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Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover

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Abstract

The production of extracellular cellulases by a newly isolated thermoacidophilic fungus, *Aspergillus terreus* M11, on the lignocellulosic materials was studied in solid-state fermentation (SSF). The results showed that the high-level cellulase activity was produced at 45 °C pH 3 and moisture 80% with corn stover and 0.8% yeast extract as carbon and nitrogen sources. 581 U endoglucanase activity, 243 U filter paper activity and 128 U β-glucosidase activity per gram of carbon source were obtained in the optimal condition. Endoglucanase and β-glucosidase exhibited their maximum activity at pH 2 and pH 3, respectively, and both of them showed remarkable stability in the range of pH 2–5. The activities of endoglucanase and β-glucosidase were up to the maximum at 70 °C and maintained about 65% and 53% of their original activities after incubation at 70 °C for 6 h. The enzyme preparations from this strain were used to hydrolyze Avicel. Higher hydrolysis yields of Avicel were up to 63% on 5% Avicel (w/v) for 72 h with 20 U FPase/g substrate.

Keywords: Solid-state fermentation (SSF); *Aspergillus terreus* M11; Corn stover

1. Introduction

Cellulose enzymes, which can hydrolyze cellulose forming glucose and other commodity chemicals, can be divided into three types: endoglucanase (*endo*-1,4-β-D-glucanase, EG, EC 3.2.1.4); cellobiohydrolase (*exo*-1,4-β-D-glucanase, CBH, EC 3.2.1.91) and β-glucosidase (1,4-β-D-glucosidase, BG, EC 3.2.1.21) (Hong et al., 2001; Li et al., 2006). Scientific communities have strong interests in cellulases because of their applications in industries as follows: starch processing, animal food production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry, and textile industry (Adsul et al., 2007; Kaur et al., 2007).

However, the major bottleneck of comprehensive application of cellulase in industry is the high cost of the enzyme production. Substantial cost reduction may be possible by exploring ways of cellulose conversion using microorganisms that produce cellulolytic enzymes. It is therefore imperative to look for microorganisms that have a high rate of cellulase production (Kotchoni and Shonukan, 2002). Besides, it has been reported that solid-state fermentation (SSF) is an attractive process to produce cellulase economically due to its lower capital investment and lower operating expenses (Xia and Cen, 1999). Another approach to reduce the cost of cellulase production is use of the cheap and easily available substrates. Several studies in the literature indicate that the carbon source used in cultivations is one of most important factors affecting the cost and yield of cellulase production. Therefore for reducing the cost of enzyme, selection of a cheap and easily available substrate appears to be essential (Beg et al., 2000; Senthilkumar et al., 2005).

The lignocellulosic biomass, especially agricultural wastes, is known to be an excellent carbon source for

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microbial enzyme production. Those lignocellulosic biomass, such as corn stover and wheat bran, are very abundant, cheap and easily available. Various agricultural substrates-byproducts and microbial cultures have been used successfully in the SSF for cellulase production (Yang et al., 2006).

The commercial cellulases are mainly extracellular enzymes produced by mesophilic or thermophilic fungi (Kim et al., 2005). Since the use of cellulose degrading enzymes is related to industrial processing and operating at high temperature, application of thermostable enzymes produced by mesophilic or thermophilic fungi appears to be advantageous. *Aspergillus* species are major agents of decompositions and decay and thus possess the capability to produce a brand range of enzymes. The genus is known to be a good producer of cellulase. Cellulase production has been described for many *Aspergillus* species (Lockington et al., 2002; Ong et al., 2004; Wang et al., 2006), but only a few reports available on the production of cellulase from *Aspergillus terreus* (Workman and Day, 1982; Emtiazi et al., 2001). Up to now, few studies have been carried out on the cellulase production of *A. terreus* in the SSF.

In this study, a thermoacidophilic strain of *A. terreus*, proved to be a good producer of cellulase, was isolated. And then, its ability of cellulases production in the SSF was investigated. Moreover, some of the critical factors affecting cellulases production by this strain in the SSF were also optimized.

2. Methods

2.1. Strain isolation and identification

A. terreus M11 was isolated from the compost containing cellulose at the factory of Zhengzhou, China. The isolation of *A. terreus* M11 was performed as described by Li et al. (2006). The used medium was following: CMC induction medium containing yeast extract, 2.5 g; tryptone, 2.5 g; CMC (low viscosity), 2.5 g; $(NH_4)_2SO_4$, 1 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g and $MgSO_4$, 0.2 g in 1 L water. The solid medium containing 1.5% agar (w/v) was used for screening the cellulolytic strain and potato dextrose agar medium (PDA medium) was used for strain maintenance.

ITS sequence of *A. terreus* M11 was amplified by PCR with the primers ITS1: 5′-AGAAGTCGTAACAAGGTTTCCGTAGG-3′ and ITS4: 5′- TCCTCCGCTTATTGATATGC-3′. The amplification was performed using the PTC-150 Minicycler with the following cycling parameter: 95 °C for 2 min, followed by 35 cycles of 1 min at 95 °C, 1 min at 50 °C, and 1 min at 72 °C with final extension for 10 min. Then, the PCR fragment was ligated into the PMD18-T vector (TAKARA, China), and transformed into *Escherichia coli* DH 5α. The amplified construct harboring the ITS rDNA was sequenced. Then, the ITS rDNA sequence was compared with GenBank and a neighbor-jointing phylogenetic tree was constructed by the PHYLIP package (Felsenstein, 1989) with related *Aspergillus*. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method with 1000 replicates.

2.2. Substrates

The lignocellulosic materials, namely wheat bran, wheat straw, corn stover, rapeseed straw, bulrush straw and sugar cane bagasse were all obtained locally. They were firstly dried and chopped into small pieces by a chopper, then ground into smaller particles in a hammer mill, and finally separated by 0.45 mm (40 meshes) sieve. The fraction that passed through the sieve was used for medium preparation in the SSF.

2.3. Inoculation and culture conditions

The strain was maintained on PDA medium at 45 °C for 5 d for spore production. The conidial suspensions were prepared by washing slant cultures with 5 ml of sterilized 0.9% NaCl (w/v) solution. Spore suspension was counted at 10^6–10^7 spores/ml by a haemacytometer.

Five grams of dry carbon source mixed with the mineral solution (gram per liter distiller water: $(NH_4)_2SO_4$ 3.5 g, KH_2PO_4 3 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2$ 0.5 g) was put into 250 ml Erlenmeyer flasks, which were all sterilized for 30 min at 121 °C in succession (Juhász et al., 2005). Two milliliters of prepared spores was inoculated and incubated at 45 °C under static conditions.

2.4. Experiment design

To optimize the process of fermentation, carbon and nitrogen sources, concentration of nitrogen source, the effect of initial moisture, initial pH and growth temperature were determined with monofactorial experiments in the screened fermentation conditions. According to the results of monofactorial experiments, three levels of four factors (growth temperature, initial pH, initial moisture and concentration of nitrogen source) were made for orthogonal test to get an optimal fermentation condition. Factors and levels of orthogonal were designed (Table 2). All tests were repeated two times with two replicates for each sample in all the experiments. Values were the average of samples in the experiment.

2.5. Enzyme extract

The solid substrate culture broth was prepared by adding 10-fold (v/w) distilled water and shaking (180 rpm) at 30 °C for 60 min. Then, the solid materials and fungal biomass were separated by centrifugation (10,000×*g*, 15 min). The clarified supernatant used for enzyme assays.

2.6. Enzyme assay

The activities of total cellulase (filter paper activity, FPA), endoglucanase and β-glucosidase were determined

as reported earlier (Grajek, 1987). Filter paper activity was assayed by incubating 1 ml the suitably diluted enzyme solution with citrate buffer (50 mM, pH 5) containing Whatman No. 1 filter paper (50 mg, 1 × 6 cm). The reaction mixture was incubated at 60 °C for 30 min.

Endoglucanase (CMCase, *endo*-1,4-β-D-glucanase; EC 3.2.1.4) activity was carried out in the total reaction mixture of 1 ml containing 0.5 ml of suitably diluted enzyme and 0.5 ml of 1% (w/v) CMC solution in citrate buffer (50 mM, pH 5). This mixture was incubated at 60 °C for 30 min. The release of reducing sugars was determined by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959).

β-Glucosidase (β-D-glucoside, glucohydrolase; EC 3.2.1.21) activity was estimated using *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) as substrate. The total of assay mixture (1 ml) consisting of 0.9 ml of *p*NPG(1 mM) and 0.1 ml of suitably diluted enzyme was incubated at 60 °C for 30 min. The *p*-nitrophenol liberated was measured at 420 nm after developing the color with 2 ml of sodium carbonate (2 M).

One unit (U) of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of glucose or *p*-nitrophenol from the appropriate substrates per minute under the assay conditions.

2.7. Characteristics of the partially purified cellulases

The crude cellulase was precipitated with ammonium sulfate (75%). The precipitate was recovered by centrifugation at 10,000×*g* for 30 min at 4 °C and dissolved in citrate buffer (pH 7.0). The enzyme solution was desalted on a Sephadex G-25 column. Then, the enzymatic characteristics were studied including optimal pH, stability of pH, optimal temperature and thermostability.

2.8. Hydrolytic potential of the cellulase system

The hydrolysis experiments were performed with 5% Avicel (microcrystalline cellulose) as substrate and different crude enzymes produced by *A. terreus* M11 at 70 °C, pH 3.0 with shaking at 150 rpm. The samples were analyzed on the reducing sugars after 72 h.

3. Results and discussion

3.1. Isolation and identification of A. terreus M11

The colonies of this isolated strain spread around with velvety, white hyphae after incubation for 2 d on PDA and became brown after 4 d. The microscopic morphology showed that hyphae were septate and hyaline, conidial heads were columnar, conidiophores were smooth-walled and hyaline and conidia were small, globose and smooth. This strain was further identified by its ITS sequence. The sequence had been deposited in Genbank database (Accession number: EF432562). Sequence analysis suggested that M11 was phylogenetically related to members of the genus *Aspergillus* (Fig. 1). Based on these, the strain M11 was identified as *A. terreus*, namely *A. terreus* M11.

3.2. Effect of carbon and nitrogen sources

Since any biotechnological process is likely to base on crude enzymes, it is important to increase their activities in the culture supernatants by selecting the best carbon and nitrogen sources and optimizing their concentrations (Gomes et al., 2000).

Cellulase production was found to be dependent on the nature of the carbon source used in the culture medium.

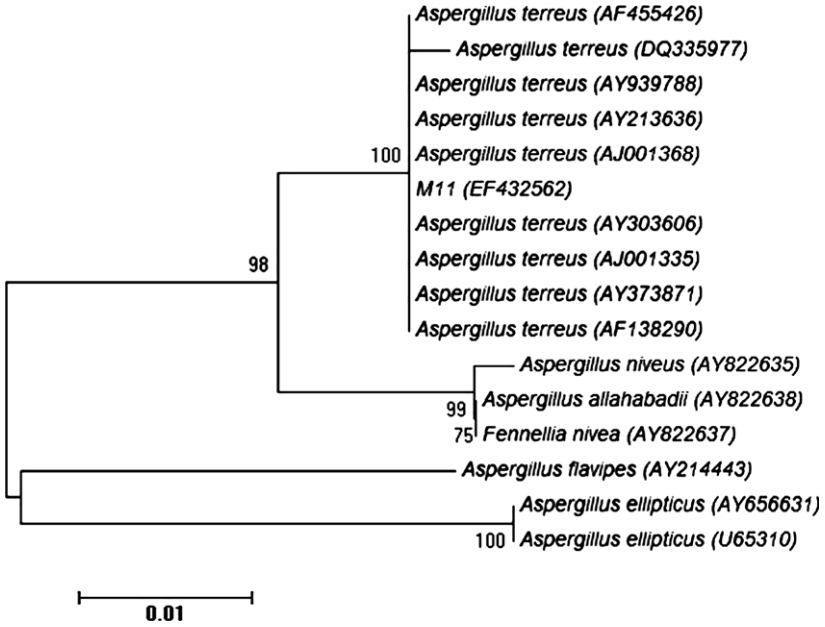

Fig. 1. Phylogenetic tree of ITS sequences of the strain M11 and the fungi most closely related to it.

Various lignocellulose carbon sources were tested for their effect on cellulase production. The influence of various carbon sources on the cellulase biosynthesis on *A. terreus* M11 was recorded in Table 1. Corn stover was the best carbon source for CMCase, FPase and β-glucosidase production among the tested lignocellulosic materials listed in Table 1. This might be attributed to its hemicellulose nature and favorable degradability, the presence of some nutrients in the carbon source (Senthilkumar et al., 2005). The composition of corn stover was identified according to the analytical procedure recommended by Yang (Yang, 2001). The result showed that corn stover was approximately composed of cellulose 39.54%, hemicellulose 25.76%, Klason lignin 17.49%, ash 5.04%. Besides, the efficiency of enzyme production also depends on the bare chemical composition of the raw material, accessibility of various components and their chemistry or physical associations. Corn stover has been known as an ideally suitable substrate for cellulase production (Panagiotou et al., 2003).

The effects of nitrogen source on production of cellulase enzyme were variable, depending on the fungi and the compound tested (Kachlishvili et al., 2006). The enzyme production was affected significantly under different concentration of nitrogen source (Panagiotou et al., 2003). When different nitrogen sources were tested, the results showed that enzyme activities were higher with organic nitrogen (Table 1). Maximum cellulase activity was obtained when yeast extract was added. These data were in accordance to previous some reports (Beg et al., 2000), though other research found that inorganic nitrogen sources were the optimal (Kalogeris et al., 2003a). Furthermore, the concentration of yeast extract was also optimized. The optimal concentration was 1% and used in subsequent experiments.

Table 1
Effect of carbon and nitrogen sources on cellulases production of *A. terreus* M11

Source	Enzyme yield (U/g dry carbon source)		
	CMCase	FPA	β-Glucanase
Carbon source			
Sugar cane	267	48	8
Rape straw	122	12	11
Bulrush straw	255	98	16
Wheat straw	417	166	87
Wheat bran	315	94	79
Corn stover	440	198	91
Nitrogen source			
Beef paste	368	183	58
Yeast extract	467	201	93
Peptone	356	177	89
Urea	318	171	65
$(NH_4)_2SO_4$	186	82	41
NH_4NO_3	142	61	25
$NaNO_3$	113	49	30
Yeast extract (%, w/v)			
0.5	432	175	86
1.0	469	207	102
1.5	443	184	96

Table 2
Factors and levels of orthogonal test

Levels	Factors			
	A	B	C	D
	Growth temperature (°C)	Nitrogen concentration (%)	Initial moisture (%)	Initial pH
1	35	0.8	70	2
2	40	1.0	75	3
3	45	1.3	80	4

3.3. Effect of moisture level

Fungi were well-known to favor a moist environment for their growth. The optimal moisture content in the SSF depends on the nature of the substrate, the requirements of the microorganism, and the type of the end product (Kalogeris et al., 2003b). Therefore, the effect of moisture content was analyzed on the enzyme production. The result showed that the increase of the initial moisture content from 60% to 80% greatly enhanced the enzyme activity in the broth, and till 80% the activity was the highest (data not shown). This could be attributed to the faster growth of microorganism at high moisture content and the subsequent early initiation of the enzyme production. As reported elsewhere, the high moisture enhanced fungal growth and cellulase production when lignocellulosic substrates were the carbon sources in the SSF (Kalogeris et al., 2003b; Panagiotou et al., 2003). But, too much water in the medium could make the medium clumped, and not benefit aeration and the growth of hyphae, which could result in decrease of enzyme production.

3.4. Effect of initial pH

The effect of initial pH of culture medium on the cellulase production was investigated. Production of cellulase did not obviously vary within an initial pH range of 2–5 (data not shown). The maximum cellulase activity was obtained when the initial pH was 2. Therefore, this optimal pH value of the strain was much lower than the reported optimal culture pH 3–6 of fungi (Romero et al., 1999; Xia and Cen, 1999; Jecu, 2000). This property suggested it could be used to degrade cellulosic materials and produce cellulase under acidic condition.

3.5. Effect of culture temperature and time

Temperature was the most important physical variables in the SSF. The temperature for the cellulase production of *A. terreus* M11 was optimized. The optimal temperature was 35–45 °C (data not shown), and the maximal cellulase production occurred at 45 °C which was in the range of the temperature of thermophilic fungi (Maheshwari et al., 2000). The temperature (35–45 °C) did not strongly affect

the enzyme production in this study, which was favorable for stabile cultivation in solid medium. As once as temperature was above 45 °C, the biosynthesis of enzymes markedly decreased.

The time course of cellulase production was further investigated. The maximum production was observed after 96 h (data not shown). Then the level of enzyme production gradually declined. Time course required to reach maximum levels of activity may be affected by several factors, including the presence of different ratios of amorphous to crystalline cellulose (Ögel et al., 2001).

3.6. Analysis of enzyme activities in orthogonal experiment

The results of the orthogonal experiment showed that the optimal culture conditions for three enzymes was No. 7, which consisted of pH 3, 45 °C, 80% moisture and 0.5% nitrogen concentration (Table 3). Under optimal culture conditions, the average activity of CMCase, FPase and β-glucanase reached 581 U/g, 243 U/g and 128 U/g, respectively. *R* values indicated that effects of four factors on endoglucanase and FPase were C > D > A > B, while influences of factors on β-glucanase were C > B = D > A.

3.7. Comparisons of cellulase production using SSF

The comparisons of cellulase activities produced by *A. terreus* M11 in the SSF with those reported for other cellulase producing fungi showed that the fungus investigated in this work compared favorably with the most of those fungi (Table 4). In fact, the comparisons of cellulase activities produced by different laboratories is not readily made in quantitative manner as no standard conditions of cellulase activity assay have yet been adopted. It only supplied a relative comparison in this paper.

3.8. Characteristics of partially purified cellulases

Endoglucanase exhibited its maximum activity at pH 2 while β-glucosidase was optimally active at pH 3 (data not shown). The optimal pH of both enzymes was much lower than those reported in other literature (Maheshwari et al., 2000; Lechner and Papinutti, 2006). The optimal temperature of β-glucosidase and endoglucanase activity was both 70 °C (data not shown). This was accordant to the temperature of industrial process. Therefore, they were suitable for industry application.

Incubation of *A. terreus* M11 cellulases for various times in buffer solutions with pH values between 2 and 7 at 70 °C. The results were showed in Fig. 2a. The endoglucanase was stable in the range of pH 2–5 and the β-Glucosidase was stable at pH 3–5. But their activities markedly decreased at above pH 5. It was remarkable that the enzymes were more stable at acidic pHs than at neutral pHs. The activities of endoglucanase and β-glucosidase were, respectively, maintained 65% and 53% after incuba-

Table 3
Analyses of cellulolases in orthogonal experiment

Run no.	A	B	C	D	Enzyme yield (U/g carbon source)		
					CMCase	FPase	β-Glucanase
1	1 (35 °C)	1 (0.8%)	1 (70%)	1 (2)	467 ± 12	196 ± 6	95
2	1 (35 °C)	2 (1.0%)	2 (75%)	2 (3)	540 ± 16	225 ± 10	119 ± 9
3	1 (35 °C)	3 (1.3%)	3 (80%)	3 (4)	552 ± 10	231 ± 5	122 ± 6
4	2 (40 °C)	1 (0.8%)	2 (75%)	3 (4)	505 ± 13	211 ± 9	106 ± 10
5	2 (40 °C)	2 (1.0%)	3 (80%)	1 (2)	544 ± 20	228 ± 5	119 ± 4
6	2 (40 °C)	3 (1.3%)	1 (70%)	2 (3)	515 ± 9	215 ± 8	113 ± 8
7	3 (45 °C)	1 (0.8%)	3 (80%)	2 (3)	581 ± 18	243 ± 12	128 ± 9
8	3 (45 °C)	2 (1.0%)	1 (70%)	3 (4)	542 ± 15	218 ± 8	109 ± 5
9	3 (45 °C)	3 (1.3%)	2 (75%)	1 (2)	513 ± 11	224 ± 13	120 ± 7
CMCase							
K1	1559	1553	1524	1524			
K2	1564	1626	1558	1636			
K3	1636	1580	1677	1599			
R	77	73	153	132			
FPase							
K1	652	650	629	648			
K2	654	671	660	683			
K3	685	670	702	660			
R	33	21	73	35			
β-Glucanase							
K1	336	329	317	334			
K2	338	347	345	360			
K3	357	355	369	337			
R	21	26	52	26			

Table 4
Comparisons of cellulases production from different fungi using SSF

Microorganism	Carbon source	Enzyme yield (U/g carbon source)			References
		CMCase	FPA	β-Glucosidase	
Fusarium oxysporum	Corn stover	304		0.140	Panagiotou et al. (2003)
Trichoderma reesei MGG77	Rice bran		2.314		Latifian et al. (2007)
Funalia trogi IBB 146	Wheat straw	356	26		Kachlishvili et al. (2006)
Sporotrichum thermophile	Beet pulp, Cellulose	109		12.1	Grajek (1986)
Trichoderma reesei ZU-02	Corncob		158		Xia and Cen (1999)
Trichoderma koningi F244	Wheat bran	287.3	94.0	184	Li et al. (2004)
Thermoascus aurantiacus	Wheat straw	1709	5.5	79	Kalogeris et al. (2003b)
Aspergillus niger KK2	Rice straw	129	19.5	100	Kang et al. (2004)
Thermoascus aurantiacus	Wheat straw	1572		101.6	Kalogeris et al. (2003a)
Aspergillus terreus M11	Corn stover	563	231	119	This work

tion at pH 2 and pH 3 at 70 °C for 6 h (Fig. 2b). The high thermostability suggested that the cellulases could be of commercial interest.

3.9. Hydrolysis of Avicel

The crude cellulase produced in the SSF was directly used to hydrolyze Avicel. When the cellulase dosage per gram substrate was changed from 5 to 20 U, the yield of enzymatic hydrolysis was increased obviously from 17% to 63%. However, further increase of the cellulase dosage did not contribute to further increase in hydrolysis yield (data not shown). It was reported 20 U FPase crude enzyme preparation produced by the mutant EU1 of *Penicillium janthinellum* NCIM1171 was used to hydrolyze 5% Avicel for 150 h to gain 61% yield enzymatic hydrolysis (Adsul et al., 2007). Comparing with this report, enzyme preparations derived from *A. terreus* M11 had comparatively high hydrolysis yield.

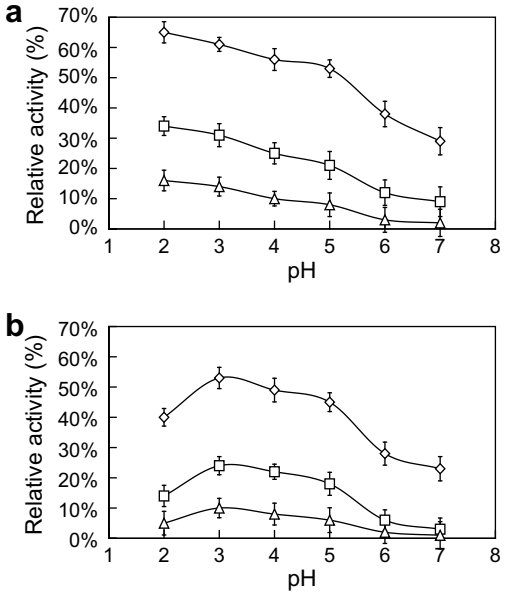

Fig. 2. Stability of the partially purified cellulases at pH 2–7. Endoglucanase (a); β-glucosidase (b). Symbols: ◇ 6 h; □ 12 h; △24 h. Relative activity was expressed as a percentage of the activity of the enzymes stored at 4 °C.

4. Conclusions

The ability of newly isolated *A. terreus* M11 to produce cellulases in the SSF with corn stover as carbon source was investigated. The results suggested the potential use of *A. terreus* M11 for cellulase production in a shorter period with a cheap medium. 581 U endoglucanase activity, 243 U FPase and 128 U β-glucosidase activity per gram of carbon source were obtained after culturing in the SSF for 96 h at 45 °C and pH 3. The cellulases were stable in the range of acidic pHs at 70 °C. The above properties suggested the cellulases might be used as key enzymes in the production of bioethanol from cellulose. Traditionally, the cellulose is hydrolyzed by acids at high temperatures for bioethanol production. In the subsequent step of enzymic hydrolysis, an acid- and heat-tolerant cellulase could simplify the reaction process and reduce the cost of production.

References

Adsul, M.G., Bastawde, K.B., Varma, A.J., Gokhale, D.V., 2007. Strain improvement of *Penicillium janthinellum* NCIM 1171 for increased cellulase production. Bioresour. Technol. 98, 1467–1473.

Beg, Q.K., Bhushan, B., Kapoo, M., Hoondal, G.S., 2000. Enhanced production of a thermostable xylanase from *Streptomyces* sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. Enzyme Microbiol. Technol. 27, 459–466.

Emtiazi, G., Naghavi, N., Bordbar, A., 2001. Biodegradation of lignocellulosic waste by *Aspergillus terreus*. Biodegradation 12, 259–263.

Felsenstein, J., 1989. PHYLIP-PHYlogeny Inference Package, version 3.2. Cladistics 5, 164–166.

Grajek, W., 1986. Temperature and pH optima of enzyme activities produced by cellulytic fungi in batch and solid state cultures. Biotechnol. Lett. 8, 587–590.

Grajek, W., 1987. Comparative studies on the production of cellulases by thermophilic fungi in submerged and solid-state fermentation. Appl. Microbiol. Biotechnol. 26, 126–129.

Gomes, I., Gomes, J., Gomes, D.J., Steiner, W., 2000. Simultaneous production of high activities of thermostable endoglucanase and beta-glucosidase by the wild thermophilic fungus *Thermoascus aurantiacus*. Appl. Microbiol. Biotechnol. 53, 461–468.

Hong, J., Tamaki, H., Akiba, S., Yamamoto, K., Kumagai, H., 2001. Cloning of a gene encoding a highly stable *endo*-β-1,4-glucanase from *Aspergillus niger* and its expression in yeast. J. Biosci. Bioeng. 92, 434–441.

Jecu, L., 2000. Solid state fermentation of agricultural wastes for endoglucanase production. Ind. Crop. Prod. 11, 1–5.

Juhász, T., Szengyel, Z., Réczey, K., Siika-Aho, M., Viikari, L., 2005. Characterization of cellulases and hemicellulases produced by *Trichoderma reesei* on various carbon sources. Process Biochem. 40, 3519–3525.

Kachlishvili, E., Penninckx, M.J., Tsiklauri, N., Elisashvili, V., 2006. Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation. World J. Microbiol. Biotechnol. 22, 391–397.

Kalogeris, E., Christakopoulos, P., Katapodis, P., Alexiou, A., Vlachou, S., Kekos, D., Macris, B.J., 2003a. Production and characterization of cellulolytic enzymes from the thermophilic fungus *Thermoascus aurantiacus* under solid state cultivation of agricultural wastes. Process Biochem. 38, 1099–1104.

Kalogeris, E., Iniotaki, F., Topakas, E., Christakopoulos, P., Kekos, D., Macris, B.J., 2003b. Performance of an intermittent agitation rotating drum type bioreactor for solid-state fermentation of wheat straw. Bioresour. Technol. 86, 207–213.

Kang, S.W., Park, Y.S., Lee, J.S., Hong, S.I., Kim, S.W., 2004. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour. Technol. 91, 153–156.

Kaur, J., Chadha, B.S., Kumar, B.A., Saini, H.S., 2007. Purification and characterization of two endoglucanases from *Melanocarpus* sp. MTCC 3922. Bioresour. Technol. 98, 74–81.

Kim, J.Y., Hur, S.H., Hong, J.H., 2005. Purification and characterization of an alkaline cellulase from a newly isolated alkalophilic *Bacillus* sp. HSH-810. Biotechnol. Lett. 27, 313–316.

Kotchoni, S.O., Shonukan, O.O., 2002. Regulatory mutations affecting the synthesis of cellulase in *Bacillus pumilus*. World J. Microbiol. Biotechnol. 18, 487–491.

Latifian, M., Hamidi-Esfahani, Z., Barzegar, M., 2007. Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour. Technol. 98, 3634–3637.

Lechner, B.E., Papinutti, V.L., 2006. Production of lignocellulosic enzymes during growth and fruiting of the edible fungus *Lentinus tigrinus* on wheat straw. Process Biochem. 41, 594–598.

Li, Y.H., Ding, M., Wang, J., Xu, G.J., Zhao, F., 2006. A novel thermoacidophilic endoglucanase, Ba-EGA, from a new cellulose-degrading bacterium, *Bacillus* sp. AC-1. Appl. Microbiol. Biotechnol. 70, 430–436.

Li, P.J., Jing, D.B., Zhou, Q.X., Zhang, C.G., 2004. Optimization of solid fermentation of cellulase from *Trichoderma koningii*. J. Environ. Sci. (China) 16, 816–820.

Lockington, R.A., Rodbourn, L., Barnett, S., Carter, C.J., Kelly, J.M., 2002. Regulation by carbon and nitrogen sources of a family of cellulases in *Aspergillus nidulans*. Fungal Genet. Biol. 37, 190–196.

Maheshwari, R., Bharadwaj, G., Bhat, M., 2000. Thermophilic fungi: their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64, 461–488.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Biochem. 31, 426–428.

Ögel, Z.B., Yarangümeli, K., Dü, H., Ifrij, I., 2001. Submerged cultivation of *Scytalidium thermophilum* on complex lignocellulosic biomass for endoglucanase production. Enzyme Microbiol. Technol. 28, 689–695.

Ong, L.G., Abd-Aziz, S., Noraini, S., Karim, M.I., Hassan, M.A., 2004. Enzyme production and profile by *Aspergillus niger* during solid substrate fermentation using palm kernel cake as substrate. Appl. Biochem. Biotechnol. 118, 73–79.

Panagiotou, G., Kekos, D., Macris, B.J., Christakopoulos, P., 2003. Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. Ind. Crop. Prod. 18, 37–45.

Romero, M.D., Aguado, J., González, L., Ladero, M., 1999. Cellulase production by *Neurospora crassa* on wheat straw. Enzyme Microbiol. Technol. 25, 244–250.

Senthilkumar, S.R., Ashokkumar, B., Raj, K.C., Gunasekaran, P., 2005. Optimization of medium composition for alkali-stable xylanase production by *Aspergillus fischeri* Fxn 1 in solid-state fermentation using central composite rotary design. Bioresour. Technol. 96, 1380–1386.

Wang, X.J., Bai, J.G., Liang, Y.X., 2006. Optimization of multienzyme production by two mixed strains in solid-state fermentation. Appl. Microbiol. Biotechnol. 73, 533–540.

Workman, W.E., Day, D.F., 1982. Purification and properties of beta-glucosidase from *Aspergillus terreus*. Appl. Environ. Microbiol. 44, 1289–1295.

Xia, L., Cen, P.L., 1999. Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem. 34, 909–912.

Yang, S., 2001. Chemistry of Plant Fibers, third ed. China Light Industry Press, Beijing.

Yang, S.Q., Yan, Q.J., Jiang, Z.Q., Li, L.T., Tian, H.M., Wang, Y.Z., 2006. High-level of xylanase production by the thermophilic *Paecilomyces themophila* J18 on wheat straw in solid-state fermentation. Bioresour. Technol. 97, 1794–1800.