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β-Xylanase production by *Aureobasidium pullulans* grown on sugars and agricultural residues

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Aureobasidium pullulans **grew well in media containing glucose, fructose, xylan or xylose but β-xylanase was only produced with xylan or xylose. Lactose and maltose were poor substrates for growth. β-Xylanase production was repressed in media containing glucose or fructose along with xylose. Agricultural residues, such as wheat bran, paddy husk and rice straw, could be used as carbon sources for growth and β-xylanase production of *Aureobasidium pullulans*. Tween 80 at 0.5% (v/v) increased the yield of β-xylanase by up to 20%.**

Key words: Agricultural wastes, *Aureobasidium pullulans*, glucose repression, hemicellulose, β-xylanase, xylose.

Xylan, a heteropolymer of (β-1–4)-linked xylose, is the next most abundant natural polysaccharide after cellulose and accounts for 20 to 30% dry weight of agricultural residues. Its abundance indicates that xylanolytic enzymes can play an important role in bioconversion, in the preparation of cellulose pulps, in fibre liberation technology, etc. (Biely 1985; Wong *et al.* 1988).

The expansion in the use of lignocellulosic agricultural and forestry waste will depend greatly on increased availability of microbial β-xylanases. The present paper deals with the production of β-xylanase from a strain of *Aureobasidium pullulans* grown on media containing sugars (xylose and non-xylose) and hemicellulosic agricultural wastes.

Materials and Methods

Microorganism and Cultivation

Aureobasidium pullulans NCIM 1050 (from the National Chemical Laboratory, Pune, India), maintained on Biely's Agar slants (Biely *et al.* 1980), was grown at 30°C for 3 days in a shaken (180 rev/min) 250-ml flask containing 50 ml of Biely's basal medium with 1% (w/v) glucose. This was used at 1% (v/v) as inoculum in all experiments.

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β-Xylanase Preparation and Assay

The organism was cultivated in a Biely's basal medium containing the indicated carbon sources at 1% (w/v) for 70 h, and then each was centrifuged (2000 × ***g*** for 25 min at 4°C) and the supernatant assayed for β-xylanase activity by the dinitrosalicylic acid method (Miller 1959) using oat spelt xylan (Sigma) at 1% (w/v). Standard β-xylanase assay conditions were 50°C and pH 5. One unit of xylanase activity was defined as that releasing 1 μmol xylose equivalent per min.

Results

Time Course of Growth and β-Xylanase Production by Aureobasidium pullulans *with Xylan as the Sole Carbon Source*

The course of β-xylanase production by the mould growing on 1% (w/v) xylan is shown in Figure 1. β-Xylanase was secreted only during active growth and ceased after 70 h.

Effect of Carbon Source on β-Xylanase Production

Highest activities of xylanase were produced when cells were grown on a medium containing xylose or xylan as carbon source (Table 1). Although glucose and fructose were utilized, β-xylanase activity was minimal with these sugars. Cells grew very poorly with maltose and lactose. Xylose induces the β-xylanase of *Aureobasidium pullulans* and negligible activity could be detected in culture supernatants when cycloheximide (25 μg/ml) was added (data not shown) in the medium containing xylose.

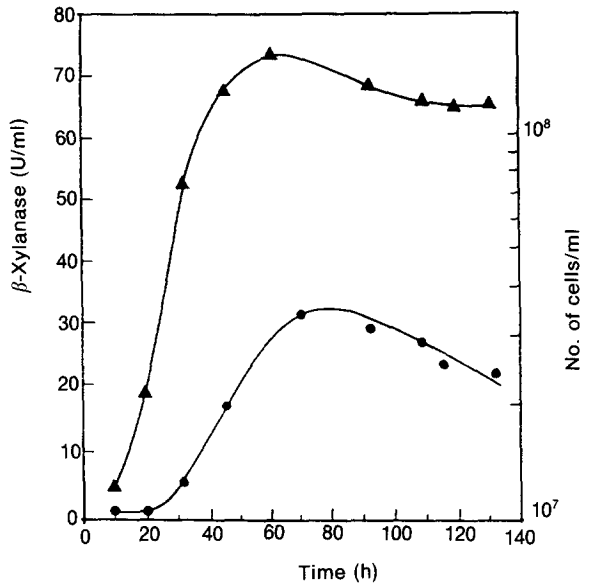

Figure 1. Time course of growth (cells per ml; ▲) and β-xylanase activity (●) of *Aureobasidium pullulans* NCIM 1050 grown in Biely's basal medium containing 1% (w/v) xylan.

With xylose as sole carbon source, the specific activity of β-xylanase per cell was maximal (Table 2) and was 60- to 360-fold less when cells were grown in media containing glucose or fructose at the same concentration.

Of a number of hemicellulosic agricultural residues, highest activities of β-xylanase (2.7 to 8.5 U/ml) were obtained with wheat bran, rice straw and paddy husk (Table 3). No activity could be detected with carboxymethyl cellulose (CMC) or solka floc.

Surfactants can stimulate secretion of enzymes (Reese & Maguire 1969). Accordingly, when Tween 80 was added at

Table 1. Effect of various carbon sources on the growth and production of β-xylanase of *Aureobasidium pullulans*.*

Supplemented carbon source [at 1% (w/v)]	Cell numbers (No. of cells $\times 10^7$/ml)	Activity of β-xylanase (U/ml)	Activity of β-xylanase (U/10^7 cells)
None	2	Nil	Nil
Glucose	26	2.2	0.08
Fructose	17	0.6	0.04
Lactose	3	0.6	0.2
Maltose	3	Nil	Nil
Xylan	10	31	3.1
Xylose	11	39	3.6

* Primary inoculum, prepared as described in Materials and Methods, was transferred to Biely's basal medium containing 0.67% (w/v) yeast nitrogen base, 0.2% (w/v) asparagine, 0.5% (w/v) KH_2PO_4 and different carbon sources. The cultures were grown for 70 h after which cell numbers and extracellular xylanase activities were measured.

Table 2. Effect of glucose and/or fructose on xylose-induced xylanase in *Aureobasidium pullulans*.*

Growth substrate [at 1% (w/v)]	Cell numbers (No. of cells $\times 10^7$/ml)	Activity of β-xylanase (U/ml)	Activity of β-xylanase (U/10^7 cells)
Xylose	11	40	3.6
Glucose	24	0.3	0.01
Fructose	12	0.7	0.06
Xylose + Glucose	25	8.2	0.3
Xylose + Fructose	27	13.3	0.9

* Primary inoculum was prepared as described in Materials and Methods. Cells were then transferred to media containing sugars [at 1% (w/v) each]. Growth and xylanase activities were determined after 70 h.

Table 3. Xylanase production by *Aureobasidium pullulans* grown on hemicellulosic agricultural residues in the presence and absence of Tween 80.*

Hemicellulosic agricultural residue [at 1% (w/v)]	Growth (No. of cells $\times 10^7$/ml)		Activity of β-xylanase (U/ml)	
	without Tween 80	with Tween 80	without Tween 80	with Tween 80
Bagasse	2.6	2.1	0.1	0.7
Carboxymethylcellulose	0.6	0.1	Nil	0.6
Solka floc	0.5	0.1	Nil	Nil
Rice Straw	4.1	3.0	2.4	5.1
Tamarind seed powder	0.2	0.5	0.5	1.6
Sawdust	1.3	0.9	0.3	0.3
Wheat bran	15.0	7.0	8.5	10.2
Paddy husk	4.0	2.3	4.5	5.8

* Cells from the primary inoculum were inoculated into Biely's basal medium supplemented with different hemicellulosic agricultural residues at 1% (w/v). At the end of 70 h of incubation, both growth and xylanase activities were measured. Tween 80 was added at a concentration of 0.5% (v/v).

0.5% (v/v) to media with different substrates, β-xylanase yields (U/ml) increased by 13%, 20% and 21% with paddy husk, wheat bran and rice straw, respectively.

Discussion

While several species of fungi have been reported to produce extracellular β-xylanase (Dekker & Richards 1976), this present species offers an advantage in the study of β-xylanase in that it produces no cellulase. For this reason, as well as the manipulative advantages offered by yeast morphology, we have used the strain of *Aureobasidium pullulans* for studies on production of β-xylanase.

The extracellular β-xylanase of *Aureobasidium pullulans* is inducible by glucose (Leathers *et al.* 1986). In the presence of cycloheximide (25 μg/ml) the amount of β-xylanase produced in the xylose medium was 10-fold less than that produced in the cycloheximide-free xylose medium. The strain could use various natural hemicellulosic agricultural residues such as wheat bran, rice straw, bagasse, paddy husk and sawdust, but yielded high activities of β-xylanase with only some of them. Since the chemical structure of natural residues is highly complex, the differential induction of β-xylanase by them is obscure.

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