XYLAN FROM SUGAR CANE BAGASSE AS INDUCER OF GLUCOSE OXIDASE GENE IN A RECOMBINANT *ASPERGILLUS NIDULANS* STRAIN

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Introduction. Many filamentous fungi are widely used in the bio industry to produce homologous and heterologous enzymes with several industrial applications. In the search for an appropriate host expression system to produce high levels of proteins, recombinant fungal strains are usually constructed (1). The ascomycete fungus *Aspergillus nidulans* is a useful model organism to study metabolic regulations. Growth of fungal strains under controlled culture conditions in bioreactors is the main strategy to improve secreted protein yields. Hemicelluloses are composed by xylans, arabinans, galactans, glucans, and mannans. From these polysaccharides, xylans are the major hemicelluloses components of wood and agriculture residues. The bagasse fibbers are industrially used as a source of hemicellulose, as combustible in the sugar factories and as raw material in the production of cellulose pulp and paper.

In this work, the recombinant *A. nidulans* strain sVAL040 (2) was selected to produce heterologous glucose oxidase by using xylan extracted from sugarcane bagasse as the sole carbon source and to compare the resulting yields with those obtained when xylose was used as the inducer.

Metodology. The strain sVAL040 (*argB2/argB*$^+$, *metG1*, *biA1*, *xlnB*$_p$*::goxC*), carrying the *Aspergillus niger goxC* cDNA gene under control of *xlnB*$_p$ promotor was used for evaluate glucose oxidase production. Batch cultures were carried out in a LH S 210 fermentor with 1L of working volume. Temperature, pH, and agitation speed were automatically controlled. Biomass was first grown at pH 5.6 with 4 g/l of fructose, which was previously reported as a substrate that neither induces nor represses enzyme expression. After 20 h of growth, xylan (equivalent to 10 g/l of xylose) was added to induce the *xlnBp* at pH 4.5. Xylose content in the xylan from bagasse was determined by the phenol-sulphuric acid method (3). In order to compare the resulting enzyme induction with that obtained with xylose, the same procedure was carried out with 10 g/l xylose instead of xylan. Glucose oxidase activity was determined in the supernatant of the culture and was expressed as percentages of that observed with sVAL040 under xylose inducing conditions (5).

Results and Discussion. Expression of *xlnB*$_p$ is controlled by at least three mechanisms: specific induction by xylan or xylose, carbon catabolite repression and regulation by ambient pH (4,5). Xylose as well as xylan from bagasse, induced the expression of glucose oxidase with the recombinant strain sVAL040. Under the experimental conditions, the highest GOX activity values were obtained with xylan from bagasse. (Figure 1).

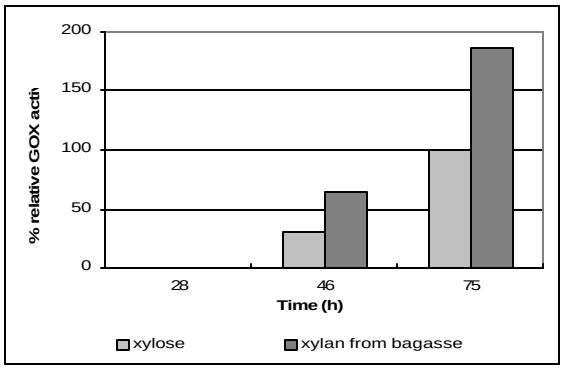

Figure 1: % Relative GOX activity with xylose and xylan from bagasse as inducers during the batch fermentation.

Conclusions Hemicellulose ranks after cellulose as the second most abundant group of renewable polysaccharides in nature. Sugarcane bagasse is an abundant by-product in our region. The possibility to use it for glucose oxidase production with the recombinant strain sVAL040 and the strategy of regulation of gene expression by ambient pH represents an interesting alternative for reducing the global cost of the industrial process.

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