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Production of thermo stable alkaline xylanase from *Bacillus* species

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ABSTRACT

The aim of the present study is to determine the effect of cultural conditions on the xylanase production from *Bacillus* sps isolated from soil contaminated with cotton mill industrial effluents of Chittoor, A.P., India, when grown on liquid media with rice bran,grass, corn cob and sugarcane baggage as carbon sources. Among the carbohydrates used in the study, glucose is found to be best inducer for xylanase production by *Bacillus* sps. Yeast extract and potassium dihydrogen phosphate were found to be best nitrogen and phosphorous sources for optimum xylanase production by the bacterial isolate at temperature 50°C with pH 9.

KEYWORDS

Bacillus species;
Thermo-stable alkaline xylanase;
Submerged.

INTRODUCTION

Xylanases are inducible enzymes which can hydrolyze xylans and xylo-oligosaccharides to D-xylose. Xylan is a major component of the cell walls of monocots and hardwoods, representing up to 35% of the dry weight of plants. Xylan is a complex polymer consisting of a β-D-1,4-linked xylopyranoside backbone substituted with acetyl, arabinosyl, and glucuronosyl side chains. Many bacterial and fungal species are able to utilize xylans as a carbon source. Xylanases have several applications it has been widely used in paper and pulp industries where breaks the hemicellulose chains that are responsible for the close adherence of lignin to the cellulose network. The use of xylanase a reduction in organo-chlorine pollutants such as dioxin from the paper making process that generates during chlorine based bleach. Addition of xylanase stimulates growth rates by improving digestibility, which also improves the quality of the animal litter. Xylanase thinsout the gut contents and allows increased nutrient absorption and diffusion of pancreatic enzymes in the digesta. It also changes hemicellulose to sugars so that nutrients formerly trapped within the cell walls are released. Addition of xylanase modifies the wheat flour arabinoxylans and can result in Making bread fluffier and keeping it fresh longer. Xylanase plays a crucial role in brewing industry where it can be able to break hemicellulose down into sugars and can also reduce the viscosity of the brewing liquid, improving its filterability. Treatment

of forages with xylanase (along with cellulase) results in better quality silage and improves the subsequent rate of plant cell wall digestion by ruminants. Xylanases improve the extraction of oil from oil-rich plant material such as corn oil from corn embryos. Xylanase production using xylan rich agro-residues such as wheat bran, eucalyptus kraft pulp, wheat straw, rice bran, rice straw, sugarcane bagasse and corn cob has been attempted by several workers using fungi[1,3,15,17], bacteria[2,4,18], actinomycetes[5,10,14] and yeasts[12]. These studies were performed with a thermophilic xylanolytic bacterial species producing thermo-stable xylanase (55°C), as thermostable xylanases are more relevant for industrial application[10,12], and also as xylanase production by thermophilic bacteria under SSF using corn cob has not been reported of the enzyme. Cultivation with alternate carbon nitrogen sources was also conducted

The objective of the present study was to investigate xylanase production by the local isolate *Bacilus sps* and to evaluate the thermo stability, alkalinity and optimization of enzyme on inexpensive materials.

EXPERIMENTAL

Materials

Oat spelt xylan (Sigma Chemicals Pvt. Ltd., India) was used for enzyme assay. Solid substrates viz. Rice bran (RB), Corn cob (CB), Grass (G), Sugar cane bagasse (SB) were obtained locally, and chopped into 1-2 cm particle size.

Microorganism

The bacterial strain used in this study was isolated from local industrial effluents, of Tirupati, Chittor dist, A.P.India and the strain was identified as *Bacillus cereus* by standard biochemical tests. The optimum growth temperature of this strain was 40°C.

Inoculum preparation

Inoculum was prepared by addition of distillted water to the nutrient agar slants, stored at 4°C and subcultured for further studies. For inolculum preparation the culture was grown at 40°C for 48h and was used to inoculate 50 ml of fermentation medium.

Fermentation medium

Two grams of finely chopped each solid substrate containing one gram of yeast extract and 2gms of bactopeptone (pH 8.0) were taken into 250 ml Erlenmeyer flasks and were autoclaved for 15min at 15 psi., cooled, inoculated with 10% (v/w) of inoculum (48 h old) and incubated at 45°C. At the desired intervals, the flasks were removed and the contents were extracted with 50 ml of 0.05 M sodium phosphate buffer (pH 7.0).

Enzyme extraction

Enzyme was extracted with 50 ml of 0.05M Sodium phosphate buffer (pH 7.0). It was centrifuged at 5000 rpm for 20 min. The clear supernatant was used for enzyme assay.

Xylan solution preparation

1% xylan solution was prepared in each of the above buffers and it was kept in boiling water bath for 5 mins. This solution was used as the substrate for carrying out the test.

Xylanase assay

Assays for crude xylanase were performed using 0.5% soluble oat spelt xylan (Sigma) in 50 mM sodium phosphate buffer, pH 7.0 with 1.8 ml substrate and 0.2 ml crude enzyme. The mixture was incubated at 60^{O} C for 15 min. The released reducing sugar was measured by the 3,5-dinitrosalicylic acid (DNS) methods[13]. The color developed was measured at 575 nm with xylose as standard. One unit (U) of xylanase activity is defined as the amount of enzyme that releases 1 umol xylose/min/ml under the above mentioned conditions.

Effect of pH on enzymatic activity

The effect of pH on enzyme activity was carried out at different pH (6.0-11.0) .The effect was studied in the following buffers: sodium-phosphate buffer, (pH 6.0, 7.0 and 8.0); glycine-NaOH, (pH 9.0 and pH 10.0) and carbonate-bicarbonate buffer (pH 11.0).

Effect of carbon source on xylanase production by *Bacillus* sps

Effect of various carbon sources on the xylanase production was assessed by culturing the bacterium in the production medium (pH 7.0-7.5) at 40°C with the supplementation of different carbon sources like corncob, sugarcane bagasse, rice bran and grass at 0.5%

TABLE 1 : Effect of substrate concentrations on xylanase production by *Bacillus* sps

Xylan concentration (%)	Enzyme activity (U/ml)
0.5	3.2
1	3.9
1.5	3.4
2	3.0

Values are presented in the table are mean of two separately conducted experiments

TABLE 2 : Effect of natural carbon sources on xylanase production by *Bacillus* sps

Natural carbon source	Enzyme activity (U/ml)
Grass	3.4
Corn cob	8.4
Sugarcane bagasse	9.8
Rice bran	8.2
control	3.9

Activity measured in terms of liberation of glucose/ml/h

TABLE 3 : Effect of Nitrogen sources on xylanase production by *Bacillus* sps

Nitrogen source	Enzyme activity (U/ml)
Urea	10.8
Peptone	10.1
Yeast extract	10.1
Control	9.8

Activity measured in terms of liberation of glucose/ml/h. Values are presented in the table are mean of two separately conducted experiments

concentration. Medium with Xylan at same concentration was used as control. Xylanase activity was measured by the method of Miller[13].

Effect of nitrogen sources on xylanase production by *Bacillus* sps

Effect of various nitrogen sources on the xylanase production was assessed by culturing the bacterium in the production medium (pH 7.0-7.5) at 40°C with the supplementation of different nitrogen sources like peptone, yeast extract and urea in the xylanase production medium. After 24 h enzyme activity was estimated.

RESULTS AND DISCUSSION

In the present investigation, xylanase production on commercial xylan and natural sources has been studied. Various parameters like substrate concentration, incubation time, temperature, pH and effect of carbon and nitrogen sources have been standardized to obtain highest yields of xylanase.

Further increase in substrate concentration above 1% was limiting factor for xylanase activity. It may be due to high substrate concentration in the reaction medium led to unfavorable for production of enzyme. The activity of xylanase was observed high (3.9IU/ml) in the medium with 1% xylan as substrate (TABLE 1). Similar results had also been employed for *streptomyces sps*[12].

TABLE 2 shows that replacement of xylan by natural 'C' sources in the production medium. The use of all these natural sources increased the enzyme activity. Of all the natural 'C' sources, the medium with sugar cane bagasse had induced 9.8U/ml, the maximum xylanase activity. Xylan rich crude fibrous biomaterials were also examined for obtain economical advantages. Corncob and Rice bran were able to induce 8.4 u/ml and 8.2U/ml respectively, where as xylanase production declined when grass was used as 'C' source. Similar levels of xylanase productin induced by crude fibrous biomaterials as well as xylan are also found for *B.coagulans*[7], *B.licheniformis* 77-2[8] and *G.thermoleovorans*[16]. In other end, purified xylan could induce more xylanase by Bacillus strain V1-4[19] than fibrous material could.

The effect of different nitrogen sources was investigated by replacement of $NaNO_3$ by biologically active 'N_2'sources like peptone, yeast extract and organic Urea to check the sutability for enzyme production by the present isolate. TABLE 3 explained the addition of all nitrogen sources tested led to an increase in the enzyme activity than compared to control. Of all, urea was found to be the best N_2 source for enzyme production, inducing significant levels of xylanase for 10.8u/ml. The above findings were also same in the studies of[6] where peptone, yeast extract and their combination could induce significant xylanase with 14.38u/ml. The results is opposite in the case of B. strain V1-4[19] with corn steep liquor as the best N_2 sources for enzyme production.

pH and temperature

The crude xylanse preparation exhibited its maximal activity at pH 9 using glycine-NaOH (Figure 1). Below or above pH 9 (6-10), using sodium phosphate buffer, and carbonate-bicarbonate buffer, had deterious

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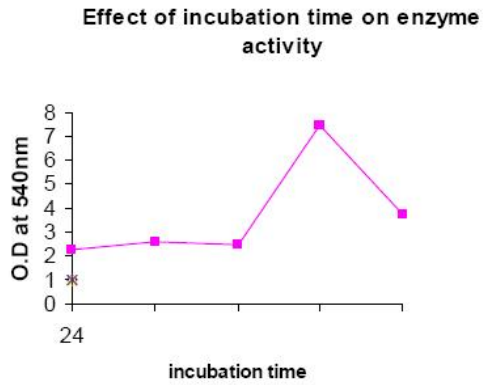

Figure 1 : Effect of incubation time on enzyme production by *Bacillus* sps

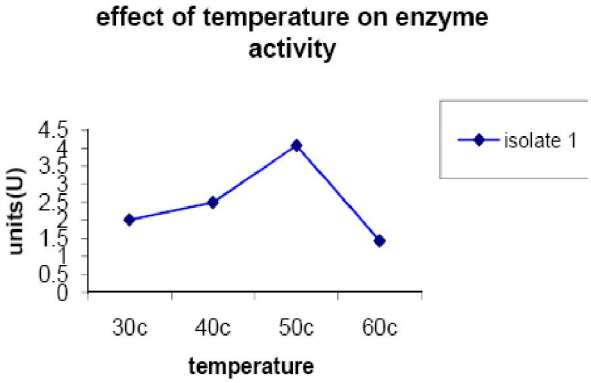

Figure 2 : Effect of temperature on enzyme production by *Bacillus* sps

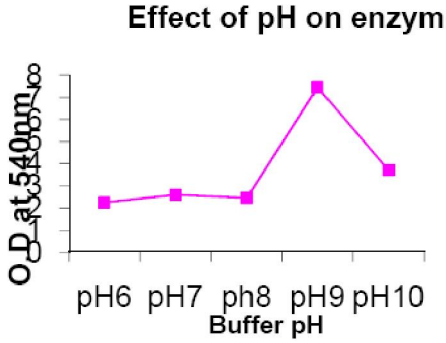

Figure 3 : Effect of pH on enzyme production by *Bacillus* sps

effect on enzyme activity. The relative xylanase activity at pH 8.0 was 7.4 U/ml and at pH 9 were 9.1%. Retained thermo stability from pH 7 to pH 9 was quite close to the Xylanase of *Geobacillus* sp.[16] and superior to the ones of *Paenibacillus* sp.[11], *Bacillus* sp.[6,9,19]. The incubation temperature range of 30 to 60ºC (Figure 2) favored xylanase production and the maximum yield was attained at 50ºC, denoting good thermostability and thermophilicity of xylanases.

CONCLUSION

The pulp and paper technology is one of the fastest growing industries and the use of thermostable xylanases seems attractive since they provide global environmental benefits. However, scaling up of the enzyme production from the respective microorganisms to the level required by the industry remains to be seen. It is also worth mentioning that, extreme thermophiles that are able to secrete xylanase are few. The search for a thermophile with high yield of enzyme and the desired characteristics is still being pursued[20]. On the other hand, thermostable xylanase were studied from a number of bacterial and fungal origins and in the majority of studies are found to be optimally active at, or near, mesophilic temperatures (approximately 40.0°C-60.0°C). Xylanases are also active at pH from 2.0 to 11.0[21]. The results obtained in the present study had an optimum pH of 9.0 and temperature of 50°C. The xylanases were stable for more than 60 min at50°C. The xylanases produced by *Bacillus* strains isolated in this study were stable over a wide range of temperature (40C-80°C). In addition, the cost of xylanases production will be greatly reduced with inexpensive agricultural substrates such as wheat bran, wheat straw and corncob.

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