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Profile of Enzyme Production by *Trichoderma reesei* Grown on Corn Fiber Fractions**

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Abstract

Corn fiber is the fibrous by-product of wet-mill corn processing. It typically consists of about 20% starch, 14% cellulose, and 30% hemicellulose in the form of arabinoxylan. Crude corn fiber (CCF) was fractionated into de-starched corn fiber (DSCF), corn fiber with cellulose (CFC) enriched, and corn fiber arabinoxylan (CFAX), and these fractions were evaluated as substrates for enzyme production by *Trichoderma reesei*. *T. reesei* QM9414 and Rut C-30 grew on CCF, DSCF, CFC, or CFAX and secreted a number of hydrolytic enzymes. The enzymes displayed synergism with commercial cellulases for corn fiber hydrolysis.

Index Entries: *Trichoderma reesei*; corn fiber; cellulase; xylanase

Introduction

Fuel ethanol production in the United States has reached an annual level of 2.8 billion gallons using over 900 million bushels of corn. Approximately 50% of corn fermented to ethanol is processed by wet-milling. A major by-product of wet milling is corn fiber, an enriched fiber fraction associated with outer hull (periderm). Annual corn fiber production in the US has been estimated at 3.4 million tons *(1).* Corn fiber is currently marketed as a low-grade animal feed ingredient, but market prices have been in decline due to rapid increase in supply. Corn fiber is typically composed of 60–70% carbohydrates, which includes 20% residual starch, 14% cellulose, and 30% hemicellulose *(2)*. There is an opportunity to reverse this trend by developing alternative uses for corn fiber. Corn fiber

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is a good candidate as a novel fermentation feedstock because of its high carbohydrate content and central location at wet mills. In this article corn fiber was investigated as a feedstock for producing cellulases and hemicellulases by *T. reesei* cultures.

Corn fiber has a complex hemicellulose structure. The hemicellulose is in the form of arabinoxylan comprising a backbone of beta-1,4-xylosidic chain with various side chains of arabinose, xylose, acetyl, and glucuronic acids *(3).* Ferulic acid and galactose are linked to arabinose and galactose to xylose side chains *(3).* Procedures to de-starch and extract hemicellulose from corn fibers have been developed *(4,5),* which makes it possible to study enzymatic hydrolysis of constitutes of corn pericarp cell walls. Each unique side-group requires its own enzyme for removal. As a result of corn fiber xylan's complex structure, microorganisms capable of growing on de-starched corn fiber will need to make a mixture of several hydrolyzing enzymes. Conversely, enzyme preparations made from microbial cultures grown on de-starched corn fiber should be much richer in hemicellulolytic activities compared to those grown on simpler substrates.

Crude corn fiber has been reported to support the growth of a number of microorganism (6–9). Enzymatic activities related to degradation of lignocellulosic biomass were produced, but hydrolysis of intact corn fiber cellulose and hemicellulose by enzymes from corn fiber-grown *Aureobasidium pullulans (6), Thermobifida fusca (9),* or of commercial sources *(10,11)* is inefficient. Successful saccharification of corn fiber carbohydrates was dependent on prior pretreatment with either dilute acid or alkaline *(6,11).*

T. reesei is the most significant microorganism for production of commercial cellulase products *(12).* The fungus and its cellulase genes and proteins are the targets of research for the development for cost-effective lignocellulose-degrading cellulases *(13)*. However, commercially available enzymes manufactured using *T. reesei* are low in hemicellulase and thus are ineffective for hydrolyzing corn fiber xylan *(2).* The fungus grown on crude corn fiber was found to produce low levels of cellulase and xylanase activities *(8).* Here we report the results of growth of cellulase hyperproducing mutants, QM9414 and Rut C-30, on fractionated corn fiber components, enzyme production, and hydrolysis of the corn fiber fractions by the enzymes.

Materials and Methods

Materials and Microbial Strains

T. reesei QM91414 (NRRL 6156) and Rut C-30 (NRRL 11485) were obtained from the Agricultural Research Service (ARS) Culture Collection, National Center for Agricultural Utilization Research, USDA/ARS (Peoria, IL) and propagated on PDA (potato, 0.4%; dextrose, 2.0%; and agar, 2.0%, w/v) plates. Corn fiber was obtained from Aventine Renewable

Energy, Inc. (Pekin, IL) and kept frozen at −20°C with 60% moisture. Other supplies including commercial Novozyme (Bagsværd, Denmark) enzymes, corn steep liquor, and reagents, if not otherwise stated, were purchased from Sigma-Aldrich (St. Louis, MO).

Enzymatic and Chemical Treatment of Corn Fiber

Wet corn fiber (1000 g, 60% moisture) was dried in a 60°C oven for 16 h. A portion of the dried corn fiber was ground using a Glen Mill A10 (Glen Mill, Inc., Clifton, NJ) and passed a sieve size of 30 (0.6 mm). This material was designated as crude corn fiber (CCF) for growth of fungal cultures.

Wet corn fiber was also used directly for preparation of corn fiber fractions. The wet corn fiber (475 g) was stirred in 1.5 L of 5 m*M* sodium citrate buffer, pH 4.5, at 55°C. Glucoamylase (320 U, Sigma) and sodium azide (0.02%, w/v) were added and stirring (150 rpm) was continued for 18 h. Liquid was drained through four layers of cheesecloth and retained fiber was washed three times with 2 L of dH_2O each. The glucoamylase-treated fiber was then dried, milled, and sieved as for the CCF above. This material was designated as de-starched corn fiber (DSCF). Alkaline (2.0%, w/v, KOH) treatment of DSCF was used to separate corn fiber cellulose (CFC) enriched and corn fiber arabinoxylan (CFAX) fractions according to Hespell *(5)* with some modifications. DSCF (150 g) was stirred intermittently in 2 L of 2.0% KOH at 70°C for 4 h. After cooling down to room temperature, the suspension was centrifuged for 15 min at 12,000*g*. The pellet was rinsed with 500 mL dH_2O and centrifuged again. After drying at 60°C overnight, the pellet was milled and sieved as for the CCF and used as corn fiber cellulose (CFC)–enriched material. The supernatant from the KOH extraction step was acidified to pH 4 with concentrated HCl and spun (12,000*g*, 15 min) to remove precipitate. Polysaccharides in supernatant were precipitated by 95% cold ethanol with supernatant to ethanol ratio of 1:2 (v/v). The harvested polysaccharides served as crude corn fiber arabinoxylan (CFAX) and were further purified with two cycles of ethanol precipitation and $Ca(OH)_2$ extraction *(5)* and the purified material was again dried, milled, and sieved as above. The resulting slightly off-white powder was used as CFAX.

T. reesei *Cultivation*

For each shake flask (250 mL) containing 50 mL potato (0.4%, w/v) dextrose (2.0%, w/v) medium, two inoculation loops of *T. reesei* mycelia with conidia were transferred. Flasks were shaken at 250 rpm and 28°C in an incubator for 72 h. To test growth and enzyme production of *T reesei* QM9414 and Rut C-30 using corn fiber fractions as carbon sources and inducers, induction media contained per liter 15 g KH_2PO_4, 20 g corn steep liquor, 5.0 g ammonium sulfate, 0.5 g $Mg(SO_4)_2 \cdot 7H_2O$, 1.0 g Tween 80,

and 40 g corn fiber fractions. All ingredients except corn fiber fractions were first dissolved and pH and volume were adjusted to 4.8 and 1 L. Each 250 mL Erlenmeyer flask received 40 mL of the basal medium and 1.8 g each of the various corn fiber fractions. Flasks were plugged with foam stoppers and wrapped with aluminum foil. Medium was autoclaved for 15 min at 121°C. After cooling, each flask was inoculated with 5 mL 72 h potato dextrose (PD)–grown fungal mycelial culture. Fungal cultures were grown at 28°C with agitation (250 rpm). Samples (2 mL) were taken from each flask daily for 8 d. Samples were chilled on ice for 30 min and centrifuged at 8000*g* for 15 min to remove mycelia and insoluble residues. Supernatant was frozen at −20°C until analyzed.

Production of Polyclonal Antibodies Against a T. reesei *CBHI Peptide*

A peptide, GPGSSFTLDTTKKLT, corresponding to amino acid residues 291–305 of *T. reesei* cellobiohydrolase I *(14)* was synthesized and conjugated to Keyhole Limpet Hemocyanin (KLH). Two rabbits received 10 mg each of the KLH conjugate over a period of 6 wk. Serum samples were drawn after wk 4, 6, and 8 and analyzed by ELISA for antibody titers. Serum (50 mL) was obtained from each rabbit and subjected to ammonium sulfate precipitation (50% saturation). Peptide synthesis and antibody production were done in Biosynthesis, Inc. (Lewisville, TX). CBHI specific antibodies were further purified using a peptide conjugated HiTrap NHS-activated column (Amersham Biosciences, Piscataway, NJ).

Analytic Procedures

All enzyme assays except for filter paper activity were done at 50°C in 250 μL volume containing either 1.0% (w/v) polysaccharide or 5 m*M* *p*-nitrophenyl conjugated substrates in 50 m*M* sodium acetate buffer, pH 4.8, and 50 μl appropriately diluted enzyme solutions. Filter paper activity was done as described *(15).* Reducing sugar concentrations were measured according to Miller *(16).* When polysaccharides were used as substrates, one unit of activity was defined as 1 μmol of either glucose or xylose equivalent generated per min. With *p*-nitrophenyl conjugated substrates, one unit of activity was defined as one (mol of *p*-nitrophenol (PNP) released per min. Protein concentration was determined using the D_C Protein Assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard.

Proteins were subjected to sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) with a separating gel containing a gradient of 8.0–16.0% (w/v) acrylamide. Protein bands were visualized using the Biosafe Commassie Blue (Bio-Rad) staining solution. Protein bands were also transferred to a polyvinylidene fluoride membrane and subjected to Western analysis using the CBHI peptide specific antibodies.

Transfer and Western blotting were done according to the protocol provided by Bio-Rad.

For hydrolysis of corn fiber fractions, enzymes in the supernatant of *T. reesei* cultures were concentrated fivefold using Centricon Plus 20 cups (Millipore, Billerica, MA). Loss of enzymatic activity was monitored by assaying activities in the filtrate. DSCF, CFC, and CFAX (10.0%, w/v) were suspended or dissolved in dH_2O containing 0.02% sodium azide. The pH of fiber substrate suspensions was adjusted to 4.8 with 6 *N* HCl. Reaction tubes containing 1.0 mL fiber substrates and 0.2 mL sodium acetate buffer (1 *M*, pH 4.8). After temperature reached 45°C, appropriately diluted enzyme preparations and dH_2O were added to the final volume of 2.0 mL. Reaction tubes were shaken at 250 rpm and 45°C. Samples (200 μL) were taken at time 0 and periodically thereafter. Enzymatic activities in the samples were destroyed by boiling the tubes for 5 min. Supernatants of the samples were obtained after centrifuging the tubes at 12,000*g* for 10 min. Reducing sugars in the supernatants were determined using the dinitrosalicylic acid (DNS) method *(16)* with glucose as standard for DSCF and CFC hydrolysis and xylose as standard for CFAX hydrolysis.

Trifluoroacetic acid (TFA, 2 *N*) hydrolysis was done by heating a sealed vial containing 200 mg biomass powder in 1.0 mL volume at 100°C for 1 h. Solid was removed by centrifugation (8000*g*, 15 min). Neutral sugar composition of fractionated corn fibers and their hydrolysates was also determined by thin layer chromatography and HPLC equipped with an Aminex 87P column (Bio-Rad) using a refractive index detector for sugar measurement *(2)*.

Results and Discussion

Preparation of Corn Fiber Fractions

To develop inexpensive substrates for the growth of *T. reesei* and effective inducers for both cellulase and hemicellulase production, we fractionated and evaluated three polysaccharide components of corn fiber from a corn wet mill ethanol plant. Dried milled corn fiber was used as crude corn fiber (CCF); the neutral sugar composition, determined by TFA hydrolysis, is presented in Table 1. Treatment with TFA completely hydrolyzed hemicelluloses and starch but not cellulose *(17).* Glucose content (30%), mostly from starch and sugars, from hemicellulose (28.5%) were within the range of previously reported values *(4,5,11).* The residual solid was 21%. Other components in corn fiber but not analyzed for this study included cellulose (15–20%), proteins (11%), crude fat (0.25%), ash, lignin, acetyl, phenolic, and glucuronic acid groups *(10,11),* some of these components ending up as the residual solid.

Partial removal of starch was achieved by treatment with glucoamylase. As shown in Table 1, TFA hydrolyzable starch decreased from 30% in CCF to 14% in DSCF, concurrent with an increase in hemicellulose-derived

Table 1
Neutral Sugar Analysis of Corn Fiber Fractions after TFA Hydrolysis [a]

Sample (g/g)	Glucose (g/g)	Xylose (g/g)	Arabinose (g/g)	Galactose (g/g)	Residual solid (g/g)	Total
CCF	0.30	0.17	0.10	0.025	0.21	0.81
DSCF	0.14	0.21	0.13	0.030	0.39	0.90
CFC	0.036	0.16	0.095	0.023	0.53	0.84
CFAX	0.003	0.42	0.27	0.11	0.013	0.82

[a]Abbreviations: CCF, crude corn fiber; DSCF, de-starched corn fiber; CFC, corn fiber with cellulose enriched; and CFAX, corn fiber arabinoxylan.

sugars. A substantial amount (14%) of TFA hydrolyzable glucose was still present in DSCF, indicating that some of the starch was resistant to glucoamylase hydrolysis and/or TFA hydrolysis was able to hydrolyze certain non-starch glucose-based polymers. Amorphous cellulose and β-glucan could be present in CF and fall into the latter category. Extraction of arabinoxylan from DSCF (200 g) with 2.0% KOH yielded a 67 g pellet (dry basis). The washed and dried pellet was found after TFA hydrolysis to contain 3.6% glucose, 16% xylose, 9.5% arabinose, and 2.3% galactose (Table 1). The alkali-insoluble fraction after arabinoxylan extraction weighed 106 g, constituted 53% of the initial weight of DSCF, and was found to be comprised of mostly glucose (92%) after cellulase/β-glucosidase hydrolysis. Therefore, the alkali-treated insoluble material was termed corn fiber with cellulose (CFC) enriched. It should be pointed out here that the 2.0% KOH extraction only solubilized approx 50% of arabinoxylan of the total arabinoxylan from DSCF. The partial xylan solubilization was also reported by extraction with 2.0% $Ca(OH)_2$ *(5).* Differences between the alkali (2.0% KOH) soluble and insoluble arabinoxylans of DSCF were not explored.

After two cycles of acidification, alcohol precipitation and $Ca(OH)_2$ extraction, the KOH-extracted CFAX was purified. Dried and milled CFAX (45 g) had a slightly off-white color and a yield of 52% of the total xylan in the starting material. Composition analysis after TFA hydrolysis gave over 80% as xylose, arabinose, and galactose and extremely low amount of glucose or solid (Table 1), characteristic of high-purity alkaline extracted CFAX *(3,5).*

Growth of and Enzyme Production by T. reesei

PD-grown *T. reesei* mycelia were inoculated into corn fiber fraction media. Both strains grew vigorously using each fraction as carbon source under these culture conditions. Growth was characterized by measuring protein-concentration changes (Fig. 1) and enzyme activities (Table 2) toward filter paper (FP), carboxymethyl cellulose (CMC), PNP β-1, 4-glucopyranoside (PNPG), PNP β-xylopyranoside (PNPX), PNP α-1,3-arabinofuranoside (PNPA), and oat spelt xylan (OSX). Over the 8 d of growth, pH dropped gradually from 4.8 to the range of 4.0–4.2 for all

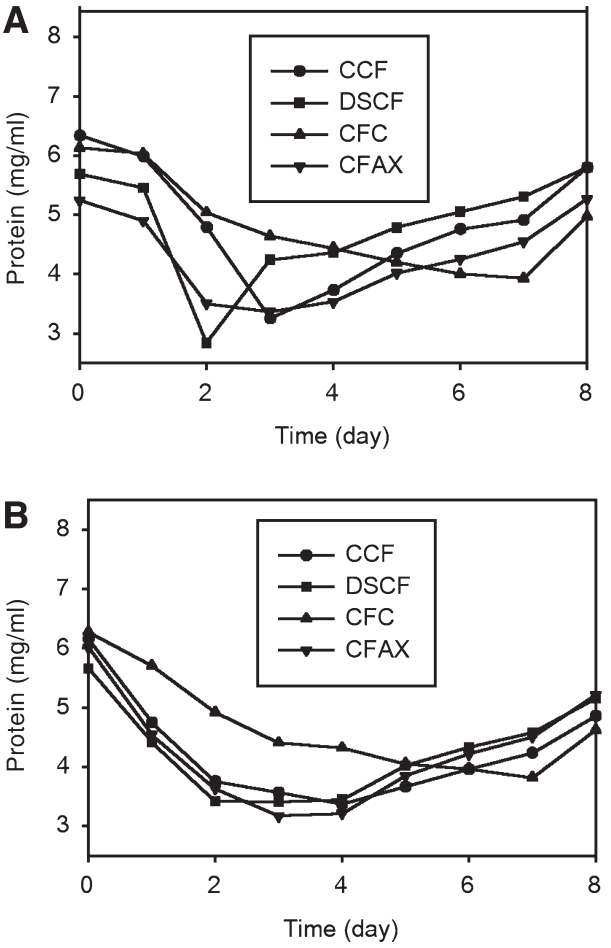

Fig. 1. Change of extracellular protein levels of *T. reesei* cultures during the growth on the four fractions of corn fiber—CCF, DSCF, CFC, and CFAX. Panes **A** and **B** are data obtained for *T. reesei* QM9414 and Rut C-30, respectively.

cultures except the CFC-containing cultures, where the pH actually increased from 4.8 to 5.4. Normally, the pH of shake flask *T. reesei* cultures drops because of acetic acid production. The pH increase observed for the CFC cultures could be from the slow release of residual KOH left within the fiber. For all cultures, protein concentrations decreased for the first 2–3 d and then increased (Fig. 1). It is suspected that the initial decrease in external protein levels reflected the utilization of proteins and organic nitrogen present in the medium by the fungus and the subsequent increase was due to the production of extracellular enzymes. The CFC cultures of both QM9414 and Rut C-30 were delayed in decreasing protein levels at the beginning and showed less increase by the end of the fermentation, possibly due to higher pH in these cultures compared to the others. Further work with better control of pH for CFC fermentation should clarify the pH effect.

Table 2
Production of Enzyme Activities by *T. reesei* after Grown on Corn Fiber Fractions for 8 d

	Substrate					
	FP (U/mL)	CMC (U/mL)	OSX (U/mL)	PNPG (U/mL)	PNPA (U/mL)	PNPX (U/mL)
QM9414						
CCF	0.22	6.25	98.5	0.43	13.0	0.79
DSCF	0.28	9.52	201	0.64	24.7	0.93
CFC	0.20	5.65	115	0.27	16.7	1.27
CFAX	0.11	1.76	221	0.39	32.0	0.56
Rut C-30						
CCF	0.31	5.28	86.1	0.49	6.80	0.76
DSCF	0.23	7.58	156	0.59	8.72	1.47
CFC	0.18	3.75	66.8	0.18	16.7	2.03
CFAX	0.35	9.35	621	0.73	23.4	0.59

Reducing-sugar concentrations in all cultures remained low (<5.0 mg/mL), suggesting the rapid uptake of sugars released from easily hydrolyzed polysaccharides and the recalcitrant nature of the corn fiber fractions. The starch and arabinoxylan fractions in CCF and CFAX, respectively, were soluble in the media, suggesting they should have been easily saccarified, but still no rapid increase of reducing sugars occurred during the early stage of fermentation. The genes coding for cell-wall-degrading enzymes, therefore, should not be repressed by high levels of glucose and xylose during the entire course of fermentation. These results support the notion that crude corn fiber even with certain levels of adherent starch left can serve as an inexpensive feedstock for *T. reesei* growth and enzyme induction.

The broths recovered from the 8-d-old fungal cultures were measured for several lignocellulose degradation related enzyme activities (Table 2). Activities toward filter paper, carboxymethyl cellulose (CMC), oat spelt xylan (OSX), PNPG, PNPX, and PNPA were present in cultures with any of the four corn fiber fractions used. The activity levels toward filter paper (0.11–0.38 FPU/mL) and CMC (1.76–9.52 U/mL) were comparable to those reported for Rut C-30 grown on wood-derived lignocellulosic substrates *(18)* and for *Trichoderma* sp. strain 414 grown on CCF *(8).* By contrast, activities toward OSX (66.8–621 U/mL, Table 2, Fig. 3) were much higher than that (approx 4.0 U/mL) previously reported *(8).* The xylanase activity, 621 U/mL, in CFAX Rut C-30 culture was nearly threefold greater than that (221 U/mL) in QM9414 culture (Table 2, Fig. 3) and most of the xylanase was produced during the last 3 d. The high xylanase activity could be associated with the heavy band of approx 20 kDa as visualized by SDS-PAGE

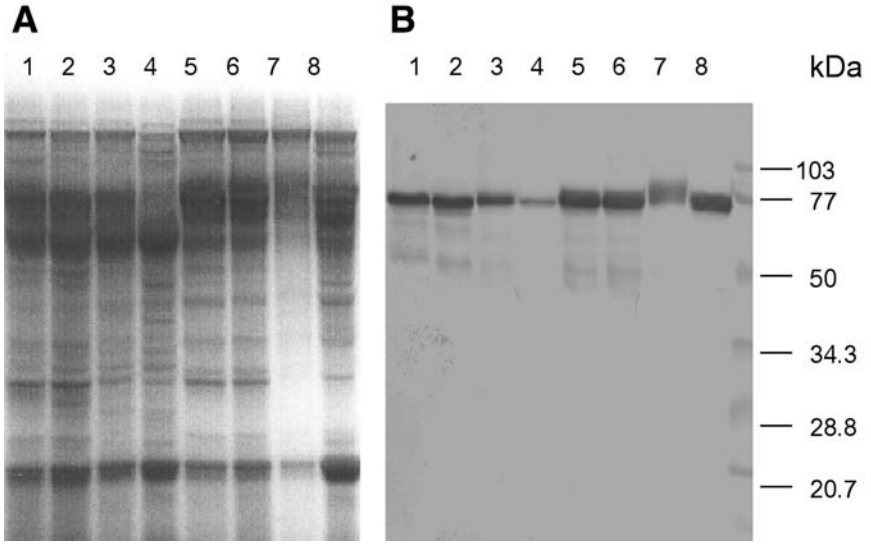

Fig. 2. Analysis of extracellular proteins by SDS-PAGE (panel **A**) and Western blotting using CBH-1 specific antibodies (panel **B**) for *T. reesei* grown on the four corn fiber fractions. Supernatant samples (10 (L for panel **A** and 2 (L for Panel **B**) of cultures grown on CCF (lane 1 for QM9414 and lane 5 for C-30), DSCF (lane 2 for QM9414 and lane 6 for C-30), CFC (lane 3 for QM9414 and lane 7 for C-30), and CFAX (lane 4 for QM9414 and lane 8 for C-30) were loaded.

analysis (Fig. 2A). This band might be actually the xylanase XylI, XylII, or both *(19).* CFAX is heavily substituted by arabinose, xylose, and galactose containing side chains *(3)* and, because of that, a purified *Fusarium* xylanase failed to hydrolyze the substrate *(20).* The presence of these sidechains would be expected to retard the hydrolysis of CFAX compared to less substituted xylans (e.g., birchwood and oat spelt xylans) during *T. reesei* cultivation, and this might have resulted in a more persistent induction and/or lack of high levels of monosaccharides for repression of the fungal hemicellulase gene system.

In addition to the 20-kDa band, many other protein bands, ranging from 20 to over 100 kDa, were also detected by SDS-PAGE (Fig. 2A). The corn fiber fraction–grown *T. reesei* cultures produced many more visible bands than cultures grown on either cellulose or lactose (data not shown), further indicating that corn fiber and its enriched components induce a wider variety of genes encoding for hemicellulases compared to solely using cellulose. Under the culturing conditions using the four corn fiber fractions, both strains produced the cellobiohydrolase I protein (Fig. 2B), although Rut 30 appeared to produce more of this enzyme than QM9414.

Hydrolysis of Corn Fiber Fractions

Concentrated DSCF-grown Rut C-30 enzyme preparation (10 U/mL CMCase), commercial Celluclast (10 U/mL CMCase), and Novozyme 188

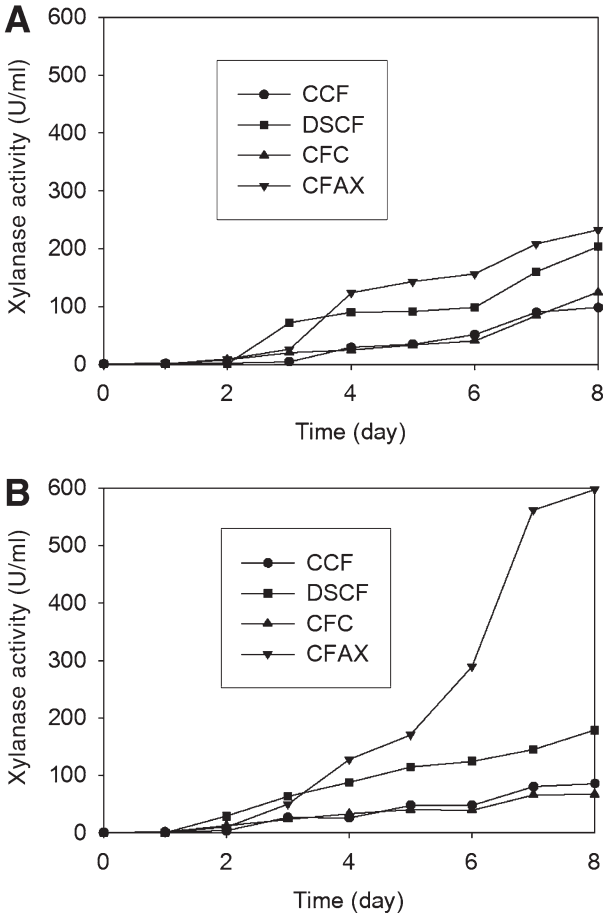

Fig. 3. Xylanase production of *T. reesei* QM9414 (panel **A**) and Rut C-30 (panel **B**) during shake-flask cultivation on corn fiber fractions.

(5.0 U/mL β-glucosidase) were used individually and in combinations for the hydrolysis of DSCF, CFC, and CFAX. Hydrolysis of CCF was not done because the high starch content could complicate the interpretation of results. Reducing sugar concentrations released from the three corn fiber fractions (5.0%, w/v) were measured over a period of 48 h (Fig. 4). A substantial amount of DSCF (8.0 mg/mL) representing around 20% of total polysaccharides was hydrolyzed by Novozyme 188 (Fig. 4A). Based on neutral sugar determinations on hydrolysates, the reducing sugars were released mostly from hemicellulose and residual starch constituents. Novozyme 188 contains high levels of activity against soluble starch (5300 U/mL) and PNPG (750 U/mL) and lesser levels of activities against CMC (21 U/mL), OSX (395 U/mL), CFAX (45 U/mL), PNPA (70 U/mL), and PNPX (62 U/mL). Little activity against filter paper was detected. In contrast, Celluclast possesses high levels of activities against CMC (2550 U/mL) and filter paper (125 U/mL) and lower (but detectable) levels of activities against

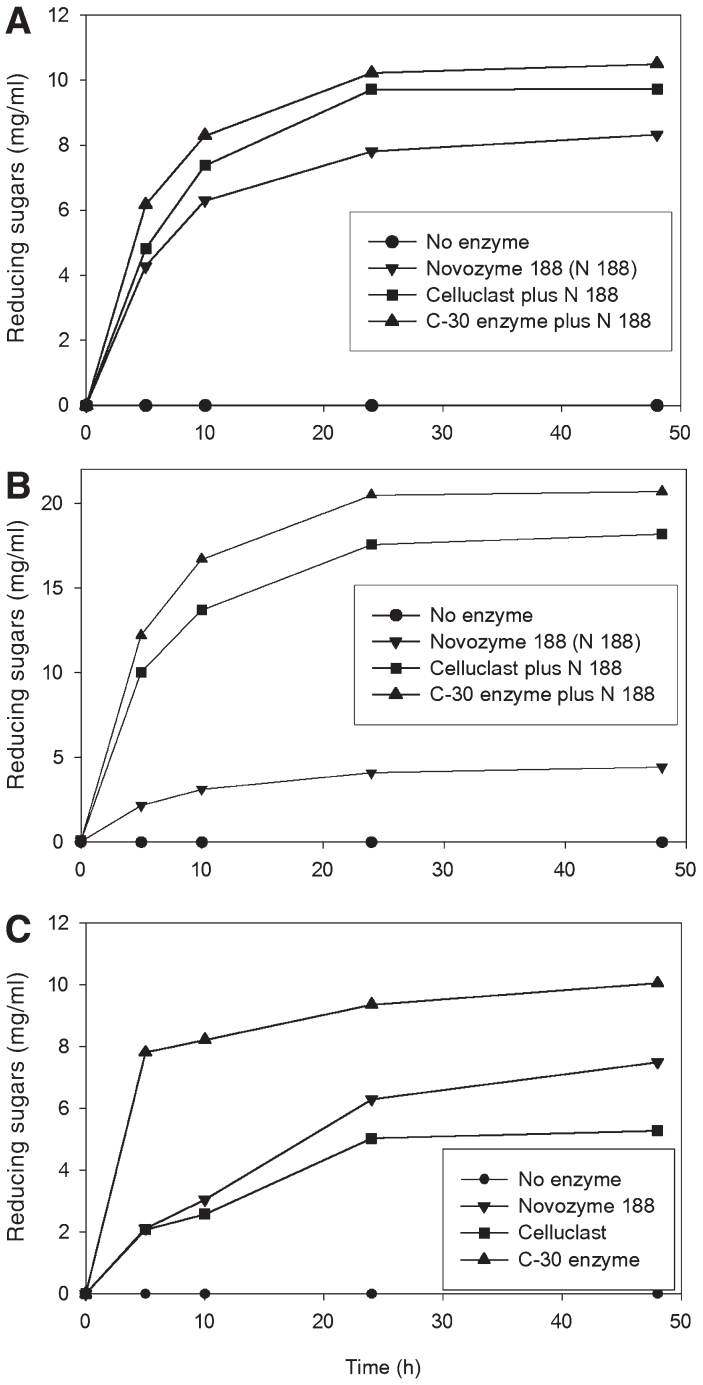

Fig. 4. Release of reducing sugars from corn fiber fractions by commercial cellulases and enzymes of *T. reesei* Rut C-30 grown on de-starched corn fiber (DSCF). Hydrolysis was done for DSCF (panel **A**), CFC (panel **B**), and CFAX (panel **C**).

Table 3
Neutral Sugar Composition in Hydrolysates of Corn Fiber Fractions [a]

Enzyme	Substrate	Glucose (mg/mL)	Xylose (mg/mL)	Arabinose (mg/mL)	Galactose (mg/mL)
No enzyme	DSCF	ND	ND	ND	ND
N 188	DSCF	10.3	0.66	0.68	0.22
CC plus N 188	DSCF	10.5	1.60	1.20	0.26
C-30 plus N 188	DSCF	10.0	2.00	1.74	0.26
No enzyme	CFC	ND	ND	ND	ND
N 188	CFC	2.52	0.74	0.92	0.10
CC plus N 188	CFC	17.3	1.76	1.48	ND
C-30 plus N 188	CFC	16.8	2.20	1.90	ND
No enzyme	CFAX	ND	ND	ND	ND
N 188	CFAX	1.30	3.50	5.60	0.52
CC	CFAX	1.28	3.16	5.18	0.63
C-30	CFAX	1.36	4.94	6.36	1.36

[a]Enzyme activity levels for the hydrolysis were 5.0 U/mL β-glucosidase of Novozyme 188 (N 188), 10.0 U/mL CMCase of Celluclast (CC), and 10.0 U/mL CMCase of Rut C-30 grown on DSCF (C-30).

hemicellulose and soluble starch. The Rut C-30 enzymes from cultures grown on DSCF have more balanced activities against both cellulose and hemicellulose (Table 2) and also displayed diverse bands on SDS-PAGE (Fig. 2). More reducing sugars were obtained during DSCF hydrolysis when Novozyme 188 was combined with either Celluclast or C-30 enzymes (Fig. 4A). The highest level of conversion was about 10.0 mg/mL, which accounts for slightly greater than 20% conversion of DSCF into glucose equivalents. Analysis of neutral sugars in the hydrolysates revealed that most of the increase after the Celluclast and C-30 enzyme additions were sugars from hemicellulose hydrolysis (Table 3), demonstrating that *T. reesei* cellulases, even with β-glucosidase supplemented, are unable to degrade the un-pretreated cellulose within corn fiber.

In comparison to DSCF, CFC was hydrolyzed much less extensively by Novozyme 188 alone, but much more rapidly and to a much higher degree (20.2 mg/mL) by either Celluclast or C-30 enzymes plus Novozyme 188 (Figs. 4A and 4B). This comparison indicates that as expected following removal of the arabinoxylan by alkaline extraction, the residual cellulose becomes more susceptible to hydrolysis by fungal cellulases.

Hydrolysis of CFAX by Novozyme 188, Celluclast, and DSCF-grown C-30 enzymes alone showed that the C-30 enzymes performed the best (Fig. 4C and Table 3). These data further suggest that *T. reesei* growing on more complex substrates, particularly those rich in hemicellulose, produces hemicellulase activities than those cultures grown in media with

simpler carbon sources. The largest amount of neutral sugars detected was less than 15 mg/mL, which corresponds to 35% of conversion of total mass in CFAX. Oligosaccharides and glucuronic, methyl glucuronic, acetic, and ferulic acids may account for some of the unaccountable mass. Oligosaccharides were present as unidentified peaks during the HPLC runs. The incomplete conversion of CFAX to monosaccharides may reflect insufficient amounts of certain activities such as those of β-xylosidase, feruloyl esterase, and β-glucuronidase.

Conclusion

Crude corn fiber (CCF) has been separated into several fractions enriched with different polysaccharides by removing residual starch and alkaline extraction of hemicellulose. Cellulase hyperproducing strains of *T. reesei* are able to grow vigorously on CCF and its cell wall–enriched fractions. Furthermore, these strains produce a more balanced enzyme profiles for the hydrolysis of both cellulose and hemicellulose. In particular, very high levels of xylanase activity were obtained when *T. reesei* Rut C-30 was cultured in corn fiber arabinoxylan medium.

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