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Potential of Cellulase and Xylanase Production by Fungal Strains Using Corn Husks as Substrate

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Abstract

Cellulase and xylanase are enzymes produced by microorganisms to breakdown a component of the plant cell walls. Particular fungal strains are interesting enzyme producers due to their higher production level of cellulase and xylanase in comparison with yeast and bacteria. In this study, the potential of the fungal strains *Aspergillus awamori* BCC13292, *A. niger* BCC7037, *A.flavus* BCC18310, *Trichoderma harzianum* BCC17752, *T.reesei* BCC7041 and *Penicillium chrysogenum* TISTR3554, were investigated for their ability to produce enzymes. Cellulase and xylanase production were detected with an agar plate containing carboxymethyl-cellulose (CMC) and xylan as the carbon source, respectively. The results showed that *A.niger* cultured in CMC, and *T.reesei* cultured in xylan, revealed the largest clear zones of 1.50 and 3.05 cm., respectively. The cellulase and xylanase activities of these fungal strains cultured in a liquid medium containing CMC and xylan were also assayed. The results showed that *A.flavus* cultured in xylan exhibited the highest xylanase activity (1.06 U/mg proteins), while the greatest cellulase activity was obtained from *T.reesei* (6.22 U/mg proteins) cultured in CMC. In addition, each fungal strain was cultured in a liquid medium consisting of corn husks to test their ability in enzyme production. The greatest cellulase and xylanase activities were acquired from *A.niger* and *T.reesei* with 0.41 U/mg proteins and 0.67 U/mg proteins, respectively.

Keywords: cellulase, xylanase, fungal strains, corn husks

1. Introduction

Cornhusks, as both an agricultural and industrial waste, are considered a very abundant organic resource and, therefore, have a low cost (1, 2). The composition of cornhusks include carbohydrate polymers (cellulose, hemicelluloses) and aromatic polymers (lignin) (3). These carbohydrate polymers are renewable resources that could be converted into other important products such as ethanol, xylitol and methane (4). The success of the utilization of these renewable resources is dependent on the development of the process and the production of the enzymes required for lignocellulosic hydrolysis (5). Enzymatic hydrolysis of lignocelluloses by cellulase and xylanase, which are hydrolytic enzymes, is one of the most effective methods for releasing the main monosaccharides

(glucose and xylose)(4). Cellulase hydrolyzes the β-glycosidic bonds of the native cellulose to glucose, while xylanase degrades the linear polysaccharide β-1, 4-xylan to xylose(6). These hydrolytic products can be converted to ethanol, xylitol and methane by microbial fermentation. Many microorganisms (fungi, yeast and bacteria), especially fungal strains, are of interest for their cellulase and xylanase production due to their high yield (7, 8, 9). Among the cellulolytic or xylanolytic microfungi, the genera *Aspergillus, Trichoderma and Penicillium* are notable cellulase and xylanase producers. Medeiros *et al*. has shown that *Pennicillium corylophilum, Aspergillus niger* and *Trichoderma longibrachiatum* could produce xylanase by being grown on oat spelt xylan(10). Chinedu *et al*. has demonstrated that *A.niger, P.chrysogenum* and *T.harzianum* could release cellulase when using sugarcane pulp, corncobs and sawdust as substrates (5). However, there have been few studies on the hydrolysis of corn husks through the use of fungal strains. Thus, the main objective of this study was to evaluate the potential of these fungal strains to produce cellulase and xylanase using corn husks as a substrate.

2. Materials and methods

2.1 Materials

Corn husks were supplied by Sun Sweet Co., Ltd. (Chiangmai, Thailand). They were cut into small pieces and air-dried for 4-6 hours. The material was then ground to a particle size of 0.5 mm in a mechanical blender and homogenized in a single lot and stored at room temperature until they were used. Xylan and carboxymethyl-cellulose that were used as substrates were obtained from Sigma-Aldrich Co. LLC, Germany.

2.2 Microorganisms

A total of 6 fungal strains, *Aspergillus awamori* BCC13292, *Aspergillus niger* BCC7037, *Aspergillus flavus* BCC18310, *Trichoderma harzianum* BCC17752 and *Trichoderma reesei* BCC7041 were obtained from the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand and *Penicillium chrysogenum* TISTR3554 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Thailand. These fungal strains were grown on Potato Dextose Agar (PDA) plates for 3-5 days at 30°C prior to screening.

2.2.1 Screening of microorganism on agar plate

Xylanase and cellulase activity were assessed through growing the fungi on malt extract agar medium (MEA) containing xylan and carboxymethyl-cellulose (CMC), respectively, as the carbon source. The composition of the medium consisted of peptone, 5.0g; yeast extract, 5.0g; K_2HPO_4, 0.2g; agar, 20.0g ; with 0.1% xylan (or 1.0% CMC), (in 1 liter of distilled water) as the substrate (13). The inoculated plates were incubated for 10-30 hours. After incubation, the plates were flooded with 0.1% aqueous congo red and destained with 1M NaCl for 15 minutes. A clear zone surrounding the colony indicated the xylanase (or cellulase) activity.

2.2.2 Enzyme production in liquid culture

The fungal strains were grown on synthetic media containing CMC (or xylan or corn husk) as a carbon source. The composition of the medium consisted of (g/l) $(NH_4)_2SO_4$, 1.3; KH_2PO_4, 0.37; $MgSO_4.7H_2O$, 0.25; $CaCl_2.2H_2O$, 0.07; $FeCl_3$, 0.02; yeast extract, 1.0; with 1% CMC (or 0.1% xylan or 0.5% corn husk) as the sole carbon source (5,11). The pH was adjusted to 6.0. Each flask (150 ml) containing 50 ml of the growth medium was inoculated with 5 discs of 5.0 millimeter diameter samples of the fungi from PDA plates using a sterile cork borer. The cultures were incubated on a rotary shaker (120 rpm) at 30±2 °C for various times. The crude enzyme supernatant was recovered by centrifugation at 7500 rpm for 15 min and used in the enzyme assays.

2.3 Enzyme assays

Cellulase activity was measured by mixing 1.0 ml of 1.0% CMC in 0.1 M sodium acetate buffer (pH 5.0) with 1.0 ml of crude enzyme (5). The mixture was incubated at 37°C for 30 min. The reducing sugar released by enzyme hydrolysis was measured as the glucose equivalent using the dinitrosalicyclic acid reagent (12). The xylanase activity was assayed by measuring reducing-sugar using 1.0% xylan as a substrate. The reaction mixture contained 1 ml of 1.0% (w/v) xylan in 0.05 M sodium citrate buffer, pH 6.0 and 1 ml of crude enzyme (13). The mixture was incubated at 37°C, while being shaken for 30 minutes. The reducing sugar released by enzyme hydrolysis was measured as the xylose equivalent using the dinitrosalicyclic acid reagent. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of glucose from the CMC substrate or xylose from the xylan substrate per 1 min under the assay conditions.

2.4 Protein assay

The protein content of the crude enzyme preparations was determined according to the method described by Lowry *et al.* (14) using bovine serum albumin (BSA) as a standard.

2.5 Data analysis

Mean values and standard deviations (S.D.) were calculated from the results using MS Excel 2007.

3. Results

3.1 Screening of enzyme activity on agar plates

Cellulase and xylanase activities from the fungal strains that were cultured on an agar medium containing CMC and xylan, were screened through measuring the clear zone on the agar. The fungal strains that produced zones of clearing around their inoculums are shown in Table 1. *A.niger* cultured in CMC and *T.reesei* cultured in xylan demonstrated the largest clear zones of 1.50 and 3.05 cm, respectively.

Table 1. Enzyme activity of fungal strains on agar plate

Organisms	Diameter of enzyme activity (cm)	
	Cellulase	Xylanase
A.awamori BCC13292	ND	1.55
A.niger BCC7037	1.50	2.30
A.flavus BCC18310	ND	2.10
T.harzianum BCC17752	ND	2.10
T.reesei BCC7041	ND	3.05
P.chrysogenum TISTR3554	1.40	2.20

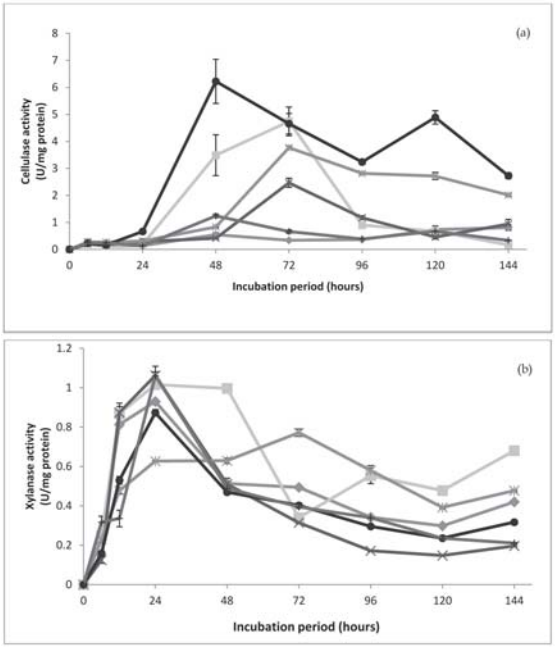

Figure 1. Cellulase (a) and xylanase (b) activities of *A.awamori* (◆), *A.niger* (■), *A.flavus* (X), *T.harzianum* (✳), *T.reesei* (●) and *P.chrysogenum* (+) incubated in CMC and xylan containing media, respectively.

3.2 Enzyme activity

Figure 1 (a-b) shows the cellulase and xylanase activities of fungal strains grown in a liquid medium containing CMC and xylan, respectively. The result showed that the highest cellulase activity of 6.22 units/mg protein was obtained from the culture broth containing CMC of *T.reesei* at 48 hours of incubation. The highest xylanase activity was found in the culture broth containing xylan of *A.flavus* at 24 hours of incubation with a value of 1.06 units/mg protein.

The potential of cellulase and xylanase production of fungal strains that were cultured on corn husks at various times is shown in Fig 2 (a-b). The maximum cellulase and xylanase activity values were found in the culture broth of *A.niger* and *T.reesei* after 72 hours of cultivation, respectively.

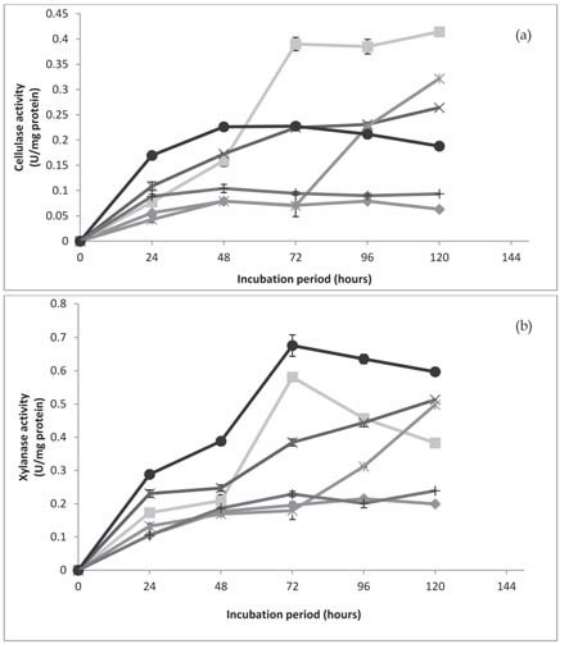

Figure 2. Cellulase (a) and xylanase (b) activities of *A.awamori* (◆), *A.niger* (■), *A.flavus* (X), *T.harzianum* (✳), *T.reesei* (●) and *P.chrysogenum* (+) incubated in corn husk containing media at various times.

4. Discussion

The production of cellulase and xylanase by the fungal strains were assayed by the digestion of substrate on the agar plate (Table 1), and liquid medium (Figures 1, 2) containing CMC and xylan, respectively. *T. reesei* produced the highest amount of cellulase enzyme when incubated in a liquid medium containing CMC for 48 hours. This result is inconsistent with the detection on the agar plate. It may be due to the effect of time and environment involved in enzyme production. This explanation is consistent with the research of Sridevi *et al*. (2011) and Tseng *et al*. (2000), whose content demonstrated that some fungal strains were identified as negative on the agar plate, but could produce enzymes in liquid medium. In contrast, some strains which were identified as being potential enzyme producer on agar medium, did not produce any or revealed low enzyme production activity in the liquid medium (13,15). For the production of xylanase, all fungal strains were able to produce xylanase on agar plates and in liquid medium containing xylan. This result is consistent with the previously recorded results of detection on agar plates. *A. flavus* showed the highest activity of xylanase production at 24 hours of cultivation. The production of cellulase and xylanase when the fungal strains were grown on corn husks offers interesting results. *A.niger* and *T.reesei* revealed significant ability in terms of cellulase and xylanase production, respectively (Figure 2). Our results agree with the previous studies of Chinedu *et al.* (2011) who reported that *A.niger* had an ability to produce cellulase using the waste cellulosic materials (sugarcane pulp, corncob and sawdust) (5). Furthermore, Xiong *et al.* (2005) showed that higher xylanase activities could be obtained from *T.reesei* cultured in oat husks and sugar beet pulp hydrolysates (16). However, all of the fungal strains showed lower amounts of cellulase and xylanase activity when grown on corn husks, than when grown on CMC and xylan. This may be due to the different concentration of substrate using as the sole carbon source in the medium. Moreover, these results have demonstrated that the time periods needed for the production of cellulase and xylanase were shorter when incubated on CMC and xylan, compared to corn husks. Based on these results, it is possible to conclude that plant cell wall material, including lignin and cellulose, may be the cause for the inducement in the production of the degrading enzymes (17). In the next experiment, chemical, physical and physicochemical lignocellulosic pretreatment would be applied to potentially increase the available substrates for the production of enzymes using fungi.

5. Conclusion

In our experiment on the production of cellulase and xylanase from certain fungal strains, *A.niger* and *T.reesei* were shown to be capable of providing the highest cellulase and xylanase production, respectively, after 72 hours in a liquid medium consisting of corn husks.

6. Acknowledgements

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