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Hanseniaspora thailandica BC9 β-Glucosidase for the Production of β-D-Hexyl Glucoside

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For biotechnological production of high-valued β-D-hexyl glucoside, the catalytic properties of *Hanseniaspora thailandica* BC9 β-glucosidase purified from the periplasmic fraction were studied, and the transglycosylation activity for the production of β-D-hexyl glucoside was optimized. The constitutive BC9 β-glucosidase exhibited maximum specific activity at pH 6.0 and 40°C, and the activity of BC9 β-glucosidase was not significantly inhibited by various metal ions. BC9 β-glucosidase did not show a significant activity of cellobiose hydrolysis, but the activity was rather enhanced in the presence of sucrose and medium-chain alcohols. BC9 β-glucosidase exhibited enhanced production of β-D-hexyl glucoside in the presence of DMSO, and 62% of β-D-hexyl glucoside conversion was recorded in 4 h in the presence of 5% 1-hexanol and 15% DMSO.

Keywords: *Hanseniaspora thailandica* BC9, β-glucosidase, transglycosylation, alkyl glycoside, β-D-hexyl glucoside

Introduction

β-Glucosidase (E.C. 3.2.1.21) catalyzes the hydrolysis of the β-1,4-glycosidic linkage between two glucose residues or that between glucose and aglycone of alkyl β-D-glycoside or aryl β-D-glycoside [1]. The enzyme is found in all living organisms, such as animals, plants, molds, yeasts, and bacteria [2–5], and the property and physiological roles are diverse depending on the organisms and the cellular locations [6]. The industrial application of β-glucosidase is also very diverse and it is utilized in various aspects; hydrolysis of cellulose for bioethanol production, hydrolysis of flavonoid glycosides for high-value aglycones, and liberation of aroma in wine [7–9]. Owing to the intrinsic chemical characteristics, β-glucosidase can also produce alkyl glycosides, disaccharides, and oligosaccharides via the reverse hydrolysis or transglycosylation reaction [10–14]. It is understood that reverse hydrolysis is driven by the thermodynamic pathway and transglycosylation is driven by the kinetic pathway [15].

Alkyl glycoside has a wide range of applications as a nonionic surfactant, particularly in cosmetics, personal care, foodstuffs, pharmaceuticals, membrane protein research, and antibacterial agents [16]. Moreover, it is favored over the other synthetic nonionic surfactants, due to its biodegradability and low toxicity. Even though alkyl glycosides can be synthesized by chemical reactions [17, 18], enzyme-catalyzed synthesis is much more desirable owing to the superior selectivity and eco-benign reaction conditions. Therefore, β-glycosidase with transglycosylation

activity has attracted considerable interests [19], and various glycosyl donors (mostly aryl glycosides and disaccharides) have been reported to produce alkyl glycosides from the reactions with different chain length of alcohols (as glycosyl acceptor) [11, 20, 21]. Until now, most alkyl glycoside production has been reported from animal or plant β-glucosidases, such as jade snail (*Achatina fulica*), almond (*Prunus amygadalus*), cassava (*Manihot esculenta* Crantz), and Thai rosewood (*Dalbergia cochinchinensis* Pierre) [22–26], because microbial β-glucosidases have a drawback of low transglycosylation activity [3, 7, 27–32]. However, industrial applications of these β-glucosidases are disadvantageous, owing to the slow growth and difficult harvest. Recently, we have isolated two yeast strains, *Pichia anomala* MDD24 and *Hanseniaspora thailandica* BC9, which enhance the aroma of wine by β-glucosidase, during alcohol fermentation. Specifically, the β-glucosidase from *H. thailandica* BC9, BC9 β-glucosidase, showed decent transglycosylation activity with medium-chain alcohols and identified as *H. thailandica* ST-464 [5]. In this study, β-glucosidase from *H. thailandica* BC9 was purified, and the β-D-hexyl glucoside production by transglycosylation activity was characterized.

Materials and Methods

Organism and Cell Culture

H. thailandica BC9 (Accession No. AB501148) was inoculated on yeast peptone dextrose agar (YPD agar), containing 10 g of yeast extract, 20 g of peptone, 20 g of glucose, and 15 g of agar in 1 L of distilled water, and incubated for 48 h at 30°C. One loopful was inoculated into YPD broth, containing 10 g of yeast extract, 20 g of peptone, and 20 g of glucose, in 1 L of distilled water, and incubated in a shaking incubator (150 rpm) at 30°C for 15 h. One percent of inoculum (approximately 10^8 cells/ml) from yeast broth was transferred into 100 ml of YPD broth with and without 1.5% (w/v) cellobiose in a 250 ml Erlenmeyer flask. The culture was grown in an incubator shaker at 150 rpm, and 30°C for 24 h [7].

BC9 β-Glucosidase Assay and Protein Determination

In the standard assay condition, the BC9 β-glucosidase activity was determined by measuring the amount of *p*-nitrophenol (*p*NP) liberated from *p*-nitrophenol-β-D-glucopyranoside (*p*NPβG; Sigma, USA) by BC9 β-glucosidase catalysis [5]. The enzyme solution (0.1 ml) was mixed with 2 mM *p*NPβG in 0.1 M citrate phosphate buffer at pH 6.0 (0.2 ml). The reaction mixture was incubated at 30°C for 30 min. The enzymatic reaction was stopped by adding 1 ml of 0.25 M Na_2CO_3 (Fluka, , Switzerland). The liberated *p*NP in the reaction mixture was measured with a spectrophotometer at 405 nm. One unit of β-glucosidase activity (U) was defined as the amount of enzyme that released 1 nmol of *p*NP per minute under the experimental conditions. The protein was measured by Lowry's method using bovine serum albumin as the standard protein [33].

Purification of BC9 β-Glucosidase

Crude enzyme from the periplasmic fraction (2.04 mg/ml, 14 ml) was adjusted with $(NH_4)_2SO_4$ to a final concentration of 1 M $(NH_4)_2SO_4$ and loaded onto the hydrophobic interaction HiPrep butyl FF column (GE Healthcare, Sweden) previously equilibrated with 1.0 M $(NH_4)_2SO_4$ in 50 mM citrate phosphate buffer, pH 6.0, equipped with a Fast Protein Liquid Chromatography system (GE Healthcare). The unbound proteins were washed from the column with 1.0 M $(NH_4)_2SO_4$ in 50 mM citrate phosphate buffer, pH 6.0. The enzyme was eluted with a 1.0 to 0.0 M $(NH_4)_2SO_4$ gradient in 50 mM citrate phosphate buffer (pH 6.0), with a flow rate of 1 ml/min. The active BC9 β-glucosidase fractions were pooled for enzyme activity assay and protein concentration determination. The specific activity of the purified protein was 2,560 U/mg protein from the 5-fold purification.

Effects of pH and Temperature on BC9 β-Glucosidase Activity

The optimal pH and stability were investigated by determination of the partially purified enzyme activities with *p*NPβG in the pH range 3.0–10.0 (0.1 M citrate phosphate buffer pH range 3.0–7.0, 0.1 M phosphate buffer pH range 6.0–8.0, and 0.1 M Tris-HCl pH range 8.0–10.0) at 30°C [34]. For the temperature-dependent activity, enzyme activity was measured at the range of 20–70°C in 0.1 M citrate phosphate buffer, pH 6.0. The relative enzyme activity at each pH or temperature was calculated as a percentage of the maximum enzyme activity. The thermal and pH stabilities of the enzyme were also investigated by measuring the relative activity at standard assay condition, after the enzyme was incubated for 1 h at different pH values and temperatures [4, 7, 34, 35].

Substrate Specificity

The substrate specificity of BC9 β-glucosidase was measured by incubating the partially purified enzyme (25.36 U, 0.1 ml) with 2.0 mM of different substrates, which were aryl glycosides (*p*NPβG, *p*-nitrophenol-β-D-galactopyranoside (*p*NPβGal), *p*-nitrophenol-β-D-fucopyranoside (pNPβFuc), and *p*-nitrophenol-β-D-arabinopyranoside (*p*NPβAra)), alkyl glucosides (β-D-hexyl glucoside and octyl glucoside), and disaccharides (sucrose and cellobiose) at standard assay conditions [7, 35]. The enzyme activities on aryl glycosides were monitored by measuring the released *p*NP product by spectrophotometry, and the activities on alkyl glucosides and disaccharide were determined by measuring the released glucose by HPLC.

Effects of Metal Ions, Reagents, Sugar, and Alcohol on BC9 β-Glucosidase Activity

The effects of metal ions, reagents, sugar, and alcohol on the enzyme activity were assayed by using *p*NPβG as the substrate. The enzyme activity was determined with various metal ions and reagents at the final concentrations of 0.2 and 2.0 mM for 30 min

[27]. The enzyme activity was also measured in the presence of sugars (glucose, sucrose, fructose, xylose, and cellobiose) [7] and alcohols (methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-hexanol, and 1-octanol) at the concentration of 0–20% (v/v) [26]. By changing the concentration of co-solvent, such as Tween 20, dimethylsulfoxide (DMSO), and dimethylformamide (DMF), from 5% to 30% (v/v), the effect of co-solvent on the enzyme activity was investigated too. The enzyme activity was measured according to standard assay conditions and the relative activity was calculated as a percentage relative to the control experiment.

β-D-Hexyl Glucoside Production

The effect of DMSO on β-D-hexyl glucoside production was studied. The 1.8 ml of reaction mixture containing 6 mM *p*NPβG, 294 U of BC9 β-glucosidase, and 0%, 5%, or 10% (v/v) 1-hexanol was incubated at 30°C under shaking condition (200 rpm) in the presence or absence of 15% (v/v) DMSO in 0.1 M citrate phosphate buffer at pH 6.0. After 24 h incubation, the samples were boiled for 5 min to stop the enzyme activity. The amounts of β-D-hexyl glucoside and glucose were analyzed by HPLC.

Effects of 1-Hexanol Concentration and Time Course on β-D-Hexyl Glucoside Production

The 1.8 ml reaction mixtures containing 6 mM *p*NPβG, 294 U of BC9 β-glucosidase, 15% (v/v) DMSO, and 0–30% (v/v) 1-hexanol in 0.1 M citrate phosphate buffer at pH 6.0 were prepared. The reaction mixtures were then incubated at 30°C for 24 h under shaking condition (200 rpm). The samples were boiled for 5 min to stop enzyme activity. The amounts of β-D-hexyl glucoside, *p*NPβG, and glucose were analyzed by using HPLC.

The incubation time for best β-D-hexyl glucoside synthesis was determined by using 5% (v/v) 1-hexanol in the presence of 15% (v/v) DMSO. The reaction mixtures were incubated at 30°C for 24 h under shaking condition (200 rpm). An aliquot of the product was withdrawn in every 4 h. The samples were heated for 5 min by boiling to stop further enzyme activity. The amounts of β-D-hexyl glucoside, *p*NPβG, and glucose were analyzed by using HPLC.

HPLC Analysis

β-D-Hexyl glucoside, *p*NPβG, and glucose were analyzed by HPLC (Waters 2414; Waters, USA) equipped with a refractive index detector and a sugar Pak column (particle size, 4 µm; dimension, 300 × 6.2 mm; Waters, USA). The sample was filtered through a nylon syringe filter (pore size, 0.22 µm; diameter, 13 mm; Whatman, USA), and 20 µl of analyte was injected. The isocratic elution was with 100% deionized water with a flow rate of 0.5 ml/min at 90°C. Standard glucose, β-D-hexyl glucoside, and *p*NPβG were used and the retention times were detected at 9.6, 19.1, and 28.6 min, respectively [36]. The percentage distribution between hydrolysis and transglycosylation in the presence of 1-hexanol was calculated from the molar ratio of glucose and β-D-hexyl glucoside.

Results

Localization of BC9 β-Glucosidase

Depending on yeast strains, β-glucosidase was reported to be found from extracellular and intracellular parts [4, 37]. When the BC9 β-glucosidase activity was measured from the growth medium and intracellular parts, the activity was detected only from the intracellular part. Further fractionation of the cell compartments and the activity measurements found that the periplasmic fraction had twice the activity of the cytoplasmic fraction (Supplementary data). The membrane fraction showed only marginal activity. The distribution of BC9 β-glucosidase activity was not changed regardless of 1.5% cellobiose.

Non-*Saccharomyces* yeast species, including *Debaryomyces vanrijiae*, *D. pseudopolymorphus*, and *D. hansenii* UFV-1, are

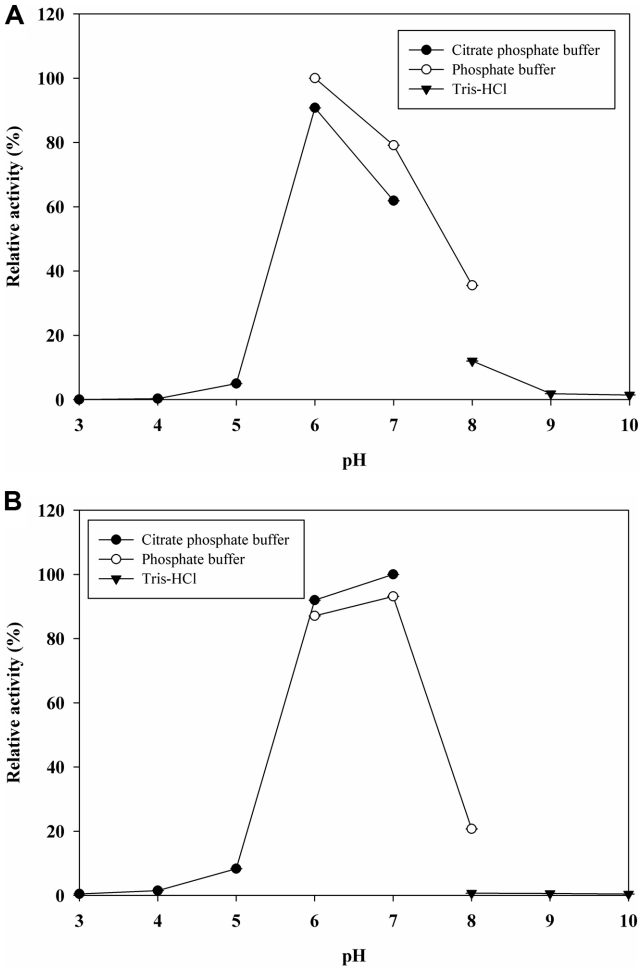

Fig. 1. Effect of pH on BC9 β-glucosidase enzyme.
(**A**) pH-dependent activity; (**B**) pH stability. Relative activity was normalized as a percentage of β-glucosidase activity.

reported to contain cellobiose transporters and produced both intracellular and extracellular β-glucosidases in the presence of cellobiose [27, 38, 39]. In contrast, BC9 β-glucosidase was produced in the cell without cellobiose, which suggested BC9 β-glucosidase is a constitutive enzyme produced in the absence of cellobiose [40].

Optimum pH and Stability of BC9 β-Glucosidase

The effect of pH on the BC9 β-glucosidase activity was studied by using *p*NPβG as the substrate (Fig. 1A). The maximum activity of BC9 β-glucosidase was found at pH 6.0, similar to the other non-*Saccharomyces* yeast β-glucosidases [31]. Yeast β-glucosidases were reported to show the optimum pH in a range of pH 4.0 to 6.5 [4, 41–43]. The pH stability of β-glucosidase revealed that the enzyme was stable from pH 6.0 to 7.0 in both 0.1 M citrate phosphate buffer and 0.1 M phosphate buffer. However, the enzyme activity decreased rapidly (more than 80%) when the pH was higher than 8.0 or lower than 5.0 after 1 h of incubation (Fig. 1B).

Effect of Temperature on BC9 β-Glucosidase Activity and Stability

The profile of the BC9 β-glucosidase activity was determined at the range of 20°C to 70°C with *p*NPβG as the substrate. The highest activity was observed at 40°C, and the enzyme activity dropped rapidly at the temperatures higher and lower than 40°C (Fig. 2). This temperature dependency was significantly different from the other β-glucosidases, which exhibited best activity at much higher temperatures. For example, the optimum temperature of β-glucosidases from *Metschnikowia pulcherrima* [44], *Aspergillus niger* [45], *Gongronella* sp. W5 [46], and *Neosartorya fischeri* P1 [29] were 50°C, 60°C, 70°C, and 80°C, respectively. The thermal stability of BC9 β-glucosidase was monitored after 1 h of incubation at various temperatures. It was stable at

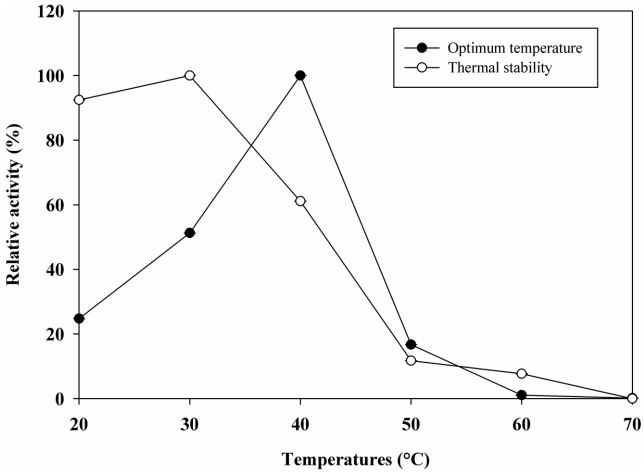

Fig. 2. Effect of temperature on the β-glucosidase activity. (●) Optimum temperature, (○) thermal stability. Relative activity was normalized as a percentage of β-glucosidase activity.

20°C and 30°C, but the activity decreased as the temperature increased to 40°C and was completely lost at 70°C. It was reported that β-glucosidase from yeasts *Candida peltata*, *Debaryomyces vanrijiae*, and *Pichia quilliermonii* were stable at the temperature of 30°C to 45°C [34, 38, 40], whereas fungal β-glucosidases from *Paecilomyces* Bainier and *Tararomyces thermophilus* exhibited high thermal stability at the temperature of 50°C to 60°C [14, 47].

Substrate Specificity of BC9 β-Glucosidase

The substrate specificity for the BC9 β-glucosidase hydrolysis activity was determined with aryl glycosides, alkyl glycosides, and disaccharides (Table 1). It was found that *p*NPβG was the best substrate for BC9 β-glucosidase. The enzyme showed very low hydrolytic activity for the other *p*NP-glycosides, such as *p*NPβGal, *p*NPβFuco, and *p*NPβAra. The enzyme exhibited high specificity for glucose

Table 1. Relative activity of BC9 β-glucosidase on various substrates.

Substrates	Type of bond	Relative activity[a] (%)
p-Nitrophenyl-β-D-glucopyranoside (*p*NPβG)	β(1,4)	100.00 ± 0.06
p-Nitrophenyl-β-D-galactopyranoside (*p*NPβGal)	β(1,4)	2.69 ± 0.05
p-Nitrophenyl-β-D-fucopyranoside (*p*NPβFuco)	β(1,4)	6.59 ± 0.07
p-Nitrophenyl-β-D-arabinopyranoside (*p*NPβAra)	β(1,4)	0.32 ± 0.03
β-D-Hexyl glucoside	β(1,4)	47.31 ± 1.36
Octyl glucoside	β(1,4)	46.14 ± 0.52
Cellobiose	β(1,4)	4.08 ± 0.26
Sucrose	β1,β2	0.00 ± 0.00

[a]Values are the means ± SD (n = 3)

Table 2. Effects of metal ions and reagents on BC9 β-glucosidase.

Metal ions and reagents	Final concentration	Relative activity[a] (%)	Final concentration	Relative activity[a] (%)
Control	0.2 mM	100 ± 0.01	2 mM	100.00 ± 0.00
Na_2SO_4	0.2 mM	91.56 ± 0.02	2 mM	96.73 ± 0.03
NaCl	0.2 mM	93.27 ± 0.01	2 mM	96.54 ± 0.03
KCl	0.2 mM	86.47 ± 0.00	2 mM	93.68 ± 0.01
$CaCl_2$	0.2 mM	95.06 ± 0.02	2 mM	106.3 ± 0.02
$CaCO_3$	0.2 mM	96.16 ± 0.00	2 mM	95.31 ± 0.02
$MgSO_4$	0.2 mM	90.38 ± 0.01	2 mM	86.02 ± 0.01
$ZnSO_4$	0.2 mM	94.08 ± 0.01	2 mM	88.3 ± 0.02
$MnCl_2$	0.2 mM	85.21 ± 0.00	2 mM	98.48 ± 0.02
$MnSO_4$	0.2 mM	98.03 ± 0.01	2 mM	98.93 ± 0.00
$FeCl_3$	0.2 mM	96.02 ± 0.00	2 mM	101.33 ± 0.00
EDTA	0.2 mM	82.52 ± 0.01	2 mM	96.81 ± 0.01
β-Mercaptoethanol	0.2 mM	95.67 ± 0.01	2 mM	93.72 ± 0.01
SDS	0.2 mM	40.45 ± 0.02	2 mM	0.00 ± 0.00

[a]Values are the means ± SD (n = 3)

with β-1,4-glucosidic linkage similar to *Paecilomyces* Bainier β-glucosidase [47]. The hydrolysis activity for the alkyl glucosides, and hexyl and octyl glucosides, was measured at around 50% compared with *p*NPβG. It was concluded that BC9 β-glucosidase flavored hydrolysis of β-1,4-glucosidic linkage in the aryl glucoside. Cellobiose exhibited about 4.0% of hydrolytic activity and no activity was observed from sucrose.

Effects of Metal Ions and Reagents on BC9 β-Glucosidase Activity

The effects of metal ions on the BC9 β-glucosidase activity was investigated (Table 2). At two different concentrations of metal ions (0.2 and 2.0 mM), they had little effect on the enzyme activity. Moreover, the chelating agent EDTA and the reducing agent β-mercaptoethanol only slightly decreased the enzyme activity. The results indicated metal ion-independency and the absence of disulfide bond of BC9 β-glucosidase [27, 46, 48, 49]. Complete loss of enzyme activity with 2.0 mM of SDS was explained by the denaturation of the enzyme [38].

The effect of sugars on the BC9 β-glucosidase activity was investigated with *p*NPβG (Fig. 3). The enzyme activity of BC9 β-glucosidase was significantly inhibited in the presence of glucose, and only 5% of relative activity was observed with 20% glucose. It is common that family 3 glycoside hydrolase (GH3) β-glucosidases are substantially inhibited by glucose [50]. It was reported that glucose acted as a competitive inhibitor with a k_i value in the range of 11.36–84 mM for the β-glucosidases from *Pseudomonas* ZD-8, *Pichia etchellsii*, *P. anomala* MDD24, *D. hansenii* UFV-1, *Wickerhamomyces anomalus* AS1, and *P. anomala* AL 112 [7, 27, 35, 51–53]. Fructose slightly inhibited BC9 β-glucosidase, and less than 20% of relative activity was inhibited by 20% of fructose. Xylose showed more inhibition on the BC9 β-glucosidase activity but less than glucose.

Whereas the β-glucosidase from *T. thermophilus* was strongly inhibited by cellobiose [14], BC9 β-glucosidase

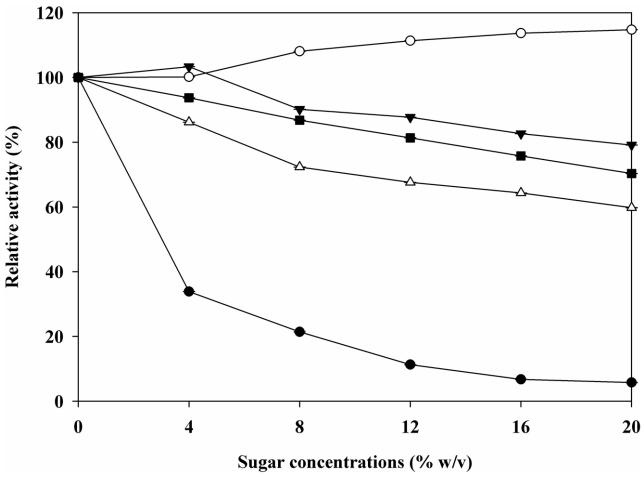

Fig. 3. Effects of sugars (●, glucose; ▼, fructose; △, xylose; ○, sucrose; and ■, cellobiose) on β-glucosidase activity.
Relative activity was normalized as a percentage of β-glucosidase activity.

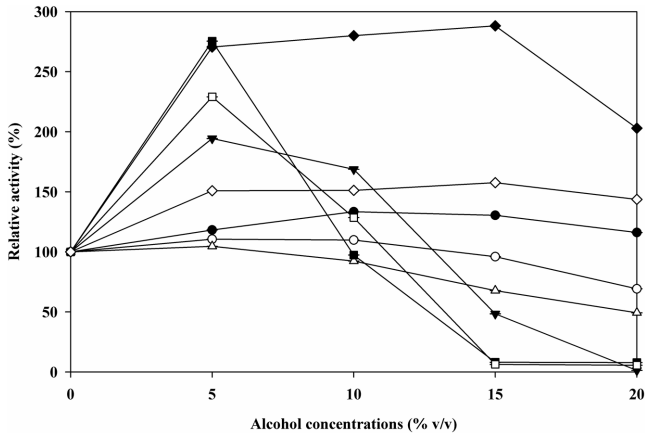

Fig. 4. Effects of alcohols (●, methanol; ○, ethanol; ▼, 1-propanol; △, 2-propanol; ■, 1-butanol; □, 2-butanol, ◆, 1-hexanol, and ◇, 1-octanol) on β-glucosidase activity.
Relative activity was normalized as a percentage of β-glucosidase activity without alcohol.

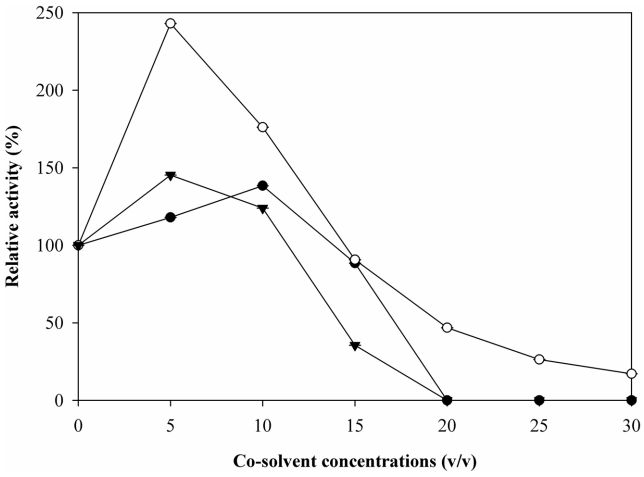

Fig. 5. Effects of co-solvents (○, DMSO; ▼, DMF, and ●, Tween 20) on β-glucosidase activity.
Relative activity was normalized as a percentage of β-glucosidase activity without co-solvent.

was slightly inhibited by cellobiose. In the presence of 20% cellobiose, it showed more than 70% of relative activity. The results indicated cellobiose either was not a good substrate (Table 1) or was an inhibitor of BC9 β-glucosidase. Interestingly, our enzyme activity was activated to 115% in the presence of 20% (w/v) sucrose. Therefore, it can be proposed that BC9 β-glucosidase could catalyze the transglycosylation activity when sucrose acts as a glucose acceptor for oligosaccharide synthesis [12].

The effects of various alcohols on the *p*NPβG hydrolysis were also studied (Fig. 4). All the tested alcohols activated the enzyme activity at 5% and the relative activity showed in the range of 104%–275%. Specifically, 1-butanol and 1-hexanol exhibited high enzyme activation by 275% and 270%, respectively. Even 15% of 1-hexanol enhanced the enzyme activity and the relative activity reached 288%. It was proposed that the activation of enzyme activity by alcohols is caused from the additional transglycosylation reaction by alcohol as a glucosyl acceptor [54].

1-Hexanol was selected for the synthesis of alkyl glucoside for the study because it activated BC9 β-glucosidase activity, and β-D-hexyl glucoside has more commercial value than other medium-chain alkyl glucosides in cosmetics and pharmaceuticals industries. However, the solubility of 1-hexanol in aqueous phase was limited, and application of co-solvents was attempted (Fig. 5). DMSO, DMF, and Tween 20 activated enzyme activity at the range of 5%–10%. However, DMF dramatically reduced the enzyme activity when the concentration was higher than 10%. In the cases of DMSO and Tween 20, the enzyme activity was only slightly inhibited with 15% of co-solvents. However, it was reported that Tween 20 strongly inhibited the recombinant β-glucosidase from *Anoxybacillus* sp. at 5% (v/v) [55]. In addition, the high viscosity of Tween 20 was not suitable for β-D-hexyl glucoside synthesis in our experiments. Since DMSO was a common co-solvent for alkyl glycoside synthesis [11, 13, 22], 15% DMSO was adopted for the BC9 β-glucosidase production of β-D-hexyl glucoside.

β-D-Hexyl Glucoside Production

To increase β-D-hexyl glucoside production, experiments were performed with 5% and 10% 1-hexanol as the glucose acceptor and *p*NPβG as the glucose donor in the presence of 15% DMSO. As shown in Table 3, 65.93% and 57.43% of β-D-hexyl glucoside were produced with 5% and 10% 1-hexanol, respectively. In the absence of DMSO, only 6% of transglycosylation activity was observed from both concentrations of 1-hexanol. Based on these results, co-solvent played an important role in decreasing the water activity and increasing the substrate solubility, thus shifting the equilibrium toward the transglycosylation reaction [13].

Optimizing the incubation time is important for obtaining the highest β-D-hexyl glucoside production as well as the cost of the process. β-D-Hexyl glucoside production was performed for 24 h by using *p*NPβG as the glucose donor with 5% 1-hexanol in the presence of 15% DMSO. The maximum β-D-hexyl glucoside production of ~62% was obtained at 4 h and substrate *p*NPβG was reduced to 3.83%

Table 3. Effect of DMSO on hexyl glucoside (HG) synthesis via transglucosylation activity.

DMSO (% v/v)	1-Hexanol (%)	Glucose concentration (mM)	% Glucose	HG concentration (mM)	% HG
0	0	5.47 ± 0.34	100.00	0.00 ± 0.00	0.00
	5	4.80 ± 0.08	87.76	0.35 ± 0.05	6.36
	10	4.94 ± 0.07	90.26	0.37 ± 0.03	6.72
15	0	5.20 ± 0.15	100.00	0.00 ± 0.00	0.00
	5	1.29 ± 0.06	24.77	3.43 ± 0.12	65.93
	10	1.21 ± 0.07	23.22	2.99 ± 0.03	57.43

(Fig. 6). However, the amount of β-D-hexyl glucoside began to slowly decrease after 4 h and approached to 50%, probably due to the secondary hydrolysis of β-D-hexyl glucoside [56].

Discussion

The limited industrial applications of β-glucosidase in food and medicinal applications have been enlarged due to emerging bioenergy production. Furthermore, the application of catalytic activity was diversified to alkyl glucoside production, which can be considered as a modified version of glucoside hydrolysis by alcohols. In this report, basic characteristics of a new β-glucosidase from *H. thailandica* BC9 were investigated for the future industrial production of β-D-hexyl glucoside. Because of the high commercial value, the catalytic reaction of 1-hexanol transglycosylation was studied in detail. In principle, transglycosylation always competes with hydrolysis and the catalytic performance can be improved by adjusting the temperature and effective substrate concentration. Practically, efficient substrate feeding and product separation can be obtained by manipulating the reaction conditions for the marginally soluble medium chain alcohol and alkyl glucoside.

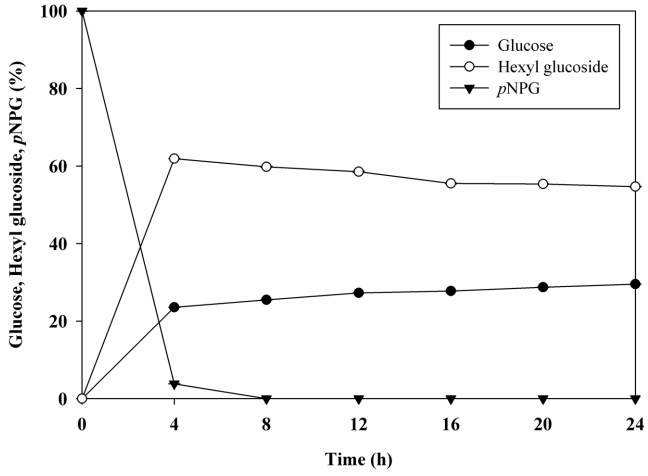

Fig. 6. Time courses for the synthesis of β-D-hexyl glucoside catalyzed by BC9 β-glucosidase with 5% 1-hexanol in the presence of 15% DMSO.

The periplasmic β-glucosidase from the new species *H. thailandica* BC9 exhibited moderate thermal and pH stability, which allow energy-efficient industrial application. It was not inhibited by various metal ions, reagents, and surfactants. Although it was inhibited by glucose, BC9 β-glucosidase was activated with sucrose and several alcohols. The BC9 β-glucosidase marginally hydrolyzed cellobiose, but produced β-D-hexyl glucoside in a decent yield. The optimized reaction conditions resulted in 62% of β-D-hexyl glucoside conversion in 4 h in the presence of 5% (v/v) 1-hexanol and 15% (v/v) DMSO.

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Conflict of interest

The authors have no financial conflicts of interest to declare.

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