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INDUCTION OF XYLANOLYTIC ACTIVITY IN *Aureobasidium pullulans* USING XEROGRAPHIC PAPER

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ABSTRACT

The possibility of using cheaper culture media for endo-1,4-β-xylanase production by the color variant-derivative strain of *Aureobasidium pullulans* Y-2311-1, one of the known best producers of this activity, was investigated. Of all the studied alternative substrates, xerographic paper showed to be the best inducer of xylanolytic activity in this strain, using different carbon sources. The highest extracellular xylanase activity obtained was 15 U/mL when using lactose as the carbon source in the presence of xerographic paper.

INTRODUCTION

One of the possible applications of hemicellulolytic enzymes, namely xylanases, in the pulp and paper industry is at deinking process of wastepaper, one of the critical steps in the recycling process. Enzymatic deinking is likely to be of industrial use since it does not only brings great environmental advantages, but it is less costly [1,2] and more efficient [1,2,3] than conventional chemical deinking as well.

Aureobasidium pullulans has previously been shown to produce extracellularly a cellulase-free endo-1,4-β-xylanase of extremely high activity [4] that might allows the use of the culture supernatant without further purification or concentration for the industrial enzymatic hydrolysis of xylan or xylan-containing materials. However, the commercially-available arabinoxylan (one of the best inducers of xylanase activity by *A. pullulans*) is too expensive to allow its use for any industrial enzymatic production [5].

Then, the aim of this work was to investigate the effectiveness of using cheaper culture media for xylanase production by the color variant-derivative strain of *Aureobasidium pullulans* Y-2311-1, described [6] as the best overproducer strain of this enzyme. In this sense, xerographic paper was used as an alternative inducer with glucose, sucrose or lactose as the carbon source. To minimise the cost of enzyme production, agro-industrial residues (whey, carob syrup, molasses and wine must) were also used as alternative carbon sources. Other alternative substrates for xylanase induction, such as brewers' spent grain, wheat germ, corn cobs and hardwood wood chips were also studied.

The efficiency of using *A. pullulans* xylanolytic system for improving toner removal from printed wastepaper is being evaluated under different operation conditions at laboratory scale.

METHODS AND MATERIALS

Strain

The organism used (*Aureobasidium pullulans* NRRL Y-2311-1) is a color variant-derivative strain and was kindly gifted by Dr. Laura Vasconcelos (Central Cer, PT). Stock cultures were maintained on potato dextrose agar slants at 4°C, in the dark, and transferred into fresh medium once a month.

Culture Media

The culture medium used in this work has the following composition (g/L): yeast nitrogen base, 6.7; asparagine, 2.0; and KH_2PO_4, 5.0. This basal medium was filter sterilised. The medium was supplemented with 1.0% (w/v) inducer substrate - oat spelts xylan, birchwood xylan, xylose, xerographic paper, brewers' spent grain, wheat germ, corn cobs or hardwood wood chips. When a carbon source was added, two concentrations were used: 0.5% (w/v) glucose, sucrose or lactose; or 1% (w/v) (as total sugars assayed by the phenol-sulphuric acid method [7]) whey from bovine milk, carob syrup (extracted from carob pods according to [8]), molasse or wine must.

Paper Furnish

The paper used in these culture media had been printed by xerography with toner containing a styrene/butadiene polymer. The printed sheets were milled to approximately 0.5-mm-diameter pieces.

Cultivation Conditions

The microrganism was grown at 28°C in 500 mL Erlenmeyer flasks containing 150 mL of the medium previously described shaken at 130 rpm on an orbital shaker, without pH control. Batch cultures were also carried out in a 6-L Biolafitte fermentor with a 4-L working volume. The aeration rate was set at 1 vvm and the stirring at 500 rpm at uncontrolled pH value.

Culture Sampling

The bacterial cells were removed from the culture broth by centrifugation (4000×g for 30 min at 4°C), and the cell-free supernatant dialysed (cut-off= 6-8 kDa) against distilled water overnight at 4°C. These dialysates were further used for the enzymatic assays.

Enzymatic Assays

Endo-1,4-β-Xylanase Activity

Endo-1,4-β-xylanase was assayed using 1% (w/v) oat spelts xylan as substrate, at 50°C and pH 5.0, according to Bailey *et al.* [9]. One unit of endo-1,4-β-xylanase activity (U) was defined as that amount of enzyme which catalyses the release of 1 micromole of xylose equivalents per minute under the assay conditions.

Filter Paper Activity (FPase)

Filter paper activity (FPase), which describes the overall cellulolytic activity, was assayed by the IUPAC method [10] using Whatman No. 1 filter paper (about 50 mg) as substrate, at 50°C and pH 4.8. Enzyme activity was reported as micromoles of glucose equivalents released per minute under standard conditions (U).

RESULTS

When growing the strain of *Aureobasidium pullulans* Y-2311-1 on conventional inducer substrates (xylan or xylose), the presence of high xylanolytic activity on culture supernatants of this strain, as previously described [4,6,11,12], could be confirmed (results shown on Table I).

Table I- Shake-flasks cultures of *A. pullulans*, using conventional inducer substrates.

Inducer Substrate (1%)	**Growth Time** (days)	**β-xylanase Activity** (U/mL)	**FP_{ase} Activity** (U/mL)
oat spelts xylan	3	46.5	0.02
	7	28.4	0.00
birchwood xylan	3	43.8	0.03
	7	51.2	0.00
xylose	7	58.6	0.04

When using xerographic paper as inducer (the target substrate in deinking), an insoluble substrate, there was the need to use an additional carbon source in the medium to ensure the growth. It was verified a significant induction effect on xylanase production whatever the carbon source added: up to 170-fold increase (Table II). The highest level of xylanase production (15 U/mL) was obtained when using lactose as carbon source.

Table II- Shake-flasks cultures of *A. pullulans*, after 3 days, using xerographic paper as inducer.

Carbon Source	**Inducer Substrate** (1%)	**β-xylanase Activity** (U/mL)	**FP_{ase} Activity** (U/mL)
glucose	+	8.5	0.03
	-	0.1	0.01
sucrose	+	6.4	0.00
	-	0.3	0.00
lactose	+	14.8	0.00
	-	0.6	0.00
wine must	+	1.9	0.06
	-	0.4	0.00
molasse	+	1.7	0.03
	-	0.3	0.01
carob syrup	+	0.2	0.08
	-	0.0	0.02

In order to study the enzyme induction on *Aureobasidium pullulans*, simultaneous cultures using unprinted paper (**white**), ordinary xerographic paper (**xerographic**) and xerographic paper with 100% coverage (**black**), and other tested inducer substrates, were carried out. These results (Table III) demonstrate that the xylanolytic activity induction is due to the presence of paper in the culture medium. This xylanase production is reduced by the toner on the xerographic paper, which might be a result from the lower accessibility to the inducer substrate and/or from a growth inhibition provoked by toner.

Table III- Shake-flasks cultures of *A. pullulans*, after 3 days, using lactose as carbon source and different inducer substrates.

Inducer Substrate (1%)	**β-xylanase Activity** (U/mL)	**FP_{ase} Activity** (U/mL)
white paper	3.3	0.00
xerographic paper	2.8	0.00
black paper	0.1	0.00
brewers' spent grain	1.6	0.04
wheat germ	3.1	0.03
corn cobs	0.4	0.04
hardwood wood chips	0.1	0.03
-	0.0	0.03

Oxygen affects xylanase production by *Aureobasidium pullulans* Y-2311-1. This production increased when *A. pullulans* was cultured with xerographic paper, using whey from bovine milk (an agro-industrial residue) as carbon source, in batch

aerated fermentor that allows a non-limiting oxygen growth (Figure 1).

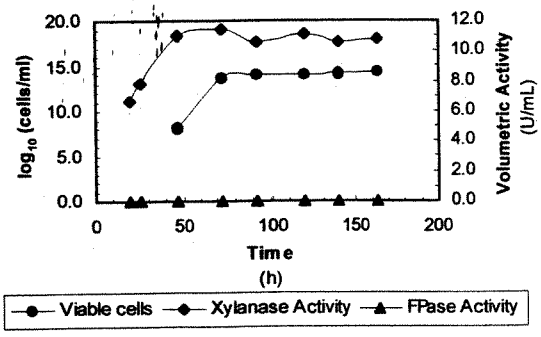

Figure 1. Batch culture of *A. pullulans* in fermentor, using xerographic paper as inducer substrate and whey as carbon source.

CONCLUSIONS

The results of this work clearly demonstrate that endo-1,4-β-xylanase production by *Aureobasidium pullulans* Y-2311-1 is induced by xerographic paper, a very cheap substrate.

Cellulase-free xylanolytic preparations were obtained for most of the culture media tested, namely when using xerographic paper with lactose as the carbon source (case in which the highest xylanase activity was obtained).

The study of endo-1,4-β-xylanase production by this *A. pullulans* strain should proceed to optimise the growth conditions in order to favour this production, namely by changing the composition of the culture medium and by making use of fermentor cultivation.

Deinking laboratory trials using this *A. pullulans* xylanolytic system for improving toner removal from xerographic wastepaper are in progress and will also be presented.

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