# Partial purification and characterization of xylanase from *Bacillus cereus* X3

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## Abstract:

Three strain of *Bacillus cereus* were obtained from soil sours Laboratories of Biology Department/ College of Science/ University of Baghdad. The bacteria secreted extracellular xylanase in liquid cultur the test ability of xylanase production from these isolates was studied semi quantitative and quantitative screening appeared that *Bacillus cereus X3* was the highest xylanase producer.

The enzyme was partial purification 191 fold from cultur by reached step by 4 U/mg proteins by ammonium sulfat precipitation 80%, Ion exchang DEAE-cellulos chromatography

Characterization study of the partial purifation enzyme revealed that the enzyme had a optimum activity pH8 and activity was stable in the pH rang (8-10) for 30min. maximal activity was attained at 50C

**Key words: *Bacillus cereus* X3, xylanase, Partial purification.**

## Introduction:

Xylan is the most abundant noncellulosic polysaccharide present in both hardwoods and annual plants and accounts for 20–35% of the total dry weight in tropical plant biomass[1–2].

In temperate softwoods, xylans are less abundantand may comprise about 8% of the total dry weight [3,4]. Xylan is found mainly in the secondary cell wall and is considered to be forming an interphase between ligninand other polysaccharides. It is likely that xylan molecules covalently link with lignin phenolic residues and also interactwith polysaccharides, such as pectin and glucan. In simples, xylans are linear homopolymers that contain D-xylosemonomers linked through $\beta$-1, 4–glycosyl bonds [5, 6].

Xylanases are of industrial importance, which can be used in paper manufacturing to bleach paper pulp, increasing the brightness of pulp and improving the digestibility of animal feed and for clarification of fruit juices. Applications of xylanase avoid theuse of chemicals that are expensive and cause pollution [7]. Microorganisms are the rich sources of xylanases, produced by diverse genera and species of bacteria, actinomycetes, and fungi. Several species of *Bacillus* and filamentous fungi secrete high amounts of extracellular xylanases [8]. Xylanase secretion often associates with low or high amountof cellulases. To use xylanase for pulp treatment, it is preferable to use cellulose-free xylanases, since the cellulase may adversely affect the quality of the paper pulp [9–10]. Industrial production of enzymes on large scale is associated mainly with substrate. The use of agriculture residues as low-cost substrates for the production of industrial enzymes is a significant way to reduce production cost.(11)

study the isolation, purifaction and cheracterzation of xylanase frome *Bacillus cerecus*.

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## Material and methods:

Six samples collected from the department of Biology\College of Science inoculated were prepared by transfer to nutrient agar and incubated for 4 days at 30 ˚C.

The growing colonies were purified by sub culturing on nutrient agar for many time units pure culture was obtained, the morphology, size, shape and margin of bacteria *Bacillus*

Culture media of *Bacillus cereus* isolation and produce xylanase :

1- Liquid media for isolation and production xylanase:(1)

yeast extract ……. 0.2 gm
Nacl …… 0.2 gm
MgSo4…….. 0.02 gm
K2Hpo4 …… 1.5 gm
Xylan ……... 0.5 %

All component were dissolved in 90 ml, pH was adjusted to 8 and then volume was completed to 100 ml and sterilize by autoclaving at 121 C for 15 minutes

2- Xylanase medium for semi quantitative method: (1)

nutrient agar……..2.8 gm
xylan ……o.5%

All dissolved in 100 ml D.W pH was 8 and sterilized by autoclave 121 C for 15 minutes.

Semi quantitative method: (2)

The activated bacteria were cultured on xylan and inocubatet at 37 C for 48 hrs. the plate zone clearness around bacteria and colonies inoculated xylanase production, the diameter of colonies was obtained which represented as semi quantitative assay of xylanase.

Estimation of xylanase activity:

Xylanase activity was measured accordin . [12]. A 900 $\mu$L of 1%solubilised birchwood xylan solution was added with 100 $\mu$L enzyme solution in a test tube. 1.5mL DNS reagent was added and incubated at 50◦C for 5min in water bath [13]. The absorbance was measured at 540 nm. The reaction was terminated at zero time in the control tubes. The standard graph was prepared using 0–500 $\mu$g xylose. was set in UV-VIS spectrophotometer using buffer solution. One unit of xylanase activity was defined as the amount of enzyme that liberates 1micro mole of reducing sugars equivalent to xylose per minute under the assay conditions described. Solubilised xylan was prepared by stirring birchwood xylan with 1M NaOH for six hours at room temperature followed by centrifugation and freeze drying the supernatant after neutralising the alkali with 1M Hcl.the protine was estimated by the method of lowry *etal*(14).

Xylanase purification:

*Bacillus cereus* was aerobically grown at 37oC. for 48 h in a liquid medium described above. The cells were separated by centrifugation at 12,000 × g for 10 min and used as crude enzyme suspention. The purification of the xylanase was done at 4oC. The crude xylanase was precipitated with ammonium sulfate at a concentration corresponding to 80% saturation. The resultant precipitate was collected by centrifigation at 15,000 × g for 20 min, dissolved in 50 mM sodium phosphate buffer (pH 7.0), and dialyzed against the same buffer and applied to the DEAE-sepharose column. The elution was done from 0-0.5 M NaCl. The xylanase active fraction was eluted at 0.25 M NaCl gradient. The active fractions from DEAE-sepharose column were combined, mixed with the same volume of 3 M ammonium sulfate and put onto Phenyl 5 PW column, which was previously equilibrated with 20 mM sodium phosphate buffer (pH 6.8) containing 1.5 M ammonium sulfate. Adsorbed proteins were eluted with a descending linear gradient of ammonium sulfate. Xylanase activities were eluted at 0.2 M ammonium sulfate. The active

fraction from phenyl 5 PW was dialyzed against 5 mM sodium phosphate buffer of pH 7.0. The dialyzed enzyme solution was put onto a hydroxyapatite column previously equilibrated with the same buffer. The absorbed protein was eluted with a linear gradient of 5-100 mM sodium phosphate(15).

Determination of conditions for xylanase production:

•Effect of temperature of xylanase:

100 ml of N.B with 2 ml of activated Bacteria and incubated at for different temperature (40, 45, 50,55,60 c.) supernatant were assayed for enzyme activity, protein concentration and specific activity.

• Effect of pH:

100 ml of NB with 2 ml of activated Bacteria at different pH values (4, 5, 6 ,7,8) adjested with the 1 N HCL or NaOH,. Supernatant were assayed for enzyme activity, protein concentration and specific activity.

## Results and discussion:

Isolation and Identification of Bacteria.

About 6 bacterial strains, which formed clear halos around their colonies on xylan agar plates, were picked up for further studies, isolated from soil collected at selected study site. The strain that showed 1-4 cm zone of clearance around the colony proved its xylanolytic ability Table (1)

**Table 1:- *Bacillus cereus* isolates in xylan agar media incubation at 37 ˚C for 72 hrs.**

| Isolation number | Zone of hydrolysis/ cm |
|---|---|
| X1 | 4 |
| X2 | 2.6 |
| X3 | 3.2 |

Concentration of Xylanase:

The culture filtrate was precipitated by fractional (35–80%) ammonium sulphate saturation.

Proteins precipitated within this range had maximum xylanase activity and was used for purification

The enzyme precipitation at 80% saturation and dialyzed give specific activity 3.1 U\mg protein (Fig. 1). The results of (3) show that the precipitation of xylanase from *streptomyces sp* was carried out with the 75% saturation of (NH4)2So4, while (4) reported that fraction of 65% (NH4)2So4 were contiant highe xylanase activity 2.3 U\mg protein produced by *Arthrobacter Spp.*

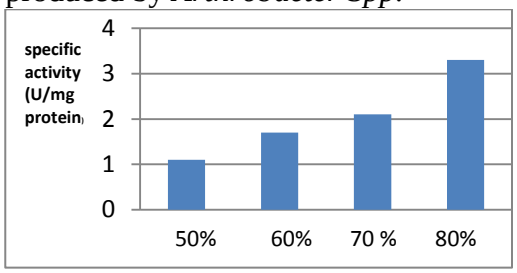



**Fig.1: Specific activity of *Bacillus cereusX3* xylanase after perciptation with ammonium sulphate**

Purification of Xylanase:

Xylanase was further purified by DEAE cellulose ion exchange column.The enzyme was eluted from DEAE cellulose column at a NaCl concentration of 0.25M (Figure 2).The fractions (no 19–25) having maximum specific activity were concentrated. Xylanase was purified 97-fold with a specific activity of 1102 U/mg (Table 2). The specific activity of xylanase produced by *Bacillus pumilus* was eviously reported as 298 U/mg (16)

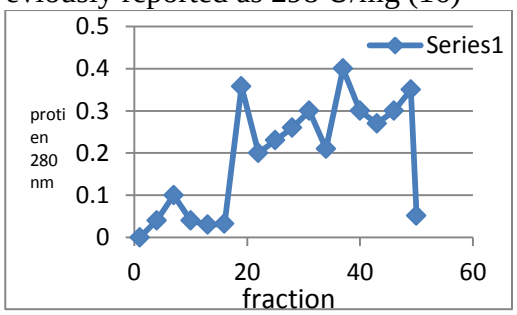
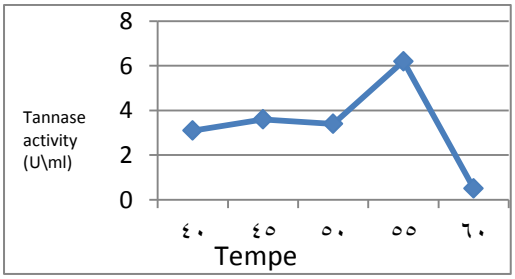



**Fig. 2: Elution profile of xylanase from DEAE-cellulose column chromatography**

**Table(2): purifaction of xylanase from *Bacillus cereusX3***

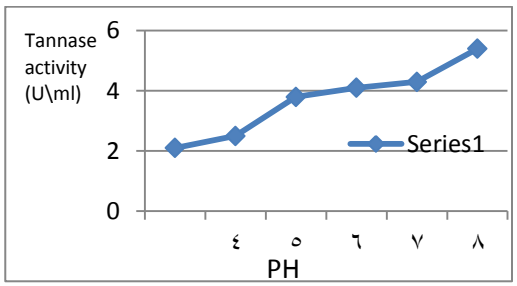
| Sample | Volum (ml) | Activity (U/ml) | Protein (mg/ml) | enzyme activity( u/ml) | Total activity (U/ml) | specific activity U/mg protein | fold | Yield % |
|---|---|---|---|---|---|---|---|---|
| Crude extract | 1000 | 1.9 | 1900 | 21.5 | 21500 | 468 | 1 | 100 |
| Preciptio n 80% | 50 | 7.85 | 392.5 | 401 | 20050 | 19.6 | 4.5 | 93.26 |
| DEAE-cellulose | 5 | 2.55 | 12.75 | 2810 | 14050 | 4.68 | 97.5 | 65.3 |

Influence of temperature:
The xylanase production at different temperatures range were examined after 3 day keeping the other fermentation constant xylanase production increase at temperature 55 C maximum production of protease 6.2 U/ml (Fig.3) also found *Bacillus spp* optimally at 50 C (17). It was revealed that temperature does not only affect growth rate of organism but also exhibited marked influence on the level of protease production




**Fig(3): Effact of tempertur regines on the production of xylanase by *Bacillus cereusX3***

Influence of pH:
the maximum xylanase production 5.4 U/ml found at pH 7 (Fig.4). The result clearly indicated neutrophilic of the bactera. The medium xylanase production by *Bacillus cereusX3* was observed in range pH 6-8. Growth and protein production cased of pH 10 optimum pH 8 has been reported for natural xylanase of (18).




**Fig(4): Effect of PH on the production of xylanase by *Bacillus cereusX3***

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# التنقية الجزئية لانزيم الزايلينيز المستخلص من بكتريا*Bacillus cereus X3*

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**الخلاصة:**

تم الحصول على ثلاثة عزلات من التربة جاهزة ومشخصة من قسم علوم الحياة- جامعة بغداد ، ودرست قدرتها على انتاج انزيم الزايلنيز حيث اظهرت نتائج الغربله الكميه والشبه الكميه ان العزله *Bacillus cereus X3* هي الاغزر انتاجا للانزيم تمت تنقية الانزيم جزئيا 191 مرة من راشح المزرعة بعدد خطوات تنقية متسلسلة من كبريتات الامونيوم بنسبة اشباع 80% وكروماتوغرافيا التبادل الايوني بطريقة الوجبة. DEAE-cellulos تم دراسة بعض صفات الانزيم المنقى جزئيا اذ وجد انه يملك فعالية مثلى في الرقم الهيدروجيني 8 والانزيم ثابتا في مدى من الرقم الهيدروجيني القاعدي (8-10) لمدة 30 دقيقة، ويكون الانزيم ثابتا حراريا بدرجة50 م°.