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SHORT COMMUNICATION

Xylanase Production by a Newly Isolated *Aspergillus niger* SS7 in Submerged Culture

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Abstract

Xylanase production by a newly isolated *Aspergillus niger* SS7 was studied in submerged culture. The optimum initial pH for xylanase production was found to be 7.0. Different agricultural and industrial wastes were evaluated for their ability to induce xylanase production by this isolate. The best xylanase production (293.82 IU/ml) was recorded at 3% (w/v) corn cob hulls after 120 h of incubation. The *Aspergillus niger* SS7 isolate grown in a simple medium, proved to be a promising microorganism for xylanase production.

Key words: *Aspergillus niger*, submerged culture, xylanase

Xylan, the major constituent of hemicellulose is considered along with cellulose and chitin to be among the most abundant polysaccharides in nature with a high potential for degradation into useful end products (Saha, 2003). The xylan molecule consists of a β-1,4-linked D-xylose backbone and can be substituted by different side groups such as L-arabinose, D-galactose, acetyl, feruloyl, *p*-coumarouyl and glucuronic acid residues (Poutanen *et al*., 1987; Subramanyan and Prema, 2002). The most important enzyme in xylan degradation is endo b-1,4-D-xylanase (EC3.2.1.8), which hydrolyzes β-1,4-glycosidic linkages between xylopyranose units (Colins *et al*., 2005).

Xylanase has attracted considerable research interest because of its potential industrial applications. In animal feed, it increases the body weight gains (Medel *et al*., 2002). In bread making, it improves the desirable texture, loaf volume and shelf life of bread (Courtin and Delcour, 2002). In pulp and paper industry, xylanase is used in the prebleaching process to reduce the use of toxic chlorine chemicals (Wong *et al*., 2000). Due to their diversity, fungi have been recognized as a target for screening and as a source of new enzymes with useful and/or novel characteristics (Singh *et al*., 2003).

Considering the industrial importance of xylanase, the objective of this study was to evaluate the production of xylanase by a new *Aspergillus niger* SS7 isolate in submerged culture.

Aspergillus niger SS7 was isolated from soil collected from the garden of the agricultural college, Damascus University, Syria. It was identified by the Paris Natural History Museum (PNHM), Paris, France. Stock cultures were maintained on potato dextrose agar at 4C°.

Xylanase production by the new isolate (*Aspergillus niger* SS7) was carried out in Erlenmeyer flasks (250 ml) containing 50 ml of basal culture medium (g/l); 2.0, yeast extract; 2.0, peptone; 1.5, K_2HPO_4; 0.5, $MgSO_4 \times 7H_2O$. Fresh fungal spores have been used as inoculum and 1 ml spore suspension (10^5–10^6 spores/ml) was added to the sterilized medium and incubated at 30°C for 5 days in a rotary shaker (120 rpm). The filtrate obtained was assayed for enzyme activity.

Evaluation of xylanase production in the basal medium supplemented with different agricultural and industrial wastes (barley straw, wheat straw, wheat bran, corn hulls, olives pulp, apple pulp, sawdust) was determined.

Xylanase activity was measured by the method described by Bailey *et al.* (1992), using 1% birchwood xylan as substrate. The solution of xylan and the enzyme at an appropriate dilution were incubated at

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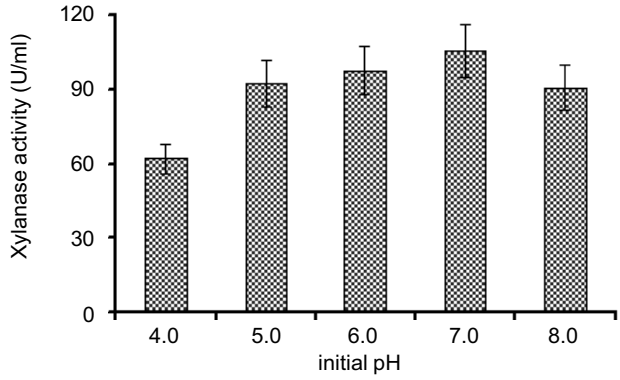

Fig. 1. Effect of different initial pH values on xylanase production by *Aspergillus niger* SS7.

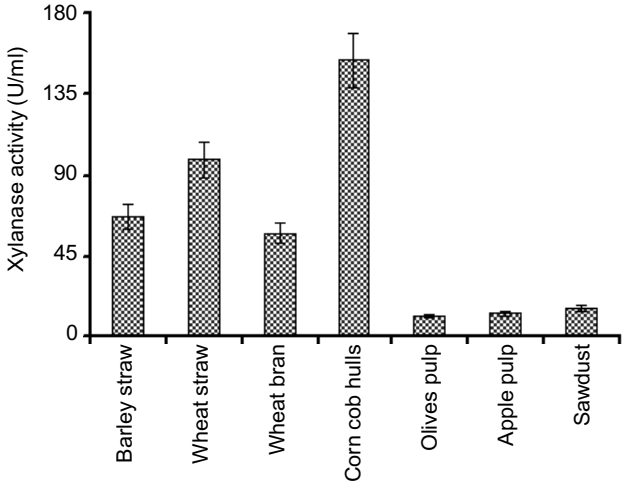

Fig. 2. Effect of different agricultural and industrial wastes at concentration of 1% on xylanase production by *Aspergillus niger* SS7.

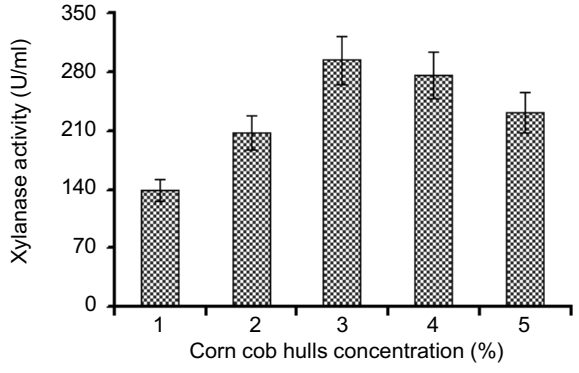

Fig. 3. Effect of corn cob hulls concentration (%) on xylanase production by *Aspergillus niger* SS7.

55°C for 5 minutes and the reducing sugars were determined by the dinitrosalicylic acid procedure (Miller, 1959), with xylose as a standard. The released xylose was measured spectrophotometrically at 540 nm. One unit (U) of enzyme activity is defined as the amount of enzyme releasing 1 µmol xylose/ml per minute under the described assay conditions. Results given are the mean of triplicate experiments.

Xylanase production has been shown to be markedly dependent on pH in several species such as *Trichoderma, Fusarium* and *Penicillium* (Silveira *et al*., 1999; Kuhad *et al*., 1998; Gaspar *et al*., 1997). The biosynthesis of xylanase by *Aspergillus niger* SS7 isolate was influenced by initial pH (4–8.0). The optimum initial pH for xylanase production was 7 (Fig. 1). These results are in agreement with those reported for other fungal species such as *Aspergillus nidulans*, 6.8 (Espinar *et al*., 1992); *Aspergillus fischeri*, 6.0–10.0 (Raj and Chandra, 1995); *Aspergillus versicolor*, 6.5 (Carmona *et al*., 1997). Cultivation of fungi at an unfavourable pH value may favour limited growth rate and xylanase production by reducing accessibility of the hemicellulosic substrate (Poorma and Prema, 2007).

The use of purified xylan as a substrate is uneconomical for large-scale production of xylanase (Seyis and Aksoz, 2005). Thus, we investigated the effect of some agricultural and industrial residues as carbon sources. These inexpensive and available agricultural and industrial residues offer cost effective substrate for xylanase production as reported by (Bakri *et al*., 2003; Poorma and Prema, 2006). Corn cob hulls have been proved to be the best agricultural and industrial waste sources (Fig. 2) and consequently used for further studies. The effect of corn cob hulls concentration was investigated in the range 1–5% (w/v) (Fig. 3). A significant increase in xylanase production was observed when the substrate concentration in the medium was increased from 1 to 5%. The highest titre (293.82 U/ml) was achieved with 3% (w/v) corn cobs hulls. Increasing the concentration further to 3% (w/v) resulted in a decrease in xylanase production. A similar situation has been encountered by other researchers using high concentration of lignocellulosic materials as substrates for enzyme production (Singh *et al*., 1995; Kuhad *et al*. 1998).

The results obtained from the submerged culture indicates that significant xylanase production from *Aspergillus niger* SS7 isolate could be achieved by selective use of nutrients and growth conditions. Since xylan is an expensive substrate for commercial scale production of xylanase, the possibility of using agricultural residues for xylanase production was investigated. Corn cob hulls could be used as a less expensive substrate for efficient xylanase production.

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