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Production of Xylanase on Natural Substrates by *Bacillus subtilis*

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ABSTRACT

Bacillus subtilis, locally isolated, produced significant level of xylanase activity in the presence of different carbon sources. However, *B. subtilis* performed well when grown on pretreated natural substrates such as rice straw, wheat straw, bagasse, wheat bran and kraft pulp at 2% concentration. The organism produced higher level of xylanasse activity when bagasse was used as a carbon source followed by rice straw, wheat straw, wheat bran and kraft pulp. Xylanase activity was enhanced when different supplements were added in the bagasse medium. The maximal induction was observed when medium supplemented with 0.2% sucrose.

Key Words: Xylanase; Production; Natural substrates; *Bacillus subtilis*

INTRODUCTION

There has been growing interest in xylanase production and its application because xylanase is important in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material. However, very useful application of xylanase is in the pulp and paper industry (Viikari *et al*., 1991; Bajpai & Bajpai, 1992). Different natural lignocellulasic materials were used as substrate for xylanase production rather than easily metabolizable carbohydrates. Alam *et al*. (1994) reported the production of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* on various agro-industrial wastes. Singh *et al*. (2000) reported the production of xylanase activity when *T. lanuginosus* strain was cultivated on a medium containing corn cobs as substrate. Elisashvili *et al*. (1999) reported that the synthesis of cellulasses and xylanases were induced when grown on medium containing crystalline cellulose and plant raw materials.

This paper reports the xylanase production by using lignocellulosic materials.

MATERIALS AND METHODS

Enzyme production. *B. subtilis* isolated from wheat straw compost was grown in minimal salt medium as described by Saleem *et al*. (1997). Different natural lignocellulosic materials e.g. rice straw, wheat straw, bagasse, kraft pulp and wheat bran were milled and sieved to 20 mesh size. The ground substrates were pretreated with 2% NaOH at room temperature for two hours followed by washing several times with water untill neutral and then dried in an oven at 500ºC to obtain a constant weight. *B. subtilis* was cultivated in medium having the composition in g/100 mL: magnesium sulphate 0.02, dipotassium hydrogen phosphate 0.05, potassium dihydrogen phosphate 0.05, calcium chloride 0.01, yeast extract 0.2. Two per cent alkali treated substrates were used as carbon source. The fermentation medium inoculated with *B. subtilis* was incubated in a Galenkamp Incubator Shaker at 500ºC and 180 rpm for 14 h and culture supernatant obtained after centrifugation of culture medium was used as a extracellular enzyme source.

Xylanase assay. To determine the xylanase activity, 0.5 mL of an appropriately diluted culture supernatant with 0.5ml of 1% solubilised birchwood xylan (Sigma Chemical Co. USA) in 0.05 M Mcllvaine bufferm pH 6.0, was incubated at 600ºC for 10 min. The reducing sugars liberated were estimated as xylose equivalents by DNS method (Ghose, 1987).

One unit of the enzyme activity is defined as the amount of enzyme that released one micromole of reducing sugars equivalent to xylose per minute under the assay conditions

Protein estimation. Protein in the culture supernatant was measured by dye binding method (Bradford, 1976) using bovine serum albumin as standard.

Effect of supplements. Effect of different supplements like Tween 80, Triton X–100, peptone, xylan and sucrose on xylanase production was studied by adding 0.2% each in the culture medium. Effect of sucrose was further studied by adding its different concentration (0.1-0.3%) in the fermentation medium.

RESULTS AND DISCUSSION

Effect of natural substrates on xylanase production. The effect of agro-industrial substrates on xlanase production was studied rather than using easily metabolizable carbohydrates. Table I shows the levels of xylanase production using various lignocellulosic materials. When *B.*

subtilis was grown in fermentation medium containing 0.2% pre-treated bagasse as carbon souce, 4.0 U/mL of xylanases activity was obtained after 14 h of fermentation at 50ºC (Fig. 1). Significant amounts of xylanase activity i,e. 3.2 and 3.5 U/mL were also produced after 14 h of fermentation at 50ºC when wheat straw and rice straw were used as carbon sources, respectively. However, lesser amount of enzyme activities were produced when wheat bran and kraft pulp were used as carbon source in the growth medium. *T. lanuginosus* SSBP, isolated from soil produced significant level of xylanase activity when grown in the presence of corn cobs as carbon source. On the contrary, lower levels of xylanase activity were produced after growth on other xylan substrates, sugars and soluble starch (Singh *et al.* 2000). Elisashvili *et al.* (1999) has also reported the induction of cellulase and xylanase activities when microorganism was grown in the media containg crystalline cellulose and plant raw materials. However, xylan was found to be less effective for xylanase production. Gaspar *et al.* (1997) had reported the best production of xylanase when *P. canescens* was grown in the presence of wheat straw and soya meal and the expression was repressed by glucose, xylose and lactose.

Table I. Effect of alkali treated lignocellulosic materials on xylanase production by *B. subtilis* after 14 hours of fermentation at 50ºC

Lignocellulosic substrates 0.5%	Soluble proteins (mg/mL)	Xylanase activity (U/mL)
Wheat straw	0.010	3.2
Rice straw	0.011	3.5
Bagasse	0.012	4.0
Wheat bran	0.010	2.0
Karaft pulp	0.012	2.0

Table II. Effect of different supplements on xylanase production

Supplements	Relative Xylanase activity
Unsupplemented	100%
Xylan suspension	118%
Tween 80	110%
Triton X 100	105%
Peptone solution	100%
Sucrose	125%

The reaction mixture containing 2.0% substrate and 0.2% supplements was incubated at 50C° for 14 hours.

To further optimize the bagasse medium, the effects of various supplements in combination with bagasse were studied as shown in Table II. Supplementation with peptone did not lead to improved enzyme production. However, Tween 80 had positive effect on xylanase production. 18% increase in xylanase activity observed when 0.2% xylan suspension was added in the medium. Alam *et al.* (1994) reported that *T. lanuginosus* and *T. aurantiacus* produced xylanase activity when grown on various lignocellulosic substrates, maximum xylanase production was obtained with wheat bran. Enzyme activity was enhanced (19%) when wheat bran medium supplemented with xylan and only 5% increase in enzyme activity was observed when Tween 80 was added in the medium.

The effect was more pronounced (25%) when bagasse medium was supplemented with 0.2% sucrose. However, a little increase in xylanase production was observed when sucrose was used at the concentration greater than 0.2% as shown in Fig. 2.

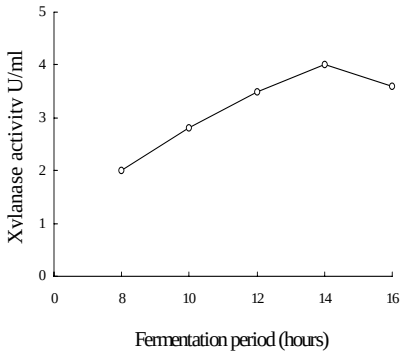

Fig. 1: Effect of fermentation period on the production of xylanase activity.

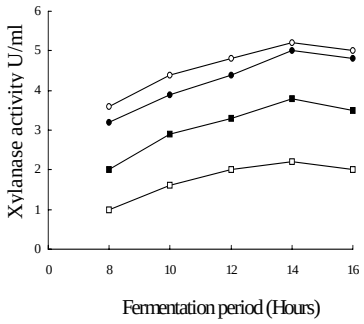

Fig. 2: Effect of different sucrose concentration on the production of xylanase activity.

0.05%, 0.1%, 0.2%, 0.3%

The results of our study conclude that bagasse which is inexpensive as well as abundantly produce higher amount of activity among the natural substrates can be used. Since pure commercial xylan is too expensive to be used as substrate suggest that bagasse may be a good alternative for xylanase production from industrial point of view.

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