JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9833–9837 9833
DOI:10.1021/jf1019104

Isolation of Cellulase-Producing Bacteria and Characterization of the Cellulase from the Isolated Bacterium *Cellulomonas* Sp. YJ5

Li-Jung Yin,*,† Po-Shin Huang,§ and Hsin-Hung Lin#

†Department of Sea Food Science, National Kaohsiung Marine University, No. 142 Hai-Chuan Road, Nan-Tzu, Kaohsiung 81143, Taiwan, §Department of Food and Nutrition, Providence University, 200 Chung-Chi Road, Salu, Taichung, 43301, Taiwan, and #Nugen Bioscience (Taiwan) Company, Ltd., No. 29 40th Road, Taichung Industrial Park, Taichung 40768, Taiwan

A cellulase-producing bacterium was isolated from soil and identified as *Cellulomonas* sp. YJ5. Maximal cellulase activity was obtained after 48 h of incubation at 30 °C in a medium containing 1.0% carboxymethyl cellulose (CMC), 1.0% algae powder, 1.0% peptone, 0.24% $(NH_4)_2SO_4$, 0.20% K_2HPO_4, and 0.03% $MgSO_4 \cdot 7H_2O$. The cellulase was purified after Sephacryl S-100 chromatography twice with a recovery of 27.9% and purification fold of 17.5. It was, with N-terminal amino acids of AGTKTPVAK, stable at pH 7.5−10.5 and 20−50 °C with optimal pH and temperature of 7.0 and 60 °C, respectively. Cu^{2+}, Fe^{2+}, Hg^{2+}, Cr^{3+}, and SDS highly inhibited, but cysteine and β-mercaptoethanol activated, its activity. Substrate specificity indicated it to be an endo-β-1,4-glucanase.

KEYWORDS: Cellulase; *Cellulomonas* sp.; purification; characterization

INTRODUCTION

Cellulases can effectively hydrolyze cellulose into glucose via three enzymes' synergistic actions including endo-β-1,4-glucanase (EC 3.2.1.4, EG; randomly cleaving internal linkages), cellobiohydrolase (EC 3.2.1.91, CBH; specifically hydrolyzing cellobiosyl units from nonreducing ends), and β-D-glucosidase (EC 3.2.1.21; hydrolyzing glucosyl units from cellooligosaccharides) (*1*). Currently, they are frequently applied in the animal feeds, textile, wastewater treatment, and brewing and winemaking industries (*2*, *3*). These enzymes are composed of independently folding, structurally and functionally discrete units called domains or modules, making cellulases modular (*4*). Although cellulases act on polymers via either exo- or endocleavages, they target specific cleavage of β-1,4-glycosidic bonds (*5*). A carbohydrate binding domain (CBD) is the most common accessory module of cellulases. The major function of CBDs is to deliver and bring the catalytic domain into much closer contact with crystalline cellulose for efficient hydrolysis. Binding of cellulase via CBD close to cellulose is extremely stable, yet still allows the enzymes to diffuse laterally across the surface of substrates. Some CBDs are preferential to binding noncrystalline cellulose (*6*−*8*).

Over the past decades, cellulase-producing bacteria have been isolated from a wide variety of sources such as composts, decayed plant materials, feces of ruminants, soil and organic matters, and extreme environments such as hot springs (*9*). Screening for bacterial cellulase in microbial isolates is typically performed on carboxymethyl cellulose (CMC) containing plates (*10*). This method can be timely, but zones of hydrolysis cannot be easily discernible. Now, the new substrates, 2-(2′-benzothiazolyl)phenyl (BTP) cellooligosaccharides with two to four polymerizations (BTPG2−4) were synthesized for the screening of microbial cellulolytic activity. The usefulness of BTP substrates was shown during the purification of *Bacillus polymyxa* cellulolytic complex, which consists of at least three types of enzymes: cellobiohydrolase, endo-β-D-glucanase, and β-glucosidase (*11*).

A number of cellulolytic enzymes, especially cellulases produced by fungi and bacteria, have been isolated and characterized (*12*). Recently, the bacterial strain B39, isolated from poultry manure in Taichung, Taiwan, was identified as a novel cellulose-degrading *Paenibacillus* sp. strain. A high molecular weight (148 kDa) cellulase with both CMCase and avicelase activities was found to be secreted by this isolate. The CMCase activity, with maximum activity at 60 °C, pH 6.5, of the isolated cellulase was much higher than that on Avicel or filter paper (*13*). On the other hand, another novel cellulase-producing *Paenibacillus campinasensis* BL11 was also isolated from the black liquor of brownstock at washing stage of the Kraft pulping process. It was a thermophilic, spore-forming bacterium, which could be grown between 25 and 60 °C over a wide pH range (*14*). More recently, a thermostable cellulase was found in newly isolated *Bacillus subtilis* DR from a hot spring. The high-temperature environment allowed for the production of a thermostable endocellulase CelDR with an optimum temperature at 50 °C. This CelDR retained 70% of its activity (CMCase) even after 30 min of incubation at 75 °C (*15*).

Whole green microalgae, *Chlorella* and *Scenedesmus*, have poor digestibility and assimilability in human intestine due to their rigid cell wall (*16*). The enzymatic hydrolysis of cell walls and plasma proteins is considered as a promising method to improve the protein digestibility of algae, which consequently makes them

*Author to whom correspondence should be addressed (phone +886-7-3617141, ext. 3608; fax +886-943299201; e-mail ljyin@mail.nkmu.edu.tw).

Published on Web 08/05/2010

useable in human nutrition and medical applications (*17*, *18*). Accordingly, this study aimed to isolate the cellulase-producing bacteria and characterize the cellulase produced by the isolated strain.

MATERIALS AND METHODS

Isolation of Cellulase-Producing Bacteria. The cellulase-producing bacterium was isolated from the soil in northern Taiwan. Ten percent of sample suspended in sterile phosphate-buffered saline (PBS, 10 mM potassium, 150 mM NaCl, pH 7.4) was inoculated and incubated in a 1.0% CMC broth at 30 °C for 7 days. Ten percent of the resulting broth was inoculated to a medium (CMC broth, CMCB) containing 1.0% CMC, 1.0% algae powder, 1.0% peptone, 0.24% $(NH_4)_2SO_4$, 0.20% K_2HPO_4, and 0.03% $MgSO_4 \cdot 7H_2O$ and incubated at 30 °C for 3 days. The resulting broth was then spread on a CMC agar plate (CMCA, CMCB + 1.5% agar) and incubated at 30 °C for 3 days. The colonies with clear zone (indicating those with CMC hydrolyzing ability) on CMCA were selected and inoculated onto a fresh CMCA. After 3 days of incubation at 30 °C, the colonies with the largest CMC hydrolyzing haloed ring were picked up and subjected to identification. The isolated strain was stored at −80 °C in CMCB with 20% (v/v) glycerol for further studies. CMCB was employed for further production of cellulase under aerobic incubation conditions at 30 °C with 150 rpm shaking. The viable cell counts (CFU/mL) and cellulase activity were measured during cultivation.

Bacterial Identification. According to the method described by Yoon and Choi (*19*), the sequence of 16s rDNA of the isolated strain was determined after genomic DNA extraction and PCR amplification by using the forward primer [5′-(AGT TTG ATC CTG GCT CAG GAC GAA CG)-3′] and reverse primer [5′-(AGC CGG TCC CCC TGC AAG)-3′]. Comparison of the sequence with homologous strains in GenBank was performed using the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was inferred from BLAST at the National Center for Biotechnology Information (NCBI).

The cellular fatty acids of the organism grown on a TSA agar for 2 days were saponified, methylated, and extracted according to the protocol of Sherlock Microbial Identification System (MIDI, Newark, DE).

Purification of Cellulase. After 2 days of cultivation at 30 °C with shaking (150 rpm), the broth was centrifuged at 8000*g* for 20 min, passed through a 0.45 μm membrane to remove cells, and then concentrated by ultrafiltration with a 5 kDa cutoff membrane. The resulting crude enzymes were then eluted on Sephacryl S-100 HR (2.6 × 100 cm) with 20 mM Tris-HCl buffer (pH 8.0) at a flow rate of 12 mL/h and a fraction volume of 2.0 mL. Fractions with cellulase activity were collected and concentrated by ultrafiltration with a membrane (MW cutoff: 5 kDa) to a minimal volume. They were passed through Sephacryl S-100 HR again with the same conditions. Fractions with cellulase activity were again collected and subjected to the following assays.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). Electrophoresis was performed mainly according to the method of Laemmli (*20*). To the purified enzyme an equal volume of sample buffer (0.05% bromophenol blue, 5% β-Me, 10% glycerol, and 2% SDS in 0.25 M Tris-HCl buffer; pH 6.8) was added and boiled at 100 °C for 2 min. The resulting samples were then subjected to SDS-PAGE (resolving gel, 12.5% polyacrylamide; stacking gel, 4% polyacrylamide) by using a Mini-Protein II system (Bio-Rad, Hercules, CA). Electrophoresis was performed at room temperature for 1.5 h at 100 V. A low molecular weight calibration kit (GE Healthcare BioSciences Corp., Westborough, MA) was used as markers [phosphorylase B subunit (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean inhibitor (20.1 kDa), and α-lactalbumin (14 kDa)].

Endo-β-1,4-glucanase Activity Assay. Endo-β-1,4-glucanase activity was determined by incubation of 900 μL of 1% CMC in 20 mM phosphate buffer (pH 7.0) with 100 μL of the appropriate concentration of enzyme at 50 °C. After 30 min of reaction, 1 mL of dinitrosalicylic acid (DNS) was added and boiled in a water bath for 5 min to stop the reaction. The resulting samples were then cooled to room temperature, and the absorbance was measured at 540 nm (A_{540}). One unit of endo-β-1,4-glucanase activity was defined as the amount of enzyme that could hydrolyze CMC and release 1 μg of glucose within 1 min of reaction at 50 °C (*21*).

Determination of Protein Concentration. Protein concentration was determined by Lowry's method, using bovine serum albumin as a standard (*22*).

N-Terminal Amino Acid Sequence Analysis. Purified cellulase was subjected to SDS-PAGE analysis and then electrotransferred onto polyvinylidene difluoride membrane (PVDF). Proteins were stained with Coomassie blue R-250 after electrophoresis. N-Terminal amino acid sequences were analyzed by Edman degradation (*23*) after being electrotransferred onto PVDF.

Temperature Optimum. To 900 μL of 1% CMC in 20 mM phosphate buffer (pH 7.0) was added 100 μL of the appropriate concentration of enzyme, and the activity was measured at various temperatures (20, 30, 40, 50, 60, 70, 80, 90 °C) according to the method of Miller (*21*).

Temperature Stability. Purified cellulase in 20 mM phosphate buffer (pH 7.0) was incubated at various temperatures (20, 30, 40, 50, 60, 70, 80, 90 °C) for 30 min. The residual activity was measured at 50 °C according to the method of Miller (*21*).

pH Optimum. To 900 μL of 1.0% CMC at various pH values (pH 3.0−7.5, 50 mM citrate buffer; pH 7.5−9.5, 50 mM Tris-HCl buffer; pH 9.0−10.5, 50 mM carbonate buffer) was added 100 μL of cellulase, and the activity was measured at 50 °C according to the method of Miller (*21*).

pH Stability. Purified cellulase was incubated in buffers with various pH values (pH 3.0−7.5, 50 mM citrate buffer; pH 7.5−9.5, 50 mM Tris-HCl buffer; pH 9.0−10.5, 50 mM carbonate buffer) at 50 °C for 30 min. The residual activity was then measured at 50 °C according to the method of Miller (*21*).

Effects of Metals and Inhibitors. Purified cellulase in 20 mM Tris-HCl buffer (pH 7.0) with various metals (K^+, Li^+, Na^+, NH_4^+, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Ni^{2+}, Zn^{2+}, Cr^{3+}, Fe^{3+}) or chemicals [cysteine; dithiothreitol (DTT); ethylenediaminetetraacetic acid (EDTA); iodoacetic acid (IAA); β-Me; phenylmethanesulfonyl fluoride (PMSF); SDS; urea] were incubated at room temperature for 30 min. The final concentrations of metals and inhibitors were 1.0, 5.0, and 10.0 mM. After 30 min of incubation, the residual activity was measured at 50 °C according to the method of Miller (*21*).

Substrate Specificity. The hydrolytic ability of purified cellulase against 1% Avicel, cellulose, chitin, chitosan, CMC, locust bean gum, and xylan and 5 mM *p*NP-Glu in 20 mM phosphate buffer (pH 7.0) was determined to evaluate the substrate specificity.

Statistical Analysis. One-way analysis of variance (ANOVA) was run using the Statistical Analysis System (SAS/STAT), release 8.0 (Cary, NC). Duncan's multiple-range test was used to determine the significance of differences within treatments. For each treatment, three replicates were measured, and the mean values were calculated. Values were considered to be significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Isolation of Cellulase-Producing Bacterium and the Production of Cellulase. The cellulase-producing bacteria surviving after 7 days of incubation in a selective medium containing 1.0% of CMC at 37 °C were isolated from soil. After being transferred to CMCB, only 18 colonies had CMC-degrading abilities among the total 50 colonies of these strains (data not shown). The strain with the highest hydrolytic activity was subjected to identification. According to the fatty acids analysis and 16s rDNA sequencing (data not shown), this strain was identified as *Cellulomonas* sp. YJ5, a catalase-positive, oxidase-negative, and non-endospore-forming Gram-positive bacterium. The alignment of 16s rDNA by BLAST from GenBank indicated about 92.0% sequence similarity with those from *Cellulomonas* sp. d20 (AJ298927) and *Cellulomonas* sp. EMB (DQ413153). As shown in **Figure 1**, the logarithm phase of *Cellulomonas* sp. YJ5 was observed between 0 and 48 h of incubation; however, a rapid increase in cellulase activity occurred after 24 h of incubation at 30 °C. Broth after 2 days of incubation was collected for cellulase purification and characterization.

The biological degradation of cellulose has been studied for many years. Many cellulolytic enzymes, especially cellulases from *Cellulomonas* sp., have been purified and characterized (*19*).

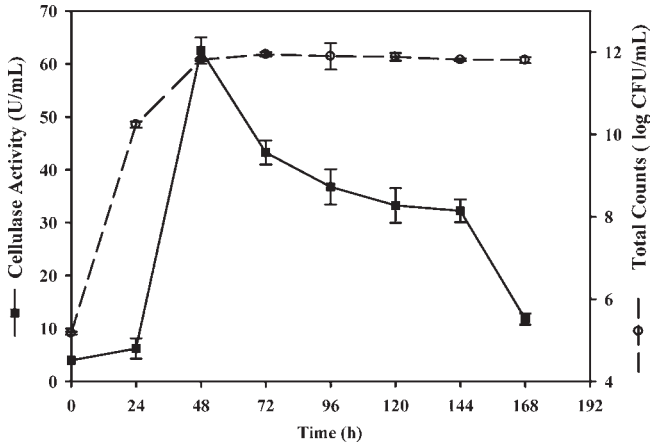

Figure 1. Changes in total count and cellulase activity of *Cellulomonas* sp. YJ5 during 7 days of incubation at 30 °C.

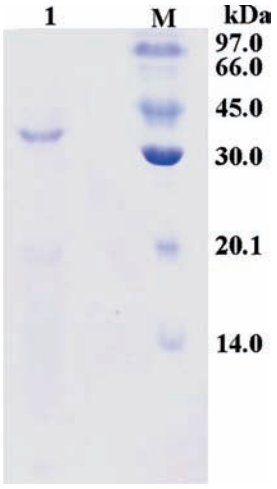

Figure 2. SDS-PAGE of purified cellulase from *Cellulomonas* sp. YJ5. The concentration of gels was 15% acrylamide. Lanes: 1, purified endoglucanase; M, markers phosphorylase B (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

Algae powder was used not only for the complex nutrient for bacterial growth but also as an inducer for the production of cellulase, due to its hydrolytic ability on microalgae. Because algae powder or CMC consists of numerous celluloses, they could be degraded into small oligosaccharides by cellulase before bacterial utilization (*12*). Accordingly, the existence of nitrogen or carbon sources, such as peptone, CMC, and algae powder, could induce the production of cellulases and provide for growth.

Enzyme Purification. *Cellulomonas* sp. YJ5 was cultivated in the medium and produced only the endo-β-1,4-glucanase at 30 °C for 2 days. However, the activities of the other two enzymes, avicelase and β-glucosidase, were very low during cultivation (data not shown). After removal of the cells, the cellulase was purified to electrophoretical homogeneity after two Sephacryl S-100 chromatographies (**Figure 2**). At this stage, the specific activity and purification fold were 168.4 units/mg and 17.5, respectively (**Table 1**).

Molecular Mass. According to the SDS-PAGE profile of the purified cellulase (**Figure 2**), no subunit was observed. It was, therefore, considered that the purified enzyme was a monomer. The molecular mass (*M*) was estimated to be 43.7 kDa (**Figure 2**). The *M* of the purified cellulase was lower than that of some other endoglucanases obtained from *Cellulomonas* spp. such as 50 and 52 kDa from *Cellulomonas uda* CS1-1(*19*), 49 kDa from *Cellulomonas flavigena* (*24*), 46.7 kDa from *Cellulomonas fimi* (*25*), and 66 kDa from *C. uda* (*26*).

Table 1. Summary of the Purification of Cellulase from *Cellulomonas* Sp. YJ5

procedure	total activity (U)	total protein (mg)	specific activity (U/mg)	purification (fold)	recovery (%)
crude enzyme	13770.0	1429.8	9.6	1.0	100.0
first Sephacryl S-100	6655.0	52.0	128.1	13.3	48.3
second Sephacryl S-100	3839.1	22.8	168.4	17.5	27.9

The N-terminal amino acids of the purified cellulase were AGTKTPVAK. By comparison of the N-terminal sequence with those from other species in GenBank, the purified cellulase was the same as those from *Bacillus subtilis* (ABV45393), *Bacillus amyloliquefaciens* (ABS70711), and *Bacillus licheniformis* (ABK63476), but only moderately similar to β-1,4-β-mannosidase (77.8%) from *Lycopersicon esculentum* (AAK56557) and endoxylanase (55.6%) from *C. fimi* (CAA90745) (**Table 2**).

Temperature Effects. The optimal temperature of purified cellulase was found to be 60 °C at pH 7.0 (**Figure 3A**). The purified cellulase was stable at temperatures < 50 °C and could retain 70% activity even after 30 min of incubation at 60 °C (**Figure 3A**). The optimum temperature of purified cellulase was the same as that of some of *C. flavigena* (60 °C) (*27*) and higher than that of *C. flavigena* (50 °C) (*24*) and *Mucor circinelloides* (55 °C) (*28*). However, it was lower than that of some other *Bacillus* strains [65 °C (CH43) and 70 °C (RH68)] (*29*). The thermal stability of purified cellulase (< 50 °C) was similar to those from other *Bacillus* strains at 0−50 °C (*29*), but lower than that from *M. circinelloides* (0−70 °C) (*28*).

pH Effects. *Cellulomonas* sp. YJ5 cellulase exhibited highest activity at pH 7.0 and was stable at pH 7.5−10.5 (**Figure 3B**). According to previous studies, the optimal pH values of cellulases were 6.0 (*24*) and 7.5 (*27*) from *C. flavigena*, 5.0−6.5 for those from *Bacillus* strains (*29*), 6.0−7.0 from *Aspergillus niger* (*30*), and 5.0−7.0 from *Lysobacter* sp. (*31*). The purified cellulase was stable at pH 7.5−10.5, which was higher than those from *M. circinelloides* (4.0−7.0) (*28*) and *Bacillus circulans* (4.5−7.0) (*32*). This pH stability range is good for this enzyme to be utilized in alkaline conditions such as paper pulp processing (*3*).

Effect of Metal Ions, Inhibitors, and Other Reagents. As shown in **Tables 3** and **4**, most metal ions such as K^+, Li^+, Na^+, and NH_4^+ did not affect the activity, whereas cysteine, β-Me, and 1 mM Co^{2+} and Cu^{2+} activated the purified cellulase. The purified cellulase activity was greatly inhibited by Cu^{2+}, Fe^{2+}, Hg^{2+}, Cr^{3+}, and SDS, but only moderately inhibited by Cd^{2+}, Mg^{2+}, Fe^{3+}, EDTA, and PMSF. The activation of cellulase by reducing agents might be due to the reduction of disulfide bonds, which was caused by autoxidation or aggregation during purification or storage (*32*). In this study, inactivation by Cu^{2+}, Fe^{2+}, Hg^{2+}, and Cr^{3+} was almost similar to that from *Catharanthus roseus* (*33*) and *Chalara paradoxa* (*34*). Among these metals and chemicals, Hg^{2+} and Cu^{2+} can bind the thiol groups and also interact with imidazole or carboxyl groups of amino acids (*34*), whereas SDS can interact with the hydrophobic group of amino acids (*29*). The data obtained from this study were quite similar to those investigated by Saha (*28*), Lucas et al. (*34*), and Murashima (*35*); that is, K^+, Na^+, and NH_4^+ did not affect the cellulase from *Rhizopus oryzae* (*35*), Co^{2+} activated that from *M. circinelloides* (*28*), but Fe^{2+} and Mg^{2+} inhibited that from *Chalara paradoxa* (*34*).

Substrate Specificity. The purified enzyme showed highest activity against CMC (100%) and moderate activity on locust

Table 2. Comparison of N-Terminal Amino Acid Sequences of Cellulase of *Cellulomonas* Sp. YJ5 with Those from Other Strains

Strain (NCBI number; enzyme)													
Cellulomonas sp.YJ5 (cellulase)	1	A	G	T	K	T	P	–	–	–	V	A	K	9
B. subtilis (ABV45393, cellulase)	30	A	G	T	K	T	P	–	–	–	V	A	K	38
B. amyloliquefaciens (ABS70711, cellulase)	30	A	G	T	K	T	P	–	–	–	V	A	K	38
B. licheniformis (ABK63476, cellulase)	30	A	G	T	K	T	P	–	–	–	V	A	K	38
Lycopersicon esculentum (AAK56557, β-1,4-beta-mannosidase)	321	K	S	T	K	T	P	G	Y	T	V	A	K	332
Cellulomonas fimi (CAA90745, endoxylanase)	39	D	P	T	T	T	P	–	–	–	V	A	T	47

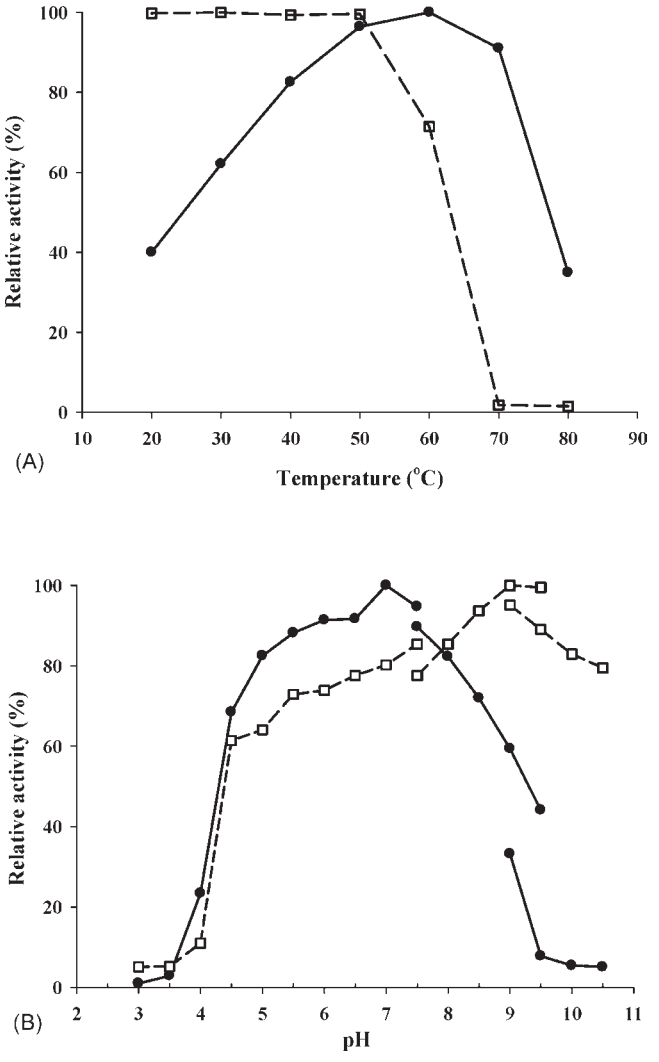

Figure 3. Effects of temperature (**A**) and pH (**B**) on cellulase: (●) optimal; (□) stability.

bean gum (27%) and xylan from oat spelt (13%) (**Table 5**). It could not hydrolyze the crystalline substrates of Avicel, chitin, and cellulose. According to substrate specificity results and alignment of the N-terminal sequence, these phenomena indicated that the substrate binding domain of cellulase had very high affinity to glucose from CMC and moderate affinity to mannose from locust bean gum and xylose from xylan, which was further confirmed by the result from N-termimal sequence alignment and proved its hydrolytic ability to those substrates. This suggests that

Table 3. Effect of Metal Ions on the Cellulase from *Cellulomonas* Sp. YJ5

	relative activity (%)		
metal[a]	1 mM	5 mM	10 mM
none	100.0	100.0	100.0
K^+	103.0	99.0	96.0
Li^+	101.0	100.0	94.0
Na^+	105.0	101.0	96.0
NH_4^+	103.0	99.0	94.0
Ba^{2+}	105.0	99.0	96.0
Ca^{2+}	105.0	101.0	93.0
Cd^{2+}	92.0	83.0	70.0
Co^{2+}	119.0	110.0	89.0
Cu^{2+}	116.0	32.0	1.0
Fe^{2+}	87.0	0.0	0.0
Hg^{2+}	0.0	0.0	0.0
Mg^{2+}	95.0	89.0	60.0
Ni^{2+}	98.0	93.0	84.0
Zn^{2+}	102.0	103.0	106.0
Cr^{3+}	85.0	50.0	31.0
Fe^{3+}	100.0	85.0	78.0

[a] The counterion of these metals is chloride.

Table 4. Effect of Chemicals on the Cellulase from *Cellulomonas* Sp. YJ5

	relative activity(%)		
chemical[a]	1 mM	5 mM	10 mM
none	100.0	100.0	100.0
cysteine	114.0	126.0	132.0
DTT	104.0	104.0	104.0
EDTA	94.0	89.0	76.0
IAA	103.0	94.0	92.0
β-Me	108.0	117.0	125.0
PMSF	106.0	96.0	89.0
SDS	100.0	64.0	46.0
urea	103.0	101.0	104.0

[a] DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; IAA, iodoacetic acid; β-Me, β-mercaptoethanol; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

Table 5. Substrate Specificity of the *Cellulomonas* Sp. YJ5 Cellulase

substrate	initial velocity (μg of glucose/min)	relative activity (%)
CMC	59.2	100
locust bean gum	16.0	27
xylan from oat spelt	8.0	13
Avicel PH-101	5.4	9
chitin	2.7	5
cellulose	2.1	4
chitosan (≥75% deacetylated)	0	0
5 mM *p*NP-Glu	0	0

the purified celleulase could break natural agri-wastes for further industrial application. So far, most of the cellulases from fungi and bacteria were found to be CMCase (*12*). However, that from *Brevibacillus* sp. strain JXL could hydrolyze cellulose, CMC, and xylan (*36*). According to the substrate specificity found in this study, the purified cellulase revealed high hydrolytic activities on CMC and was considered to be an endo-1,4-glucanase.

From the results obtained in this study, this novel *Cellulomonas* sp. YJ5 could utilize chlorella powder as substrate for growth and subsequently produce high levels of cellulase. This characteristic highly benefits microalgae industrial applications such as hydrolyzing the cell wall of *Chlorella* (data submitted to *J. Food Sci.* for publication). Further optimization of commercial scale production for cellulase and biohydrolysis of *Chlorella* cell walls using this cellulase are ongoing in our laboratory.

LITERATURE CITED

(1) Perez, J.; Munoz-Dorado, J.; de la Rubia, T.; Martinez, J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *Int. Microbiol.* **2002**, *5* (2), 53–63.

(2) Mandels, M. Applications of cellulases. *Biochem. Soc. Trans.* **1985**, *13* (2), 414–6.

(3) Beguin, P.; Aubert, J. P. The biological degradation of cellulose. *FEMS Microbiol. Rev.* **1994**, *13* (1), 25–58.

(4) Henrissat, B.; Teeri, T. T.; Raj, W. A. A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. *FEBS Lett.* **1998**, *425*, 352–354.

(5) Wood, T. M.; McCrae, S. I. Synergism between enzymes involved in the solubilization of native cellulose. *Adv. Chem. Ser.* **1979**, *181*, 181–209.

(6) Jervis, E. J.; Haynes, C. A.; Kilburn, D. G. Surface diffusion of cellulases and their isolated binding domains on cellulose. *J. Biol. Chem.* **1997**, *272* (38), 24016–24023.

(7) Johnson, P. E.; Tomme, P.; Joshi, M. D.; McIntosh, L. P. Interaction of soluble cellooligosaccharides with the N-terminal cellulose-binding domain of *Cellulomonas fimi* CenC. 2. NMR and ultraviolet absorption spectroscopy. *Biochemistry* **1996**, *35*, 13895–13906.

(8) Din, N.; Gilkes, N. R.; Tekant, B.; Miller, R. C. J.; Raj, W. A.; Kilburn, D. G. Non-hydrolytic disruption of cellulose fibres by the binding domain of a bacterial cellulase. *Bio/Technology* **1991**, *9*, 1096–1099.

(9) Doi, R. H. Cellulases of mesophilic microorganisms: cellulosome and nocellulosome producers. *Ann. N.Y. Acad. Sci.* **2008**, *1125*, 267–279.

(10) Hankin, L.; Anagnostakis, S. Solid media containing carboxymethyl cellulose to detect CM celllulase activity of microorganisms. *J. Gen. Microbiol.* **1977**, *98*, 109–115.

(11) Ivanen, D. R.; Rongjina, N. L.; Shishlyannikov, S. M.; Litviakova, G. I.; Isaeva-Ivanova, L. S.; Shabalin, K. A.; Kulminskaya, A. A. Novel precipitated fluorescent substrates for the screening of cellulolytic microorganisms. *J. Microbiol Methods* **2009**, *76* (3), 295–300.

(12) Tomme, P.; Warren, R. A.; Gilkes, N. R. Cellulose hydrolysis by bacteria and fungi. *Adv. Microbial Physiol.* **1995**, *37*, 1–81.

(13) Wang, C. M.; Shyu, C. L.; Ho, S. P.; Chiou, S. H. Characterization of a novel thermophilic, cellulose-degrading bacterium *Paenibacillus* sp. strain B39. *Lett. Appl. Microbiol.* **2008**, *47* (1), 46–53.

(14) Ko, C. H.; Chen, W. L.; Tsai, C. H.; Jane, W. N.; Liu, C. C.; Tu, J. *Paenibacillus campinasensis* BL11: a wood material-utilizing bacterial strain isolated from black liquor. *Bioresour. Technol.* **2007**, *14*, 2727–2733.

(15) Li, W.; Zhang, W. W.; Yang, M. M.; Chen, Y. L. Cloning of the thermostable cellulase gene from newly isolated *Bacillus subtilis* and its expression in *Escherichia coli*. *Mol. Biotechnol.* **2008**, *2*, 195–201.

(16) Morris, H. J.; Almarales, A.; Carrillo, O.; Bermudez, R. C. Utilisation of *Chlorella vulgaris* cell biomass for the production of enzymatic protein hydrolysates. *Bioresour. Technol.* **2008**, *99* (16), 7723–7729.

(17) Stoilov, I. L.; Georgiev, T. D.; Taskov, M. V.; Koleva, I. D. Oral preparation for patients with chronic renal insufficiency and other protein metabolic diseases. WO Patent WO/1995/029,252, 1995.

(18) Tchorbanov, B.; Bozhkova, M. Enzymatic hydrolysis of cell proteins in green algae Chlorella and Scenedesmus after extraction with organic solvents. *Enzyme Microb. Technol.* **1988**, *10*, 233–238.

(19) Yoon, M. H.; Choi, W. Y. Characterization and action patterns of two β-1,4-glucanases purified from *Cellulomonas uda* CS1-1. *Journal of Microbiology and Biotechnology* **2007**, *17* (8), 1291–1299.

(20) Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **1970**, *227* (5259), 680–685.

(21) Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, 31.

(22) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **1951**, *193* (1), 265–275.

(23) Edman, P.; Begg, G. A protein sequencer. *Eur. J. Biochem.* **1967**, *1*, 80–91.

(24) Perez-Avalos, O.; Sanchez-Herrera, L. M.; Salgado, L. M.; Ponce-Noyola, T. A bifunctional endoglucanase/endoxylanase from *Cellulomonas flavigena* with potential use in industrial processes at different pH. *Curr. Microbiol.* **2008**, *57* (1), 39–44.

(25) Jing, H.; Cockburn, D.; Zhang, Q.; Clarke, A. J. Production and purification of the isolated family 2a carbohydrate-binding module from *Cellulomonas fimi*. *Protein Express. Purif.* **2009**, *64* (1), 63–68.

(26) Nakamura, K.; Kitamura, K. Purification and some properties of a cellulase active on crystalline cellulose from *Cellulomonas uda*. *J. Ferment. Technol.* **1983**, *61* (4), 379–382.

(27) Mejia-Castillo, T.; Hidalgo-Lara, M. E.; Brieba, L. G.; Ortega-Lopez, J. Purification, characterization and modular organization of a cellulose-binding protein, CBP105, a processive β-1,4-endoglucanase from *Cellulomonas flavigena*. *Biotechnol. Lett.* **2008**, *30* (4), 681–687.

(28) Saha, B. C. Production, purification and properties of endoglucanase from a newly isolated strain of *Mucor circinelloides*. *Process Biochem.* **2004**, *39*, 1871–1876.

(29) Mawadza, C.; Hatti-Kaul, R.; Zvauya, R.; Mattiasson, B. Purification and characterization of cellulases produced by two *Bacillus* strains. *J. Biotechnol.* **2000**, *83* (3), 177–187.

(30) Akiba, S.; Kimura, Y.; Yamamoto, K.; Kumagai, H. Purification and characterization of a protease-resistant cellulase from *Aspergillus niger*. *J. Ferment. Bioeng.* **1995**, *79* (2), 125–130.

(31) Ogura, J.; Toyoda, A.; Kurosawa, T.; Chong, A. L.; Chohnan, S.; Masaki, T. Purification, characterization, and gene analysis of cellulase (Cel8A) from *Lysobacter* sp. IB-9374. *Biosci., Biotechnol., Biochem.* **2006**, *70* (10), 2420–2428.

(32) Kim, C. H. Characterization and substrate specificity of an endo-β-1,4-D-glucanase I (Avicelase I) from an extracellular multienzyme complex of *Bacillus circulans*. *Appl. Environ. Microbiol.* **1995**, *61* (3), 959–965.

(33) Smriti; Sanwal, G. G. Purification and characterization of a cellulase from *Catharanthus roseus* stems. *Phytochemistry* **1999**, *52* (1), 7–13.

(34) Lucas, R.; Robles, A.; Garcia, M. T.; Alvarez De Cienfuegos, G.; Galvez, A. Production, purification, and properties of an endoglucanase produced by the hyphomycete *Chalara* (Syn. *Thielaviopsis*) *paradoxa* CH32. *J. Agric. Food Chem.* **2001**, *49* (1), 79–85.

(35) Murashima, K.; Nishimura, T.; Nakamura, Y.; Koga, J.; Moriya, T.; Sumida, N.; Yaguchi, T.; Kono, T. Purification and characterization of new endo-1,4-β-D-glucanases from *Rhizopus oryzae*. *Enzyme Microb. Technol.* **2002**, *30* (3), 319–326.

(36) Liang, Y.; Yesuf, J.; Schmitt, S.; Bender, K.; Bozzola, J. Study of cellulases from a newly isolated thermophilic and cellulolytic *Brevibacillus* sp. strain JXL. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*(7), 961−970.

Received for review May 18, 2010. Revised manuscript received July 26, 2010. Accepted July 26, 2010.