J. Microbiol. Biotechnol. (2013), **23**(3), 351–356
http://dx.doi.org/10.4014/jmb.1210.10060
First published online January 25, 2013
pISSN 1017-7825 eISSN 1738-8872

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Optimization of β-Glucosidase Production by a Strain of *Stereum hirsutum* and Its Application in Enzymatic Saccharification[S]

Ramachandran, Priyadharshini[1], Ngoc-Phuong-Thao Nguyen[1], Joon-Ho Choi[2], Yun Chan Kang[1], Marimuthu Jeya[1]*, and Jung-Kul Lee[1]*

[1]*Department of Chemical Engineering, Konkuk University, Seoul 143-701, Korea*
[2]*Department of Food Science and Biotechnology, Wonkwang University, Iksan-city, JeonBuk 570-749, Korea*

Received: October 23, 2012 / Revised: December 3, 2012 / Accepted: December 4, 2012

A high β-glucosidase (BGL)-producing strain, *Stereum hirsutum*, was identified and isolated and showed a maximum BGL activity (10.4 U/ml) when cultured with Avicel and tryptone as the carbon and nitrogen sources, respectively. In comparison with other BGLs, BGL obtained from *S. hirsutum* showed a higher level of activity to cellobiose (V_{max} = 172 U/mg, and k_{cat} = 281/s). Under the optimum conditions (600 rpm, 30°C, and pH 6.0), the maximum BGL activity of 10.4 U/ml with the overall productivity of 74.5 U/l/h was observed. BGL production was scaled up from a laboratory scale (7-L fermenter) to a pilot scale (70-L fermenter). When *S. hirsutum* was cultured in fed-batch culture with rice straw as the carbon source in a 70-L fermenter, a comparable productivity of 78.6 U/l/h was obtained. Furthermore, *S. hirsutum* showed high levels of activity of other lignocellulases (cellobiohydrolase, endoglucanase, xylanase, and laccase) that are involved in the saccharification of biomasses. Application of *S. hirsutum* lignocellulases in the hydrolysis of *Pinus densiflora* and *Catalpa ovata* showed saccharification yields of 49.7% and 43.0%, respectively, which were higher than the yield obtained using commercial enzymes.

Key words: Biomass, β-glucosidase, production, pilot scale, saccharification

Cellulose is the main constituent of wood tissue and is the most abundant renewable biomass on Earth [12]. Microbial cellulases that hydrolyze cellulose are industrially important and are used in industries such as food, animal feed, brewing and wine making, agriculture, biomass refining, pulp and paper, textile, and laundry [10]. At least 3 types of enzymes, endoglucanase (EG, E.C.3.2.1.4), cellobiohydrolase (CBH, E.C.3.2.1.91), and β-glucosidase (BGL, 3.2.1.21), are involved in the conversion of cellulose to glucose. EGs act randomly along the cellulose chains to produce cellulose fragments. CBHs act as exoglucanase to release cellobiose, and BGLs hydrolyze cellobiose to yield glucose. Cellobiose exerts product inhibition on both CBH and EG. BGL not only produces glucose but also reduces the product inhibition exerted by cellobiose, allowing efficient functioning of the other cellulolytic enzymes. Therefore, high BGL activity is essential for efficient enzymatic saccharification of a lignocellulosic biomass [5,8,16].

Many bacteria, fungi, and yeast produce BGL. The enzyme system of *Trichoderma* spp. has been the most studied and reviewed among those of the cellulose-producing fungi. The amount of BGL produced by this fungal species is insufficient for the effective conversion of cellulose to glucose. High levels of BGL are important for the complete conversion of cellulose because of the product inhibition by cellobiose on CBH and EG. In the flavor industry, BGLs are the key enzymes in the production of aromatic compounds from the glucosidic precursors present in fruit and fermentation products. BGL is also useful in a de-inking process for removal of printing ink from wastepaper.

In the present study, we isolated and identified a potent BGL-producing fungus, *Stereum hirsutum* SKU512. In a previous study, a woody biomass was biologically pretreated using *S. hirsutum*. This species of white rot fungus could effectively degrade lignin and other lignin-like compounds, such as chlorine-containing aromatic compounds [11].

*Corresponding authors
J.-K. L.
Phone: +82-2-450-3505; Fax: +82-2-458-3504;
E-mail: jkrhee@konkuk.ac.kr
M. J.
Phone: +82-2-458-3501; Fax: +82-2-458-0879;
E-mail: jeya@konkuk.ac.kr

[S]Supplementary data for this paper are available on-line only at http://jmb.or.kr.

However, the cellulolytic activity of *S. hirsutum* has not been reported. Here, we evaluated the optimal culture parameters for maximum BGL production by *S. hirsutum*. We also studied the enzymatic saccharification of lignocellulosic substrates using lignocellulases from *S. hirsutum*.

Materials and Methods

Screening, Isolation, and Identification of the Microorganism

The soil samples were collected from Sorak Mountain, Korea by using the capillary tube method and were diluted in 0.9% saline. The aliquots were spread on potato dextrose agar (PDA) plates, which were then incubated for 3 days. Morphologically distinct colonies were inoculated into 3 ml of growth medium (composition in g/l: peptone, 8; yeast extract, 2; KH_2PO_4, 5; K_2HPO_4, 5; $MgSO_4 \cdot 7H_2O$, 3; and cellulose, 20; Sigma, St. Louis, MO, USA). The colonies were cultured at 28°C with agitation at 200 rpm for 5 days. The BGL activity of the culture broth was analyzed using a previously described method using *p*-nitrophenyl-β-D-glucopyranoside (pNPG, Sigma). On the basis of the results of the analyses, the strain with the highest BGL activity was selected. The isolated strain was identified by using internal transcribed spacer (ITS) rDNA sequence analysis. The sequence was then submitted to GenBank with the accession number HM004553. The identified strain *S. hirsutum* SKU512 was added to the list of organisms at the Korean Culture Center of Microorganisms (KCCM) and was given the KCCM accession number 10982P.

Culture Conditions and Shake Flask Cultures

The fungal strain was subcultured every 3 weeks and stored at 4°C on PDA plates. A 500-ml flask containing 50 ml of PDB was used to seed the culture. After 4–5 days of incubation, 5 ml of this seed culture was inoculated in 50 ml of standard production medium (composition in g/l: peptone, 8; yeast extract, 2; KH_2PO_4, 5; K_2HPO_4, 5; $MgSO_4 \cdot 7H_2O$, 3; thiamin·HCl, 0.02; and cellulose, 20) with the pH adjusted to 5.0. The flasks were incubated at 25°C with agitation at 150 rpm for 5 days.

The optimal concentrations of the best carbon and nitrogen sources for BGL production were evaluated by varying the concentration from 1% to 5% and from 0.5% to 2.5%, respectively. The effects of vitamins in BGL production were studied using various vitamins (inositol, riboflavin, pyridoxine hydrochloride, biotin, calcium pantothenate, folic acid, and thiamin hydrochloride) at different concentrations ranging from 1 to 50 mg/l. All fermentation trials were performed in triplicate, and the enzyme activity was assayed after 5 days of culturing. The activities of other lignocellulases such as cellobiohydrolase, endoglucanase, and endoxylanase were determined according to standard procedures.

Laboratory-Scale and Pilot-Scale Cultures

The fermentation trials were performed in a 7-L fermenter (working volume, 3.5 L) and the agitation rate, pH, and temperature were examined. For these experiments, 1% (v/v) of 5-day-old preculture was used as the inoculum. The standard medium was used, and the aeration rate was fixed at 0.5 vvm. Antifoam agent was added when required. Samples were drawn from the fermentative broth at regular intervals and analyzed to determine the enzyme activity. For the 70-L stirred fermenter, the precultures were used at 1.3% (v/v) with a 35-L working volume. The optimal conditions including medium compositions determined in shake flask scale and fermentation in 7-L jar fermenter scale were applied for the scale-up production.

Pretreatment and Enzymatic Saccharification of Lignocellulosic Biomass

Two lignocellulosic biomasses (*Catalpa ovata* and *Pinus densiflora*) were used as the substrates for hydrolysis. The lignocellulosic content of the biomasses was analyzed according to the method described by Sluiter *et al*. [17]. Glucose and xylose contents were analyzed using a high performance liquid chromatography (HPLC, UltiMate 3000, Dionex) instrument equipped with an evaporative light scattering detector (ELSD; ESA Inc., USA). The sugars were separated using a Shodex Sugar SP0810 column at 30°C with 70% acetonitrile as the eluent at a flow rate of 0.5 ml/min.

The lignocellulosic materials were pretreated with NaOH prior to the hydrolysis. Therefore, 20 g of the biomass was suspended in 80 ml of a 2% aqueous solution of NaOH at 85°C for 1 h, with a solid-to-liquid ratio of 1:4. The solid residue was collected through filtration and extensively washed with distilled water until the pH was neutral. The pretreated biomass was dried in an oven at 70°C to maintain a constant weight and used as the substrate for the saccharification experiments. A typical hydrolysis mixture consisted of 0.2 g of the substrate, 20 FPU (filter paper units) of the enzyme [Celluclast 1.5L (Novozyme) or *S. hirsutum* BGL], and 10 ml of sodium acetate buffer (pH 5.0). This mixture was incubated at 37°C in a rotary shaker at 150 rpm and was sampled at different time intervals. The samples were immediately heated to 100°C to denature the enzymes, cooled, and centrifuged for 10 min at 8,000 rpm. The supernatant obtained was used for determining the reducing sugar content, which was measured using the 3,5-dinitrosalicylic acid (DNS) method. The conversion efficiency was calculated by using the following equation: % Saccharification = reducing sugars × 0.9 × 100/amount of carbohydrates in the substrate. The results obtained for enzymatic conversion using *S. hirsutum* cellulase were compared with those obtained during enzymatic conversion using Celluclast 1.5L.

Results and Discussion

Identification of the Isolated Strain

A high-BGL-producing fungus was isolated from the soil. The ITS rDNA of the isolate was sequenced, and the results showed that the isolated strain exhibited maximum identity with *S. hirsutum* HM004553. Morphological analysis showed that the fruiting bodies were often fused together and were semicircular or irregular, hairy, and laterally attached, without a stem. In addition, the isolated strain showed a similar cellular fatty acid composition as that of *S. hirsutum* (data not shown). On the basis of the morphology, fatty acid composition, and rDNA gene sequence, the isolated strain was identified as a variant of *S. hirsutum* and was named *S. hirsutum* SKU512.

Table 1. Effects of various carbon sources on the production of β-glucosidase (BGL) by *S. hirsutum*.

Carbon source (20 g/l)	BGL specific activity (U/mg-protein)	BGL activity (U/ml)
Avicel	7.21 ± 0.67	5.32 ± 0.39
Cellobiose	0.51 ± 0.04	0.10 ± 0.01
Maltose	2.17 ± 0.33	0.16 ± 0.01
CMC	5.51 ± 0.45	1.99 ± 0.12
Xylan	0.54 ± 0.04	0.21 ± 0.01
Lactose	1.86 ± 0.12	0.13 ± 0.01
Glucose	0.53 ± 0.03	0.03 ± 0.01
Rice straw	2.85 ± 0.11	1.47 ± 0.03
Bagasse	1.18 ± 0.13	0.86 ± 0.06
Palm kernel	1.55 ± 0.11	0.38 ± 0.04
Cellulose	6.70 ± 0.50	3.29 ± 0.21

Tryptone (8 g/l) and yeast extract (2 g/l) were used as the nitrogen sources.

Effects of Carbon, Nitrogen, and Vitamin Sources on BGL Production

We studied the effects of different carbon sources on BGL production by *S. hirsutum* SKU512. After 12–13 days of incubation, the highest BGL activity (5.31 U/ml; specific activity, 7.20 U/mg) was obtained when Avicel was used as the sole carbon source (Table 1) at a concentration of 20 g/l. In an attempt to reduce the enzyme production cost, we also tested BGL production with different concentrations of rice straw. In a medium with rice straw concentration of 30 g/l, a maximum BGL activity of 6.52 U/mg was observed; this was comparable to the BGL activity obtained using Avicel as the carbon source. Similarly, among the nitrogen sources that were tested for BGL production, tryptone was found to be the most significant source (data not shown). In a medium with tryptone concentrations between 10 and 15 g/l, BGL total and specific activities of 5.48 U/ml and 8.12 U/mg, respectively, were recorded. To examine the effects of different vitamins on BGL production, the medium containing Avicel (20 g/l) and tryptone (10 g/l) was supplemented with folic acid, pyridoxine hydrochloride, riboflavin, biotin, calcium pantothenate, or inositol (0–50 mg/l) (Table 2). The control culture without any vitamin supplementation showed a lower BGL activity (3.13 U/ml). The addition of vitamins such as thiamin hydrochloride, folic acid, pyridoxine hydrochloride, and riboflavin was shown to increase the BGL activity. A 1.6-fold increase in BGL activity (4.86 U/ml; 9.96 U/mg) was observed when the culture medium was supplemented with 20 mg/l of inositol. In a medium with rice straw concentration of 30 g/l instead of Avicel and supplemented with inositol, a BGL activity of 8.80 U/mg was observed. This high BGL activity could be attributed to the increased BGL production after inositol supplementation. Similarly, supplementation of thiamine was shown to increase the BGL production in *Fomitopsis pinicola*, which could be because the expression of BGL was higher than that of other proteins in the fungal proteome [6].

Optimization of Process Parameters for BGL Production

The fermentation conditions, including the agitation rate, temperature, and pH, were evaluated for BGL production on a laboratory-scale 7-L fermenter. The highest BGL activity was obtained when culturing was performed at an agitation speed of 600 rpm (Table 3). When the agitation speed was increased, the BGL total and specific activity decreased because of reduced protein concentration. Similar to our observations, Mendoza *et al.* [13] reported that a high agitation rate of 600 rpm was necessary for the optimal production of xylanase by *Thermomyces lanuginosus* C1a. A previous report [2] also showed that cellulase production by *Thermobifida fusca* increased with increasing aeration and agitation speed, because aeration and oxidative respiration are interconnected.

The cultures were incubated at different pH and temperature values to evaluate the optimal criteria for maximum BGL production in the 7-L fermenter. The optimal pH was 6.0, and growth of the organism at higher pH values resulted in decreased BGL production (Table 3). *S. hirsutum* was cultured at different temperatures ranging from 24°C to 32°C, and the optimal temperature for maximum BGL production was 30°C. An increase in temperature to 32°C resulted in decreased enzyme production (Table 3).

Table 2. Effects of various vitamins on the production of BGL by *S. hirsutum*.

Vitamin (mg/l)	1	5	10	20	30	50
Thiamin HCl	3.12	3.29	3.31	3.44	3.47	3.37
Inositol	3.54	4.35	4.71	4.86	4.01	3.5
Riboflavin	3.62	3.67	3.71	4.18	3.74	3.15
Pyridoxine HCl	3.29	3.71	3.86	3.53	3.50	3.26
Biotin	3.71	4.03	3.89	3.79	3.68	3.55
Calcium pantothenate	3.43	3.47	3.79	3.9	3.82	3.27
Folic acid	3.13	3.19	3.43	3.49	3.19	3.11
Control			3.13			

All the values represent the BGL activity in U/ml. Each value is the mean of triplicate measurements, and no measurement varied from the mean by more than 15%.

Table 3. Effects of the agitation rate, pH, and temperature on BGL production.

Parameter	Value	BGL specific activity (U/mg-protein)	BGL activity (U/ml)
Agitation rate[a] (rpm)	500	7.43 ± 0.32	1.85 ± 0.24
	600	9.18 ± 0.31	2.01 ± 0.23
	700	6.57 ± 0.44	0.85 ± 0.08
	800	6.52 ± 0.56	0.46 ± 0.05
pH[b]	4	4.37 ± 0.57	1.46 ± 0.07
	5	10.2 ± 0.3	1.99 ± 0.27
	6	15.5 ± 1.9	2.73 ± 0.12
	7	14.0 ± 1.3	1.65 ± 0.11
Temperature[c] (°C)	24	14.4 ± 0.8	2.71 ± 0.20
	27	18.4 ± 1.8	3.14 ± 0.14
	28.5	23.3 ± 1.6	3.96 ± 0.26
	30	25.6 ± 1.5	5.52 ± 0.11
	32	8.00 ± 1.02	2.25 ± 0.19

[a]Other parameters were pH 5.0 and temperature of 24°C.
[b]Other parameters were 600 rpm and temperature of 24°C.
[c]Other parameters were 600 rpm and pH 6.0.

At the optimal conditions, *S. hirsutum* exhibited maximum BGL total (10.4 U/ml) and specific (27.7 U/mg) activities, which were respectively 3.1- and 4.2-fold higher than those of the shake flask cultures before optimization (Fig. 1). The total activity obtained (10.4 U/ml) was comparable to that obtained for *Aspergillus wentii* (10 U/ml) [18] and higher than those for *Trichoderma harzianum* (1.7 U/ml) [7], *Aspergillus niger* (7.2 U/ml) [3], *Thermoascus aurantiacus* (6.1 U/ml), and a mixed culture of *Trichoderma reesei* and *Aspergillus ochraceus* (1.6 U/ml). The overall BGL productivity achieved using *S. hirsutum* was 74.5 U/l/h, which was higher than those obtained for *A. wentii* (61.9 U/l/h) [18], *T. aurantiacus* (31.9 U/l/h), and a mixed culture of *A. ochraceus* and *T. reesei* (10.9 U/l/h).

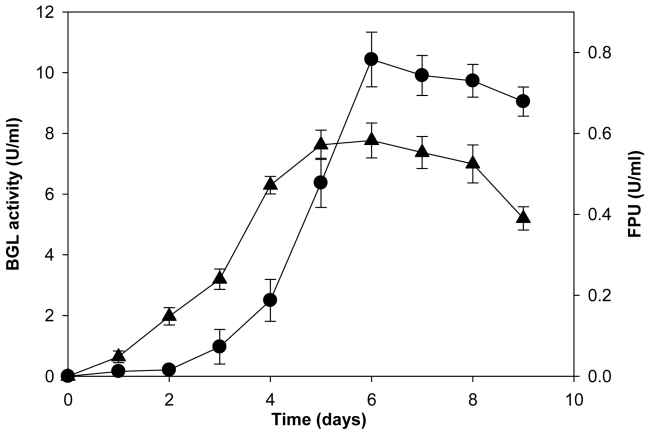

Fig. 1. Time required for expression of maximum total cellulase activity (in FPU) and β-glucosidase (BGL) production by *S. hirsutum*.
S. hirsutum was cultured for 9 days in a 7-L fermenter with 3 L of culture medium containing rice straw and tryptone as the carbon and nitrogen sources, respectively. Cultures were maintained at a temperature of 30°C and agitated at 600 rpm. (●) BGL activity; (▲) enzyme activity in FPU.

Scale-Up of BGL Production from a Laboratory Scale to a Pilot Scale

BGL production by *S. hirsutum* was scaled up from a laboratory scale to a pilot scale. Fig. 3 shows the time course of BGL production in a typical batch cultivation mode with working volume of 35 L. The optimal media components used in the 7-L fermenter were used. Fermentation was performed at 200 rpm, 30°C, and pH 6.0. Maximum activity (6.1 U/ml) was obtained at 96 h of cultivation. After 96 h, the volumetric activity decreased significantly from 6.1 U/ml to 3.4 U/ml. To improve the enzyme productivity, a fed-batch culture followed. Feeding of medium components at 10% (v/v) was performed at 96 h. The highest BGL activities (12.3 U/ml and 15.3 U/mg) were obtained after 156–165 h of fermentation, with the overall productivity of 78.6 U/l/h, which was comparable to that achieved in a 7-L fermenter. When one more feeding cycle was used at 165 h, there was no more increase in BGL activity at later stages (data not shown).

Purification and Assessment of the Kinetic Parameters of *S. hirsutum* BGL

The BGL obtained by culturing *S. hirsutum* was about 200-fold purified to homogeneity (Fig. 2) from the culture supernatants by performing a single-step chromatography on a gel filtration column [14]. The relative molecular mass of BGL from *S. hirsutum* was determined as 98 kDa

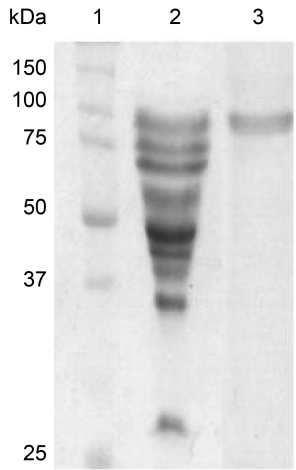

Fig. 2. Polyacrylamide gel electrophoresis (PAGE) of BGL purified from the culture supernatant of *S. hirsutum* showing lane 1, molecular marker; lane 2, cell extract; and lane 3, purified BGL.

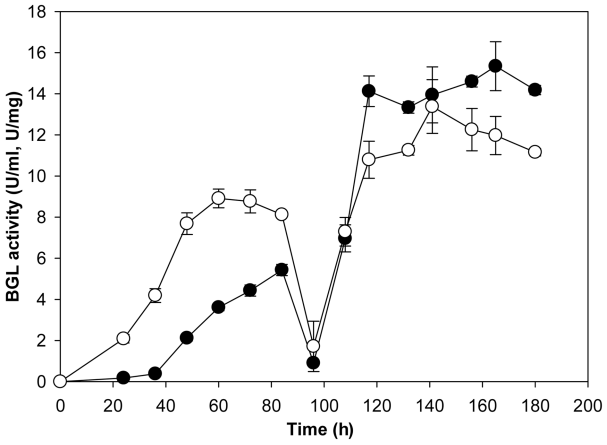

Fig. 3. Time course of BGL production by *S. hirsutum* under optimized condition in a 70-L fermenter (working volume of 35 L) with one feeding cycle at 96 h.
(●) Volumetric activity (U/ml); (○) specific activity (U/mg). Values are the means of two replicate determinations; error bars indicate the standard deviations. When not shown, the error bars fall within the symbols.

by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The purified BGL was incubated in a 100 mM sodium acetate buffer (pH 5.0) at 65°C with different concentrations of cellobiose (range, 1–100 mM). Values for K_m and V_{max} were determined by nonlinear regression analysis of the data using Prism 5 (Graphpad Software, Inc., CA, USA). In comparison with other fungal BGLs (Supplementary Table S1), BGL from *S. hirsutum* showed a higher level of activity toward cellobiose (K_m = 1.2 mM, V_{max} = 172 U/mg, k_{cat} = 281/s).

Lignocellulases in *S. hirsutum* Culture

The highest levels of FPU (0.58 U/ml), CBH (2.4 U/ml), and EG (0.5 U/ml) were also observed in *S. hirsutum* cultures under the same optimal conditions for BGL production. In addition, *S. hirsutum* showed prominent endoxylanase and laccase activities; the highest endoxylanase and laccase activities were 29.9 and 0.12 U/ml, respectively. Hence, the enzyme cocktail observed in *S. hirsutum* culture could be used for the saccharification of lignocellulosic biomasses.

Saccharification of Woody Biomass Using *S. hirsutum* Lignocellulases

Table 4 shows the reducing sugar concentration and the saccharification yield after hydrolysis of pretreated and untreated plant materials by *S. hirsutum* cellulase. Without a pretreatment procedure, saccharification of the lignocellulosic biomass did not produce high yields, because the lignin in the plant cell wall acted as a barrier to the enzyme action [4]. The conversion efficiency increased with the use of pretreated substrates. The highest saccharification yield using the *S. hirsutum* cellulase was achieved for pretreated *P. densiflora* (49.7%) and *C. ovata* (43.0%). Although the cellulose content of *C. ovata* was higher than that of *P. densiflora*, the lower saccharification yield and reducing sugar concentration were obtained from *C. ovata*; this could be attributed to the rigid structure of the lignin content in *C. ovata*, which protected the cellulose and hemicellulose contents from enzymatic hydrolysis [9]. The reducing sugar concentrations and the saccharification yield obtained by *S. hirsutum* cellulase were higher than those obtained by using the commercial enzyme Celluclast 1.5L (Table 4). Celluclast 1.5L is the most frequently used enzyme in various studies on lignocellulose hydrolysis [1, 15], and this enzyme contains an almost complete set of cellulases but lacks BGL. However, *S. hirsutum* produced a significant level of BGL along with other lignocellulases; and hence, it can be used for enzymatic hydrolysis of various lignocellulosic biomasses.

In conclusion, a potent BGL-producing strain (*S. hirsutum*) was isolated and identified, and applied in the saccharification of lignocellulosic biomasses. The conditions for maximum production of BGL were optimized and production was

Table 4. Reducing sugar concentration and saccharification yield after hydrolysis of pretreated and untreated plant materials.

Plant species			*P. densiflora*	*C. ovata*
Untreated biomass	*S. hirsutum* lignocellulase	Reducing sugar (mg/g-substrate)	161	133
		Saccharification yield (%)	17.8	14.2
	Celluclast	Reducing sugar (mg/g-substrate)	118	104
		Saccharification yield (%)	13.1	11.1
Pretreated biomass	*S. hirsutum* lignocellulase	Reducing sugar (mg/g-substrate)	435	405
		Saccharification yield (%)	49.7	43.0
	Celluclast	Reducing sugar (mg/g-substrate)	422	381
		Saccharification yield (%)	46.7	40.7

Each value represents the mean of triplicate measurements, and no measurement varied from the mean by more than 15%.

scaled up from a laboratory scale to a pilot scale. The saccharification rates for woody biomasses treated with the fungal enzyme were higher than those obtained by the commercial enzyme. Thus, *S. hirsutum* has been proven for use in the enzymatic saccharification of lignocellulosic materials.

Acknowledgment

This paper resulted from the Konkuk University research support program.

References

1. Cara, C., E. Ruiz, I. Ballesteros, M. J. Negro, and E. Castro. 2006. Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. *Process Biochem.* **41:** 423–429.
2. Deng, Y. and S. S. Fong. 2010. Influence of culture aeration on the cellulase activity of *Thermobifida fusca*. *Appl. Microbiol. Biotechnol.* **85:** 965–974.
3. Garcia Kirchner, O., M. Segura-Granados, and P. Rodriguez-Pascual. 2005. Effect of media composition and growth conditions on production of β-glucosidase by *Aspergillus niger* C-6. *Appl. Biochem. Biotechnol.* **121–124:** 347–359.
4. Gupta, R., G. Mehta, Y. P. Khasa, and R. C. Kuhad. 2011. Fungal delignification of lignocellulosic biomass improves the saccharification of cellulosics. *Biodegradation* **22:** 797–804.
5. Horn, S., G. Vaaje-Kolstad, B. Westereng, and V. G. Eijsink. 2012. Novel enzymes for the degradation of cellulose. *Biotechnol. Biofuels* **5:** 45.
6. Joo, A. R., K. M. Lee, W. I. Sim, M. Jeya, M. R. Hong, Y. S. Kim, *et al*. 2009. Thiamine increases β-glucosidase production in the newly isolated strain of *Fomitopsis pinicola*. *Lett. Appl. Microbiol.* **49:** 196–203.
7. Kocher, G. S., K. L. Kalra, and G. Banta. 2008. Optimization of cellulase production by submerged fermentation of rice straw by *Trichoderma harzianum* Rut-C 8230. *Int. J. Microbiol.* **5**.
8. Kostylev, M. and D. Wilson. 2012. Synergistic interactions in cellulose hydrolysis. *Biofuels* **3:** 61–70.
9. Kovacs, K., S. Macrelli, G. Szakacs, and G. Zacchi. 2009. Enzymatic hydrolysis of steam-pretreated lignocellulosic materials with *Trichoderma atroviride* enzymes produced in-house. *Biotechnol. Biofuels* **2:** 14.
10. Kuhad, R. C., R. Gupta, and A. Singh. 2011. Microbial cellulases and their industrial applications. *Enzyme Res.* **2011**. DOI: 10.4061/2011/280696.
11. Lee, S. M., B. W. Koo, J. W. Choi, D. H. Choi, B. S. An, E. B. Jeung, *et al*. 2005. Degradation of bisphenol A by white rot fungi, *Stereum hirsutum* and *Heterobasidium insulare*, and reduction of its estrogenic activity. *Biol. Pharm. Bull.* **28:** 201–207.
12. Lynd, L. R., M. S. Laser, D. Bransby, B. E. Dale, B. Davison, R. Hamilton, *et al*. 2008. How biotech can transform biofuels. *Nat. Biotechnol.* **26:** 169–172.
13. Mendoza, N. S., Y. Katakura, and S. Shioya. 2004. Influence of agitation and dissolved oxygen tension on the growth and cellulase-free xylanase production by *Thermomyces lanuginosus* C1a in bioreactor. *Biotechnol. Sustain. Util. Biol. Resour. Trop.* **17:** 284–289.
14. Nguyen, N. P., K. M. Lee, I. W. Kim, Y. S. Kim, M. Jeya, and J. K. Lee. 2010. One-step purification and characterization of a β-1,4-glucosidase from a newly isolated strain of *Stereum hirsutum*. *Appl. Microbiol. Biotechnol.* **87:** 2107–2116.
15. Saha, B. C. and M. A. Cotta. 2008. Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol. *Biomass Bioenergy* **32:** 971–977.
16. Singhania, R. R., A. K. Patel, R. K. Sukumaran, C. Larroche, and A. Pandey. 2013. Role and significance of β-glucosidases in the hydrolysis of cellulose for bioethanol production. *Bioresour Technol*. **127:** 500–507.
17. Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, *et al*. 2008. *Determination of Structural Carbohydrates and Lignin in Biomass*. National Renewable Energy Laboratory, Golden, CO.
18. Srivastava, S. K., K. B. Ramachandran, and K. S. Gopalkrishnan. 1981. β-Glucosidase production by *Aspergillus wentii* in stirred tank bioreactors. *Biotechnol. Lett*. **3:** 477–480.