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ORIGINAL PAPER

Analysis of the saccharification capability of high-functional cellulase JN11 for various pretreated biomasses through a comparison with commercially available counterparts

Tetsushi Kawai · Hikaru Nakazawa · Noriko Ida · Hirofumi Okada · Shuji Tani · Jun-ichi Sumitani · Takashi Kawaguchi · Wataru Ogasawara · Yasushi Morikawa · Yoshinori Kobayashi

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Abstract Although the capabilities of *Trichoderma reesei* cellulases have been greatly improved, these enzymes are still too costly for commercial use. The aim of this research was to assess the biomass saccharification capability of JN11, a recombinant cellulase, compared with that of the commercially available cellulases Accellerase 1500 and Cellic CTec. The activities of JN11, Accellerase 1500, and Cellic CTec were compared by using various types of cellulosic biomass, including rice straw, Erianthus, eucalyptus, and Japanese cedar. JN11 had higher saccharification capability for rice straw, Erianthus, eucalyptus, and Japanese cedar compared with the commercial cellulases. The JN11 saccharification of cellulosic biomasses, including hemicellulose (NaOH-pretreated biomasses), resulted in high glucose and xylose yields because of the high xylanase/xylosidase activity of JN11. Moreover, even JN11 saccharification of hemicellulose-free biomasses (sulfuric acid-, hydrothermally, and steam exploded-pretreated biomasses) resulted in high glucose yields. The cellulase activity of JN11, however, was comparable to that of its commercial counterparts. These findings indicate that the saccharification ability of cellulase is unrelated to its cellulase activity when measured against Avicel, CMC, *p*NP-lactoside, and other substrates. JN11 showed high activity for all types of pretreated cellulosic biomasses, indicating its usefulness for saccharification of various cellulosic biomasses.

Keywords *Trichoderma reesei* · Recombinant cellulase · Bioconversion · Cellulosic biomass · Enzymatic saccharification

T. Kawai · N. Ida · Y. Morikawa · Y. Kobayashi (✉)
Tsukuba Laboratory, Japan Bioindustry Association, AIST Tsukuba Central 6, 1-1-1, Higashi, Tsukuba, Ibaraki 305-8566, Japan
e-mail: yn-kobayashi@aist.go.jp

H. Nakazawa · H. Okada · W. Ogasawara · Y. Morikawa
Department of Bioengineering, Nagaoka University of Technology, 1603-1 kamimomioka, Nagaoka, Niigata 940-2188, Japan

S. Tani · J. Sumitani · T. Kawaguchi
Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-cho, Sakai, Osaka 599-8531, Japan

Introduction

A great deal of research has focused on the enzymatic degradation of cellulosic biomass into monomeric sugars that can then be converted into biofuels and biochemicals [12]. Cellulase, a cellulose degradation enzyme, consists of various component enzymes, such as cellobiohydrolase (CBH; releases cellobiose from a reducing or non-reducing end of the cellulose chain), endoglucanase (EG; cleaves inside the amorphous cellulose chain), and β-glucosidase (BGL; releases glucose from cellooligomers such as cellobiose). Cellulose is hydrolyzed by the synergistic actions of these components [28]. However, large amounts of cellulase are required because crystalline cellulose is refractory to hydrolysis. The resulting high cost of saccharification therefore restricts the industrial use of cellulosic biomass. Cellulosic biomass includes hemicelluloses together with cellulose. The hydrolysis of hemicellulose is performed by hemicellulase; for example, in the hydrolysis of xylan, xylanase cleaves the β-1, 4 bond of xylan, and β-xylosidase hydrolyzes the xylo-oligosaccharides released. Cellulose degradation is promoted by the removal of hemicellulose, which overlies the cellulose [19]. To use

cellulosic biomass industrially, it is important that cellulase and hemicellulase work in concert.

Many organisms produce cellulase and hemicellulase, including bacteria [5], fungi [6], and insects [27]. The fungus *Trichoderma reesei* secretes large quantities of many relating enzymes and efficiently degrades cellulose and hemicellulose in biomass [1, 15]. Mutant strains obtained by treatment with ultraviolet light and nitrosoguanidine show high cellulase production, especially strains PC-3-7 [11] and RUT-C30 [25]. However, the BGL of the cellulase from *T. reesei* has little activity [21]. To reduce the cost of the enzymes used in the saccharification of biomass, Genencor and Novozymes partnered with the National Renewable Energy Laboratory (NREL) and the Department of Energy to engineer a new cellulase. This seminal work resulted in a 20- to 30-fold cost reduction [3]. Similarly, by using PC-3-7, we constructed a recombinant strain (X3AB1) that expresses *Aspergillus aculeatus* BGL1 (AaBG1) under the control of the *xyn3* promoter [16]. The BGL activity of JN11, the cellulase produced by X3AB1, is over 60 times more active than the wild type (WT). The saccharification enzymes of this strain enhance the extent of saccharification considerably, without compromising the cellulase activity because of the use of the *xyn3* promoter [29].

Although the capabilities of *T. reesei* cellulases have been greatly improved, the enzymes are still too costly, therefore, research has continued but has been limited due to the lack of detailed information regarding the management of cellulase [9]. For example, the individual activities of the component enzymes are difficult to determine and the interactions between cellulosic biomass and cellulases are very complicated [8, 13]. Furthermore, if the type of cellulosic biomass changes, the cellulase reaction also changes [2]. In this study, we therefore focused on evaluating saccharifying activity using various types of cellulosic biomass, including rice straw, Erianthus, eucalyptus, and Japanese cedar. The decomposition difficulty for these biomasses increases in the order of rice straw, Erianthus, eucalyptus, and Japanese cedar. In addition to assessing the cellulase using different types of biomasses, we carried out various pretreatments, including NaOH/sulfuric acid or hydrothermal or steam explosion, because the character of cellulosic biomass depends on the type of pretreatment. For example, NaOH-pretreated biomass contains low lignin and high hemicellulose, whereas dilute sulfuric acid- and steam explosion-pretreated biomasses contain high lignin and a non-hemicellulose constituent [23]. Hydrothermally pretreated biomass contains high lignin and low hemicellulose levels [17]. We used these various pretreated cellulosic biomasses to comprehensively evaluate the activity of JN11. The aim of this research was thus to demonstrate the biomass saccharification capability of JN11 and to compare it with that of the commercially available cellulases Accellerase 1500 and Cellic CTec. The information gained from this assessment of JN11 will be of value in future modifications and improvements of recombinant cellulases.

Materials and methods

Strain and enzyme preparation

The *T. reesei* PC-3-7 and X3AB1 strain was maintained on potato dextrose agar and 10^7 conidia were inoculated into 50 ml of a basal medium [11] containing 1 % Avicel as a carbon source. This inoculum was then incubated with shaking at 220 rpm for 1 week. Crude commercial cellulase preparations, that is, Accellerase 1500 and Cellic CTec, from *T. reesei* were purchased from Genencor Incorporation (a division of Danisco, USA) and Novozymes (Denmark), respectively.

The component enzymes used in this study were produced by heterologous expression of *T. reesei* genes using *Aspergillus oryzae* and *Schizosaccharomyces pombe* as the host. *A. oryzae*, for the production of CBH I and EG I, was purchased from Ozeki (Japan). The stocked mycelium was pre-cultured in 100-ml flasks containing 4 % glucose medium (4 % glucose, 1 % peptone, 0.1 % Tween 80, 0.5 % KH_2PO_4, 0.1 % NH_4Cl, and 0.05 % $MgSO_4$) for 3 days at 30 °C with shaking at 200 rpm. The pre-culture (12 ml) was inoculated in a 2-l fermentor containing 1.2 l of 4 % glucose medium and incubated for 3 days at 30 °C with shaking at 400 rpm. The obtained culture was filtrated through a Miracloth (Calbiochem, USA) and purified via 80 % saturated ammonium sulfate precipitation. After centrifuging for 30 min at 9,000 rpm, 10 g of phosphoric acid-swollen cellulose (PSC) was added to the supernatant for purification by the adsorption of cellulase on cellulose. PSC was prepared as previously described [26], except that we used a batch size of 80 g of cellulose. The supernatant with PSC was kept on ice for 1 h and centrifuged for 10 min at 4,000 rpm. The pellet was then suspended in 40 ml of 500 mM sodium acetate buffer (pH 5.0) containing 1 M ammonium sulfate and centrifuged for 10 min at 4,000 rpm. This operation was repeated five times. The pellet was then suspended in 20 ml of 1 mM sodium acetate buffer and incubated for 1 h at 37 °C with shaking at 150 rpm. The suspension was centrifuged for 10 min at 4,000 rpm. This operation was repeated three times. The generated supernatant was used as the purified enzyme. The culture medium of *S. pombe*, for the production of CBH II, was purchased from Asahi Glass (Japan). The culture supernatant was purified with ammonium sulfate at 80 % saturation. The precipitate was suspended with

10 mM citrate buffer (pH 5.5). The suspension was then ultrafiltered and purified by means of column chromatography using DEAE-toyopearl 650 S, HiTrap Phenyl HP and Mono Q columns. The active fractions were collected, concentrated, and used as the purified enzyme.

Enzyme assays and protein analysis

Reducing sugar was measured by using the 3, 5-dinitrosalicylic acid reagent method (DNS method) [14] to determine the polysaccharide degradation activity of cellulase, xylanase, and Avicelase. The protein concentration was determined with a Quick Start Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The cellulase activity, that is, the Avicelase and *p*-Nitrophenyl-*β*-lactopyranoside degradation activity (*p*NPLase), was determined by using 0.5 % CEOLUS PH-101 (Avicel; Asahi Kasei Corporation, Japan) and 1 mM *p*NP-L (Sigma–Aldrich, St. Louis, MO, USA) as substrates in 50 mM sodium acetate buffer (pH 5.0) at 50 °C for 60 and 10 min, respectively. One unit of activity was defined as the amount of enzyme that produced 1 μmol of reducing sugar and *p*NP per minute from the substrate. When the component enzymes were added to JN11, they were used at 0.2 μM to measure the Avicelase activity. The activities of CMCase, cellobiase, xylanase, and *β*-xylosidase (*p*NPXase) were determined as described previously [16].

Biomass pretreatment and composition analysis

The pretreated biomass for saccharification was prepared with acid/alkaline or hydrothermally treatment of rice straw, Erianthus, and eucalyptus. In total, 21 g of micromilled biomass with a diameter of 100–200 μm was treated with 1 % (*w/v*) sulfuric acid in a batch pretreatment reactor (Toyo Koatsu, Japan) under operating conditions of 5 min at 170 °C for rice straw and Erianthus, and 5 min at 180 °C for eucalyptus. Similarly, the alkaline-pretreated cellulosic biomass was prepared with 0.5 % (*w/v*) sodium hydroxide in a flow-through reaction test apparatus (Toyo Koatsu, Japan) for rice straw under operating conditions of 5 min at 120 °C, and using a batch pretreatment reactor for Erianthus and for eucalyptus under operating conditions of 12 min at 140 °C and 10 min at 200 °C, respectively. In total, 21 g of cellulosic biomass was hydrothermally pretreated with water under the flow-through reaction testing conditions of 10 min at 200 °C for rice straw, and using the batch pretreatment reactor of 15 min at 200 °C for Erianthus and 15 min at 220 °C for eucalyptus. The solid fractions were harvested from the slurry by use of filtration and were washed with water.

Pretreated biomass for saccharification was also prepared with Japanese cedar. Two hundred grams of micromilled Japanese cedar with a particle diameter of 100–200 μm was treated in an explosion reaction test apparatus (Nippon Dennetsu, Japan) under operating conditions of 240 °C for 10 min (3.35 MPa). The solid fraction was harvested from the slurry by means of filtration and was washed with water.

The composition of the pretreated cellulosic biomass was determined by using high-performance liquid chromatography (HPLC) after two-step acid hydrolysis, according to the procedure published by the NREL [24].

Biomass saccharification

Biomass saccharification by the cellulases was performed in a 20-ml plastic bottle containing 5 % dry biomass mass (*w/v*) in 100 mM sodium acetate buffer (pH 5.0), with enzyme loading at 3–10 mg of protein per gram of dry biomass. The reaction was performed at 50 °C, with shaking at 150 rpm for 72 h. Biomass saccharification by the component enzymes plus JN11 was performed in a 1.5-ml microtube with 5 % dry mass (*w/v*) of the steam-exploded Japanese cedar in 100 mM sodium acetate buffer (pH 5.0) and enzyme loading of 0.2 μM component enzymes and 1 mg/g-biomass JN11. The reaction was performed at 50 °C with shaking at 1,500 rpm for 72 h. The supernatants were boiled for 5 min to inactivate the enzymes, and the produced glucose and xylose were measured by using HPLC.

The degree of biomass saccharification was calculated as a percentage of the total sugar (g) produced for the glucose and xylose in the dry biomass.

HPLC analysis

The 0.2-μm-filtrated samples obtained from the saccharification supernatant and the acid hydrolysis supernatant were applied to the HPLC system Prominence equipped with a Shimadzu refractive index detector (Shimadzu, Japan). Glucose and xylose for saccharification analysis were analyzed by means of HPLC using a Shimpak ISA-07/S2504 (Shimadzu GLC, Japan) with guard cartridges ISA-07 and OPTI-GUARD DVB (Lab Lab Company, Japan) operated at 70 °C at a flow rate of 0.5 ml/min. The mobile phase underwent gradient elution by mixing solvent A (0.15 M boric acid, pH 8.0) with solvent B (0.7 M boric acid, pH 9.0). The gradient program was as follows: 0–30 min, linear change from A–B (30:70, v/v) to A–B (20:80, v/v). Glucose and xylose for the biomass composition analysis were analyzed by use of HPLC with an Asahipak NH2P-50 4E (Showa Denko, Japan) with guard cartridges Asahipak NH2P-50G 4A (Showa Denko) and OPTI-GUARD DVB operated at 45 °C at a flow rate of 0.8 ml/min. The mobile phase was 0.3 % phosphoric acid:acetonitrile (15:85, v/v).

Results and discussion

Comparison of the activity of JN11 with that of commercially available enzymes

Before biomass saccharification, the activity of Avicelase, *p*NPLase, CMCase, cellobiase, xylanase, and β-xylosidase was measured to characterize JN11, WT, Accellerase 1500, and Cellic CTec (Table 1).

JN11 showed high *p*NPLase activity, represented by the CBH I and EG I activity, as well as high Avicelase activity. As the reactivity of EG I for *p*NPL was higher than that of CBH I [7], JN11 also showed high CMCase activity, which represents endo-cleavage activity. Similar results were observed for Accellerase 1500. In contrast, Cellic CTec showed the lowest Avicelase and *p*NPLase activity. The cellobiase activity of JN11 was 65 times higher than that of the WT and in between the activities of the commercially available enzymes. This high cellobiase activity has a strong impact on cellulase activity. The products of Avicelase and CMCase activity were determined by using the DNS method. This method could not distinguish between monosaccharides and oligosaccharides. For example, the degree of coloring of cellobiose, from which two glucose are produced when degraded by BGL, was only about 1.5 times higher than that of glucose. Moreover, the addition of AaBL1 to the WT increased the CMCase activity (data not shown). In the case of JN11, the extremely weak cellobiase activity of the WT and the use of the xyn3 promoter, which was irrelevant for cellulose degradation, led to the above-mentioned activity [20]. On the other hand, though Cellic CTec showed the highest cellobiase activity, that is, 2.5-fold higher than that of Accellerase 1500, the Avicelase and CMCase activities of Cellic CTec were approximately 30–60 % lower than that of Accellerase. Thus, the overall cellulase and endo-cleavage activity of Cellic CTec was very weak. The xylanase and β-xylosidase activities of JN11 were 3.2–5.6 times and 1.1–4.5 times higher than that of the commercially available enzymes. In conclusion, JN11 generally showed high cellulose and xylan degradation activity. Accellerase 1500 appeared to be more effective at degrading cellulose than xylan. Cellic CTec had high cellobiase activity but low cellulase and xylanase activities.

Saccharification of cellulosic biomasses by four cellulases

To evaluate the saccharifying activity of the cellulases, we performed the saccharification of rice straw, Erianthus, eucalyptus, and Japanese cedar. These cellulosic biomasses were pretreated with sulfuric acid or NaOH, hydrothermally or by steam explosion. Their components are shown in Table 2.

JN11 showed the highest glucose release ratio during the saccharification of NaOH-pretreated rice straw, followed by Accellerase 1500, Cellic CTec, and the WT (Fig. 1). Glucose release by JN11 seemed to be accelerated because of its high cellulase and xylanase activities. The WT gave the highest xylose yield, followed by JN11, Cellic CTec, and Accellerase 1500. This pattern can be attributed to the high xylanase and β-xylosidase activities of the WT and JN11 [4]. The commercial enzymes with enhanced cellobiase activity gave low glucose yields because the remaining xylan inhibited the degradation of cellulose. These results agree with the finding that the addition of a commercially available hemicellulase (Cellic HTec; Novozymes) to Accellerase 1500 increases not only xylose yield but also glucose yield (data not shown); however, the glucose yield did not exceed that of JN11. The difference between JN11 and the commercial enzymes was less pronounced with respect to the hydrolysis ratio of the hydrothermally pretreated rice straw (Fig. 2). Because hydrothermally pretreated rice straw contains about half the hemicellulose of NaOH-pretreated rice straw, the effect of the xylanase activity on the biomass saccharification was probably reduced. The saccharification of sulfuric acid-pretreated rice straw resulted in the same glucose yields for JN11 and Cellic CTec at 72 h, followed by Accellerase 1500, and then the WT (Fig. 3a). The high glucose yield for JN11 can be attributed to its high cellulase activity. One reason for the high glucose yield for Cellic CTec, which

Table 1 Specific activity (U/mg-protein) of enzyme preparations

	Avicelase	*p*NPLase[a]	CMCase	Cellobiase	Xylanase	*p*NPXase[b]
WT	0.45 ± 0.02	0.33 ± 0.01	35 ± 2.0	0.12 ± 0.0	88 ± 0.0	0.59 ± 0.01
JN11	0.68 ± 0.06	0.38 ± 0.01	53 ± 1.0	7.8 ± 0.5	67 ± 6.0	0.54 ± 0.02
Accellerase 1500	0.77 ± 0.02	0.41 ± 0.0	56 ± 3.0	5.6 ± 0.3	21 ± 1.0	0.12 ± 0.01
Cellic CTec	0.27 ± 0.03	0.30 ± 0.01	41 ± 2.0	14 ± 1.0	12 ± 0.0	0.51 ± 0.03

[a] *p*NP-lactopyranoside activity

[b] *p*NP-xylopyranoside activity

Table 2 Components (w/w)% in the pretreated cellulosic biomass

Cellulosic biomass	Pretreatment	Cellulose	Hemicellulose	Lignin	Ash
Rice straw	nt[a]	30.8	17.0	9.50	18.9
	Sulfuric acid	49.4	0.33	20.9	23.9
	Sodium hydroxide	52.6	24.2	3.80	5.40
	Hydrothermal	47.6	9.57	14.7	14.8
Erianthus	nt	34.3	21.5	18.6	5.26
	Sulfuric acid	51.6	2.19	35.4	5.49
	Sodium hydroxide	56.0	22.2	6.20	1.58
	Hydrothermal	50.9	7.20	29.8	2.17
Eucalyptus	nt	40.3	15.8	19.8	0.77
	Sulfuric acid	57.8	0.23	36.1	0.76
	Sodium hydroxide	64.8	12.1	9.70	1.10
	Hydrothermal	60.9	0.56	34.1	0.33
Japanese cedar	nt	36.0	13.5	42.7	0.35
	Steam explosion	40.1	0.41	53.4	0.09

[a] *nt* not treated

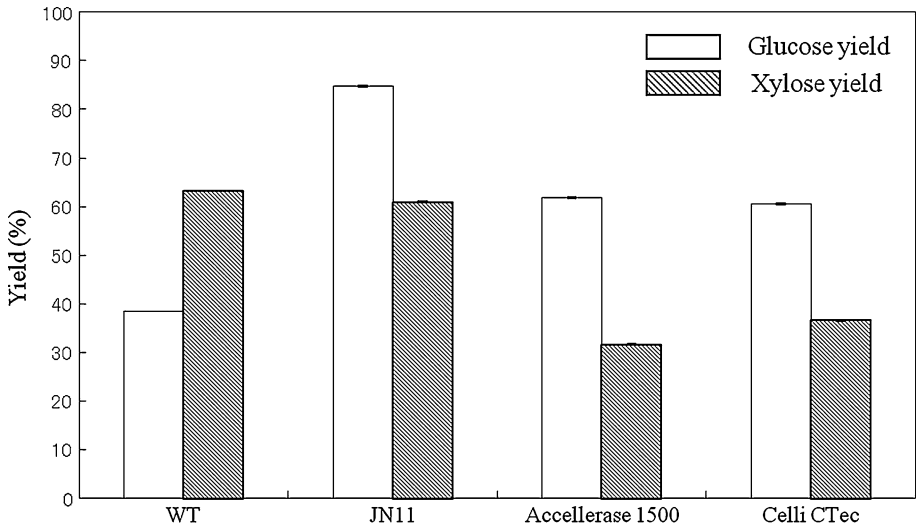

Fig. 1 Saccharification of alkaline-pretreated rice straw by cellulases. Protein was loaded at 3 mg/g-biomass and the reaction time was 72 h. Other conditions are shown in the "Materials and methods" section. *White*: glucose yield, *diagonal lines*: xylose yield

has low cellulase activity, may be that its high cellobiase activity affected its saccharification ability. Cellobiase activity clearly affected the saccharification of sulfuric acid pretreated rice straw, because the yield of JN11 for saccharification of the biomass was 2.8 times higher than that of the WT, although JN11 was only enhanced with BGL. Furthermore, when AaBG1 (0.025 mg/g of biomass) was added to the WT, saturation occurred for the NaOH-pretreated rice straw. In contrast, saccharification of sulfuric acid-pretreated rice straw using the WT required twice the amount of BGL (data not shown). These results can be attributed to the amount of lignin content [30]. The lignin content of sulfuric acid-pretreated rice straw is 5.5 times higher than that of NaOH-pretreated rice straw (Table 2). Lignin in biomass appears to inhibit the cellobiase activity, or absorb BGL to reduce the active one in the solution. To elucidate the differences between JN11 and Cellic CTec, we prepared the sulfuric acid-pretreated rice straw under mild conditions (0.5 % sulfuric acid at 150 °C). The saccharification by JN11 of the mildly conditioned pretreated biomass resulted in a glucose yield that was 1.2 times higher than that obtained with Cellic CTec (Fig. 3b). The mild condition-pretreated rice straw increased the effect of cellulose degradation activity because of its decomposition difficulty. Thus, JN11 showed high saccharifying activity for NaOH-pretreated and hydrothermally pretreated rice straw because of its high xylanase and β-xylosidase activity. In the case of sulfuric acid pretreatment, JN11 was comparable to Cellic CTec because of the high cellobiase activity of Cellic CTec.

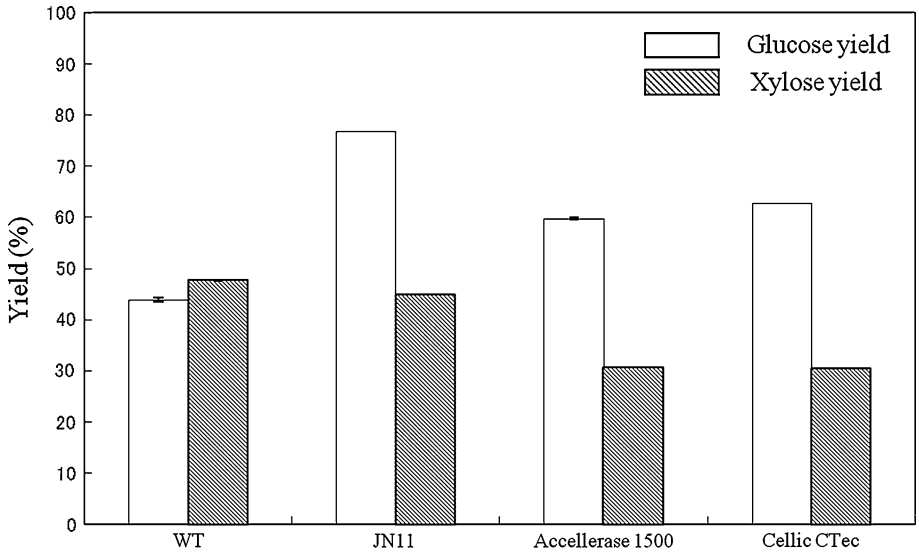

Fig. 2 Saccharification of hydrothermally pretreated rice straw by cellulases. Saccharification conditions are the same as those of Fig. 1. *White*: glucose yield, *diagonal*: xylose yield

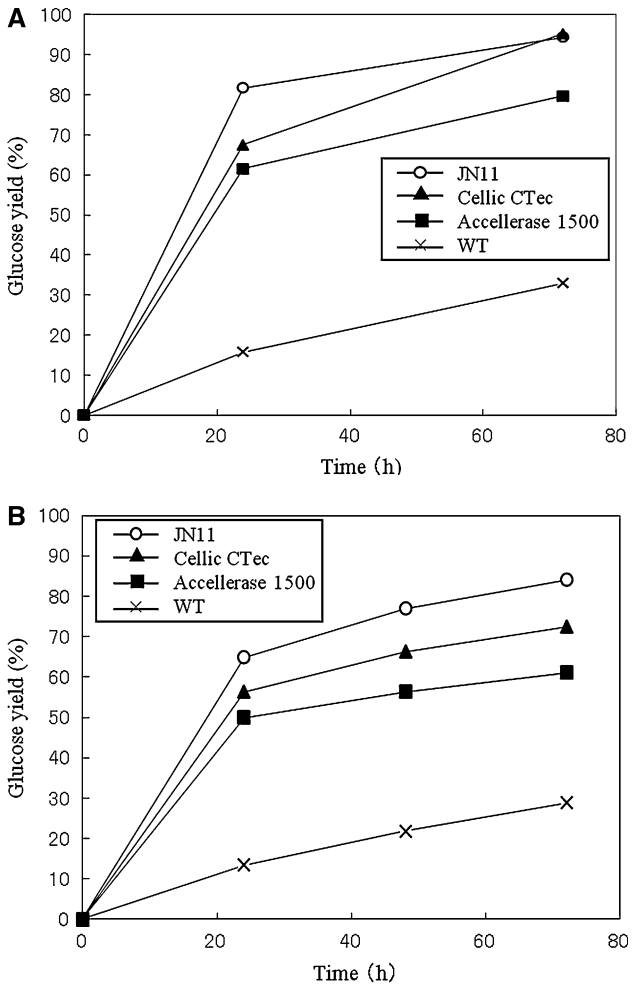

Fig. 3 Saccharification of acid-pretreated rice straw by cellulases. Protein was loaded at 3 mg/g-biomass. Biomass pretreatment conditions were **a** 1 % sulfuric acid at 170 °C for 5 min and **b** 0.5 % sulfuric acid at 150 °C for 5 min. *Open circles*: JN11; *filled triangles*: Cellic CTec; *filled squares*: Accellerase 1500; *crosses*: WT

Similar to rice straw, the saccharification of NaOH-pretreated and hydrothermally pretreated Erianthus, containing hemicellulose, by JN11 resulted in the highest glucose and xylose release (Table 3a, b). The WT also released more xylose than the commercially available enzymes. Furthermore, JN11 released more glucose than Cellic CTec from the sulfuric acid-pretreated Erianthus probably because it is more difficult to decompose the cellulose in Erianthus than in rice straw. As a result, JN11 showed high saccharifying activity for all types of pretreated Erianthus because of its high cellulase and xylanase activities.

The saccharification of eucalyptus produced a more accentuated trend than that of Erianthus. JN11 had the highest glucose release rate, followed by Cellic CTec, Accellerase 1500, and finally the WT (Table 3a). JN11 and the WT showed the highest release of xylose (Table 3b).

JN11 displayed high saccharification ability for pretreated rice straw (except for that pre-treated with sulfuric acid), Erianthus, and eucalyptus. This result can be attributed to the fact that JN11 has high cellulase activity in addition to high xylanase and β-xylosidase activities. The correlation between enzyme activities and the ability to saccharify biomass for the WT and JN11 was the result of the extremely weak cellobiase activity of the WT. However, considering the high cellulase activity of Accellerase 1500, whose cellobiase activity was about 47 times higher than that of the WT, cellulase activity alone cannot explain the saccharifying activity of the enzyme. Therefore, we saccharified steam-exploded Japanese cedar, which is extremely refractory to degradation (Fig. 4). JN11 showed the highest hydrolysis yield, followed by Cellic CTec, Accellerase 1500, and the WT. The lack of correlation between the cellulase activities (Table 1) and the saccharification of

Table 3 Saccharification yield (%) using 3 mg/g-biomass enzymes for 72 h

Cellulosic biomass	Pretreatment	WT	JN11	Accellerase 1500	Cellic CTec
Glucose yield					
Erianthus	Sulfuric acid	20	65	51	49
	Sodium hydroxide	26	76	40	45
	Hydrothermal	35	76	43	52
Eucalyptus	Sulfuric acid	11	55	35	42
	Sodium hydroxide	28	73	48	48
	Hydrothermal	11	71	40	40
Xylose yield					
Erianthus	Sulfuric acid	–	–	–	–
	Sodium hydroxide	56	62	32	36
	Hydrothermal	62	61	38	40
Eucalyptus	Sulfuric acid	–	–	–	–
	Sodium hydroxide	69	74	44	56
	Hydrothermal	–	–	–	–

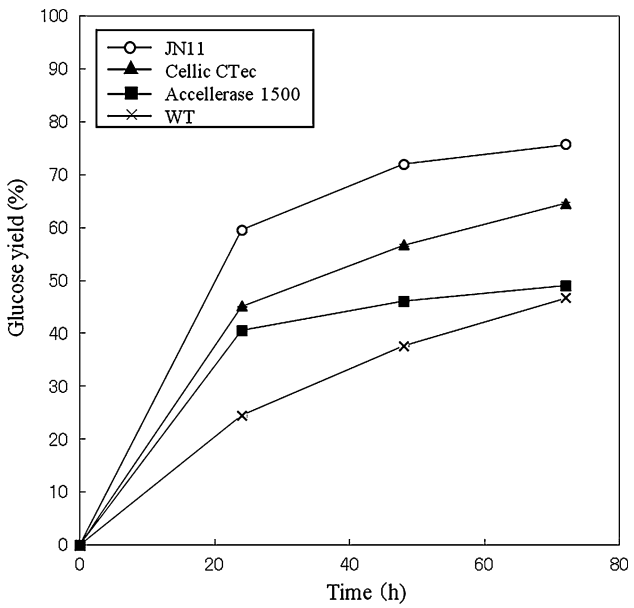

Fig. 4 Saccharification of steam-exploded Japanese cedar by cellulases. Protein was loaded at 10 mg/g-biomass. *Symbols* are the same as those used in Fig. 3

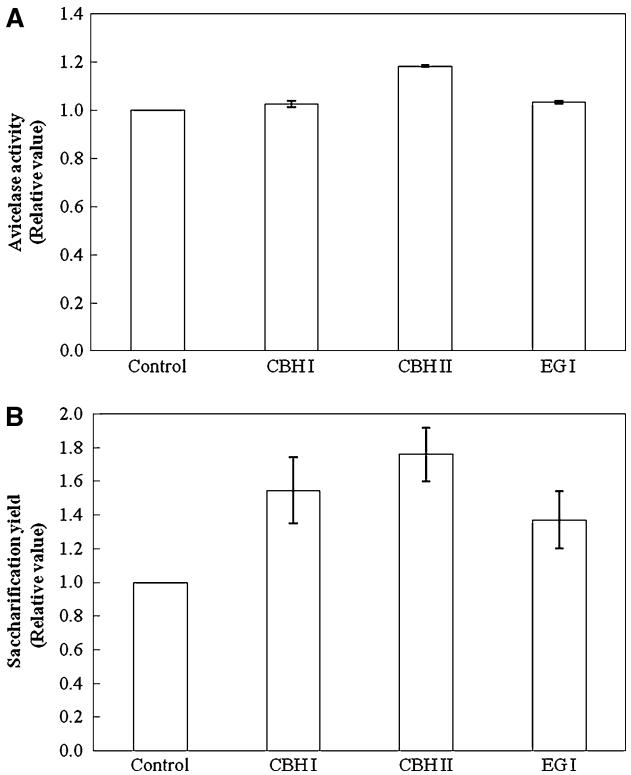

Fig. 5 **a** The measurement of Avicelase activity was carried out by using JN11. The component enzymes were loaded at a protein concentration of 0.2 μM. **b** The saccharification of steam-exploded Japanese cedar was carried out by using JN11. Protein was loaded at 1 mg/g-biomass and the reaction time was 72 h. The component enzymes were also loaded at a protein concentration of 0.2 μM. The control was JN11; CBH I, CBH II, and EG I were added to JN11

cellulosic biomass suggests that the factors that affect the degradation of the substrates used in this study differ from those that affect the degradation of cellulosic biomass.

To test our hypothesis that the factors that affect enzyme activity differ from those that affect cellulosic biomass degradation, we prepared CBH I, CBH II, and EG I, which play a central role in cellulose degradation. These three component enzymes were expressed in a heterologous host to prevent contamination by other component enzymes. These component enzymes were added to JN11 when the activity of Avicelase was measured and when the saccharification of steam-exploded Japanese cedar was performed (Fig. 5). For the Avicelase activity, the addition of

CBH I and EG I hardly had an effect. In contrast, the addition of CBH I and EG I increased the saccharification yield of steam-exploded Japanese cedar. On the other hand, the addition of CBH II was the most effective both in Avicelase activity and the saccharification activity. These results clearly show that the effect of component enzymes on Avicelase activity and on the saccharification reaction differs with the type of component enzyme present. Avicel and Japanese cedar differ by many factors, including structure, composition, and crystallinity. In addition, the reactivity of the component enzymes appears to differ with the type of pretreatment and cellulosic biomass. The structure of lignin and hemicellulose also differ depending on the pretreatment [22]. Compositional differences, such as the presence of lignin, lead to disparities in enzymatic reactivity [18]. The effects of the other component enzymes contained in *T. reesei* for degradation also likely differ with the types of substrates [10]. Hence, the types and proportions of component enzymes must be adapted for the target pretreated cellulosic biomass to produce the most functionally effective cellulase. JN11 showed the greatest saccharification ability for the pretreated cellulosic biomass assessed in this study. The reason for this is that JN11 contained the best balance of component enzymes or enzymatic activities such as xylanase and cellobiase. This problem, however, needs more investigation and is still open to discussion. Although we must consider the possibility that other potential factors may affect the saccharification of biomass, our results clearly show that differences in the effects of the major component enzymes of a cellulase are related to the biomass degradation capability of that cellulase. JN11 appears to be of value for use with a variety of pretreated cellulosic biomasses, and its functionality can be improved by adjusting its component enzymes according to the cellulosic biomass being degraded.

Conclusions

The saccharification of various types of pretreated biomass was carried out using BGL-enhanced cellulase preparations. JN11 effectively saccharified all types of pretreated biomasses. Our study shows that for high saccharification ability, cellulases need high hemicellulase activity as well as high BGL activity, and that the reactivity of component enzymes differ with the enzymatic activity and the biomass saccharification. JN11 is effective for saccharification because it includes the best balance of these activities. JN11 is, therefore, a useful tool for the enzymatic hydrolysis of cellulosic biomass and its use may reduce the enzymatic costs associated with biomass conversion.

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