cysteine hydrochloride (0.5% (W/V)) (Sigma, USA) was added into all the broths as a shared oxygen scavenger for the strictly anaerobic media condition. The pH for all culture broths was adjusted to  $7.0 \pm 0.1$  using 1 N sodium hydroxide. Naturally occurring microbiota were removed by microfiltration (0.2 micron, Ersatz-Membrane filter: BRAND, Germany).

In each experiment, the LGG in the frozen stock ( $-80^{\circ}\text{C}$ ) was activated by streaking on MRS agar followed by anaerobic incubation in fresh MRS + 0.5% (w/v) cysteine hydrochloride at  $37^{\circ}\text{C}$  for 24 h. The activated cells were inoculated into the 51 kinds of modified MRS broths. After 24 h of cell incubation, the cell pellets were collected by centrifugation at  $3,000 \times g$  for 15 min at  $4^{\circ}\text{C}$  and then the collected cells were washed twice with phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM  $\text{Na}_2\text{HPO}_4$ , 1.47 mM  $\text{KH}_2\text{PO}_4$ , pH  $7.4 \pm 0.1$ ). Cell growth rates were optically measured by a spectrophotometer at 600 nm on a microplate reader (Model Benchmark; Bio-Rad, Japan). The cell growth rates were evaluated by measuring the optical density (OD) and by the plating method.

#### Enzyme Assay

Cell lysates were prepared after evaluating the cell growth rates from the 52 sample varieties. The cell lysis solution (acetone:toluene = 9:1 (v/v)) was treated into each cell pellet in order to weaken and degrade the cell walls [30, 31]. These lysed cell suspensions were applied to analyze the varied enzyme patterns by different microbial culture conditions. The disrupted cell suspensions were produced by a cell sonication process. To evaluate changed enzyme induction patterns, 5 mM *p*-nitrophenyl- $\beta$ -D-glucopyranoside (Sigma, USA) was mixed with 80  $\mu\text{l}$  of lysed LGG suspension collected from each medium and incubated for 30 min at  $37^{\circ}\text{C}$  [30, 31]. The enzyme reaction was stopped with the addition of 100  $\mu\text{l}$  of 0.5 M  $\text{Na}_2\text{CO}_3$  solution.

#### Enzyme Characterization

The standard reaction to investigate the effects of the temperature, pH, physical disruption, and cellobiose concentration in media on the  $\beta$ -glucosidase activity were performed as aforementioned methods [29–31]. The following equation was used for assessing the decreased  $\beta$ -glucosidase activities during the cell disruption process [3, 55];

$$dA/dt = -kA$$

where A is the enzyme activity of LGG cultured in modified MRS media at time t; k = rate constant; and t = disruption time.

This equation is given by

$$\frac{dA}{A} = -kdt$$

and hence

$$\int_{A_0}^A \frac{dA}{A} = -k \int_0^t dt$$

or

$$\ln A \Big|_{A_0}^A = -kt \Big|_0^t$$

followed by the simplification of the equation results in

$$\ln \frac{A}{A_0} = -kt$$

The enhanced LGG enzyme activity by the addition of cellobiose is given by

$$dA/dc = kA$$

where A is the enzyme activity of LGG cultured in modified MRS media at time t; k = rate constant; and c = level of cellobiose in media.

The decreased  $\beta$ -glucosidase activity by the addition of glucose into the modified MRS is given by

$$dA/dg = -kA$$

A is an enzyme activity of LGG cultured at modified MRS at time t; k = rate constant; and g = level of glucose in modified MRS.

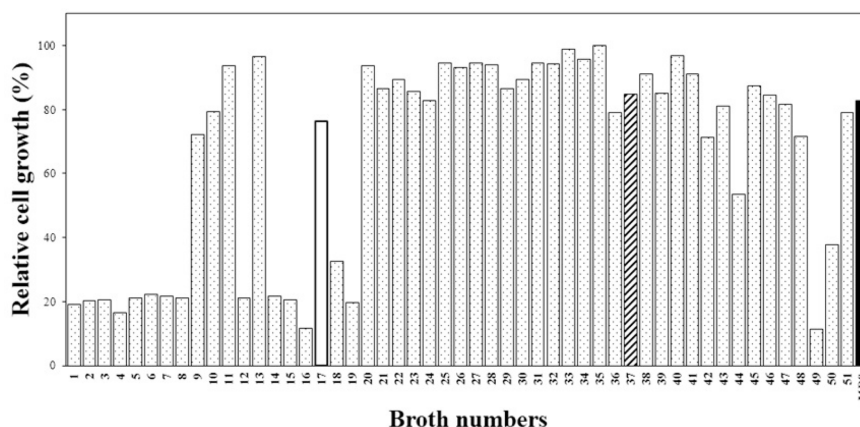
To determine the optimal concentration of cellobiose, LGG was cultured anaerobically in modified MRS media with 0.5–5% (w/v) of cellobiose at  $37^{\circ}\text{C}$  for 22 h. To evaluate the effect of the cell disruption, 1 ml ( $6 \times 10^8$  CFU/ml) of the LGG suspension was harvested from the modified MRS medium with 2% (w/v) of cellobiose and washed in ice-cold PBS. The cell pellet was resuspended in 4 ml of PBS and disrupted by cell sonication at 45 amplitude for 30 sec at  $4^{\circ}\text{C}$ . The degree of cell disruption was optically measured at 600 nm. The effect of temperature on the whole-cell  $\beta$ -glucosidase of LGG was investigated at pH  $7.4 \pm 0.1$  at the range of  $4^{\circ}\text{C}$  to  $60^{\circ}\text{C}$ . Four milliliters of 100 mM acetate buffer (pH 4 and 5) and 100 mM phosphate buffer (pH 6, 7, and 8) were treated to 1 ml ( $6 \times 10^8$  CFU/ml) of the whole LGG suspension in order to investigate the effect of pH.

#### Ginsenoside Conversion Using Whole LGG Cell

The whole-cell suspensions ( $6 \times 10^8$  CFU/ml) with phosphate buffer (100 mM, pH 6) were mixed with the ginseng extracts at the ratio of 19:1 (v/v) and incubated at  $40^{\circ}\text{C}$ . The cell-ginseng extract suspensions were collected after 2 and 4 days. TLC analysis was performed to evaluate the bioconversion of ginsenosides [30, 31].

#### Statistical Analysis

All research data were the average of triplicate tests. Significant differences were evaluated by *t*-tests using Microsoft Excel 2013 and analysis of variance with the help of Minitab 16 followed by post hoc comparisons using Tukey's test. Differences at  $p < 0.05$  were considered to be statistically significant [30].



**Fig. 1.** Relative growth rates of *L. rhamnosus* GG cultured in the various broths ( $n = 3$ ).

Commercially available MRS medium (black bar: ■) was used as a control group. Media #17 (white bar: □) and #37 (upward diagonal bar: ▨) are glucose-free MRS medium containing 4% (w/v) of cellobiose and normal MRS medium containing 2% (w/v) of cellobiose, respectively.

## Results and Discussions

### LGG Growth in Modified MRS Media

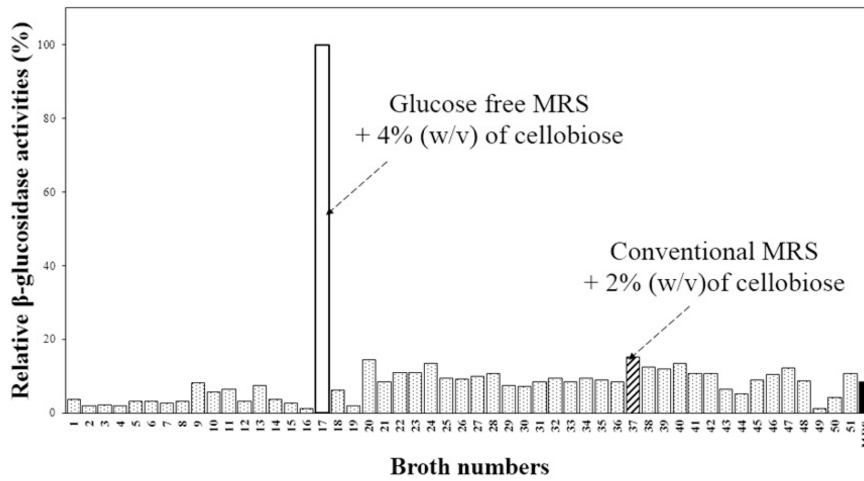
Conventional MRS medium has been commonly recommended for use in the cultivation of lactic acid bacteria (*e.g.*, *Bifidobacterium* spp., *Lactobacillus* spp.) in industry [10]. Based on the ratio of MRS medium compositions, 51 types of modified MRS media containing 4% (w/v) of different carbons, acids, and other chemicals were tested (Table 1). In order to check the effect of specific nutrients on LGG growth, we added 4% (w/v) of different nutrients to glucose-free MRS medium (See Table 1; #1–20) and 2% (w/v) sources into the conventional MRS medium, which originally contained 2% (w/v) glucose as a basal carbon source (media #21–51). Commercially available MRS medium was used for a control group (medium #52). As a single carbohydrate, L-sorbose, L-arabinose, L-rhamnose, lactose, raffinose, myoinositol, chiroinositol, pinitol, maltose, sucrose, xylose, 2-deoxy-D-glucose, ribose, and sucralose (media #1–8, 12, 14–16, 18, and 19) instead of glucose was ineffective for the growth of LGG ( $p < 0.05$ ) (Fig. 1). The growth rate of LGG cultured in the modified MRS medium containing 4% (w/v) cellobiose (medium # 17) did not have statistically significant differences from the control group ( $p > 0.05$ ). In our previous work, ascorbic acid and phytic acid induced significant morphological changes and/or enzyme activities from *Bifidobacterium* spp. [29, 30]. Accordingly, multiple organic acids were also added into the media to evaluate their effects on cell growth. However, the addition of 2% (w/v) glucuronic acid, formic acid, and maleic acid to commercial MRS medium exhibited statistically significant growth inhibiting effects on LGG ( $p < 0.05$ ).

Multiple researchers previously reported that LGG was unable to decompose certain carbon sources (*i.e.*, sucrose [36], lactose [38, 44] rhamnose, ribose, maltose [23], L-sorbose, xylose, methyl- $\alpha$ -D-glucoside [44], arabinose, and raffinose [18, 44]). However, in contrast to the report from Balejko *et al.* [4], LGG successfully fermented fructose (media #10 and 30) as a carbon source. LGG also used mannitol (medium #9), glucose (media #11, 31, and 52), galactose (media #13 and 33), cellobiose (media #17 and 37), and mannose (media #20 and 40) as carbon sources and the results of this work are in accord with Saxelin [44].

### Role of Cellobiose

Among the various carbon sources, glucose is the primary carbon source from which general chemoheterotrophic organisms can produce energy and provide enriched nutrients and act as metabolic precursors for cell growth [9]. Generally, the addition of glucose to microbial broth increases cell growth rates and cell biomass yield. If high cell recovery is the major goal, then the use of glucose is advantageous for industrial purposes. On the other hand, if the major goal is enzyme recovery or induction of glycolytic enzymes, then glucose is one of the major inhibitory reagents for that purpose when the concentration is too high [52]. Multiple studies have shown that the presence of high levels of glucose or certain carbon sources in the media can significantly decrease enzyme activities in microorganisms [14, 16]. Increased concentrations of glucose or other sugar supplements have shown decreased microbial enzyme activities.

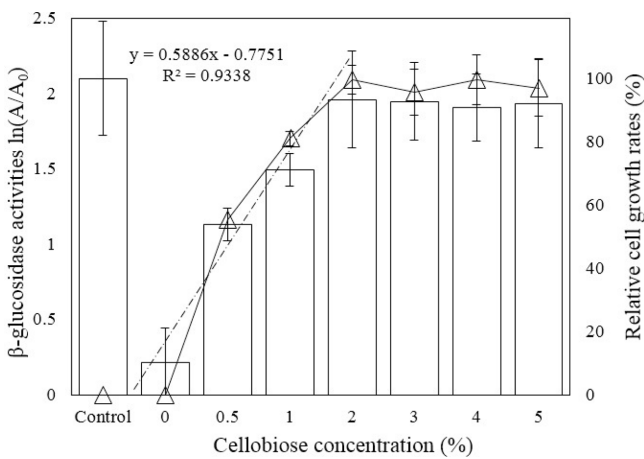
In our study, the glucose-free MRS containing 4% (w/v) cellobiose was the most fertile medium for the induction



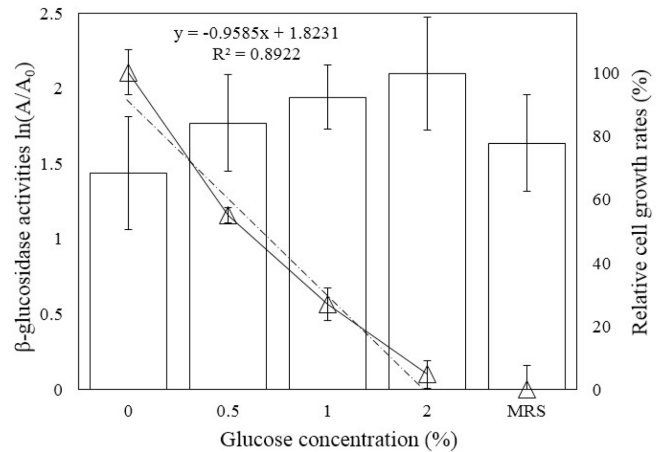
**Fig. 2.** Relative  $\beta$ -glucosidase activities of *L. rhamnosus* GG cultured in the various broths ( $n = 3$ ). Cell lysis solution was treated to samples to degrade cell walls. Black bar (■) denotes commercially available MRS medium. Media #17 (white bar: □) and #37 (upward diagonal bar: ▨) are glucose-free MRS medium containing 4% (w/v) of cellobiose and normal MRS medium containing 2% (w/v) of cellobiose, respectively.

of  $\beta$ -glucosidase from LGG (See Fig. 2; medium #17).  $\beta$ -glucosidase activity was increased markedly with the addition of cellobiose into the medium, rather than glucose. This indicates that  $\beta$ -glucosidase is undoubtedly an inducible enzyme. For further examination of the effect of cellobiose, 0–5% (w/v) of cellobiose was added to glucose-free MRS broth (Fig. 3). As a result, the degree of enzyme activity increased significantly as the concentration of cellobiose

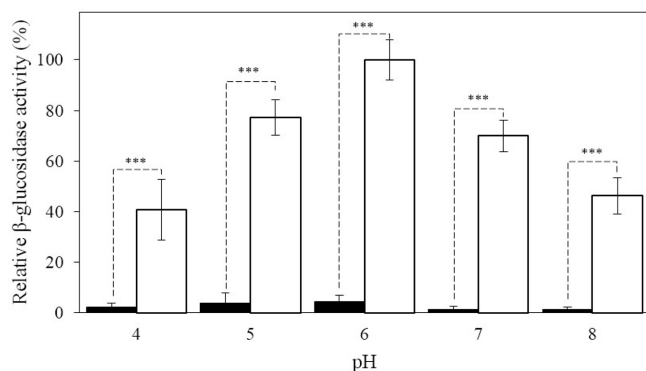
increased up to 2% ( $p < 0.05$ ). There were no statistically significant differences across the four samples containing 2% to 5% (w/v) of cellobiose ( $p > 0.05$ ). Additional cellobiose treatment into the media did not enhance enzyme activity and cell growth rates. However, the addition of glucose into the modified MRS broth containing 2% (w/v) of cellobiose instead of glucose notably decreased enzyme induction ( $p < 0.05$ ) (Fig. 4).



**Fig. 3.** Effect of cellobiose on the production of  $\beta$ -glucosidase (triangles) from *L. rhamnosus* GG ( $n = 3$ ). Cell lysis solution was treated to samples. Bar graphs denote cell growth rates. Error bars represent standard deviation. The solid line represents changed enzyme activities. The dotted line represents the intercept from 0 to 2% (w/v) of cellobiose groups. Commercially available MRS broth (Criterion, USA) was used as a control group.



**Fig. 4.** Effect of glucose on the induction of  $\beta$ -glucosidase (triangles) from *L. rhamnosus* GG ( $n = 3$ ). Cell lysis solution was treated to samples. Bar graphs denote cell growth rates. Error bars represent standard deviation. The solid line represents changed enzyme activities. The dotted line represents the intercept from 0 to 2% (w/v) of glucose groups. Commercially available MRS broth (Criterion, USA) was used as a control group.



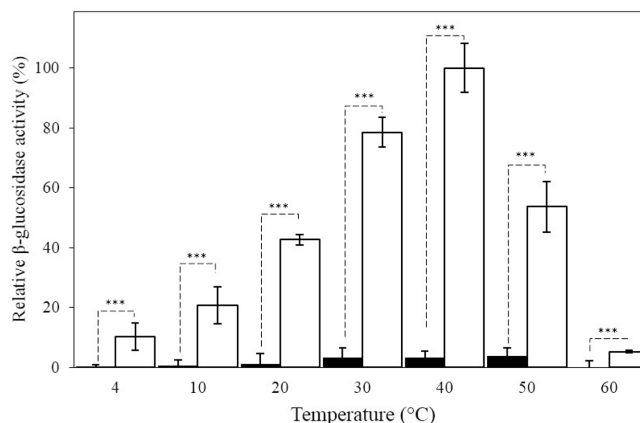
**Fig. 5.** Effect of pH on the whole-cell  $\beta$ -glucosidase of *L. rhamnosus* GG cultured in normal MRS (black) and modified MRS (white) media ( $n = 3$ ) at 37°C.

For the experiments, cell lysis or disruption process was not applied. Bars with \*\*\* represent statistically significant differences between groups at  $p < 0.001$ .

Media components act as substrates or substrate analogs for microbial enzymes, and these function as enzyme inducers [49]. When polysaccharides or disaccharides are used as carbon sources, probiotic bacteria have shown a tendency to hydrolyze their glycosidic bonds to take glucose for their energy source [43]. Therefore, introducing these strictly glucose-limited conditions to bacteria is a prerequisite to studying microbial enzyme physiology under such controlled conditions. Despite the routines of using MRS medium for the cultivation of probiotic bacteria, including *Lactobacillus* and *Bifidobacterium*, MRS medium is not an essential medium for the production of microbial enzymes because it only contains glucose as a carbon source. The medium composition-dependent induction of microbial enzymes has significant implications. As our previous data clearly showed, each medium supplement (*i.e.*, galactose, phytic acid, ascorbic acid) was used to induce different enzymes and metabolic compounds, thereby confirming a relationship exists between glycosidic enzymes and the composition of the medium [29–31]. Therefore, the use of MRS broth in enzyme induction, particularly without gene work, is limited. It was further confirmed that medium composition significantly affects enzyme induction levels and glucose acts as an inhibitor of  $\beta$ -glucosidase production (Fig. 4). We suggest that ongoing medium development should be carried out as a routine process for the production of microbial enzymes that can later be employed for the bioconversion of phytochemicals.

#### Optimal Conditions for $\beta$ -Glucosidase

In terms of an optimal condition for enzyme hydrolysis,



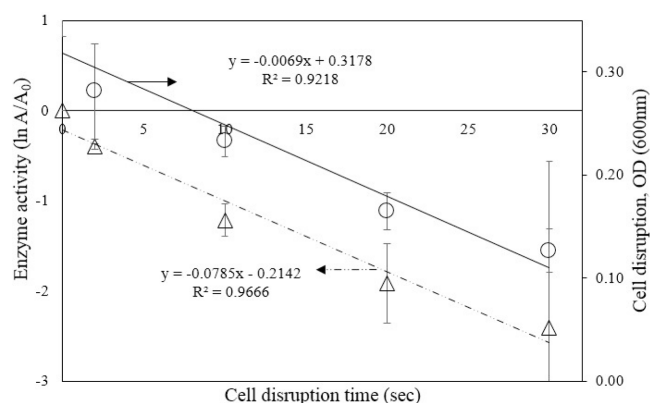
**Fig. 6.** Relative  $\beta$ -glucosidase activity of whole-cell *L. rhamnosus* GG from normal MRS (black) and modified MRS (white) media ( $n = 3$ ) at various temperatures at pH 5.0.

For the experiments, cell lysis or disruption processes were not applied. Bars with \*\*\* represent statistically significant differences between groups at  $p < 0.001$ .

pH and temperature are key factors. Moreover, optimal  $\beta$ -glucosidase conditions may vary according to the host cell. Unlike a normal assay, without using a cell disruption process, we directly applied a whole-cell suspension to evaluate the optimal condition of  $\beta$ -glucosidase owing to its distinctive enzyme characteristics. In Figs. 5 and 6,  $\beta$ -glucosidase activity from *Lactobacillus rhamnosus* GG was evaluated at different pH and temperatures in the range of 4 to 8 and 4°C to 60°C, using *p*-nitrophenyl- $\beta$ -D-glucopyranoside as a substance. The enzyme activity reached a maximum value at pH 6 and 40°C. There are various reports that related how  $\beta$ -glucosidases produced from multiple probiotic bacteria express their maximum enzyme activity at somewhat acidic conditions [35, 37, 46]. The whole-cell suspension of *Lactobacillus rhamnosus* NRRL 442 showed the highest  $\beta$ -glucosidase activity at pH 6.5 [28].

#### Whole-Cell Biocatalyst

The  $\beta$ -glucosidase of LGG was evaluated as a whole-cell fraction and a disrupted cell fraction (Fig. 7). Cell activity in the whole-cell fraction was highest; however, the degree of  $\beta$ -glucosidase was proportionally decreased over the cell disruption step with approximately 10% of total activity. The remaining  $\beta$ -glucosidase activity in the disrupted cell suspension was approximately 10% of the activity. Without the cell disruption process, the whole-cell suspension efficiently hydrolyzed *p*-nitrophenyl- $\beta$ -D-glucopyranoside. These results indicate that  $\beta$ -glucosidase apparently exists in a cell-wall-associated structure and is not resistant to

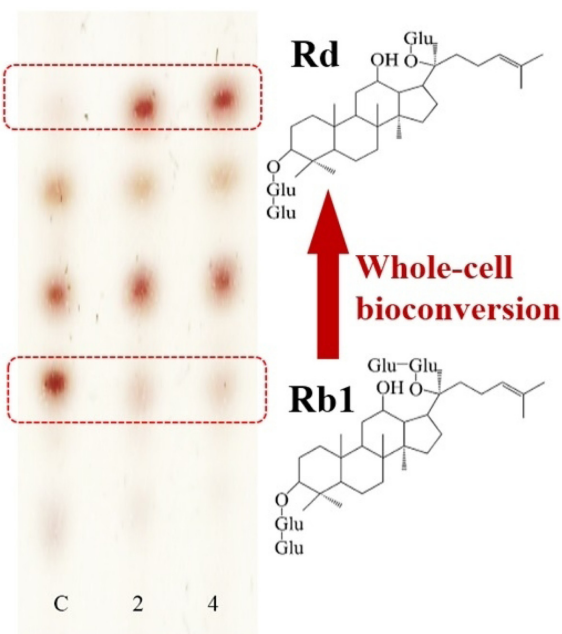


**Fig. 7.** The decreased  $\beta$ -glucosidase activities (triangles) and OD values (circles) as a function of sonication time at pH 5.0 ( $n = 3$ ).

The solid and dotted lines denote intercepts of OD values and enzyme activities. Error bars represent standard deviation.

physical disruption since in the strains examined in this work, the enzyme activity in the whole-cell suspension was always higher than  $\beta$ -glucosidase measured in the lysed or disrupted cell suspensions and cell-free extracts (data not shown). Relevant glycosidase activities have also been reported for the *Lactobacillus delbrueckii* mutant Uc-3 [2] and *Lactobacillus lactis* mutant RM2-24 [47] responsible for cellobiose, cellotriose, and cellulose fermentation. In their work, the  $\beta$ -glucosidase activity in a disrupted cell was lower than those of the whole cells. No activities were detected in the supernatant, suggesting that these activities are cell wall/membrane bound. Their experimental design and results are similar to the findings presented in this work.

According to Schüürmann *et al.* [45], when using whole-cell biocatalysts and cell cytosolic enzymes, it is necessary for target substrates and products to be able to enter the outer membrane barrier effectively. Multiple cell ingredients



**Fig. 8.** TLC analysis of  $\beta$ -glucosidase conversion of ginsenoside Rb1 into Rd by whole-cell *L. rhamnosus* GG.

Analysis was carried out under optimized conditions: addition of 2% cellobiose into glucose-free MRS broth; restriction of additional glucose supplements during cell growth; maintaining the pH and temperature of 6.0 and 40°C, respectively; and no cell disruption step for optimal enzyme activity.

and metabolites can inhibit the desired reaction. Using the whole cell in the production of pharmaceutical compounds or intermediates is therefore a restriction for this particular phenomenon. Therefore, multiple researchers have used cell disruption and centrifugation processes to produce cell-free extracts or cytosolic enzyme solutions for ginsenoside biocatalysis [7, 57]. Enzymes containing these extracts are able to effectively access target substrates and demonstrate reaction-rate limited kinetics [48]. However, a whole-cell

**Table 2.** Pros and cons of using whole-cell suspensions as microbial enzyme versus using conventional (purified and processed) enzymes.

Advantages	Disadvantages
Only limited by the number of organisms that can be cultured, whereas enzymes are limited by the number that are commercially available.	User needs to evaluate enzyme manufacturing phase using cell growth rates and substrate conversion phase.
The price of cell biomass is cheaper than that of commercially available enzymes (saving the purification costs associated with the purchase of an enzyme).	Unwanted by-products, media ingredients, and non-target enzymes can be present in whole-cell suspensions. These ingredients can affect the downstream purification.
Providing the exogenous co-factor for redox reactions is not necessary since the cells will recycle the co-factor with existing machinery even if cells are not growing.	If the enzyme is not secreted or of membrane binding form, the membrane lysis process or the transportation of substrates across the membrane is required for substrate conversion.

Adapted and modified from Prather [41].

biocatalyst with surface-displayed enzymes is able to overcome the restrictions generated by physical barriers [45]. According to Prather [41], there are several advantages of using a whole-cell catalyst for substrate conversion (Table 2). The use of whole-cell bacteria as an enzyme for biocatalytic process avoids the more time-consuming and laborious processes of enzyme production, purification, and perhaps increased production costs [50].

### Bioconversion of Ginsenoside Rb1

Multiple researchers have reported the effect and function of cell surface-displayed enzymes (*i.e.*,  $\beta$ -glucanase [21], xylanase [20], and lipase [51]). However, these works have not been practically applied with commercially relevant products and industrial application [45]. For industrial purposes, we used this whole-cell catalysis ability for ginsenoside conversion. After LGG was cultured in modified MRS medium with 2% (w/v) cellobiose, the whole-cell suspension (pH 6 and 40°C) was used for biocatalyst of ginsenoside Rb1 into Rd as shown in Fig. 8. The TLC shows that ginsenoside Rb1 was successfully converted by the whole-cell suspension. Multiple researchers have reported the gene expression of surface display enzymes for the purpose of application to carbohydrate degradation [1, 50]. However, the practical applications of these strategies in the food industry may be difficult owing to their use of GM organisms. In summary, the high correlation coefficient suggests that the level of the cellobiose and glucose have a significant impact on enzyme production. The addition of cellobiose into the culture media (rather than glucose) can be used for  $\beta$ -glucosidase production from LGG. The  $\beta$ -glucosidase activity is notably decreased by the cell disruption process and with the addition of glucose to the media. The whole-cell  $\beta$ -glucosidase has been applied for converting ginsenoside Rb1 into Rd without the use of gene work and enzyme purification processes (*i.e.*, cell disruption, centrifugation to produce cell-free extracts or purified enzyme). To our best knowledge, this is the first study with the aim to investigate the properties of whole-cell  $\beta$ -glucosidase from LGG and its use for ginsenoside conversion.

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