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PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (3): 372-379, 2004
ISSN 1028-8880


# Screening, Purification and Characterization of Xylanase from *Paenibacillus* sp.

Narayan Roy and A.T.M. Salah Uddin
Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh

**Abstract:** Xylan the major portion of the hemicellulose of plant cell walls are heterogeneous polysaccharides. Xylanases are enzymes obtained from different species of microoragnisms that degrade the xylosidic linkages of xylans backbone producing xylose with other monoresidues. Five Xylanase-producing strains ($St_1$, $St_2$ and $So_1$, $So_2$, $So_3$) were isolated from soil and water at Rajshahi region. The strains were isolated on xylan agar media and screened by β-xylanolysis method. Zymogram analysis was confirmed the xylanolytic activity. The isolated xylanase-producing strains were further tested by DNS (Dinitrosalisylic acid) method to confirm their xylanase activity. Five of the isolated strains were identified as *Paenibacillus* on the basis of catalase, oxidase, gram staining and morphological cultural characteristics as well as by different biochemical test. The strains excrete xylanase enzyme extracellularly in the media. Xylanase enzyme was purified from culture supernatant of *Paenibacillus* sp. by ammonium sulfate precipitation, gel filtration on Sephadex G-100 followed by ion-exchange chromatography on DEAE. The purified enzyme gave single band on SDS-polyacrylamide gel electrophoresis indicating its homogeneity. The molecular weight of purified xylanase was determined by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. The molecular weight of the purified xylanase was 48 kDa. The optimum temperature and pH of the purified enzyme was 55°C and pH 7.0, respectively. The 30 $\mu g^{-1}$ $disc^{-1}$ of the ethyl acetate extract of the cultural broth of *Paenibacillus* showed no antibacterial activity against the tested pathogenic bacteria but showed poor antibacterial activity at the concentration of 200 $\mu g^{-1}$ $disc^{-1}$ compared to those of standard kanamycin (30 $\mu g^{-1}$ $disc^{-1}$).

**Key words:** *Aeromonas* sp., xylanase, zymogram, $K_m$

## INTRODUCTION

Hemicellulose is the second most abundant renewable polysaccharide in nature after cellulose[1]. Xylan is the main constituent of hemicellulose and has a backbone of β-1,4-linked D-xylopyranoside residues substituted with acetyl, arabinosyl and uronyl side chains[2]. Complete breakdown of xylan requires the action of several hydrolytic enzymes[3], the most important of which is the endo-1,4-β-D-xylanase (EC 3. 2. 1. 8). Xylanases have been widely detected in bacteria and fungi and have been well characterized for their properties. Some of the xylanases have been characterized extensively with respect to their physicochemical, hydrolytic and molecular properties. Xylanases have considerable potential in several biotechnological applications. Commercial applications suggested for xylanases involve the conversion of xylan, which is present in wastes from agricultural and food industry, into xylose[4]. Similarly, xylanases could be used for the clarification of juices, for the extraction of coffee, plant oils and starch[5] and for the production of fuel and chemical feedstocks[6]. Recently, the use of xylanolytic enzymes in pulp bleaching has been considered as one of the most important new biotechnological applications of these enzymes. Due to industrial application of xylanase, our laboratory started to investigate xylanase from micriorganism. Xylanase occur widely in bacteria, yeasts and fungi. Many reports on xylanase from *Bacillus* sp., *Clostridium* sp., *Streptomyces* sp., *Aspergillus* sp., *Trichoderma* sp. and other microorganisms are available[5]. As far our knowledge, only very few report was published about xylanase from *Paenibacillus* sp. So, our special interest was xylanase from *Paenibacillus* sp. Recently, Tomomi Sumida[7] reported the molecular cloning and characterization of a glucocerebrosidase from *Paenibacillus* sp. This study showed the screening and physicochemical characterization of xylanase producing bacteria from soil and water which degraded oat spelt xylan and belong to *Paneibacillus* genus. This study also showed the isolation and some properties of xylanase from this new strain.

## MATERIALS AND METHODS

**Bacterial sample collection:** For the screening of xylanase-producing bacteria soil and water were collected

**Corresponding Author:** Naraya Roy, Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh, Tel: 750041/4109, Fax: 88-0721-750064 E-mail: nroy_64@yahoo.com

from the Rajshahi University Campus, Maharchandi and Benodpur village of Rajshahi.

**Screening of the xylanase-producing bacteria:** 0.2 gm of soil and water were dissolved in 100 ml sterile distilled water in two separate 100 ml volumetric flask and shaked vigorously. 0.2 ml of diluted sample solution was then spread on two different xylan agar plates for isolation and rapid identification of the xylanase-producing bacteria from their colonical growth as clear one and xylanolytic properties. The plates were then incubated at 37°C for 48 h.

**Zymogram analysis:** Ramozol Brilliant Blue (R. B. B) stained xylan was added in the media in place of oat spelt xylan to observe bacterial colonical growth as clear zone and xylanolytic activities. The plates were incubated at 37°C for 48 h.

**Isolation and characterization of bacteria:** All the xylanase-producing bacterial strains were isolated by their growth on xylan agar media as clear zone and xylanolytic properties were characterized according to the biochemical tests described in the "Bergey's Manual of Determinative Bacteriology.

**Biochemical tests used to characterize the isolated strains**

**Catalase test:** Catalase is an enzyme generally found in aerobes. Catalase test performed to determine the ability of the organism to liberate gas ($O_2$) from hydrogen peroxide ($H_2O_2$) by enzymatic (catalase) degradation.

**Oxidase test:** In oxidase test, the redox dye, tetramethyl-para-phenyline-diamine-dihydrochloride was reduced to a deep purple colour by the microorganism.

**Citrate utilization test:** The test organism was cultured in a medium which contains sodium acetate, an ammonium salt and the indicator bromophenol blue.

**Fermentation test:** This test is used to differentiate the organisms that ferment carbohydrate (such as mannitol, lactose). Oxidation fermentation medium is used in the fermentation test of carbohydrates.

**Lactose fermentation:** Lactose fermentation test was performed according to the procedure as described in fermentation test using 10% (w/v) sterile lactose solution.

**Indole test:** This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Indole is then tested by a calorimetric reaction with p-dimethyl-amino benzaldehyde.

**Preparation of crude enzyme extract:** A single colony of the isolated bacteria was inoculated into the xylan broth media in the conical flask by sterile loop and incubated at 37°C for 48 h with shaking. When the strain grown vastly, this culture was transferred into centrifuge tubes and the clear supernatant was collected after centrifugation at 8000×g for 15 min at 4°C. This supernatant was then used for the further experiment as the crude enzyme (xylanase) extract.

**Purification steps of xylanase**

**Treatment with ammonium sulfate:** Solid ammonium sulfate was added to the crude enzyme solution to give 80% saturation at 0°C. The mixture was stirred for 30 min and the resulting precipitates were collected at 8000 Xg for 15 min at 4°C. The precipitate was collected and dissolved in minimum volume distilled water and dialyzed against 50 mM sodium phosphate buffer pH 7.0 for 24 h at 4°C.

**Gel filtration:** The crude extract was after dialysis was applied to Sephadex G-100 column (1.2X50 cm) which was equilibrated with 50 mM sodium phosphate buffer at pH 7.0 containing 1M NaCl. The column was washed with 250 ml of the same buffer at a flow rate of 15 $ml^{-1}$ $h^{-1}$. 1.5 ml fractions were collected. The enzyme passed through the column and separated with three peak.

**DEAE-cellulose chromatography:** The active fraction from Sephadex G-100 column chromatography were pooled, concentrated and dialyzed against 50 mM sodium phosphate buffer, pH 7.0 and then put on a DEAE-cellulose column (4.0X20 cm) equilibrated with 50 mM sodium phosphate buffer at pH 7.0. The column was washed by five bed volumes of the same buffer and then elution was done with a step wise of sodium phosphate buffer, pH 7.0, from 0 M to 0.2 M. Xylanase activity was detected in the second peak, which was eluted at about 0.1 M sodium phosphate buffer, pH 7.0.

**Assay for xylanase activity:** Xylanase activity was assayed by measuring the amount of reducing sugars liberated from oat spelt xylan in 50 mM sodium phosphate buffer pH 7.0. The reducing sugars formed were measured by the Dinitro-salisylic acid (DNS) method[8]. One unit of xylanase was defined as the amount of enzyme which liberated 1 μmole of xylose per minute.

**Determination of protein concentration:** Protein concentration was determined following the method of Lowry[9] using BSA as the standard.

**Electrophoresis:** Protein-containing fractions were analyzed by Slab electrophoresis with SDS polyacrylamide gel as described by Laemmli method[10]. The stacking and resolving gels were 5 and 12% acrylamide, respectively. Proteins were stained with Coomassie brilliant blue R-250.

**Characterization of enzyme**

**Determination of molecular weight:** The molecular weight of the purified xylanase was determined by slab electrophoresis with SDS polyacrylamide gel.

**Determination of effect of temperature on the activity of xylanase:** The activity of the purified xylanase enzyme obtained from the bacteria of strain no. $St_1$ was determined at different temperature ranging from 20 to 80° C.

**Determination of effect of pH on the activity of xylanase:** The activity of purified xylanase obtained from strain no. $St_1$ was determined at different pH values (50 mM sodium acetate buffer at pH 4.0 to 5.5, 50 mM sodium phosphate buffer at pH 6.0 to 8.0 and 50 mM Tris-HCl buffer at pH 8.5 to 9.0) ranging from 4.0 to 9.0.

**Determination of $K_m$ value of xylanase:** The initial velocity is equal to the amount of product formed per unit time. The initial velocity (Vi) is determined by quantitatively measuring the amount of one of the products at various time intervals[11].

**Determination of substrate specificity on xylanase activity:** To determine the substrate specificity of xylanase towards substrates including birch wood xylan, cellulose, carboxy methyl-cellulose, starch were used as substrates in the assay instead of oat spelt xylan.

**Test of antibacterial activity:** Five pathogenic bacteria were selected for the antibacterial studies, three of which were Gram negative and the other two were Gram positive.

**Preparation of the test plates:** The 15 ml of nutrient agar medium was poured in the test tubes and plugged with cotton under the laminar airflow. The test tubes were sterilized and allowed to cool to about 40° C. By means of a sterile inoculating loop, the culture of the test organisms, were inoculated in the medium to the test tube and was agitated to ensure uniform dispersion of the organism into the medium. The medium was poured into the sterile petridish and agitated clockwise, anticlockwise, left to right, right to left for uniform dispersion.

**Preparation of the sample discs:** Solution of the ethyl acetate extract of the cultural broth of *Paenibacillus* bacteria was prepared in ethyl acetate such a manner so that 10 µl contained 50 µg of the compound. Filter paper discs were taken in a petridish and sterilized in oven at 110° C for 1 h. By means of a micropipette 6 and 40 µl of the solution were placed on the discs. Thus discs containing 30 and 200 µg of the compounds were prepared.

**Preparation of standard discs:** Prepared kanamycin (K) disc containing 30 $\mu g^{-1}$ $disc^{-1}$ of kanamycin compound was used as standard disc.

**Measurement of the zone of inhibition:** After incubation, the antibacterial activities of the test samples were determined by measuring the diameter of the zone of inhibition in term of mm with a transparent scale.

## RESULTS

**Isolation and characterization of xylanase–producing bacteria:** For a preliminary experiment of the study five bacterial samples were collected from soil and straw ($St_1$ $St_2$ and $So_1$ $So_2$ $So_3$) as a source of xylanolytic organisms. β-Xylanolytic clear and transparent zone (on xylan agar plate) producing bacterial strains were collected by spreading diluted sample solution on different xylan agar plates. The β-xylanolysis was observed (only the strain $St_1$) on the xylan agar plate after 36 to 48 h' incubation at 37°C (Fig. 1). On xylan agar plate, the colonies were circular, transparent and the color of the colonies was white. The β-xylanolytic bacterial strains were collected and were then further purified by pure culture technique. For more confirmation of xylanolytic activities, Ramozol Brilliant Blue (R.B.B) Stained xylan was added in the media in place of oat spelt xylan and the clear zone was formed in Fig. 2. Isolated bacterial colonies from soil and water were primarily screened by staining method, microscopic examination, oxidase and catalase test. The isolated strains exhibited positive oxidase test because it produced dark purple color with tetramethyl-para-phenyline-diamine-dihydrochloride by enzymatic (oxidase) degradation. The isolated strains exhibited positive catalase test because it liberated gas ($O_2$) from hydrogen peroxide ($H_2O_2$) by enzymatic degradation.

After primary isolation, several other morphological and biochemical tests were performed to characterize the isolated bacteria. It was found that, five strains were

Table 1: Biochemical tests for the identification of 5 collected strains

| Sample No. | Motility test | Urease test | Citrate utilization test | Indole test | Xylanase activity test | Fermentation test | | Comments |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Mannitol | Lactose | |
| $St_1$ | + | - | - | + | + | + | + | *Paenibacillus* |
| $St_2$ | + | - | - | + | + | + | + | *Paenibacillus* |
| $So_1$ | + | - | - | + | + | + | + | Paenibacillus |
| $So_2$ | + | - | - | + | + | + | + | Paenibacillus |
| $So_3$ | + | - | - | + | + | + | + | Paenibacillus |

Symbols: + = Positive test - = Negative test

Table 2: Summary of purification of xylanase from the bacteria of strain no $St_1$

| Steps | Total protein (mg) | Total activity (units) | Specific activity (unit/mg) | Yield (%) | Purification fold |
|---|---|---|---|---|---|
| Crude extract | 425 | 700 | 1.64 | 100.00 | 1.00 |
| Ammonium sulfate saturated | 200 | 500 | 2.50 | 71.42 | 1.52 |
| After gel filtration on Sephadex G-100 | 6.5 | 400 | 61.53 | 57.14 | 37.51 |
| DEAE-cellulose ion exchange chromatography | 1.5 | 130 | 86.66 | 18.57 | 52.84 |

Table 3: Substrate specificity of xylanase

| Substrate | Relative hydrolysis activity (%) |
|---|---|
| Oat spelt xylan | 90 |
| Birch wood xylan | 85 |
| Cellulose | 0 |
| Carboxymethyl cellulose | 0 |
| Starch | 0 |

Table 4: Antibacterial activity of the ethyl acetate extract of the cultural broth of *Paenibacillus* bacteria and Kanamycin standard

| Test organisms | Strain No. | Diameter of zone of inhibition (in mm) | | |
|---|---|---|---|---|
| | | EA 30 µg $disc^{-1}$ | EA 200 µg $disc^{-1}$ | Kanamycin 30 µg $disc^{-1}$ |
| Gram positive | | | | |
| *Bacillus subtilis* | QL-40 | 0 | 13 | 25 |
| Streptococcu S-β-haemolyticus | CRL | 0 | 12 | 26 |
| Gram negative | | | | |
| *Shigella dysenteriae* | AL-35587 | 0 | 11 | 25 |
| *Escherichia coli* | FPFC-1407 | 0 | 12 | 27 |
| *Pseudomonas aeruginosa* | CRL | 0 | 10 | 26 |

Table 5: Effect of carbon source on xylanase production

| Carbon source | Relative activity (%)[a] |
|---|---|
| Oat spelt xylan | 100.0 |
| Glucose | 0.0 |
| Xylan+Glucose | 2.2 |
| Cellulose | 1.2 |
| None | 1.0 |

a. The rate of hydrolysis of oat spelt xylan was taken as 100%

xylanolytic and were found to be *Paenibacillus* by final identification. The best growth and clear zone production of the bacteria was observed on xylan agar plate, peptone water (broth) without NaCl and nutrient agar plate supplemented with starch for 24 h incubation at 37°C. Most of the samples produced clear zone around the colonies on xylan agar plate. In nutrient broth, growth was turbid and after sedimentation bacterial growth was found like ring pellicle. Under the microscope the cells were arranged in singly, in pairs or chains. The cells were straight, rod-shaped with rounded ends to coccoid. They were motile and gram-negative because of retaining safranine color in staining reaction.

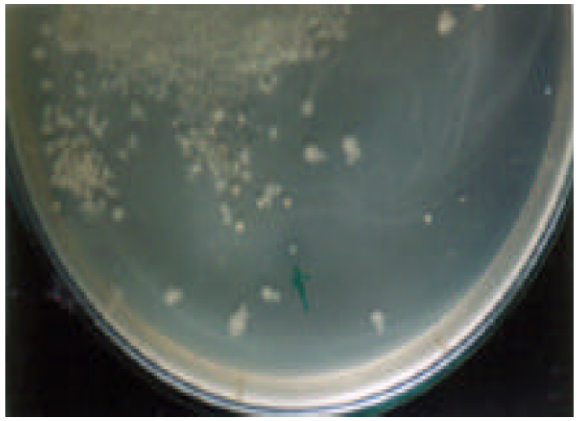

Fig. 1: Photograph showing the growth of strain $St_1$

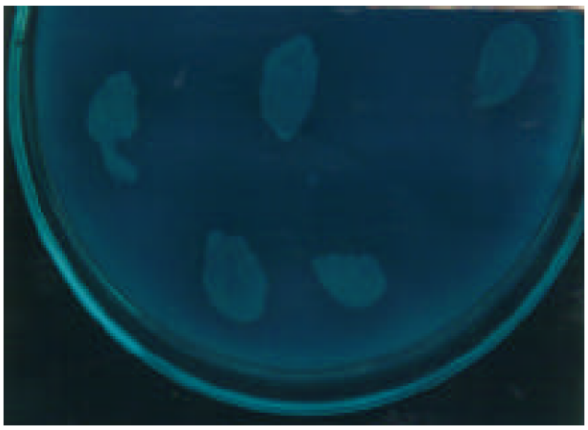

Fig. 2: Photograph showing the zymogram analysis of strain $St_1$

From the β-xylanase production, gram staining, catalase and oxidase, five strains were selected for further morphological, growth characteristics, microscopic examination and biochemical test. The strains showed negative urease test because they are unable to decompose urea to ammonia, which causes alkaline reaction. The isolated strains showed negative citrate

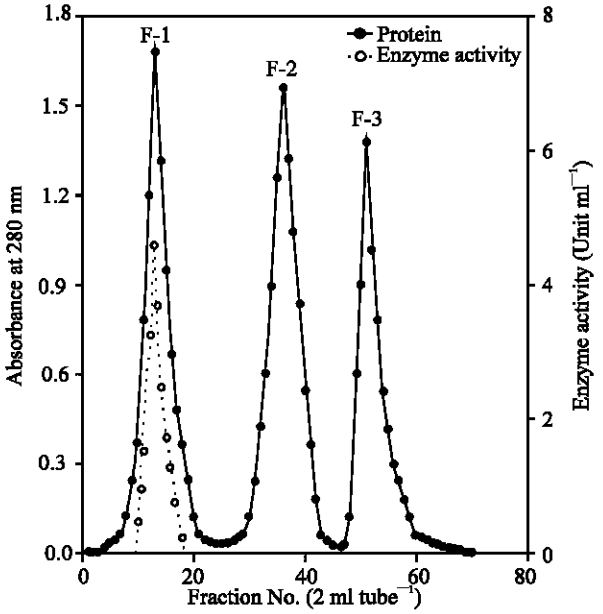


Fig. 3: Gel filtration of 100% $(NH_4)_2SO_4$ saturated crude protein exact on sephadex G-100. The crude extract (200 mg protein) was applied to the column (2.5x100 cm) pre-equilibrated with 50 mM phosphate buffer, pH 7.0 at 4°C. The flow rate 24 ml h$^{-1}$

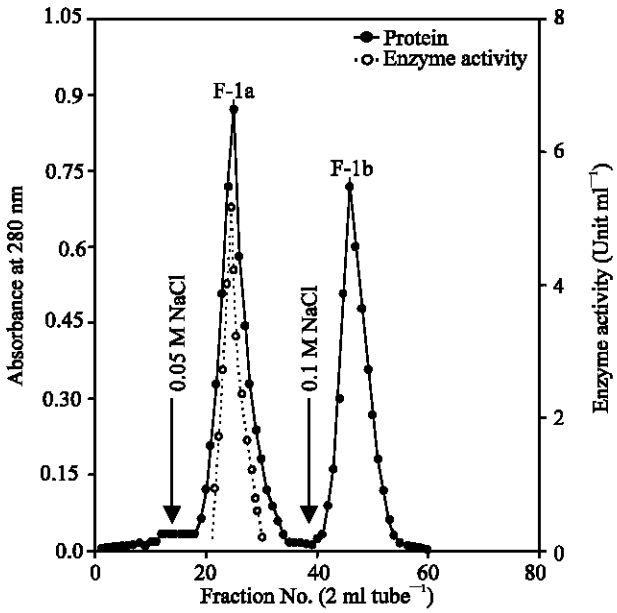


Fig. 4: Ion-exchange chromatography of F-1 fraction (6.5 mg protein) on
DEAE-cellulose column at 4°C
Size of the column: (1.5x 28 cm)
Buffer: 50 mM phosphate buffer. pH 7.0
Flow rate: 24 ml h$^{-1}$

utilization test and showed positive indole test. These five strains were labeled as $St_1$ $St_2$ and $So_1$ $So_2$ $So_3$. Results of the biochemical test have shown in the Table 1.

**Purification of xylanase**

**Gel filtration:** The ammonium sulfate (100%) precipitated of the crude enzyme extract from the bacteria of strain no. $St_1$ was dialyzed against 50 mM sodium-phosphate buffer, pH. 7.0, was applied on to the column of sephadex G-100 which was previously equilibrated with 50mM sodium-phosphate buffer, pH 7.0 at 4° C. The protein was eluted from the column with the same buffer. The crude protein extract was eluted three peaks namely F-1, F-2 and F-3 (Fig. 3). It was found that the F-1 fraction contained the xylanase activity while the F-2 and F-3 pecks no activity. Hence F-2 and F-3 fraction were not used for further investigation. The active fraction F-1 pooled separately and dialyzed against 50 mM Na-P buffer overnight. The dialysis sample was applied to the DEAE cellulose.

**DEAE-cellulose chromatography of F-1 fraction:** The active peak from gel filtration chromatography was applied to DEAE-cellulose column, which was previously equilibrated with the 50 mM Na-P buffer pH 7. A very little amount of protein containing no xylanase activity was firstly eluted with 50 mM Na-P-buffer. Then two major

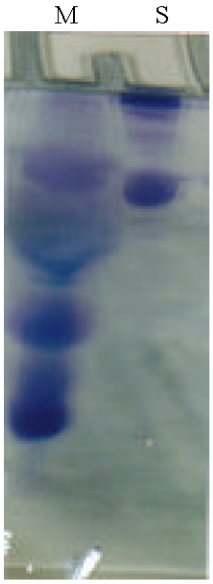


Fig. 5: Photographic representation of SDS polyacrylamide slab gel electrophoresis of purified xylanase. Lane M: Molecular mass maker: Lyosozyme (14 kDa), β-lactoglobulin (18.4 kDa), Trypsinogen (24 kDa), Pepsin (36 kDa), Albumin (45 kDa) and Albumin (BSA, 67 kDa). S: Purified xylanase

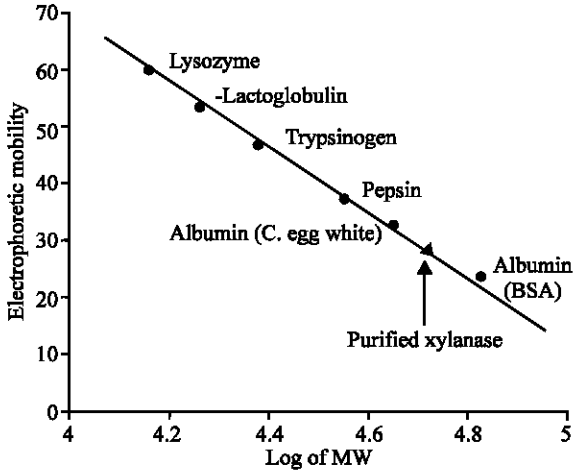


Fig. 6: Standard curve of the determination of molecular weight of purified xylanase by SDS-polyacrylamide slab electrophoresis

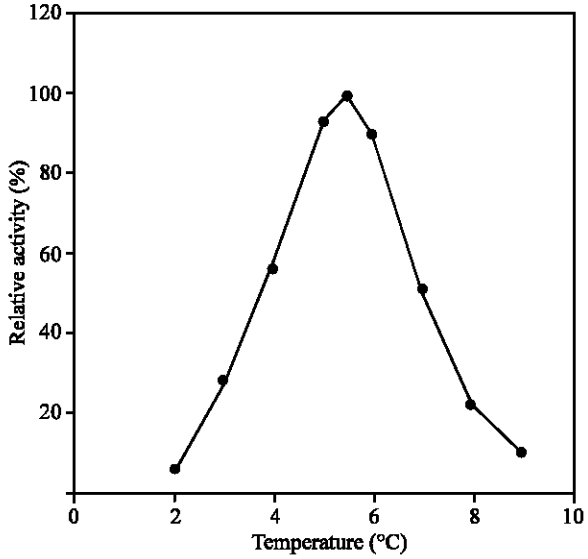


Fig. 7: Effect of temperature on the activity of xylanase obtained from the bacteria of strain on $St_1$. Enzyme solution in 50 mM phosphate buffer pH 7.0 were incubated at various temperatures for 10 min and the activities were measured as described in Materials and Methods. The highest activity was expressed as 100%

fractions of protein were eluted step-wisely with increasing concentration of the NaCl with the same buffer. The components of F-1 fraction were separated into two peaks F-1a and F-1b with the buffer containing 0.05M, 0.1M gradients respectively (Fig. 4). The enzymatic activity of all these fraction were investigated and it was found that only the fraction F-1a contained xylanase activity while the fraction F-1b possessed no xylanase

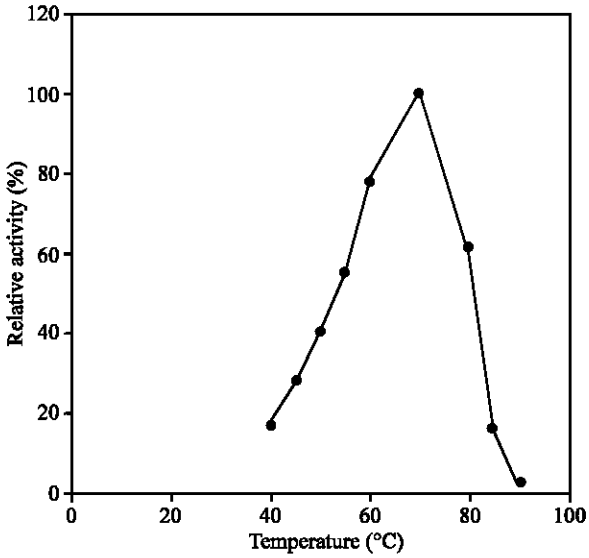


Fig. 8: Effect of pH on the activity of xylanase. The buffers were 50 mM Na-acetate buffer, pH 4.0-5.5, 50 mM Na-Phosphate buffer pH 6-8.0, 50 mM Tris-HCl buffer pH 8.5-9.0. The highest activity was expressed as 100%

activity. The active fraction came out at 0.05 M NaCl gradient.

The specific activity of xylanase was increased at each purification step. Finally the specific activity of enzyme was 86.66 and the purification fold was 52.84 (Table 2). The yield might be decreased due to denaturation of enzymes during the lengthy purification procedures or some other reasons. For the purity check the active fraction from DEAE-cellulose chromatography were applied on slab gel electrophoresis and showed single protein band (Fig. 5).

The specific activity of xylanase was increased at each purification step. Final the specific activity of enzyme was 86.66 and the purification fold was 52.84. The yield might be decreased due to denaturation of enzymes during the lengthy purification procedures or some other reasons (Table 2).

**Characterization of xylanase**

**Determination of molecular weight of purified xylanase:** The molecular weight of purified xylanase was determined by sodium dodecyl sulfate–polyacrylamide slab gel electrophoresis according to the Laemmli method. Lysozyme, (C. egg white), β-Lactoglobulin (Bovine milk), Trypsinogen (Bovine pancreas) pepsin (Hog stomach), Albumin (C. egg white) , Albumin (Bovine serum) were used as standard proteins. The molecular weight of the purified xylanase was determined from the standard curve shown in Fig. 6. This standard curve was constructed by plotting the log of molecular weight of standard protein

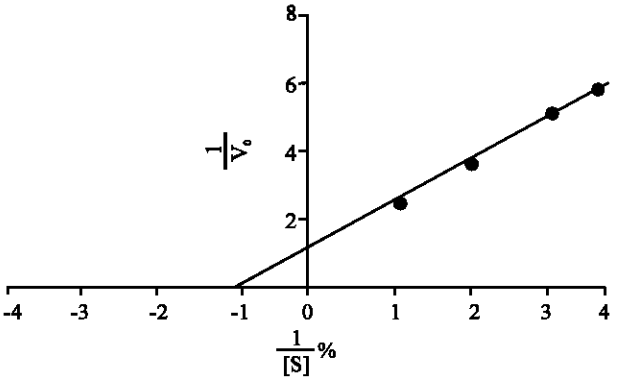


Fig. 9: A Line weaver-Burk double reciprocal plot for the determination of $k_m$ value of purified xylanase enzyme

against their electrophoretic mobility on the 10% polyacrylamide gel. The molecular weight of the purified xylanase was 48 kDa. The molecular weight of purified xylanase was found to be similar to *Fibroiobactor succinogens* S 85[12] which was 52 kDa.

**Effect of temperature on the activity of xylanase:** The effect of temperature on activity of xylanase against xylan obtained from strain no $St_1$ was examined in the temperature range of 20–90°C. The enzyme showed maximum activity around 50-55°C. From the graph it was shown that optimum temperature was observed at 55°C. With further rise of the temperature the activity of the enzyme was decreased more sharply and 94% of the activity was destroyed at 85°C (Fig. 7).

**Effect of pH on the activity of xylanase:** To determine the optimum pH of the enzyme activity against xylan, 50 mM sodium acetate buffer at pH 4.0 to 5.5 and 50 mM sodium phosphate buffer at pH 6.0 to 8.0 and 50 mM Tris-HCl buffer at pH 8.5 to 9.0 were used. The optimal pH of the enzyme activity was 7.0 when the activity was measured over a pH range from 4.0 to 9.0 (Fig. 8). Xylanase from *Bacillus* sp. WI-II showed maximum activity at pH 7.0-9.0 reported by[13]. reported that xylanases (1 and 2) produced by *Fibrobactor succinogenes* S85 showed optimum activity at pH 7.0 and 6.3, respectively.

**Substrates specificity on xylanase activity:** The substrates specificity of purified xylanase was studied using various polysaccharides as the substrates (Table 3). From the Table 3 it was shown that purified xylanase was hydrolyzed strongly oat spelt and birch wood xylan. However, xylanase did not hydrolyze cellulose, carboxymethyl cellulose, starch. Thus it was a true xylanase.

**Determination of $K_m$ of xylanase:** A Line weaver-Burk's double reciprocal plot of the data showed the Km value for xylanase enzyme against oat spelt xylan as substrate to be 0.91% which described in the Fig. 9.

**Antibacterial activity:** As shown in the Table 4, 30 $\mu g^{-1}$ $disc^{-1}$ of the ethyl acetate extract of the cultural broth of *Paenibacillus* bacteria showed no antibacterial activity against the tested pathogenic bacteria but showed low antibacterial activity at the concentration of 200 $\mu g^{-1}$ $disc^{-1}$ compared to those of standard kanamycin (30 $\mu g^{-1}$ $disc^{-1}$). The maximum and minimum antibacterial activities were observed against *Bacillus subtilis* and *Pseudomonus aeruginosa* for the tested extract.

Present investigation suggests that the ethyl acetate extract of the cultural broth of *Paenibacillus* bacteria contains some antibacterial components which shows activity against both gram positive and gram negative bacteria.

## DISCUSSION

In this study the bacterial strains were isolated from soil and water, which degraded oat spelt xylan and belong to *Paneibacillus* genus. In the primary step of this study, the xylanase-producing bacteria were isolated from the collected samples by screening procedure from their growth as clear zone and xylanolytic properties on xylan agar plate. the xylanase activity in each strain was confirmed by measuring the amount of reducing sugars liberated from xylan by the dinitrosalisylic acid method using crude extract after isolation of xylanase-producing strains, the strains were identified as *Paneibacillus* on the basis of catalase, oxidase, gram-staining reaction, morphological and culture characteristics and different biochemical tests. Xylanase of *Paneibacillus* was induced by oat spelt xylan, but the induction was inhibited by glucose (Table 5). The trace quantity of xylanase formed in the presence of xylan plus glucose could be attributable to the exhaustion of glucose followed by growth of xylan (Table 5). Then xylanase was purified to homogeneity from culture fluid by ammonium sulfate precipitation followed by gel filtration and ion-exchange chromatography. The molecular mass of purified xylanase was estimated to be 48 kDa on SDS-PAGE under reducing conditions. Similar result was obtained on xylanase from *Fibroiobactor succinogenes* 85[12] and the molecular mass was 52 kDa. The xylanase had no activity on cellulose, carboxymethyl cellulose, or starch; thus it was true xylanase. These properties were also found in the reported xylanases from *B. pumillus*[14] and *B. substilis*[15].

## REFERENCES


1. Wood Ward, J., 1984. Xylanases, structure, functions, properties and applications, Top. Enzyme. Ferment. Biotechnol., 8: 9-30.
2. Whistler, R.L. and E.L. Richards, 1970. Hemicelluloses. In: The carbihydrates, Academic Press, New York, 2a: 441-469.
3. Dekker, R.F.H. and G.N. Richards, 1976. Hemicellulases: their occurrence, purification, properties and mode of action. Adv. Cabohydr. Chem. Biochem., 32: 277-352.
4. Biely, P., 1985. Microbial xylanolytic systems Trend Biotechnol., 3: 286-290.
5. Wong, K.K.Y., L.U.L. Tan, J.N. Saddler and M. Yaguchi, 1993. Purification of a third distinct xylanase from the xylanolytic system of *Trichoderma harzianum* Can. J. Microbiol., 32: 570-576.
6. Linko, M., K. Poutanen and L. Viikari, 1989. New developments in the application of enzymes for biomass processing, In: enzyme Systems for Lignocellulose Degradation, pp: 331-346.
7. Sumida, T., N. Sueyoshi and Makota Ito, 2002. Molecular Cloning and Characterization of a Novel Glucocerebrosidase of *Paenibacillus* sp. TS 12, J. Biochem., 132: 237-243.
8. Miller, G., L.R. Blum, W.E. Glennon and A.l. Burton, 1960. Use of dinitrosalisylic acid reagent for determination of reducing sugars, Analytical Chem., 31: 426-428.
9. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin-phenol reagent, J. Biol. Chem., 193: 265-275.
10. Laemmli, U.K., 1970. Cleaves of structural proteins during the assembly of the head of bacteriophage $T_4$. Nature, (London), 227: 680-685.
11. Robyt, J.F. and B.J. White, 1990. Biochemical Techniques: Theory and Practical Wav Land Press, USA., pp: 295-296.
12. Allan, M. and Cecil W. Forsbery, 1992. Purification, characterization and mode of action of endo xylanase 1 and 2 from *Fibrobacter succinogenes* S85. Applied Environ. Microbiol., 58: 157-168.
13. Okazaki, W., T. Akiba, K. Hirokoshi and R. Akahushi, 1985. Purification and characterization of xylanases from alkalophilic thermophilic *Bacillus* spp. Agric. Biol. Chem., 49: 2033-2039.
14. Panbangred, W., T. Shinmyo, S. Kinoshita and H. Okada, 1985. Purification and properties of endoxylanase produced by *Bacillus pumilus* Agric Biol. Chem., 47: 957-963.
15. Bernier, R., M. Desrochers, M.L. Jurasek and M.G. Paice, 1983. Appli. Environ. Micrbiol., 46: 511-514.