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CELLULASE AND XYLANASE PRODUCTION BY TRICHODERMA REESEI RUT C-30

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SUMMARY

When grown on cellulose or xylan, Trichoderma reesei (strain Rut C-30) produced extra-cellular enzymes which could hydrolyze both cellulose and xylan to their respective monosaccharides. At low O_2 saturation, β-glucosidase activity is greatly reduced for cellulose-grown but not xylan-grown cells.

INTRODUCTION

Lignocellulosic biomass has potential for substituting for petroleum as a large source of chemical feedstocks (Ng et al, 1983). To become economical, maximum utilization of the feedstock is necessary. It would therefore be attractive for all of the polysaccharide components of biomass to be hydrolyzed into fermentable, monomeric sugars. In pursuit of this goal, we have been studying the fungus Trichoderma reesei Rut C-30, which has high cellulolytic activity, and its ability to hydrolyze cellulose and xylan, the two major polysaccharide components of lignocellulosic biomass.

MATERIALS AND METHODS

Trichoderma reesei (strain Rut C-30) was obtained from the Northern Regional Research Laboratories in Peoria, Ill. Rut C-30 is a hypercellulolytic strain of T. reesei isolated by Montenecourt and Eveleigh, 1979. Rut C-30 was grown at 28°C in a 7 L fermenter using the Natick media (Sternberg, 1976a). The pH was kept above 3.0 with NaOH. The O_2 was controlled relative to an O_2 probe (Ingold) measuring % saturation of dissolved O_2. The fermenter was bubbled with a combination of nitrogen and air at approximately 10% v/v min. The O_2 controller regulated air bubbling such that dissolved O_2 levels of ±1% of saturation could be maintained. Fermentations were initiated with a 4% seed culture grown in flasks with the same substrate. Enzyme assays were performed on the broth supernatant after centrifugation at 37,000xg for 30 minutes.

Carboxymethyl cellulase (CMCase) activity was assayed by measuring the reducing sugars produced (Miller, 1959) from 2% carboxymethyl cellulose after 20 minutes of incubation with extracellular enzyme at 50°C. The buffer was 0.2M sodium citrate (pH=4.8). Xylanase was measured using the same conditions and 2% xylan. The units for CMCase and xylanase are μmoles reducing sugar equivalent (glucose or xylose)/minute. Extracellular protein was measured as in Lowry et al, 1951. β-Glucosidase was assayed by measuring the amount of glucose produced by enzyme incubated with 1% cellobiose for 19 hrs at 50°C. Units are μmoles glucose produced per minute. Hydrolyses of 5% cellulose or xylan were carried out at 50°C in the presence of 0.01N NaN_3. Glucose, cellobiose and xylose were quantified using high pressure liquid chromatography. A Bio-Rad Aminex HPX 87H column was used. Samples were eluted in 0.01N H_2SO_4 and measured with a refractive index detector.

Baker TLC Grade or Solka Floc BW 200 were used as a cellulose substrate. No difference was seen for either growth or hydrolysis and both contained about 4% xylan as measured by xylose production from the enzyme hydrolysis. Xylan (Sigma Chemical Co.) was from larchwood and is partially soluble in water. No other major products from the enzyme hydrolysis of xylan besides xylose were identified on HPLC. Cellobiose, xylose and carboxymethyl cellulose (low viscosity) were from Sigma.

RESULTS AND DISCUSSION

Trichoderma reesei (Rut C-30) was grown on 1% cellulose or xylan at oxygen levels of 10, 20, 35 and 50% saturation. The levels of extracellular enzymes for 10 and 50% O_2 saturation are shown in Table I. The enzyme levels for 20% and 35% O_2 saturation were not significantly different than those produced at 50%. At 10% O_2 saturation, however, all levels as well as extracellular protein were markedly reduced.

Table I: Extracellular Enzyme Production by Rut C-30 After 9 Days Growth

Growth (1%) Substrate	O_2 (%Sat.)	Activity (units/ml) CMCase	Xylanase	β-glucosidase	Protein(mg/ml)
Cellulose	10%	6.8	-	< 0.005	0.70
Xylan	10%	7.5	-	0.06	0.73
Cellulose	50%	70	108	0.08 (0.18)[a]	3.32
Xylan	50%	47	130	0.10	1.70

[a] pH=5.0

To more accurately measure the activity of cellulase, hydrolyses of cellulose and xylan by extracellular enzymes from cellulose-

and xylan-grown cells were performed and the final products glucose, cellobiose and xylose were measured directly using high pressure liquid chromatography (Table II). Enzyme produced by cellulose-grown cells saccharified cellulose to give a 42% yield of glucose after two days and 66% after 14 days. The yield was slightly lower with enzyme produced by xylan-grown cells, 31% after two days and 60% after 14 days.

Enzymes from cellulose- and xylan-grown cells were also tested for their ability to hydrolyze xylan (Table II). As with cellulose hydrolysis, the yield of xylose was slightly higher for enzyme from cellulose-grown cells (78% yield after 14 days) than from xylan-grown cells (69% yield after 14 days). The 10% O_2 level decreased considerably the ability of enzymes from cellulose- and xylan-grown cells to produce glucose and xylose (Table II). When incubated with cellulose, enzyme from cellulose-grown cells at 10% O_2 produced more cellobiose than glucose whereas the reverse was true for enzyme from xylan-grown cells at 10% O_2.

Table II: Hydrolysis of Cellulose and Xylan by Rut C-30 Enzymes

		Cellulose Hydrolysis					
		After 2 Days		After 14 Days		Xylan Hydrolysis	
Growth Substrate	O_2 (%Sat.)	%Glu-cose	%Cello-biose	%Glu-cose	%Cello-biose	% Xylose 2 Days	14 Days
Cellulose	10%	0.30	0.72	0.52	0.89	-	-
Xylan	10%	0.38	0.31	1.00	0.22	-	-
Cellulose	50%	2.08	0.75	3.30	0.36	2.77	3.90
Cellulose (pH=5.0)	50%	2.06	0.16	3.55	0	-	-
Xylan	50%	1.53	0.84	3.01	0.44	1.78	3.43

Cells were grown for 9 days and extracellular enzyme incubated with 5% cellulose or xylan at 50°C for the time periods indicated above.

Therefore, extracellular enzyme from Rut C-30 grown on either cellulose or xylan can hydrolyze both cellulose or xylan to their respective monosaccharides. With each substrate, levels from cellulose-grown enzyme are higher than xylan-grown, but the difference is slight (10-20%). This suggests that the induction of a single enzyme system, by either cellulose or xylan, may be responsible for the hydrolysis of both substrates. Cross-reactivity of CMCase and xylanase activities has been previously observed with a cellulase component of *T. viride* grown on wheat bran (Toda et al, 1971). Dekker (1983) has observed much lower yields of xylanase (1.5 units/ml) when *T. reesei* QM 9414 was grown on xylan or hemicellulose from sugarcane bagasse. This could be due to differences in the two strains or oxygen limitation in the QM 9414 growth.

The β-glucosidase activity at 10% O_2 saturation was much higher when grown on xylan than on cellulose (Table I). β-glucosidase produced by xylan-grown cells at 10% O_2 was 80% as active as that produced at 50% O_2 whereas for cellulose-grown cells it was less than 10%. This could explain the report (Chaudhary and Tauro, 1982) that T. reesei β-glucosidase is selectively induced by xylan. In our hands, enzyme production by T. reesei in shake flasks (as used by Chaudhary and Tauro) was limited by O_2. It may be that xylan is more effective in inducing β-glucosidase activity than cellulose at low O_2 levels because it is partly soluble whereas cellulose is not.

The β-glucosidase levels produced by cellulose-grown cells was also sensitive to low pH at high O_2 levels (Table I). With cellulose-grown cells, the pH held at 5 rather than above 3 produced higher β-glucosidase activity and yielded less cellobiose during hydrolysis. This effect has been observed previously for strain QM 9414 (Sternberg 1976b). Keeping the pH above 5 enables the cellulase complex to hydrolyze cellulose to glucose with no cellobiose remaining (Table II).

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