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Characterization of yeast cell surface displayed *Aspergillus oryzae* β-glucosidase 1 high hydrolytic activity for soybean isoflavone

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Abstract

The yeast *Saccharomyces cerevisiae* GRI-117-UK was transformed to either display or secrete β-glucosidase 1 (BGL1) from the koji mold, *Aspergillus oryzae*. The β-glucosidase activity of BGL1-displaying yeast strains reached 405.9 U/g dry cell mass after 72 h of cultivation in YPD medium. The optimal pH and temperature for BGL1 displayed on the cell surfaces of the yeast were 5.0 and 55 °C, while the optima for BGL1 secreted by the yeast were 4.0 and 55 °C. The displayed BGL1 was stable at higher pH compared with the secreted BGL1. In addition, the thermostability of BGL1 was improved by displaying the enzyme on the yeast cell surfaces. In addition, the displayed and secreted forms of BGL1 had similar substrate specificity. β-Glucosidase hydrolyzes daidzin and genistin, which are the glycoside forms of soybean isoflavones, to the aglycones. Isoflavone aglycones were efficiently produced by BGL1-displaying yeast from an isoflavone mixture; at optimal temperature and pH the rate of aglycone production was at least 15.8 g/(l h). After 144 h of reaction, almost isoflavones were converted to its aglycone by BGL1-displaying yeast. The results of the present study demonstrate that BGL1-displaying yeast strains are effective whole cell biocatalysts of isoflavone aglycone production.

Keywords: β-Glucosidase; Isoflavone; *Saccharomyces cerevisiae*; *Aspergillus oryzae*; Cell surface display

1. Introduction

Facile bioconversion processes require enzyme immobilization on a solid support to separate the enzyme from the reaction mixture, thereby allowing the enzyme to be recycled. Various immobilization methods have been proposed and their advantages discussed [1,2]. During the past decade, microorganisms have been developed for the display of functional peptides and proteins on microorganism cell surfaces by fusion with cell wall-anchored proteins [3–5]. Enzymes displayed on the cell surfaces of microorganisms are regarded as enzymes immobilized to solid supports. The utilization of microorganisms as solid supports for enzymes is attractive because immobilization of the enzymes on the cell surface occurs spontaneously during cultivation. The yeast *Saccharomyces cerevisiae*, which has "generally regarded as safe" (GRAS) status and can therefore be used in the production of food and pharmaceuticals, is the most suitable microorganism for many applications. In yeast-based cell-surface display systems, the gene encoding target protein with the secretion signal sequence is fused the cell surface proteins has been used as an anchor protein to successfully display many proteins immobilized on the cell surface.

Isoflavones are secondary metabolites of plants that are produced in abundance in soybeans (*Glycine max*). Recently, isoflavones have attracted considerable attention for their cardioprotective and cancer-preventive effects and for their steroid-like actions [6]. In soybeans, isoflavones rarely exist in the aglycone forms, such as daizein, genistein and glycitein, or in the glucose-, 6″-*O*-malonylglucoside- and 6″-*O*-acetylglucoside-conjugated forms [7]. The isoflavone aglycones, which are produced by β-glucosidase hydrolysis of glycoside isoflavones, have high bioactivity due to their effective absorption [8].

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The enzyme, β-glucosidase (β-D-glucoside glucohydrolase; EC 3.2.1.21), is a ubiquitous enzyme with multiple isoforms that catalyzes the hydrolysis of isoflavone glucosides, disaccharides, oligosaccharides, aryl-glucosides and alkyl-glucosides from the non-reducing end of the glycoside molecule. The hydrolytic and transferase activities of these enzymes have been used in various biotechnological applications. For example, the hydrolytic activity of β-glucosidase has been used to degrade cellulosic biomass and to synthesize alkyl glucosides [1,2]. β-Glucosidase has been successfully displayed on the cell surfaces of yeast and the cell surface-displayed enzyme was shown to be as effective as free β-glucosidase in a variety of applications. However, a comparison of the characteristics of displayed β-glucosidase and free β-glucosidase remains to be published.

Recently, five genes (BGL1∼5) encoding β-glucosidase were identified in *Aspergillus oryzae* chromosomal DNA. Each of these genes was fused with the gene of C-terminal half region of α-agglutinin containing the putative glycosylphosphatidylinositol (GPI) anchor attachment signal sequence, which was immobilized at the C-terminal on the yeast cell surface efficiently. Each of β-glucosidase displayed on the surface of yeast catalyzed the production of soybean isoflavones aglycone, and BGL1 exhibited most high conversion yield [9]. The present study compared the enzymatic properties of BGL1 displayed on yeast cell surfaces with those of secreted BGL1. At optimal pH and temperature, BGL1-displaying yeast strains efficiently produced isoflavones aglycone from a mixture of soybean isoflavones.

2. Materials and methods

2.1. Chemicals

p-Nitrophenyl-β-D-glucopyranoside (*p*NPβG) was from Nacalai Tesque Inc. (Kyoto, Japan). Laminaribiose, cellobiose, cellotriose and cellopentaose were from Seikagaku Corp. (Tokyo, Japan). Daidzin, genistin, and Fujiflavone P40 (Fujiflavone P40 contains 26.50% of daidzein conjugated, 5.80% of genistein conjugated, and 12.57% of the other isoflavone, according to the material data sheet) were from Fujicco (Kobe, Japan). All other substrates used were from Sigma (St. Louis, MO, USA).

2.2. Yeast strains and growth media

The gene encoding BGL1 from *A. oryzae* registered in the genome database of *A. oryzae* (DOGAN ID: AO090003001511, http://www.bio.nite.go.jp/dogan/Top) was cloned, and an expression plasmid was created for both the cell surface-displayed and secreted forms of BGL1 [9]. For C-terminal immobilization of BGL1 on the cell surface by part of α-agglutinin, the gene encoding BGL1 was fused with the gene encoding the secretion signal sequence of the *Rhizopus oryzae* glucoamylase gene at the 5′ end of the BGL1 gene and the 3′-half region of the α-agglutinin gene at the 3′ end of the BGL1 gene. This fused protein was expressed under the *SED1* promoter. For secretion of BGL1, the gene encoding BGL1 was fused with the gene encoding the secretion signal sequence of the *R. oryzae* glucoamylase gene at the 5′ end of the BGL1 gene, and expressed under the *SED1* promoter. The yeast, *S. cerevisiae* GRI-117-UK (*MATa/α ura3/ura3 lys2/lys2*), was transformed with the linearized plasmids, resulting in two recombinant yeast strains: one displaying BGL1 (GRI-117-UK/BGL1) on the yeast cell surfaces and the other secreting BGL1 (GRI-117-UK/BGL1s). BGL1 displayed on yeast was used as a whole cell catalyst. A 3-ml pre-cultivated yeast cell suspension in YPD medium (10 g/l yeast extract, 20 g/l glucose, and 20 g/l peptone) was added to 100 ml of fresh YPD and incubated under aerobic conditions at 30 °C.

2.3. Preparation of BGL1-displaying yeast cells and measurement of BGL1 activity

The recombinant *S. cerevisiae* GRI-117-UK/BGL1 displaying BGL1 was grown in 3 ml YPD medium at 30 °C. The yeast cells were collected by centrifugation at 3000 × *g* for 5 min at 4 °C, and then were washed twice with distilled water.

BGL1 activity was assayed in 1 ml of 50 mM sodium acetate buffer (pH 5.0) containing 1 mM *p*NPβG as the substrate at 30 °C for 10 min with continual shaking at 150 rpm. The cell concentration of the reaction mixture was adjusted to give an optical density at 600 nm (OD_{600}) of 0.05. The reaction was stopped by the addition of an equivalent volume of 1 M Na_2CO_3. The supernatant was separated by centrifugation at 20,000 × *g* for 3 min at 4 °C, and the released *p*-nitrophenol was quantified by spectrophotometry using a wavelength of 400 nm. One unit of β-glucosidase activity was defined as the amount of enzyme that released 1 μmol of *p*-nitrophenol from the substrate per minute [10].

2.4. Determination of optimal pH and temperature for enzymatic activity and stability

BGL1-displaying yeast cells were cultivated in YPD medium for 72 h at 30 °C, collected, and washed twice with distilled water. BGL1 secreted from yeast was collected by centrifugation at 6000 × *g* for 5 min at 4 °C. The optimal pH for displayed and secreted BGL1 was assayed by measuring β-glucosidase activity with 1 mM *p*NPβG as the substrate in the pH range of 2.0 using 50 mM glycine–HCl buffer, 3.0–6.0 using 50 mM sodium acetate buffer, and in the pH range of 7.0–8.0 using 50 mM potassium phosphate buffer at 30 °C. The stability of BGL1 at various pH was determined by pre-incubating the BGL1 at various pH, ranging from 3.0 to 7.0, at 30 °C for 12 h. Any remaining BGL1 activity was assayed at same pH of the incubation using *p*NPβG as the substrate at 30 °C. The optimal temperatures for the two forms of BGL1 were determined using *p*NPβG at pH 5.0 over the temperature range of 30–70 °C. The thermostability of the enzyme in a 50 mM sodium acetate buffer (pH 5.0) was determined by incubating the enzyme at various temperatures, ranging from 30 to 70 °C, for 4 h or 12-h incubation period. Any remaining activity was measured using *p*NPβG as the substrate. The same activity of the secreted form of BGL1 (5.48 U/ml supernatant) and BGL1 displayed on the

yeast surface (5.48 U/ml suspension) were used in all experiments.

2.5. Determination of substrate specificity

The substrate specificity of the secreted form of BGL1 and the cell surface-displayed BGL1 was determined using various substrates as follows: aryl-glycosides, such as xylopyranoside (*p*NPβX), galactopyranoside (*p*NPβGal), glucopyranoside (*p*NPαG) and cellobioside ($pNP\beta G_2$), tested the specificity for *p*-nitrophenyl derivatives; alkyl-glucosides, such as methyl-glucoside (C1-G), hexylglucoside (C6-G) and octylglucoside (C8-G), examined carbon chain length; oligosaccharides with various linkages (shown in parentheses), such as sophorose (1 → 2), laminaribiose (1 → 3), cellobiose (1 → 4), cellotriose (1 → 4), cellotetraose (1 → 4), cellopentaose (1 → 4) and gentiobiose (1 → 6); and, isoflavones (daidzin and genistin). Hydrolysis of aryl-glycosides by BGL1 was determined using 1 mM substrate. Hydrolysis of alkyl-glucosides and oligosaccharides by BGL1 was measured by adding 20 mM substrate, and BGL1 activity on isoflavones was measured using 200 μM isoflavones. Each hydrolysis experiment was performed in 50 mM sodium acetate buffer (pH 5.0) at 30 °C with continual shaking at 150 rpm. After cultivation in YPD medium for 72 h at 30 °C, the yeast cells were collected by centrifugation at 3000 × *g* for 5 min at 4 °C, and washed twice with distilled water. Then, the cells were re-suspended in the reaction mixture, and the OD_{600} was adjusted to 1.0. After the reaction was stopped, the supernatant was separated by centrifugation for 3 min at 15,000 × *g* at 4 °C, and analyzed by high-performance liquid chromatography (HPLC). The activity of the secreted form of BGL1 was expressed relative to that of BGL1 displayed on the yeast surface.

2.6. Isoflavone aglycone production

Production of aglycones by BGL1 was evaluated using Fujiflavone P40, which contains 45% (w/w) isoflavone. BGL1-displaying yeast cells were grown in YPD medium, collected by centrifugation at 3000 × *g* for 5 min at 4 °C, and washed twice with 50 mM sodium acetate buffer (pH 5.0). The cells were re-suspended in 6 ml 50 mM sodium acetate buffer (pH 5.0) containing 0.73 g Fujiflavone P40 to adjust the OD_{600} to 10.

The reaction mixture was continually shaken at 150 rpm at 30, 40 or 50 °C. The reaction was terminated and the isoflavone aglycone extracted by adding 0.5 μl reaction mixture to 999.5 μl 99.5% ethanol, followed by incubation at 30 °C with continual shaking at 150 rpm. The supernatant was separated by centrifugation for 5 min at 15,000 × *g* at room temperature, and the isoflavone aglycone content was measured using HPLC.

2.7. High-performance liquid chromatography (HPLC) analysis

For HPLC analysis of alkyl-glucosides and oligosaccharides, a refractive index detector (RID-10A; Shimadzu, Kyoto, Japan) was used. A Cosmosil 5NH2-MS packed column (Nacalai Tesque Inc., Kyoto, Japan) was used for oligosaccharides, and a ODS column was used for alkyl-glucosides. The column temperature was 30 °C. An acetonitrile–water mixture (60:40, v/v) at a flow rate of 1.0 ml/min was used as the mobile phase for HPLC of both oligosaccharides and alkyl-glucosides.

Isoflavones were measured using an ODS column and a UV detector (CDD10A; Shimadzu, Kyoto, Japan) at 260 nm. The HPLC conditions were as follows: the column oven temperature was 40 °C; and, the mobile phase consisted of a linear gradient from solvent A (15% acetonitrile in 0.1% acetic acid) to solvent B (35% acetonitrile in 0.1% acetic acid) at a flow rate of 0.5 ml/min for 70 min. By using the retention time and peak area information of standard products (daidzein, glycitein, and genistein; 26, 28, and 41 min, respectively), each concentration in the experimental sample was calculated.

3. Results

3.1. Effect of cultivation time on BGL1-displaying yeast cells

Change in activity of BGL1 displayed on the yeast cell surfaces was measured over an 80-h incubation period (Fig. 1). BGL1-displaying yeast exhibited β-glucosidase activity, while 0.023 U/g (dry cell weight) was detected in parent yeast (GRI-117-UK). β-Glucosidase activity correlated strongly with yeast cell concentration at all time points. Maximal β-glucosidase activity was 405.9 (U/g dry cell mass) at 72 h of incubation. Therefore, yeast cells that had been cultivated for 72 h were used as whole cell biocatalysts in subsequent experiments.

3.2. Effect of pH and temperature on BGL1 activity

Enzyme characteristics of both the displayed and secreted forms of BGL1 were evaluated. The effects of pH (2.0–8.0)

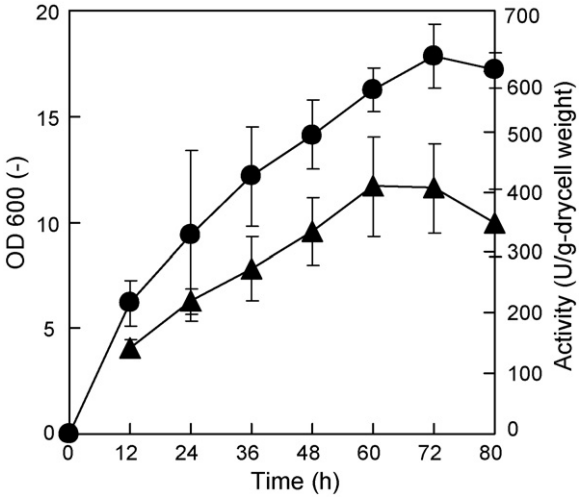

Fig. 1. Time course of displayed BGL1 activity (circles) and cell growth (optical density at 600 nm) (triangle). Each data point represents the mean of three independent experiments and the error bar indicates the standard deviation.

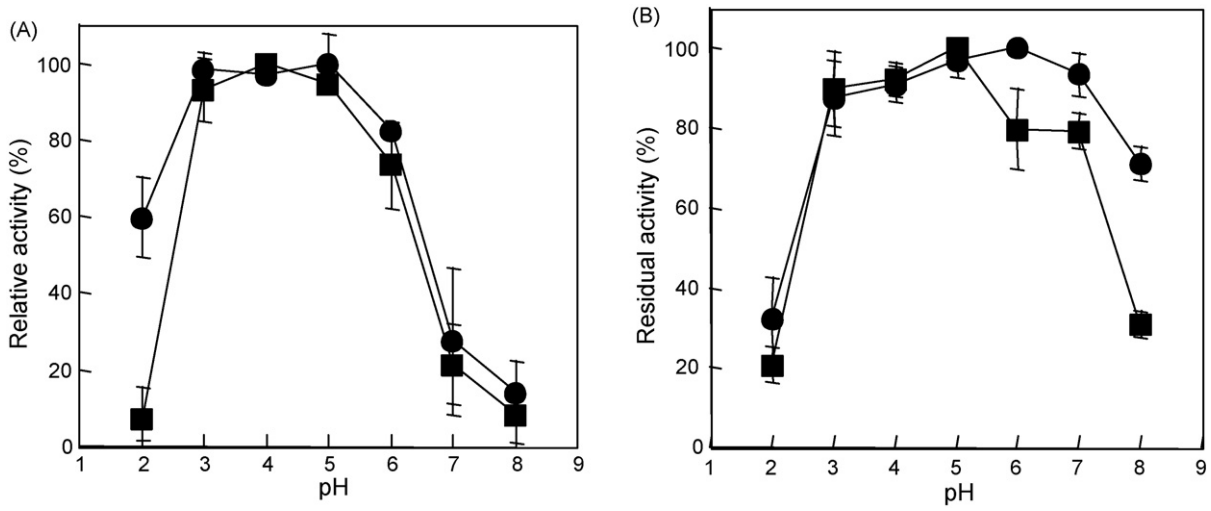

Fig. 2. (A) Effect of pH on secreted BGL1 (square) and displayed BGL1 (circle) activity at 30 °C. Relative activity was measured using 50 mM glycine–HCl buffer (pH2.0), 50 mM acetate buffer (pH 3.0–6.0) and 50 mM phosphate buffer (pH 7.0–8.0). (B) Effect of pH on secreted BGL1 (stripe) and displayed BGL1 (white) stability at 30 °C. 50 mM glycine–HCl buffer (pH 2.0), 50 mM acetate (pH 3.0–6.0) and 50 mM phosphate (pH 7.0–8.0) buffers were used. The activity of the secreted form of BGL1 (5.48 U/ml supernatant) and BGL1 displayed on the yeast surface (5.48 U/ml suspension) were used in all experiments. Each data point represents the mean of three independent experiments and the error bar indicates the standard deviation.

and temperature (30–70 °C) on β-glucosidase activity were investigated using displayed BGL1 (5.48 U/ml suspension) and secreted BGL1 (5.48 U/ml supernatant). The effect of pH on BGL1 activity on the substrate, *p*NPβG, was determined at 30 °C in various buffers ranging from pH 2.0–8.0. The optimal pH for activity of secreted BGL1 was 5.0 (Fig. 2A); this form of the enzyme was stable at pH ranging from 3.0 to 6.0 (Fig. 2B). The optimal pH for activity of displayed BGL1 was 4.0 (Fig. 2A) and this form of the enzyme also was stable at pH 3.0–6.0 (Fig. 2B). In addition, the relative activity of displayed BGL1 was higher than that of secreted BGL1 at alkaline pH.

Next, the temperature dependence of BGL1 activity using *p*NPβG as the substrate was determined by measuring activity at various temperatures at pH 5.0. Maximal activity of the secreted enzyme was observed at 55 °C (Fig. 3A); the secreted enzyme after a 4-h incubation was stable at 30–40 °C (Fig. 3B). The optimal temperature for β-glucosidase activity of displayed BGL1 also was 55 °C (Fig. 3A); the displayed BGL1 was stable in the temperature range of 30–50 °C (Fig. 3B). The thermostabilities of secreted and displayed BGL1 were investigated in detail by monitoring changes in activity during a 12 h incubation at 50 °C using *p*NPβG as the substrate (Fig. 4). After 1 h, the activity of secreted BGL1 decreased dramatically—by

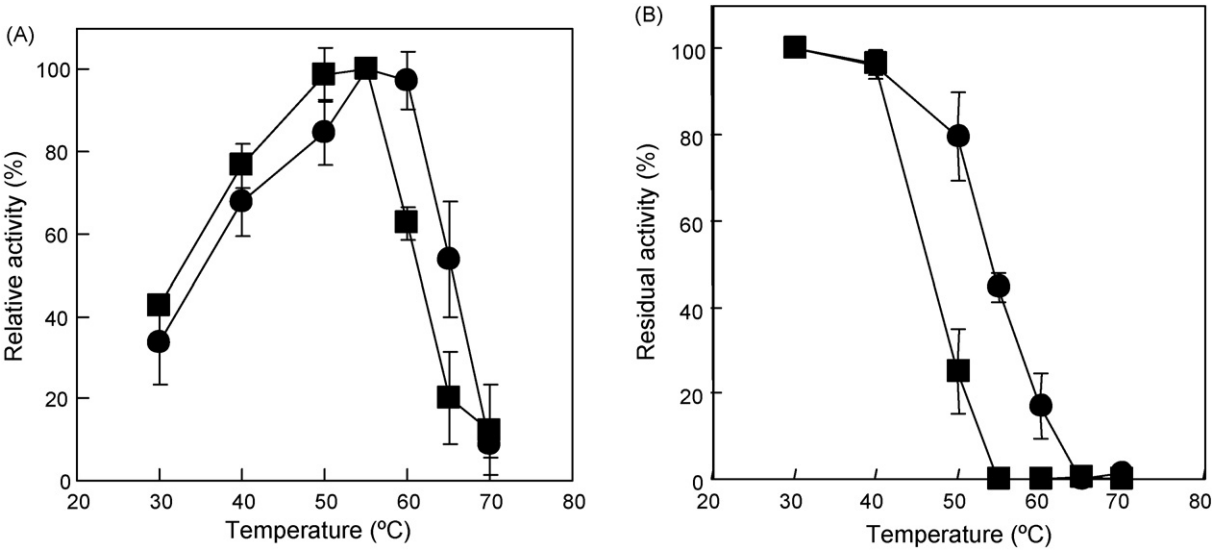

Fig. 3. (A) Effect of temperature on secreted BGL1 (square) and displayed BGL1 (circle) activity in a 50 mM sodium acetate buffer (pH 5.0). (B) Effect of temperature on secreted BGL1 (stripe) and displayed BGL1 (white) stability in a 50 mM sodium acetate buffer (pH 5.0). The activity of the secreted form of BGL1 (5.48 U/ml supernatant) and BGL1 displayed on the yeast surface (5.48 U/ml suspension) were used in all experiments. Each data point represents the mean of three independent experiments and the error bar indicates the standard deviation.

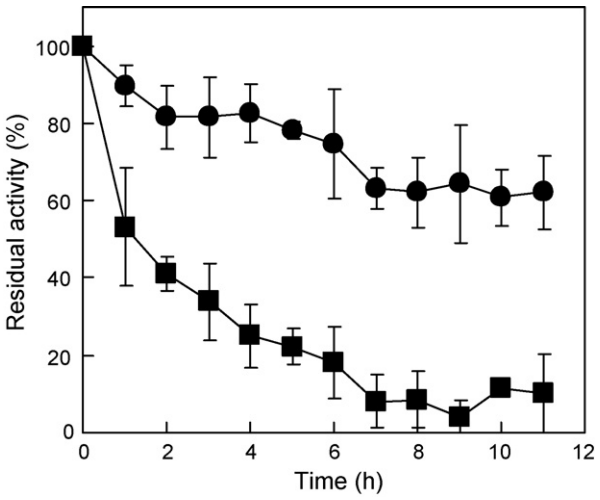

Fig. 4. Time course of residual activity of secreted BGL1 (square) and displayed BGL1 (circle) in a 50 mM sodium acetate buffer (pH 5.0) at 50 °C. The activity of the secreted form of BGL1 (5.48 U/ml supernatant) and BGL1 displayed on the yeast surface (5.48 U/ml suspension) were used in all experiments. Each data point represents the mean of three independent experiments and the error bar indicates the standard deviation.

approximately 50%. In contrast, the activity of displayed BGL1 exhibited greater thermostability; approximately 50% of the initial activity was preserved even after 12 h of incubation.

3.3. Substrate specificity

Substrate specificity of BGL1 displayed on yeast cell surfaces and BGL1 secreted by yeast was examined by comparing enzymatic activities for various substrates as follows: aryl-glycosides, alkyl-glucosides, oligosaccharides, and isoflavone conjugates (Table 1). There were no differences in substrate specificity between displayed and secreted BGL1. Both BGL1-displaying yeast cells and secreted BGL1 hydrolyzed *p*NPβG with highest substrate specificity, followed by daidzin and genistin. Oligosaccharides with -1,2,β-1,3, β-1,4 and β-1,6 linkages were not hydrolyzed by either form of BGL1.

Table 1
Hydrolysis of various substrates by cell surface-displayed or secreted BGL1

Substrate	Displayed BGL1	Secreted BGL1
p-Nitrophenyl-β-D-glucopyranoside	100	100
p-Nitrophenyl-α-D-glucopyranoside	n.d.	n.d.
p-Nitrophenyl-β-D-xylopyranoside	2.7	3.0
p-Nitrophenyl-β-D-galactopyranoside	n.d.	n.d.
p-Nitrophenyl-β-D-cellobioside	0.072	0.079
Cellobiose (Glc × 2, β-l,4)	0.46	0.30
Cellotriose (Glc × 3, β-1,4)	0.76	0.36
Cellotetraose (Glc × 4, β-1,4)	0.39	0.19
Cellopentaose (Glc × 5, β-1,4)	n.d.	n.d.
Sophorose (Glc × 2, β-1,2)	1.7	0.44
Laminaribiose (Glc × 2, β-1,3)	2.9	1.47
Gentiobiose (Glc × 2, β-1,6)	n.d.	n.d.
Methylglucoside (*C* = 1)	0.091	1.9
Hexylglucoside (*C* = 6)	3.9	4.7
Octylglucoside (*C* = 8)	7.2	5.8
Daidzin	23.2	21.7
Genistin	15.0	12.8

The substrate specificity of the secreted form of BGL1 (5.48 U/ml supernatant) and the cell surface-displayed BGL1 (5.48 U/ml suspension) was determined using various substrates in 50 mM sodium acetate buffer (pH 5.0) at 30 °C. Data are relative activity of each substrate. Each activity toward *p*-nitrophenyl-β-D-glucopyranoside was set as 100%. n.d. means not detected.

Table 2
Hydrolysis of Fujiflavone P40

Isoflavone aglycone	A	B	C	D	E	F
Daidzein	1.04	1.20	1.02	29.4	28.0	34.4
Glycitein	0.92	0.84	0.73	4.52	4.57	4.60
Genistein	0.17	0.19	0.16	6.56	7.39	8.46

Concentration (g/l) of isoflavone aglycone was shown in the table. Reaction mixtures after 3 h reaction in a 50 mM sodium acetate buffer (pH 5.0) with GRI-117-UK at 30 °C (A), 40 °C (B), or 50 °C (C), and BGL1-displaying yeast at 30 °C (D), 40 °C (E), or 50 °C (F) were analyzed according to the methods.

3.4. Isoflavone aglycone production

Isoflavone aglycone production by BGL1-displaying yeast was measured at various temperatures (Table 2). After 3 h of reaction at temperatures ranging from 30 to 50 °C the control yeast did not produce any isoflavone aglycones. However, BGL1-displaying yeasts produced isoflavone aglycones at all temperatures tested. Production of isoflavone aglycones from their glycosides increased when the reaction temperature was raised to 50 °C after 3 h of incubation, and the resulting daidzein, genistein and glycitein concentrations were 34.4, 8.46 and 4.60 g/l, respectively. Based on these results, it was estimated that the isoflavone aglycones production rate of BGL1-displaying yeast was estimated at least 15.8 g/(l h). Although Fujiflavone P40 was almost completely converted to isoflavone aglycone after 144 h of reaction (Fig. 5C), there were still remained substrates at 3 h of reaction (Fig. 5B).

4. Discussion

Because β-glucosidase plays important roles in bioconversion, various forms of the enzyme present in plants, bacteria and fungi have been isolated and characterized [11–14]. The present study used a novel β-glucosidase from *A. oryzae* to construct a BGL1-displaying yeast system for the production of isoflavone aglycones from soybean isoflavone conjugates.

The activity of BGL1 displayed on yeast cell surfaces was increased by optimization of the cultivation conditions (Fig. 1). Because expression of the BGL1 gene was under the control of the *SED1* promoter [9], maximal BGL1 activity was observed after 72 h of cultivation, consistent with native expression of the *SED1* promoter in stationary-phase cells [15]. Comparison of pH optima and stabilities for the displayed and secreted forms of BGL1 indicated that the two forms of the enzyme have different physiological characteristics. The pH optimum and stability range of BGL1 displayed on yeast cell surfaces were slightly shifted toward more alkaline pH compared with secreted BGL1 (Fig. 2A and B). The thermostability range of displayed BGL1 was higher than those of secreted BGL1 (Fig. 3B). Enzymes immobilized on solid supports by chemical binding, physical

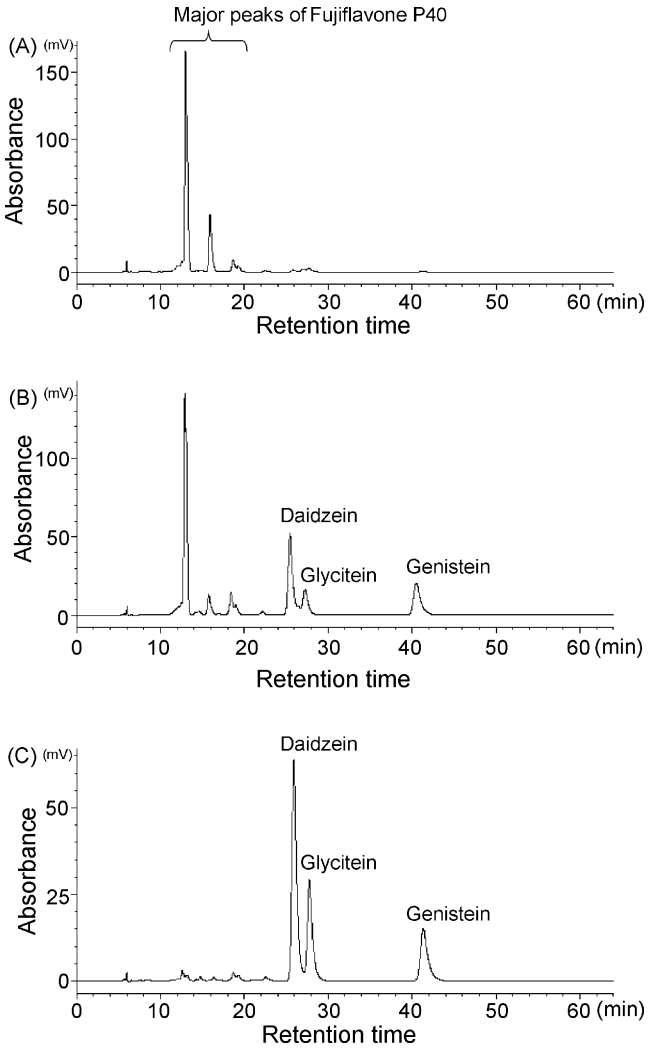

Fig. 5. HPLC analysis of hydrolysis products released from Fujiflavone P40. (A) Before reaction of Fujiflavone P40. (B) After 3-h, and (C) After 144-h reaction of Fujiflavone P40 with BGL1-displaying yeast strain in a 50 mM sodium acetate buffer (pH 5.0) at 50 °C.

retention or biomolecular affinity-binding exhibit shifts in pH and temperature optima, stability ranges and increased resistance to inactivation by high temperature and pH [16–20]. Based on these previously reported results, the enhanced stability of BGL1 displayed on yeast cell surfaces observed in the present study is assumed to be due to immobilization of the enzyme via an anchor protein, similar to covalent immobilization on a solid support. Thus, the utilization of yeast-based expression systems to display BGL1 on cell surfaces is advantageous for industrial bioconversion processes because of the elevated thermostability.

The hydrolytic activities of BGL1-displaying yeast and secreted BGL1 on a variety of glycoside substrates are summarized in Table 1. The BGL1-displaying yeast hydrolyzed a range of glucosides and synthetic xyloside; *p*-nitrophenyl-β-D-glucoside was the most highly specific hydrolyzed substrate. Moreover, daidzin and genistin, which are major components of soy isoflavones, also were hydrolyzed relatively well. These results indicate that displaying BGL1 on the yeast cell surfaces did not alter substrate specificity. Furthermore, BGL1-displaying yeast strains efficiently converted conjugated isoflavones into the aglycones.

Although parent strain GRI-117-UK could not convert Fujiflavone P40, BGL1-displaying yeast efficiently converted Fujiflavone P40 into its aglycone form. Maximal enzymatic activity on the Fujiflavone P40 substrate was at least 15.8 g/(l h) and was observed at 50 °C, which is near the optimal temperature of BGL1-displaying yeast. The isoflavone production potential of BGL-1 was comparable with that of β-glucosidase from almond and *E. coli* [21]. After 144 h reaction with BGL1-displaying yeast at 50 °C, Fujiflavone P40 was converted completely. BGL1-displaying yeast can be prepared by simple cultivation. The proposed methodology allows for effective hydrolysis of conjugated isoflavones.

The present study characterized yeast-displaying BGL1 from *A. oryzae* with high hydrolytic activity toward conjugated soybean isoflavones. This is the first report to characterize in detail BGL1 displayed on yeast cell surfaces. Thermostability was enhanced by immobilizing the enzyme to the yeast cell surface. At 50 °C, BGL1-displaying yeast hydrolyzed soy isoflavones with remarkable efficiency. Use of BGL1-displaying yeast strains may be an effective way of reducing the cost of producing isoflavone aglycones.

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