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Xylanase Production Under Submerged Fermentation by Newly Isolated *Bacillus cereus* BSA1: Parametric Optimization of Cultural Conditions

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Extracellular xylanase production by the newly isolated *Bacillus cereus* BSA1 was optimized under submerged fermentation. The growth of bacteria and its enzyme production were increased upto 72 h and after that bacteria turned to its stationary phase but enzyme production reached its maximum level at 84 h. Incubation temperature of 35°C and initial medium pH of 6.0 were found to be optimum for xylanase production. Pure xylan induced higher amount of xylanase production in respect to other tested agricultural wastes. Maximum enzyme production by the organism was obtained with a xylan concentration of 0.5% (w/v). The xylanase production became repressed when readily available monosaccharides like glucose, xylose, arabinose (0.5% w/v) were added to the xylan containing medium. This article sounds for the first time of smart and successful production of xylanase from a mesophilic soil bacterium, *Bacillus cereus* BSA1.

Keywords: *Bacillus cereus*, xylanase, optimization, submerged fermentation.

Xylan, is a β, 1-4-linked polymer of xylose often with 4-*O*-methyl glucorunate and arabinose side chains and located in the cell walls of higher plants as a major hemicellulosic compound [1]. Xylanases are extracellular enzymes produced mainly by microorganisms and can catalyze hydrolysis of this complex polymer. A complete and efficient enzymatic hydrolysis of xylan depends mainly of two types of enzymes: endo 1-4-β-D xylohydrolases (EC 3.2.1.8) which hydrolyze the xylanopyranose of the central chain, and the β- xylosidases (EC 3.2.1.37) [2, 3, 4] which hydrolyze xylobiose and other xylooligosaccharides resulting from the action of endoxylanases. The major industrial uses of this enzyme are found in biopulping and biobleaching processes[5]. Other potential applications are bioconversion of lignocellulose materials to fermentative products (e.g. ethanol), clarification of vegetable and fruit juices, improvement of consistency of beer[6] and digestibility of animal feed stock [7].

Although fungi are the potent producers of xylanase[8], their large-scale production is often limited because of slow generation time and co-production of highly viscous polymers and other cellulolytic enzymes [9]. Xylanases are also being produced by many bacteria and production by several *Bacillus* species is noteworthy[10]. A number of extremophilic xylanase producers have been isolated in particular those from alkalophiles, acidophiles [11] and salt tolerant to meet

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the industrial demands. Xylanases are usually inducible enzyme, secreted by microbes in presence of pure xylan or xylan rich residues. Synthesis of the enzyme is regulated by several environmental factors such as the substrate concentration, composition of media, adjuncts of growth factors, pH, temperature, cultivation time etc.

Present communication deals with the isolation of a potent xylanase producing bacteria and optimization of the cultural conditions for its maximum biosynthesis of enzyme.

MATERIAL AND METHODS

Isolation of bacterial strain

The bacterium was isolated from the municipal garbage of Medinipur town, Paschim Medinipur, West Bengal, India on selective xylan-agar medium by dilution plate technique. The composition of the isolating medium was (g/l) $(NH_4)_2SO_4$, 1.0; $MgSO_4$, 0.2; K_2HPO_4, 0.2; $CaCl_2$, 0.2; $MnCl_2$, 0.02; yeast extract, 0.1, xylan, 10; agar, 15.0. Before sterilization, xylan was completely dissolved in water by sonication (at 7.0 hz for 2 min).

Xylan agar plate was generally look whitish and opaque in appearance. Xylanase producing bacterium was isolated on the basis of clear and transparent zone around the colony in xylan agar plate [12] and preserved in nutrient agar slant at 4°C for further study.

Production of xylanase

Enzyme production was made in 250 ml Erlenmeyer flasks containing 50 ml of liquid media having the identical composition as stated for isolating media except agar. The sterilized medium was inoculated with 1% (v/v) freshly prepared inoculum. Fermentation was carried out in a rotary shaker (120 rpm) at 35°C for desired time. The cell-free supernatant obtained after centrifugation (5000g×5 min) was used as the crude source of xylanase. Growth of the organism was determined by counting viable cell number through dilution plate technique and represented as colony forming unit (c.f.u.).

The optimization of fermentation process was done by altering one parameter at a time in order to get maximum enzymatic yield. Parameters like cultivation time (24 - 96 h), incubation temperature (25 - 50 °C), initial medium pH (3.0 - 10.0) and concentration of xylan and effect of some other carbon sources including the agro-residues were tested for maximum enzyme production. All the experiments were done in triplicate and data presented here as mean ± SE.

Enzyme assay

Xylanase activity was assayed by measuring the released reducing sugar[13] from the birch wood xylan (Fluka) after catalysis by xylanase. The reaction mixture containing 0.3 ml enzyme solution, 0.3ml of 1% (w/v) xylan and 0.4ml phosphate buffer (0.2M, pH 7.0). The reaction was carried out for 30 min at 50ºC and terminated by the addition of l.0 ml 3, 5 - dinitrosalicylic acid (3% w/v). The solution was boiled for 15 min for colour development and the absorbency was measured at 540nm (Systronics spectrophotometer 105, India) against the enzyme blank.

One unit of xylanase activity (U/ml) was defined as the amount of enzyme required to produce 1mmol of reducing sugars as xylose by hydrolyzing xylan per minute under the above assay conditions.

RESULTS AND DISCUSSION

Identification of the isolated strain

A potent xylanase producing bacterial strain has been isolated from the municipality garbage of Medinipur town. West Bengal, India. The identification of bacterial strain was made after studying various morphological and biochemical characteristics (Table 1) according to Bergey's Manual of Systematic Bacteriology [14] as *Bacillus cereus* BSA1 and it was further confirmed from MTCC (Microbial Type Culture Collection Center), Chandigarh, India. The soil bacterium plays a significant role in nutrient recycling and its xylan degrading ability by secretion of xylanolytic enzymes is reported here. The application of the xylanolytic enzyme in pulp pre-bleaching process is noteworthy, which can ultimately decrease the pulp and paper industrial waste mediated water pollution. True bacteria belong to genus *Bacillus,* actinomycetis and few fungi have been considered as predominant producers of hemicellulases [15].

Table 1. Characterization of *Bacillus cereus* BSA1

Parameters	Result
Gram Reaction	+
Shape	Rods
Endospore	+, Central
Flurosence (UV)	-
Motility	+
Growth at temperature	30-45°C
Growth at pH	3-9
Growth on NaCl (%)	10
Growth under Anaerobic Condition	+/-
Growth on Mac-Conky agar	-
Indole Test	-
Methyl Red Test	-
Voges Prokauer Test	+
Citrate Utilization	+
Casein hydrolysis	+
Starch hydrolysis	+
Urea hydrolysis	-
Nitrate reduction	+
Nitrite reduction	-
Cytochrome Oxidase Test	+
Catalase Test	+
Gelatin liquefaction	+
Acid Production from Carbohydrates	
Arabinose	-
Dextrose	+
Fructose	+/-
Galactose	-
Inositol	-
Lactose	-
Maltose	-
Mannitol;	-
Raffinose	-
Salicin	-
Sorbitol	-
Sucrose	-
Xylose	+
Cellibiose	-
Inulin	-
Rhamnose	-

Table 2. Effect of complex carbohydrate sources on xylanase production from *Bacillus cereus* BSA1 at 35°C and pH 6.0 under shaking (120 rev/min) conditions.

Source of Raw Xylan	Xylanase (U/ml)
Oat bran,	3.707 ± 0.350
Rice bran,	1.493 ± 0.558
Wheat bran	5.147 ± 0.237
Rice straw.	2.040 ± 0.548
Sugarcane bagasse	2.387 ± 0.185
Xylan	6.427 ± 0.173

Table 3. Effect of additional carbon sources on xylanase production from *Bacillus cereus* BSA1 at 35ºC and pH 6.0 under shaking (120 rev/min) conditions.

Carbon sources (0.5%, w/v)	Xylanase (U/ml)	
	In absence of xylan	In presence of xylan
Sucrose	0.02 ± 0.01	2.27 ± 0.21
Lactose	0.01 ± 0.01	1.04 ± 0.20
Cellulose	0.02 ± 0.00	3.14 ± 0.27
Xylose	0.06 ± 0.01	0.82 ± 0.19
Rhamnose	0.02 ± 0.01	0.70 ± 0.16
Mannitol	0.00 ± 0.00	2.52 ± 0.38
Raffinose	0.01 ± 0.00	2.14 ± 0.36
Maltose	0.01 ± 0.01	1.08 ± 0..37
Glucose	0.02 ± 0.01	0.69 ± 0.36
Galactose	0.02 ± 0.01	0.93 ± 0.31
Arabinose	0.02 ± 0.01	0.30 ± 0.01
Control	ND	6.02 ± 0.58

ND: Non detectable

Optimization of cultural conditions

The time course of xylanase production in relation to growth of *Bacillus cereus* BSA1 was studied for 96h (Fig. 1). Significant enzyme production was noted after 24h of fermentation and maximum enzyme production (5.59 ± 0.29 U/ml) was observed at 84h with the cell count of 4.96 ± 0.54 × 10^8/ml, when bacteria reached to its stationary phase of growth. The level of xylanase production by this newly isolated bacterium is comparable or higher than that reported for most of the mesophilic bacteria. Although a number of reports are available regarding the yields of xylanases, it is difficult to compare them, since the enzyme producing microorganisms, production conditions, substrate and assay conditions, and the way of defining the units vary greatly [2].

Xylanase production by *B. cereus* BSA1 was studied at various incubation temperatures (25°C-50°C) and represented in Fig. 2. The bacterium was able to grow at temperatures ranging from 30°C to 55°C but maximum enzyme production (6.05 ± 0.32 U/ml) was noted at 35°C. This type of effect was also seen in case of *Bacillus pumilus* [17], which is taxonomically very close to

arabinose and galactose were tested for xylanase production as in presence well as in absence of xylan in the basal media. These tested carbohydrates had no stimulatory effects on xylanase synthesis by *B. cereus* BSA1 (Table 3). This indicated that xylanase biosynthesis in this bacterium is not under the control of catabolic stimulation and it is only induced by the xylan or xylan containing carbon source. Our result is comparable with other *Bacillus* strains, where xylan showed a strong inducing effect [4, 19].

CONCLUSION

The newly isolated soil bacterium *Bacillus cereus* BSA1 is a potent xylanase producer, it is mesophilic and it has a wide range of pH tolerance. Hence it is qualified for use in biotechnological applications and it must be useful in biobleaching process in pulp and paper industry. However, further detail study on the biobleaching process needs to be carried out.

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