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Production of Xylanase From an Alkalitolerant *Streptomyces* sp. 7b Under Solid-State Fermentation, Its Purification, and Characterization

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Abstract *Streptomyces* sp. 7b showed highest xylanase activity among 41 bacterial isolates screened under submerged fermentation. The organism grew over broad pH (5–11) and temperatures range (25–55 °C) and displayed maximum xylanase production on wheat bran (1230 U/g) under solid-state fermentation. Xylanase production was enhanced substantially (76%–77%) by inclusion of trypton (2180 U/g) or beef extract (2170 U/g) and moderately (36%–46%) by yeast extract (1800 U/g) or soybean meal (1670 U/g). Inclusion of readily utilizable sugars such as glucose, maltose, fructose, lactose or xylose in the substrate repressed the xylanase production. The optimum initial pH of the medium for maximum enzyme production was 7 to 8; however, appreciable level of activity was obtained at pH 6 (1,680 U/g) and 9 (1,900 U/g). Most appropriate solid to liquid ratio for maximum xylanase production in solid-state fermentation was found to be 1:2.5. The organism produced a single xylanase of molecular weight of approximately 30 kDa as analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis after purification with ammonium sulfate precipitation, and carboxy methyl sephadex chromatography. The enzyme was purified to the extent of 5.68-fold by salt precipitation and ion-exchange chromatography. Optimum temperature and pH for maximum xylanase activity were 50 °C and 6, respectively.

Keywords Xylanase · Solid-state fermentation · *Streptomyces* sp. 7b · Wheat bran · Optimization · Purification

Introduction

Xylan, the major component of the hemicellulosic fraction of lignocellulosic materials, is a heterogeneous polysaccharide composed of a homopolymeric backbone of 1,4-linked-β-D-xylopyranose residues, which are substituted with acetyl groups, arabinose, and

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methylglucuronic acid residues [1]. Due to heterogeneity of structure, many different enzymes are required for complete hydrolysis of xylan, but two enzymes, endo-1, 4-β-xylanase (EC 3.2.1.8), and β-xylosidase (EC 3.2.1.37) are sufficient to break down the xylan backbone [2]. Xylanases have found immense applications in various industrial sectors such as in biopulping and biobleaching, food and feed, fruit and vegetable processing, lignocellulose bioconversion, detergent, and textile [3]. In pulp and paper industries, cellulase-free xylanases provides an environment-friendly alternative for chlorine-based toxic chemical bleaching compounds, and the process is less severe, economic and less toxic as compared with conventional chemical bleaching [3].

Xylanases have been reported to be produced by wide range of microorganisms including bacteria, yeast, and fungi [2, 4–6]. One of the major hurdles in industrial production of enzymes is the high cost of the substrate [7]; utilization of agricultural-based crude substrates for industrial production of enzymes particularly by means of solid-state fermentation (SSF) can play a crucial role in the reduction of overall cost of enzyme production. SSF has got renewed interest in recent years partly due to the huge potential of low-cost agricultural byproducts as substrates, which are available in quite abundance and can be successfully utilized for production of state-of-art high-value industrial products. Besides, SSF has got numerous advantages over submerged fermentation such as high productivity, lower capital and operational costs, lower energy requirements, among others [8]. Wheat bran, sugarcane bagasse, rice bran, and corn cobs are available in plentiful in several countries particularly where economy is agricultural-based like India and can be employed for production of biotechnology-based products of industrial importance [9]. There are many reports of microbial xylanase production by using agricultural wastes as substrates by submerged fermentation [5, 10, 11] and SSF [9, 12, 13]. However, relatively fewer studies have been conducted on xylanase production by actinomycetes, particularly in SSF using crude agricultural substrates [14, 15]. In the present study, xylanolytic *Streptomyces* sp. 7b was isolated from cow dung manure, and fermentation process was optimized for maximum production of xylanase using wheat bran as the substrate by SSF and the enzyme produced was subjected to purification and characterization.

Materials and Methods

Chemicals, Media, and Media Components

All the chemicals, media, and media components use in this study were procured from HiMedia Laboratories Ltd., Ranbaxy Fine Chemicals Ltd., Qualigens Fine Chemicals Ltd. (India) and Sigma Chemicals Ltd. and Merck and Co. Inc. (USA). Nutrient broth consisting of (wt/vol) beef extract (0.3%), peptone (0.5%), and sodium chloride (0.5%), pH 8–11, was used for initial enrichment of the organisms from the raw samples. Mineral salt solution (MSS; 100×), used as source of minerals in medium for cultivation of the organism, consisted of (wt/vol) NH_4NO_3 (1%), KH_2PO_4 (0.5%), $MgSO_4.7H_2O$ (0.1%), $CaCl_2.2H_2O$ (0.01%), NaCl (0.01%), and $MnSO_4.H_2O$ (0.01%). MSS used in the medium, while studying the effect of nitrogen source(s) on xylanase production, consisted of all the above-mentioned components except NH_4NO_3 (1%). Xylan agar (pH 8–11) used for initial screening of xylanase producing organisms by Congo red staining consisted of oat spelt xylan (0.5%, wt/vol), MSS (1.0%, vol/vol), and agar (2%, wt/vol). The same medium without agar was used for analyzing xylanase producing ability of microorganisms in submerged fermentation in shake flask. SSF was carried out in medium, which consisted of

either of the carbon source viz. wheat bran, rice bran, sugarcane bagasse, or saw dust (10 g); tris buffer, 50 mM, pH 8 (10 ml); and MSS, 10× (10 ml). Composition of xylanase production medium (XPM) used for studying effect of various parameters on xylanase production was wheat bran (10 g); nitrogen source, 2% solution in 50 mM tris buffer, pH 8.0 (10 ml); MSS 100× (1 ml) without NH_4NO_3 (1%), and tris buffer, 50 mM, pH 8 (9 ml).

Xylanolytic Microorganism and Submerged Fermentation

Samples of poultry waste, humus, soil under rice or wheat straw, cow dung manure, degrading rice husk, saw dust, paper industry waste, and others were inoculated into nutrient broth having pH 8–11 (1.0 g/100 ml), and incubated at 50 °C under shaking conditions (200 rpm). Enriched samples were withdrawn after different time intervals (24–72 h) and spread on xylan agar plates and plates were incubated at 50 °C for 48 h. The colonies appeared on xylan agar plates were subjected to Congo red staining [5]. Colonies showing clear halo around them were picked up, purified further by streaking on xylan agar. Purified isolates were picked up and maintained and subjected to xylanase production in submerged fermentation. Activated culture was inoculated in xylan broth (50 ml) and incubated at 50 °C under shaking conditions (200 rpm). Samples were withdrawn periodically after 24 through 96 h, and enzyme was assayed. A total of 41 bacterial isolates was screened for xylanase production in submerged fermentation, and finally, an isolate 7b, identified as *Streptomyces* sp. and designated as *Streptomyces* sp. 7b, was found to be the highest producer and selected for further studies.

Preparation of Crude Carbon Source

Crude carbon sources viz. wheat bran, rice bran, sugarcane bagasse, or saw dust were subjected to steam treatment by autoclaving them twice for 20 min each at 121 °C, before using them as substrate for xylanase production. Sugarcane bagasse was washed several times with distilled water, dried and crushed into powder by using blender, and then subjected to steam treatment.

SSF and Xylanase Extraction

Spores were harvested from 5-day-old culture of *Streptomyces* sp. 7b grown on xylan agar into 5 ml of tris buffer (50 mM, pH 8) containing a few drops of Tween-80. One milliliter of spore suspension (2×10^7 spores) was inoculated into XPM, mixed well and incubated at 50 °C with intermittent shaking for 72 h. For xylanase extraction after 72 h, 50 ml of tris buffer was added in the fermented medium and put on shaker for 1 h, and the contents were filtered through double-layered muslin cloth. To the residues, again 50 ml of tris buffer was added and again enzyme was extracted and filtered in the same way. Filtrate from both the extractions was mixed and centrifuged ($10{,}000\times g$ for 15 min) to remove suspended debris, and the supernatant was used as the source of enzyme.

Xylanase and Cellulase Assay

Xylanase activity was assayed by measuring the release of reducing sugars from 0.5% xylan solution prepared in tris buffer (50 mM, pH 8) by 3,5-dinitro-salicylic acid method [16] at 50 °C, using standard curve for xylose. One unit of xylanase activity was defined as the amount of enzyme producing 1 μmol xylose equivalents per minute under assay

conditions. Cellulase assay was conducted under same conditions except that the substrate used was Whatman filter paper, carboxy methylcellulose, or cellulose powder. One unit of cellulase activity was expressed as the amount of enzyme required to produce 1 μmol of glucose equivalent per minute under assay conditions. Protein estimation was carried out using Lowry's method [17]. Specific activity was expressed as the enzyme activity per milligram of protein.

Optimization of Medium Nitrogen Source

For studying the effect of different nitrogen sources on xylanase production, XPM (without NH_4NO_3) was used, which consisted of various nitrogen sources viz. urea, soybean meal, gelatin, beef extract, albumin, yeast extract, or tryptone. The medium was inoculated with the spore suspension, and SSF was carried out and enzyme was extracted and assayed as described earlier.

Effect of Different Additives

Different additives viz. glucose, maltose, lactose, xylose, xylan, fructose, Tween-80, or sodium dodecyl sulfate (SDS) were included in the XPM (9 ml of 2% solution in tris buffer, 50 mM, pH 8) for studying their effect on xylanase production.

Optimization of Inoculum Size

Different volumes of spore suspension (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ml), prepared from sporulated 5-day-old culture of *Streptomyces* sp. 7b having 2×10^7 spores/ml, were used to inoculate the XPM.

Optimization of Initial Medium pH

The initial pH of XPM containing trypton as the nitrogen source was adjusted to pH 5, 6, 7, 8, 9, and 10 using different buffers (50 mM) viz. acetate, phosphate, or tris buffer, and was inoculated with spores, and SSF was run at 50 °C with intermittent shaking for 72 h.

Effect of Moisture Level in SSF Medium on Xylanase Production

Effect of moisture level of medium on xylanase production was studied by varying the ratio of wheat bran and liquid solution (1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5) in XPM during SSF. Moisture level of the production medium was varied by using sterilized distilled water.

Xylanase Purification

Crude enzyme preparation obtained after extraction of enzyme from fermented medium was centrifuged at $10{,}000\times g$ for 10 min (Sigma 3K30 rotor 12150). The supernatant was subjected to ammonium sulfate precipitation of protein at different saturation levels (25%–75%). Salt-precipitated protein was dissolved in small quantity of tris buffer (50 mM, pH 8.0) and dialyzed overnight against the same buffer.

The dialyzed enzyme preparation was applied to ion-exchange column (20×1 cm) packed with carboxymethyl sephadex (CM-sephadex), which is preequilibrated with 10

volumes of 50 mM tris buffer (pH 8). The column was washed with 50 ml of tris buffer (pH 8) to remove the unbound protein components. Then elution was carried out by different salt concentrations ranging from 0.1 to 0.5 M NaCl, and different fractions were collected. Protein suspected to be still bound was eluted with 1 M NaCl solution. Fractions collected were tested for xylanase activity and the protein content.

Native and SDS-PAGE, Zymogram Study, and Molecular Weight Determination

Native and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by using method as described by Sambrook et al. [18]. SDS-PAGE was performed at 220 V for 45 min, while native page was performed at 175 V for 90 min. Acrylamide concentration in resolving gel and stacking gel was 12% and 5%, respectively. After casting and solidification of the gel, ammonium sulfate-precipitated fraction and protein purified by ion-exchange chromatography were loaded in appropriate wells with 5 μl of loading dye. The electrophoresis was performed for 1 h in tris glycine buffer (pH 8.3).

For zymogram study, the polyacrylamide gel was overlayered on 1.5% agar gel, preseeded with 0.5% xylan, and incubated for 1 h at 45 °C. After incubation, agar gel was stained with 0.1% Congo red for 15 min and then washed three times with 1 M NaCl for 15 min each, and the zymogram was observed for clear halos by placing it on the white illuminator. The polyacrylamide gel was dipped in fixative agent (ethanol/acetic acid/water, 30:10:60) for overnight. Then the gel was washed twice each with 30% ethanol and with deionized water and then stained with 1% $AgNO_3$ for 30 min in dark conditions with gentle shaking. The staining solution was then removed, and the gel was dipped in solution of 2.5% sodium carbonate and 0.02% formaldehyde for few minutes. The dark bands appeared within few minutes. The reaction was stopped by 1% acetic acid, and the gel was observed on white illuminator. SDS-PAGE was performed to determine the molecular weight of xylanase using standard molecular weight markers (Fermentas).

Effect of Temperature and pH on Enzyme Activity

For studying the effect of temperature on enzyme activity, the enzyme assay mixture was incubated at different temperatures viz. 30, 40, 50, 60, and 70 °C, and enzyme activity was determined by measuring the amount of reducing sugars released by the enzyme using 3,5-dinitro-salicylic acid method. For determining the effect of pH on enzyme activity, the enzyme was assayed using different buffers (50 mM) such as citrate, phosphate, or tris buffer, which have different pH (4.0–10.0).

To study the thermal stability, enzyme preparation was incubated at 50 °C for different time intervals (15, 30, 45, and 60 min), and residual activity was assayed.

All the analytical experiments were conducted in triplicates, and data presented here are the mean of three different experiments. The coefficient of variation was within 7%.

Results

Xylanolytic Microorganism and Initial Submerged Fermentation

Among 41 bacterial isolates screened for xylanase production on agar medium and in submerged fermentation, the isolate 7b showed the highest xylanase-producing ability and

was selected for further studies. The organism was studied morphologically, physiologically, and biochemically for its identification. On xylan agar, the organism formed discrete, smooth, slightly upright, leathery, initially off-white later turning gray colonies of approximately 8 to 10-mm diameter; reverse was off-white to slight brown with no diffusible pigments; mycelium was nonfragmented; aerial mycelium possessed chains of spores; and spores had smooth surface. The isolate 7b showed good growth over pH range of 5 to 11 and at temperature 25 to 55 °C. Organism strongly hydrolyzed starch and lipids, but could not hydrolyze gelatin and casein, and possessed L-diaminopimelic acid. Based on the criteria of Bergey's manual of systemic bacteriology [19], the organism 7b was keyed to the genus *Streptomyces* sp. and designated as *Streptomyces* sp. 7b.

Xylanase Production Under SSF and Cellulase Activity

Agriculture-based crude substrates like wheat bran, sugarcane bagasse, rice bran, and sawdust were subjected to SSF by *Streptomyces* sp. 7b after their pretreatment with steam. Maximum xylanase production was obtained in wheat bran containing medium (1,230 U/g) after 48 h of fermentation (Fig. 1). Xylanase production in rice bran containing medium was found to be 740 and 840 U/g after 24 and 48 h, respectively. Saw dust did not induce xylanase production at all, while sugarcane bagasse induced very low level of xylanase (200–225 U/g).

Enzyme extract obtained from production medium containing wheat bran as carbon source was assayed for cellulase activity. No cellulase activity was observed on cellulose powder at all. However, negligibly small activity was observed on carboxymethyl cellulose (3.1 U/g) and on filter paper solution (2.1 U/g).

Effect of Various Organic Nitrogen Sources on Xylanase Production

Xylanase production medium (XPM) was supplemented with different organic nitrogen sources such as urea, soybean meal, gelatin, beef extract, albumin, yeast extract, or tryptone, and SSF was carried out. All the nitrogen sources except urea and albumin, enhanced xylanase production as compared with that in control. Maximum improvement in xylanase titer was supported by tryptone (77%) and beef extract (76%), while yeast extract (46%) and soybean meal (36%) did so moderately; gelatin could enhance xylanase production only marginally (Fig. 2).

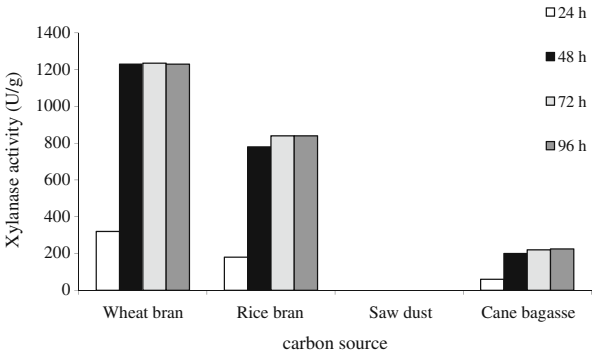

Fig. 1 Xylanase production by *Streptomyces* sp. 7b using different substrates under solid-state fermentation

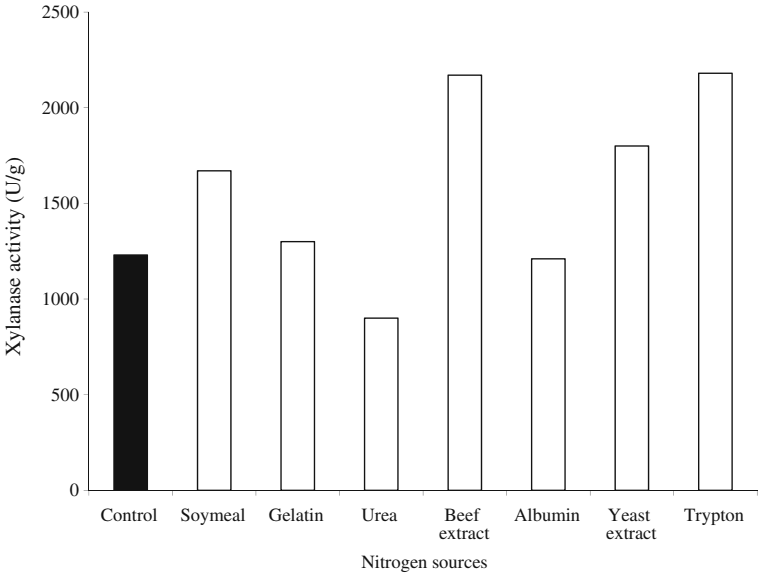

Fig. 2 Effect of different nitrogen sources on xylanase production. Ammonium nitrate of the fermentation medium was replaced with various organic nitrogen sources, and fermentation was conducted

Effect of Different Additives on Xylanase Production

Different additives viz. glucose, maltose, lactose, xylose, xylan, fructose, SDS, or Tween-80, were included in the XPM to analyze their effect on enzyme production in SSF. All the additives lead to reduction in xylanase titer as compared with that of control (Fig. 3), except xylan, addition of which caused a little increase in the xylanase production (7.33%). Xylanase production was more drastically reduced with the addition of SDS, Tween-80, lactose, and xylose as compared with the reduction caused by glucose, maltose, and fructose.

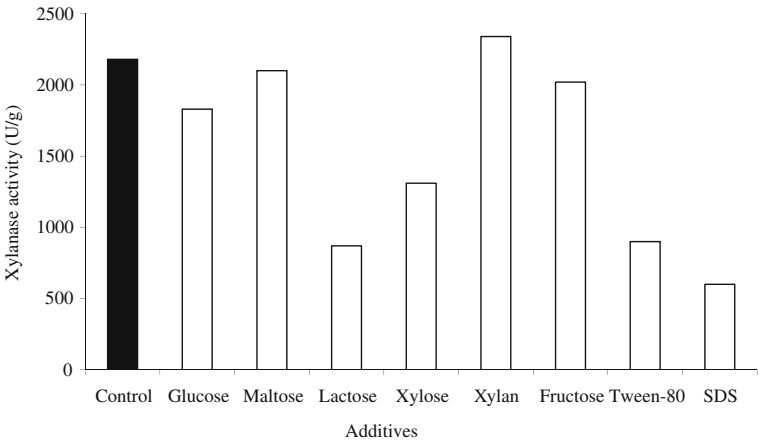

Fig. 3 Effect of supplementation of medium with different additives on xylanase production

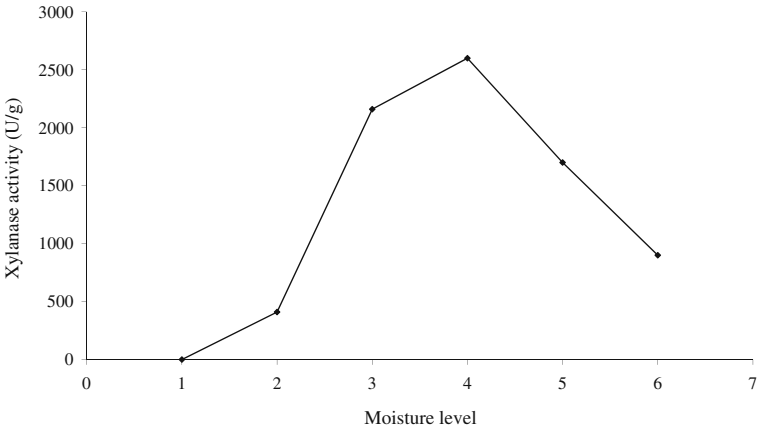

Fig. 4 Effect of moisture level of the medium of xylanase production. Numbers 1 to 6 on *x*-axis indicate solid/liquid ratio of 1:1,1:1.5, 1:2, 1:2.5, 1:3, and 1:3.5, respectively

Effect of Medium Moisture Level on Xylanase Production

Various ratios of solid substrate to moisturizing agent were tested, and highest titer of xylanase was obtained in medium having wheat bran to liquid solution ratio of 1:2.5 (Fig. 4).

Effect of Initial pH of the Medium on Xylanase Production

The organism was cultivated in XPM having wheat bran as the carbon source and tryptone as nitrogen source, adjusted at different pH levels (5, 6, 7, 8, 9, and 10) by using acetate, phosphate or tris buffer. Highest xylanase titer was obtained in production medium having pH 7 (2,620 U/g) and pH 8 (2,680 U/g) as shown in Fig. 5. However, considerable enzyme activity was found in production medium at pH 9 also (1,900 U/g).

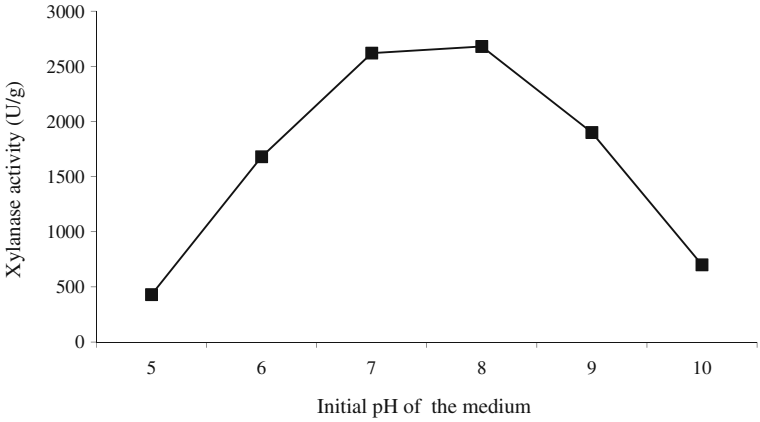

Fig. 5 Effect of initial pH of the medium on xylanase production

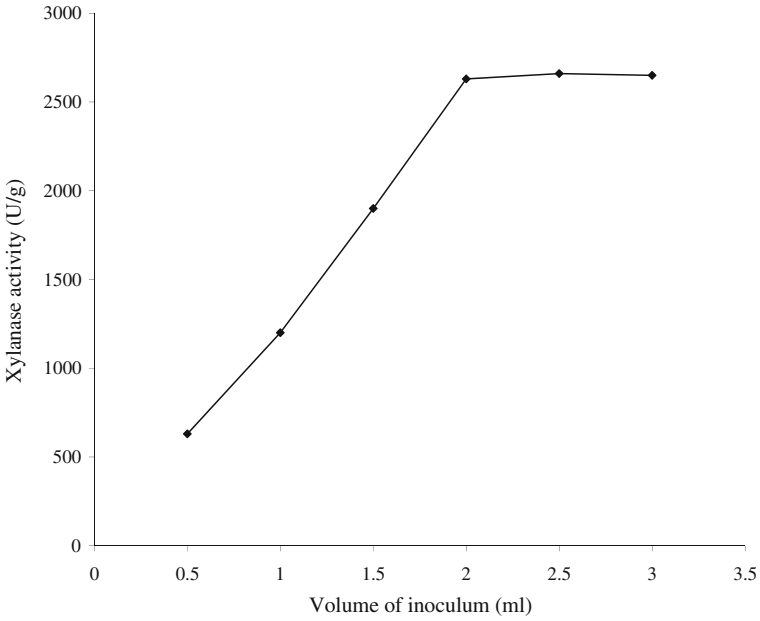

Fig. 6 Effect of inoculum size on xylanase production. The inoculum consisted of spore suspension having 2×10^7 spores/ml

Effect of Inoculum Size on Xylanase Production

Different volumes of spore suspension having 2×10^7 spores/ml were inoculated in the production medium to analyze the effect of inoculum size on xylanase production. Enzyme production increased linearly with the increase in size of the inoculum up to inoculation level of 2.0 ml (Fig. 6), and thereafter, a little or no increase in enzyme production was observed. Thus, inoculum level of 2.5 ml of spore suspension was considered to be the optimum for getting maximum xylanase titers (2,660 U/g).

Purification of Xylanase, Zymogram Analysis, and Molecular Weight Determination

Crude enzyme preparation was purified by 2.46-fold using ammonium sulfate precipitation (50%–75% saturation). Salt-precipitated enzyme preparation was dialyzed and purified further by ion-exchange chromatography using carboxymethyl sephadex (CM-sephadex), to the extent of 5.68-fold (Table 1). Specific activity of xylanase was increased with each

Table 1 Purification of xylanase from *Streptomyces* sp.7b.

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Purification fold	Recovery (%)
Crude enzyme	254	8,200	32.28	1	100
$(NH_4)_2SO_4$ precipitation (50%–75%)	49.35	3,920	79.43	2.46	47.80
CM-sephadex chromatography	19.4	3,560	183.50	5.68	43.41

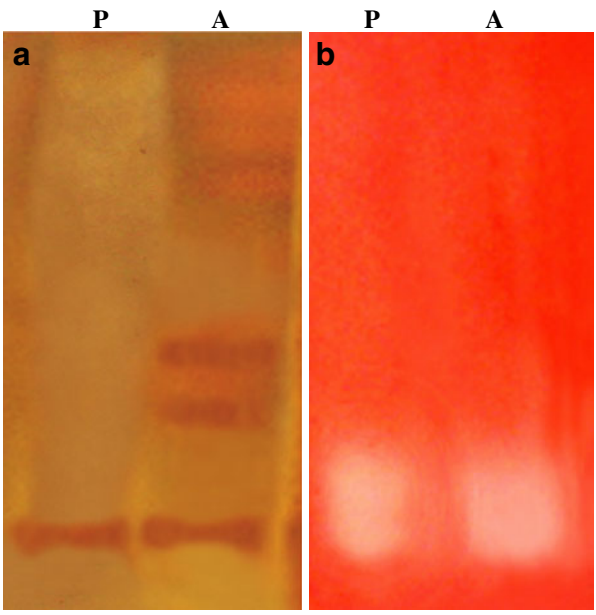

Fig. 7 Zymogram analysis of purified *Streptomyces* sp. 7b xylanase. *A* ammonium sulfate precipitated, *P* CM-sephadex-purified xylanase. **a** Native PAGE. **b** Zymogram

purification step and reached to a maximum of 183.50 U/mg of protein after ion-exchange chromatography as shown in Table 1.

In ammonium sulfate-fractionated xylanase preparation, there were three bands, but only one corresponded to xylanase as indicated by the zymogram analysis; however, in CM-sephadex-purified enzyme preparation, there was only single band as analyzed on native gel as well as on the zymogram (Fig. 7). SDS-PAGE analysis of CM-sephadex-purified preparation showed a single xylanase of molecular weight of approximate 30 kDa (Fig. 8).

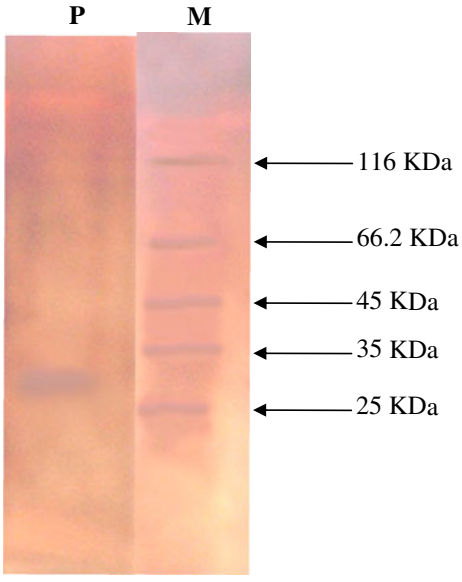

Fig. 8 SDS-PAGE analysis of *Streptomyces* sp. 7b xylanase. *P* CM-sephadex-purified xylanase, *M* standard molecular weight markers

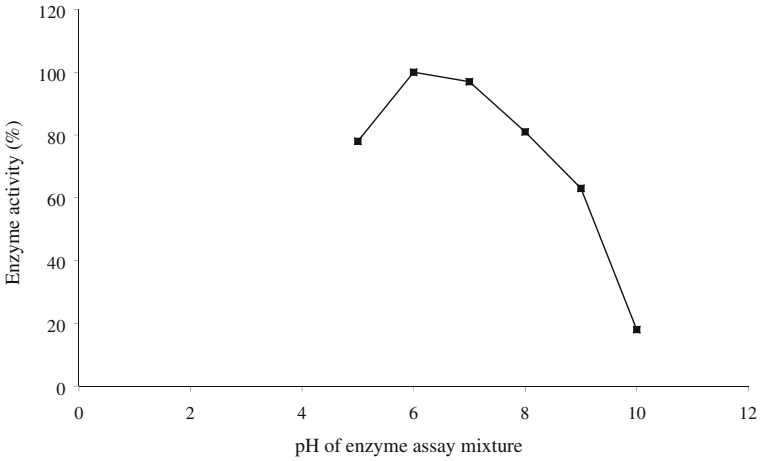

Fig. 9 Effect of pH on activity of *Streptomyces* sp. 7b xylanase. Xylanase assay was conducted at different pH using citrate, phosphate, and tris buffers

Characterization of Xylanase for Some Properties

The purified xylanase preparation was used for studying some properties of enzyme. For determining the effect of pH on enzyme activity, assay was done at different pH (5, 6, 7, 8, 9, and 10) using citrate, phosphate, and tris buffer at concentration of 50 mM. Highest xylanase activity was obtained at pH 6 to 7 (Fig. 9). Considerable amount of enzyme activity was present at pH 5 (78%), 8 (81%), and 9 (63%). However, at pH 10, the enzyme activity was decreased drastically.

Effect of temperature on xylanase activity was studied by assaying the activity at different temperatures viz. 30, 40, 50, 60, and 70 °C, by using water bath (Amersham Biosciences, Buckinghamshire, UK). Maximum xylanase activity was found at 50 °C (Fig. 10). The enzyme activity was reduced to 66%, 86%, and 73% of the maximum activity at 30, 40, and 60 °C, respectively. Enzyme lost its activity more drastically at 70 °C and retained only 10% of the initial activity.

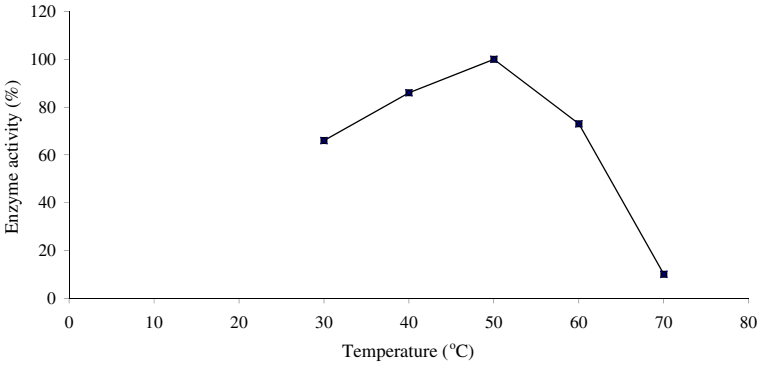

Fig. 10 Effect of temperature on xylanase activity. Xylanase assay was conducted at different temperatures at pH 7

For testing the thermostability of xylanase, enzyme was preincubated at 50 °C for time intervals of 15, 30, 45, and 60 min before using it for assaying the activity. The enzyme showed high stability for up to 30 min at 50 °C; however, after 45 min, enzyme activity was decreased to 76% of the maximum activity (Fig. 11). Further incubation caused more drastic reduction in enzyme activity, and after 60 min of incubation, the residual activity was just 10% of initial activity.

Discussion

Xylanases that are active and stable at high temperature and alkaline pH are desirable for many biotechnological applications including bleaching of kraft pulp [3, 4]. Organisms may be subjected to genetic engineering to make tailor-made xylanases, or natural diversity could be exploited to get the organism that produce industrially suitable xylanases. Submerged fermentation has largely been used, but SSF has numerous advantages [8], particularly by employing cost-effective crude carbon and nitrogen sources as substrates.

A moderately thermotolerant and alkalitolerant isolate *Streptomyces* sp. 7b was found to be a good producer of xylanase under SSF on crude substrates. Variation in xylanase production on different lignocellulosic materials may be due to differential accessibility of xylan backbone in different substrates [9]. There are many reports of xylanase production from wheat bran or other lignocellulosic materials under submerged and SSF [5, 12, 15, 20–26]. Rice bran and soybean residues served as excellent SSF substrates for xylanase production by *Bacillus* spp. [9, 13]. For bulk production of industrial enzymes, the cost of the substrate constitutes one of the most important factors in determining the overall economy of the process [7]. Agriculture-based wastes such as wheat bran, rice bran, hay, sugarcane bagasse, saw dust, wheat straw or rice straw, and others not only provide excellent low-cost substrates but also help combating environment pollution [13]. However,

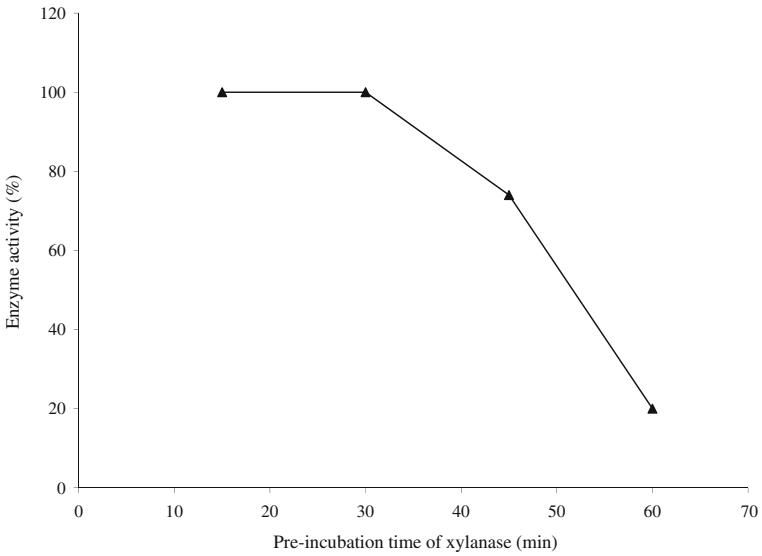

Fig. 11 Thermostability of xylanase at 50 °C. Xylanase was preincubated at 50 °C and then activity assay was conducted at different time intervals

these substrates are not easily degradable because of lignin and other complex components and therefore need pretreatment by alkali or steam [8, 9, 27], which results in breaking of side cross-linkages and make the xylan backbone more accessible to enzymes.

Streptomyces sp. 7b showed negligible cellulase production. Xylanase preparations intended to be employed in pulp and paper industries must be totally free from cellulase contamination, else the cellulose fibers may be damaged. However, such enzyme preparations could be successfully utilized in fruit and vegetable processing industries and in pretreatment of forage crops to improve the digestibility of ruminant feeds and to facilitate composting [3]. Totally cellulase-free xylanase production has been reported by many workers for different bacterial species including *Bacillus* and *Streptomyces* [15, 28, 29]. However, negligible contamination of xylanases with cellulase has also been documented [5, 30, 31].

We reported tryptone as the most efficient nitrogen source. Yeast extract, beef extract, peptone, or soybean meal has been reported to serve as good nitrogen sources for getting high yield of xylanase by different bacteria [5, 9, 22, 23, 32, 33]. However, maximum production of xylanase by *Streptomyces cuspidosporus* was reported when ammonium sulfate was used as the nitrogen source [15].

Inclusion of readily utilizable sugars (glucose, maltose, fructose, lactose, and xylose) in the medium leads to decreased production of xylanase by *Streptomyces* 7b, which may be due to catabolite repression [9]. We have reported that addition of detergents (SDS and Tween-80) in the medium caused reduction in enzyme yield, as the detergents might have affected the growth of the organism by causing cell lysis or might have caused changes in enzyme structure and conformation leading to reduction of activity [10]. However, contrary to our observations, xylanase production was reported to be doubled with the addition of Tween-80 in the culture medium [32]. Also, there are reports that indicate that SDS has no influence on xylanase activity from *Streptomyces* and *Bacillus* species [22, 34].

Proper moisture level, due to its ability to effect physical properties of the substrate, has profound influence on microbial growth and xylanase biosynthesis in SSF. Excess moisture causes decrease in porosity of the substrate, alters particle structure of the substrate, poses diffusional limitations, and leads to poor oxygen transfer, while low moisture content leads to poor solubility of the nutrients of the substrate, less swelling, and higher water retention [8, 27]. *Streptomyces* sp. 7b produced maximum xylanase at wheat bran to liquid solution ratio of 1:2.5. *Bacillus* sp. JB-99 displayed maximum xylanase production at moisture levels of 1:2 (wt/vol) [9], while *Aspergillus terreus* produced maximum xylanase when moisture level of 1:3 (wt/vol) was used [35]. The optimum moisture level in SSF depends on the nature of the substrate, the requirement of the microorganism, and type of end-products [8].

The pH of the medium can influence the enzyme production in two ways: one, it can affect the growth of the organism, and hence, the enzyme production would be effected; second, the produced enzyme may be subjected to inactivation or denaturation by unfavorable ionic environment caused by pH of the medium. We reported medium pH of 7 to 8 as the optimum for maximum xylanase production. Similar to our results, maximum xylanase production at pH 6 to 8 has been reported for different bacterial species including *Bacillus* and *Streptomyces* [5, 15, 20, 22, 32, 33]. However, *Thermoactinomyces thallophillous* subgroup C and *Bacillus* sp. JB-99 have been found to produce maximum xylanase at highly alkaline pH 9 to 10 [9, 28].

Optimization of inoculum size is necessary for maximum production of product, as high biomass consumes extra substrate and may produce more toxic compounds, while the low biomass leads to reduced yield of the products. We reported optimum inoculum size of

2.0 ml (2×10^7 spores/ml) for maximum xylanase production. However, an inoculum containing 5.0 ml of spore suspension (10^6 spores/ml) of *S. cuspidosporus* was documented to be the best for maximum xylanase production in wheat bran medium [15].

The organism *Streptomyces* sp. 7b is alkalitolerant and can grow at higher pH (10–11), but nonetheless, its xylanase shows maximum activity at pH 6 to 7; however, substantial activity was retained even at high alkaline pH (8–9). Optimum activity for most of the *Streptomyces* spp. xylanases has been reported at pH 5 to 7 [10, 11, 15, 20]. However, optimum pH of 8 to 10 has also been reported for xylanases from different *Streptomyces* and *Bacillus* species [5, 9, 13]. Although *Streptomyces* sp. 7b xylanase showed maximum activity at 50 °C but retained sufficiently high activity at 60 °C, most of the microbial xylanases are known to be optimally active in the range of 50 to 65 °C [5, 15, 20, 22, 32]. However, xylanases with temperature optima of 70 to 75 °C or higher have also been reported [9, 10, 13, 26]. *Streptomyces* sp. 7b xylanase was not found highly thermostable, but nonetheless, enzyme showed high stability at 50 °C for 30 to 45 min. Thermostable xylanases are of prime importance in different industries, particularly in pulp and paper where temperature reaches more than 90 °C. Varying thermostabilities of xylanases from different *Bacillus* and *Streptomyces* sp. have been reported by various researchers [5, 20, 22].

Conclusions

Streptomyces sp. 7b successfully utilized wheat bran as substrate and performed well in solid-state culture. Tryptone and beef extract enhanced the xylanase yield by 76% to 77%, while readily utilizable sugars repressed xylanase production. Furthermore, the organism is thermotolerant and alkalitolerant and capable of growing and carrying out fermentation at high alkaline pH and temperature; it would be interesting to study the organism and the enzyme further for potential commercial applications.

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