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Evaluation of cellulases produced from four fungi cultured on furfural residues and microcrystalline cellulose

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Abstract Four fungal strains—*Trichoderma viride*, *Aspergillus niger*, *Trichoderma koningii*, and *Trichoderma reesei*—were selected for cellulase production using furfural residues and microcrystalline cellulose (MCC) as the substrates. The filter paper activity (FPA) of the supernatant from each fungus was measured, and the performance of the enzymes from different fungal strains was compared. Moreover, the individual activities of the three components of the cellulase system, i.e., β-glucosidase, endoglucanase, and exoglucanase were evaluated. *T. koningii* showed the highest activity (27.81 FPU/ml) on furfural residues, while *T. viride* showed an activity of 21.61 FPU/ml on MCC. The FPA of the crude enzyme supernatant from *T. koningii* was 30% higher on furfural residues than on MCC. *T. koningii* and *T. viride* exhibited high stability and productivity and were chosen for cellulases production. The crystallinity index (CrI) of the furfural residues varied after digested by the fungi. The results indicated differences in the functioning of the cellulase system from each fungus. In the case of *T. koningii*, *T. reesei* and *T. viride*, furfural residues supported a better environment for cellulase production than MCC. Moreover, the CrI of the furfural residues decreased, indicating that this material was largely digested by the fungi. Thus, our results suggest that it may be possible to use the cellulases produced from these fungi for the simultaneous saccharification and fermentation of lignocellulosic materials in ethanol production.

Keywords Cellulase · Specific substrate · Furfural residues · Microcrystalline cellulose · *Trichoderma* · *Aspergillus*

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Introduction

Cellulases are mainly applied to produce reducing sugars from lignocellulose, which can be further utilized to generate ethanol (Chandra et al. 2009). In such cases, the cellulase system is used to degrade lignocellulosic materials such as agricultural waste biomass. In addition to their application in biofuel production, cellulases are also used in the fruit, vegetable, oil crop, tea, textile, and pulp industries (Ahamed and Vermette 2009). The cellulase system is a complex inducible enzyme system consisting of β-glucosidase, endoglucanase, and exoglucanase (or cellobiohydrolase) (Nascimento et al. 2009; Sukumaran et al. 2009), and the synergistic action of all three kind of enzyme components is required for the hydrolysis of cellulose (Lin et al. 2009; Lo et al. 2010; Shalini and Laurent 2006). Endoglucanases hydrolyze the accessible intramolecular β-1,4-glucosidic

bond of cellulose chains in the cellulose polymer to produce reducing and non-reducing ends. Moreover, exoglucanases cleave cellulose chains by removing the cellobiose unit from the reducing and nonreducing ends. Finally, β-glucosidases cleave cellobiose to glucose. The hydrolytic processes catalyzed by these three enzymes occur synchronously (Singhania et al. 2010), and the entire cellulase system acts to convert cellulose into glucose.

Cellulases are produced by several microorganisms, including bacteria (Baldrian and Valaskova 2008; Zhang et al. 2004), white rot fungi (Shrestha et al. 2009), and anaerobic fungi (Ljungdahl 2008). Most commercial cellulases are obtained from filamentous fungi such as *Trichoderma*, *Penicillium*, and *Aspergillus*, and these enzymes have been widely studied (Ahamed and Vermette 2009; Jorgensen et al. 2004; Liu et al. 2006; Picart et al. 2007). In recent continuous culture studies, considerable effort has been directed toward the development of mutants that can produce cellulases with superior properties (Kovacs et al. 2008). *Trichoderma reesei* is a fungus that produces the exoglucanase and endoglucanase components of the cellulase system but with low β-glucosidase activity (Kovacs et al. 2008, 2009a). The extracellular cellulolytic system of *T. reesei* is composed of 60–80% cellobiohydrolases or exoglucanases, 20–36% endoglucanases, and 1% β-glucosidases, and all three enzymes act synergistically to convert cellulose into glucose (Ahamed and Vermette 2008). Moreover, *Aspergillus niger* can secrete high levels of β-glucosidase (Kovacs et al. 2009b), and *Trichoderma viride* can produce extracellular cellulase and xylanase (Li et al. 2010).

The high cost of enzyme production limits the industrial utilization of cellulases in ethanol production. Although carbon sources such as crystalline cellulose can generally be selected as substrates for these fungi, such materials are expensive (Wen et al. 2005). In contrast, lignocellulosic biomass is an abundant resource that can be used for the production of fuels and chemicals, and lignocellulosic materials such as rice straw, sugar cane bagasse and corncob residue have been selected as carbon sources for cellulase production (Camassola and Dillon 2009; Hideno et al. 2011; Xia and Shen 2004). The cellulase from steam-pretreated lespedeza was found to show a very high hydrolytic capability for this substrate (Feng et al. 2011). Commercial furfural production facilities use corncobs as feedstock. In the process, corncobs are heated under acidic conditions to hydrolyze arabinoxylan (hemicellulose) to sugars, and the sugars thus released are converted into furfural. As the cellulose and lignin components of the cobs are relatively stable under these conditions, the residue is enriched in both of these compounds (Tang et al. 2011). In China, $\sim$2.3 million tones of furfural residues is produced annually, and proper utilization of this material is important both for optimizing the use of resources and for reducing environmental pollution. Moreover, furfural residues are rich in cellulose and should therefore be degradable for cellulolytic fungi.

Since enzyme production is greatly affected by the cellulose material of culture medium, it may be advantageous to produce cellulase on-site by adding parts of lignocellulosic substrates. It has been hypothesized that the cultivation of a fungal strain on a particular lignocellulosic substrate leads to the production of an enzyme mixture that is highly suitable for the hydrolysis of that particular substrate (Feng et al. 2011). To date, there are no reports on the use of furfural residues as a substrate for fungal cultivation and the associated hydrolytic performance of the produced cellulases. To reduce the cost of cellulase production, it is essential to select substrate-specific cellulase-producing mutants that are very productive on lignocellulosic materials. In the present study, four fungi were chosen and incubated on either furfural residues containing polysaccharides and lignin or on microcrystalline cellulose (MCC). The cellulose-degrading abilities of these fungi were then evaluated. The aim of this study was to examine the potential utilization of furfural residues as substrates in cellulase production. The results provide valuable information for the development of cost-effective cellulase production processes.

Materials and methods

Materials

Trichoderma viride (CGMCC no. 3.2941) was purchased from China General Microbiological Culture Collection Center, and *A. niger* (ACCC no. 30557) was from Agricultural Culture Collection of China. *Trichoderma koningii* (CICC no. 13007) and *T. reesei* (CICC no. 40360) were kindly provided by China

Center of Industrial Culture Collection. The fungi were subcultured on furfural residues and MCC to induce cellulose production.

MCC was purchased from Sigma-Aldrich. Furfural residues were kindly provided by Chunlei Co., Ltd. (Hebei, China). The furfural residues were water-rinsed, dried at 60°C, and then milled. Particles of mesh size 40–60 mesh were used for the cellulase production.

Cellulase production

The fungi were inoculated in potato dextrose agar medium, and the spores were harvested by washing the Petri plate with sterile water. Shake flask fermentations were carried out in 250-ml cotton-plugged Erlenmeyer flasks containing 50 ml Mandels medium (Zhang et al. 2007) supplemented with 20 g/l MCC or 20 g/l furfural residues. After autoclaving at 121°C for 20 min, the flasks were inoculated with 5 ml conidia suspension (1×10^8 conidia/ml). The flasks were incubated at 30°C on a rotary shaker at 150 rpm. After culturing for the desired time, samples were removed and centrifuged at 10,000×*g* for 10 min. The clear supernatants were collected and stored in sealed bottles at 4°C and for the subsequent activity assay.

Analytical methods

The filter paper activity (FPA) and endoglucanase activity of the cellulase preparations were evaluated according to the standard IUPAC method (Ghose 1986). After incubation at 50°C for 30 min, 1.5 ml of dinitrosalicylic acid (DNS) was added to the mixture. The sugars released by the digestion were measured by high performance anion-exchange chromatography (HPAEC; Dionex ICS3000; USA) using a pulsed amperometric detector (PAD) and an ion exchange column (Carbopac PA-20). One FPA unit is defined as the amount of enzyme that releases 1 μmol of glucose per minute during the hydrolysis, and activities are reported as FPU/ml. Exoglucanase activity was assayed using pNP-cellobioside as the substrate. The assay mixture contained 1.8 ml of 1 mg/ml pNP-cellobioside in Tris–HCl buffer (pH 8.0) and 0.2 ml of diluted enzyme solution. After incubation at 50°C for 30 min, 2 ml of 1 M Na_2CO_3 was added to the mixture. The amount of *p*-nitrophenol (PNP) liberated was measured at 400 nm using a PNP standard curve prepared at seven different concentrations. One international unit (IU) of exoglucanase activity was defined as the amount of enzyme that liberates one μmol PNP per minute under the assay conditions. The *β*-glucosidase activity was determined using the modified Berghem's method (Kovacs et al. 2009a).

The crystallinity index (CrI) of the furfural residue samples was determined by wide-angle X-ray diffraction (XRD) on an XRD-6000 instrument (Shimadzu, Japan). This facilitated the study of structural alterations of the cellulosic residues as a result of cellulase production. A previously reported method was used for the analysis (Feng et al. 2011). The sample was placed on an aluminum holder and analyzed under plateau conditions. Ni-filtered Cu Kα radiation ($\chi = 1.54$ Å) was generated at a voltage of 40 kV, and a current of 40 mA was applied. The specimens were scanned at a speed of 2°/min from 5° to 40° using a goniometer. To define the crystallinity, the CrI of the lignocellulosic samples was calculated using the intensity of diffraction of the crystalline structure (I_{002}, $2\theta = 22.6°$) and that of the amorphous fraction ($I_{\text{amorphous}}$, $2\theta = 18.0°$):

$$\text{CrI} = \left(I_{002} - I_{\text{amorphous}}\right)/I_{002} \times 100.$$

A minimum of three parallel samples were used in all analytical procedures, and the data are represented as the mean of the triplicates.

Results and discussion

Cellulase production on MCC

The strains were grown in mineral medium supplemented with 2% (w/v) MCC. Samples were taken at regular intervals, and the cellulase activity of the culture was determined. The results are shown in Fig. 1. During the first 7 days, the activities of the enzymes produced by *A. niger*, *T. koningii*, and *T. reesei* did not differ statistically. However, the cellulase activity of the supernatant from *T. viride* rapidly increased from day 3 to day 7. After day 7, the cellulase activity of the supernatant from *T. koningii* increased. On the 15th day, *T. viride* showed the highest activity (21.61 FPU/ml), followed by *T. koningii* (21.50 FPU/ml). Moreover, the FPA of *T. viride* was almost five times higher than that of *T. reesei*. Based on the results, the optimal culture time

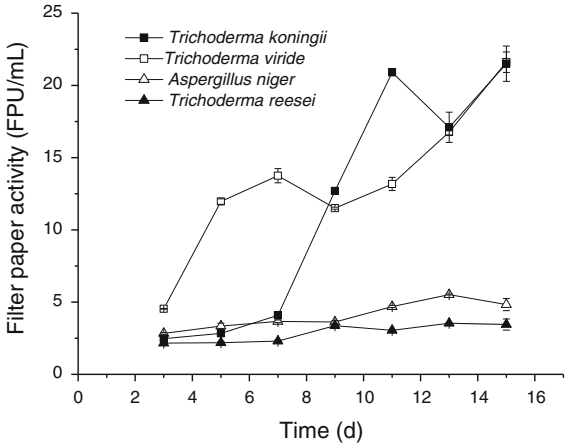

Fig. 1 FPA activities in the supernatants of the four fungi cultures. FPA measures glucose released from filter paper. The fungi were cultured in MCC medium containing 20 g/l of MCC at 30°C for 15 days

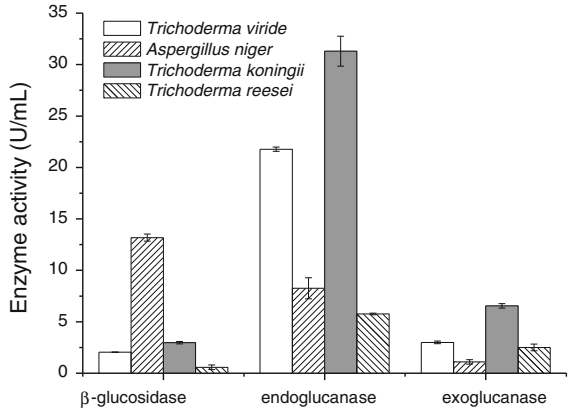

Fig. 2 Activities of β-glucosidase, endoglucanase, and exoglucanase in the supernatants of the four fungi cultures. The fungi were cultured in MCC medium containing 20 g/l of MCC at 30°C after 15 days

for cellulase production by the fungus ranged from day 13 to day 15. The activities of the β-glucosidase, endoglucanase, and exoglucanase components on the 15th day of culture are shown in Fig. 2. The results indicate that all of four fungi could produce the complete set of enzymes for the cellulase system.

Exoglucanase is a major component of the microbial cellulose degradation system and it releases cellobiose units from cellulose chains. Cellobiose units are also the products of cellulose hydrolysis by endoglucanase (Lin et al. 2009). From the results, it is obvious that the highest activity of the exoglucanase was from *T. koningii*. The lowest exoglucanase activity (1.11 U/ml) was observed from *A. niger*, which could account for the low FPA.

The endoglucanase activities varied from different fungi (Fig. 2). *T. koningii* showed the highest endoglucanase production (31.3 U/ml) followed by *T. viride*. It is noteworthy that of the three enzymes of the *T. koningii* cellulase system, endoglucanase was expressed at the highest level. Since *T. koningii* and *T. viride* showed higher FPAs than the other strains, endoglucanase appears to act an important role in the enzyme complex.

Trichoderma reesei showed poor performance in terms of β-glucosidase production, an observation noted by Chandra et al. (2009). This fungal strain also exhibited low exoglucanase and endoglucanase activities. Cellobiose accumulation reportedly results in severe feedback inhibition to the cellulase reaction (Chen et al. 2008; Martins et al. 2008). The FPA of *T. koningii* was affected by low β-glucosidase levels that could lead to cellobiose accumulation and thereby feedback inhibition. *A. niger* has been the focus of many studies due to its excellent β-glucosidase producing properties (Brumbauer et al. 2000; Hanif et al. 2004). This was also confirmed by our results that *A. niger* showed the highest β-glucosidase production. Nevertheless, the levels of endoglucanase and exoglucanase produced by *A. niger* were low. So the FPA from *A. niger* was low. These results indicate that both the endoglucanase and exoglucanase activities are important contributors to the total cellulase activity and that low β-glucosidase activity can restrict cellulase activity.

Cellulase production on furfural residues

Each of the four fungi was inoculated into liquid medium containing furfural residues, and the FPAs of the supernatant were measured with respect to the culture time (Fig. 3). The sampling time ranged from day 11 to day 19. *T. reesei* showed high cellulase production within day 13 with an FPA of 7.06 U/ml. In the case of *T. koningii*, the FPA increased continuously and reached 27.81 U/ml, which was the highest value achieved by any of the four fungi on the furfural residues medium. The glucose concentration in the *T. koningii* supernatant increased continuously during fermentation, reaching a maximum value of 0.58 g/l. The lowest glucose concentration of 0.25 g/l was

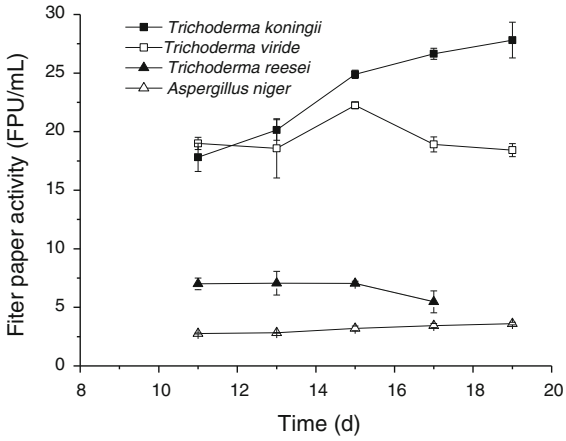

Fig. 3 FPA activities in the supernatants of the four fungi cultures. FPA measures glucose released from filter paper. The fungi were cultured in furfural residues medium containing 20 g/l of furfural residues at 30°C for 11–19 days

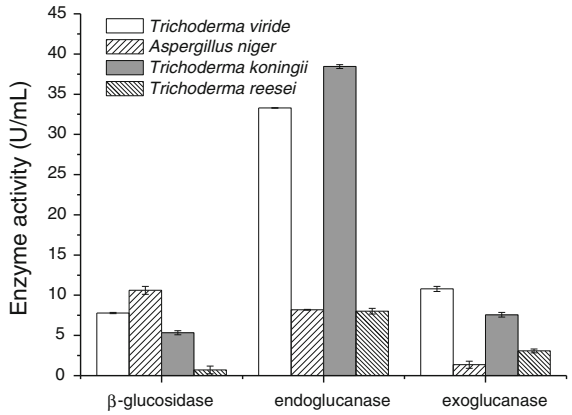

Fig. 4 Activities of β-glucosidase, endoglucanase, and exoglucanase in the supernatants of the four fungi cultures. The fungi were cultured in furfural residues medium containing 20 g/l of furfural residues at 30°C for 15 days

obtained with *A. niger*. Both *A. niger* and *T. reesei* showed low FPAs on furfural residues, indicating that the cellulose-degrading capability of these fungi requires further optimization.

As shown in Fig. 4, *T. viride* exhibited the highest exoglucanase activity (10.79 U/ml) and *A. niger* with the lowest activity (1.36 U/ml). Although the β-glucosidase activity of *A. niger* was higher than that of the other fungi, its FPA was low, and this was attributed to its low exoglucanase level. *T. viride* and *T. koningii* showed high FPAs as well as high exoglucanase and endoglucanase activities, indicating that the exoglucanase and endoglucanase levels significantly influenced the overall cellulase activity.

Cellulose degradation by cellulase requires the synergistic action of all three enzymatic components, i.e., exoglucanase, endoglucanase and β-glucosidase. Our results shows that *T. koningii* produced the highest cellulase activity, which was attributed to its excellent exoglucanase and endoglucanase activities and to the fact that sufficient amounts of β-glucosidase could be produced for the conversion of cellobiose to glucose, thereby reducing the inhibition of exoglucanase by cellobiose.

Comparison of cellulase production on furfural residues and MCC

The results of cellulase production in cellulose medium containing using 20 g/l MCC and 20 g/l furfural residues are shown in Fig. 5. Higher FPAs was obtained from *T. viride*, *T. koningii* and *T. reesei* when furfural residues was used as the substrate than MCC was used. However, the activity of the cellulase from *A. niger* was lower on furfural residues than on MCC. The FPA of the cellulase produced by *T. koningii* was 30% higher in the furfural residue medium than in the MCC medium. The results clearly showed that *T. koningii* could effectively digest furfural residues for cellulase production. Xia and Shen (2004) studied the cellulase activity of the cellulase from *T. reesei* ZU-02 and found that the cellulase concentration (5.25 IU/ml) obtained with a corncob residue substrate was similar to that achieved with purified cellulose. Our results also showed that the activity of the cellulase from *T. reesei* cultured on furfural residues was higher than that of the enzyme from *T. reesei* ZU-02. In addition, the production of β-glucosidase from *T. reesei* was promoted with the presence of furfural residues. Thus, culture of *T. reesei* on specific substrates could possibly lead to the production of higher levels of β-glucosidase.

Furfural residues consisting of cellulose, lignin and a few hemicelluloses are the by-products of furfural production from acid-catalyzed hydrolysis of corncobs (Sun et al. 2008). In contrast, MCC is a pure product obtained by the depolymerization of cellulose. As shown in Fig. 5, the higher crystallinity and better crystals structure of MCC impeded its utilization as a substrate by the fungi, which in turn influenced cellulase production. In contrast, furfural residues

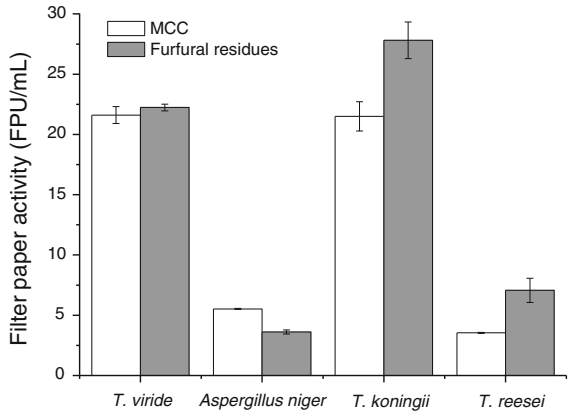

Fig. 5 Maximum FPA activities in the supernatants of the four fungi cultures. The fungi were cultured in the medium containing 20 g/l furfural residue and the medium containing 20 g/l MCC at 30°C for 10–19 days

consist of cellulose, lignin and a rather low amount of residual hemicelluloses, and the CrI of this material is low. Therefore, furfural residues were more suitable than MCC for cellulase production.

Effect of different fungi on the CrI of furfural residues

The water-rinsed furfural residue contains 42.2% cellulose, 38.7% lignin, and 1.9% hemicellulose (Tang et al. 2011). XRD was used to investigate the supramolecular order (crystallinity) of enzymatically hydrolyzed cellulose residues (Fig. 6). The CrI of the samples was determined using the diffraction intensities of the crystalline structure (I_{002}, $2\theta = 22.6°$) and the amorphous fraction ($I_{amorphous}$, $2\theta = 18.0°$). Crystalline cellulose is considered to be more difficult to degrade than the amorphous component due to the strong intermolecular hydrogen bonding between the cellulose chains.

The CrI of the residues from *A. niger* was greater than 40%, indicating that *A. niger* digested furfural residues with difficulty. The CrI of the residues on which *T. koningii* was increased from day 11 to day 13 (data are not shown). During this period, the noncrystalline regions were first degraded by *T. koningii*. As shown in Fig. 4, *T. koningii* also produced relatively high levels of exoglucanase. After day 13, the CrI decreased from 35.9 to 33.6%, indicating that the crystalline regions of the substrate was degraded during this period. However, the CrI of the substrate

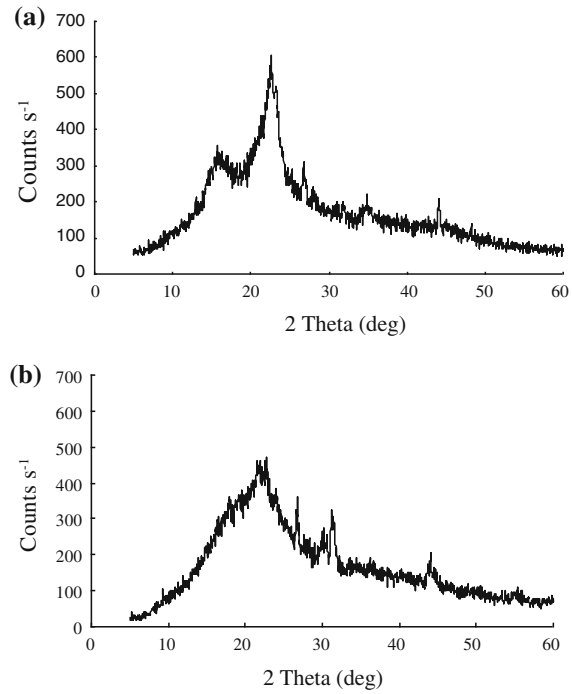

Fig. 6 X-ray patterns of furfural residue (**a**) and the sample digested by *T. koningii* on the 15th day (**b**)

subsequently increased, suggesting that the noncrystalline regions might be preferentially degraded. The change in the CrI of the residues used to cultivate *T. reesei* was similar to that observed with *T. koningii*. The CrI first increased and then decreased with the fermentation time, finally increasing to 35.8%. Thus, *T. reesei* first degraded on the noncrystalline regions, which lead to an increase in crystallinity. Subsequently, the crystallinity decreased due to degradation of the crystalline regions by *T. reesei*. At day 19, the CrI of furfural residues digested by *A. niger*, *T. koningii*, and *T. reesei* were 41.4, 34.4, and 35.8%, respectively. While the original CrI of the furfural residues was 49%.

With substrates such as furfural residues that showed a low CrI after *T. reesei* and *T. koningii* cultivation, it appears that noncrystalline regions were targeted by the fungi during the early degradation period. Thereafter, the crystalline regions were gradually degraded by the fungi, leading to a decrease in crystallinity. Additionally, the diffraction intensity of the furfural residues was lower than that of the raw material, indicating that more cellulase was produced for the digestion of the furfural residues than the

digestion of MCC. It is possible that the CrI of the residues may have changed in the acidic environment of furfural production (temperature 160°C, acid 5% H_2SO_4, and time 6–9 h). Thus, furfural residues were found to be more suitable than MCC for the cultivation of *T. reesei* and *T. koningii*.

Conclusion

In this study, the cellulase-producing capabilities of four fungi were evaluated on two different substrates, i.e., furfural residues and MCC. Culture of all four fungi on the substrates produced a complete set of cellulases. The activities of all three components of the cellulase system, i.e. endoglucanase, exoglucanase and β-glucosidase, were tested at the optimal culture time when the FPA was high. The amount of cellulase secreted by *T. koningii* was higher when it was grown on furfural residues than when it was cultured on MCC. The FPA from *T. koningii* reached 27.81 FPU/ml after it was incubated on furfural residues for 19 days, and this value was 30% higher than that obtained with MCC. The cellulase levels were also high (22.24 FPU/ml) when *T. viride* was grown on furfural residues. Therefore, culture of these fungi on suitable lignocellulosic substrates could possibly be used for cellulase production. It may also be possible to improve the cellulase production of these fungi by mutagenesis. The activities of the cellulases from the selected fungi, with the exception of *A. niger*, were higher on furfural residues than on MCC, suggesting that this medium can support a suitable cellulase production environment. Thus, the results of this study highlight the potential use of furfural residues, an abundant renewable resource, as a substrate in cellulase production.

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References

Ahamed A, Vermette P (2008) Culture-based strategies to enhance cellulase enzyme production from *Trichoderma reesei* RUT-C30 in bioreactor culture conditions. Biochem Eng J 40:399–407

Ahamed A, Vermette P (2009) Effect of culture medium composition on *Trichoderma reesei*'s morphology and cellulase production. Bioresour Technol 100:5979–5987

Baldrian P, Valaskova V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32(3): 501–521

Brumbauer A, Johansson G, Reczey K (2000) Study on heterogeneity of β-glucosidase from *Aspergillus* species by using counter-current distribution. J Chromatogr B Biomed Sci Appl 743:247–254

Camassola M, Dillon AJP (2009) Biological pretreatment of sugar cane bagasse for the production of cellulases and xylanases by *Penicillium echinulatum*. Ind Crop Prod 29(2–3):642–647

Chandra M, Kalra A, Sharma P, Sangwan R (2009) Cellulase production by six *Trichoderma* spp. fermented on medicinal plant processings. J Ind Microbiol Biotechnol 36(4):605–609

Chen M, Zhao J, Xia LM (2008) Enzymatic hydrolysis of maize straw polysaccharides for the production of reducing sugars. Carbohydr Polym 71(3):411–415

Feng Y, Liu HQ, Xu F, Jiang JX (2011) Enzymatic degradation of steam-pretreated Lespedeza stalk (Lespedeza crytobotrya) by cellulosic-substrate induced cellulases. Bioproc Biosyst Eng 34(3):357–365

Ghose TK (1986) Measurement of cellulase activities. Pure Appl Chem 59:257–268

Hanif A, Yasmeen A, Rajoka MI (2004) Induction, production, repression, and de-repression of exoglucanase synthesis in *Aspergillus niger*. Bioresour Technol 94:311–319

Hideno A, Inoue H, Tsukahara K, Yano S, Fang X, Endo T, Sawayama S (2011) Production and characterization of cellulases and hemicellulases by *Acremonium cellulolyticus* using rice straw subjected to various pretreatments as the carbon source. Enzyme Microb Technol 48(2):162–168

Jorgensen H, Morkeberg A, Krogh KBR, Olsson L (2004) Growth and enzyme production by three *Penicillium* species on monosaccharides. J Biotechnol 109(3):295–299

Kovacs K, Megyeri L, Szakacs G, Kubicek CP, Galbe M, Zacchi G (2008) *Trichoderma atroviride* mutants with enhanced production of cellulase and beta-glucosidase on pretreated willow. Enzyme Microb Technol 43(1):48–55

Kovacs K, Szakacs G, Zacchi G (2009a) Comparative enzymatic hydrolysis of pretreated spruce by supernatants, whole fermentation broths and washed mycelia of *Trichoderma reesei* and *Trichoderma atroviride*. Bioresour Technol 100(3):1350–1357

Kovacs K, Szakacs G, Zacchi G (2009b) Enzymatic hydrolysis and simultaneous saccharification and fermentation of steam-pretreated spruce using crude *Trichoderma reesei* and *Trichoderma atroviride* enzymes. Process Biochem 44(12):1323–1329

Li XH, Yang HJ, Roy B, Park EY, Jiang LJ, Wang D, Miao YG (2010) Enhanced cellulase production of the *Trichoderma viride* mutated by microwave and ultraviolet. Microbiol Res 165(3):190–198

Lin L, Meng X, Liu P, Hong Y, Wu G, Huang X, Li C, Dong J, Xiao L, Liu Z (2009) Improved catalytic efficiency of endo-beta-1,4-glucanase from *Bacillus subtilis* BME-15 by directed evolution. Appl Microbiol Biotechnol 82(4): 671–679

Liu J, Yuan XZ, Zeng GM, Shi JG, Chen S (2006) Effect of biosurfactant on cellulase and xylanase production by *Trichoderma viride* in solid substrate fermentation. Process Biochem 41:2347–2351

Ljungdahl LG (2008) The cellulase/hemicellulase system of the anaerobic fungus Orpinomyces PC-2 and aspects of its applied use. Incredible Anaerobes: From Physiology to Genomics to Fuels 1125:308–321

Lo CM, Zhang Q, Callow NV, Ju LW (2010) Cellulase production by continuous culture of *Trichoderma reesei* Rut C30 using acid hydrolysate prepared to retain more oligosaccharides for induction. Bioresour Technol 101: 717–723

Martins LF, Kolling D, Camassola M, Dillon AJP, Ramos LP (2008) Comparison of *Penicillium echinulatum* and *Trichoderma reesei* cellulases in relation to their activity against various cellulosic substrates. Bioresour Technol 99(5):1417–1424

Nascimento RP, Junior NA, Pereira N Jr, Bon EPS, Coelho RRR (2009) Brewer's spent grain and corn steep liquor as substrates for cellulolytic enzymes production by *Streptomyces malaysiensis*. Lett Appl Microbiol 48:529–535

Picart P, Diaz P, Pastor FIJ (2007) Cellulases from two *Penicillium* sp. strains isolated from subtropical forest soil: production and characterization. Lett Appl Microbiol 45(1):108–113

Shalini G, Laurent S (2006) Partitioning of β-glucosidase from *Trichoderma reesei* in poly(ethylene glycol) and potassium phosphate aqueous two-phase systems: influence of pH and temperature. Biochem Eng J 30:104–108

Shrestha P, Khanal SK, Pometto AL, van Leeuwen J (2009) Enzyme production by wood-rot and soft-rot fungi cultivated on corn fiber followed by simultaneous saccharification and fermentation. J Agric Food Chem 57(10):4156–4161

Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A (2010) Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol 46(7):541–549

Sukumaran RK, Singhania RR, Mathew GM, Pandey A (2009) Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production. Renew Energ 34(2):421–424

Sun YD, Sun R, Jiang JX, Zhu LW (2008) Process of conversion of the residue of furfural manufacture to ethanol by simultaneous saccharification and fermentation. Mod Chem Ind 28(12):48–52

Tang Y, Su ZQ, Zhao DQ, Jiang JX (2011) The effect of *Gleditsia* saponin on simultaneous saccharification and fermentation of furfural residue for ethanol production. Adv Mater Res 236–238:108–111

Wen ZY, Liao W, Chen SL (2005) Production of cellulase by *Trichoderma reesei* from dairy manure. Bioresour Technol 96(4):491–499

Xia LM, Shen XL (2004) High-yield cellulase production by *Trichoderma reesei* ZU-02 on corn cob residue. Bioresour Technol 91(3):259–262

Zhang C, Xing XH, Liu MS (2004) Production of multienzymes consisting of alkaline amylase and cellulase by mixed alkalophilic culture and their potential use in the saccharification of sweet potato. Biochem Eng J 19(2):181–187

Zhang Q, Lo CM, Ju LK (2007) Factors affecting foaming behavior in cellulase fermentation by *Trichoderma reesei* Rut C-30. Bioresour Technol 98(4):753–760