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SHORT COMMUNICATION

Purification of xylanase produced by *Bacillus pumilus*

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Abstract: This study was aimed at purifying xylanase produced by *Bacillus pumilus*. The spent medium contained 27.9 UmL^{-1} xylanase activity and 1.5 $mgmL^{-1}$ protein. The highest specific activity (33.7 Umg^{-1} protein) was achieved with 50 % $(NH_4)_2SO_4$ saturation and the xylanase recovery was 94.8 %. The dialyzed and DEAE-Sepharose purified enzyme showed 6.7-fold increase in specific activity with a yield of 84.2 %. Molecular weight of the purified xylanase was 55.4 KDa. Thus *B. pumilus* xylanase can be purified by precipitating with 50 % $(NH_4)_2SO_4$ saturation and DEAE-Sepharose ion exchange chromatography.

Keywords: Ammonium sulphate saturation, DEAE-Sepharose, dialysis, purification fold, specific activity.

INTRODUCTION

Xylanase deconstructs xylan into xylose (Nakamura *et al*., 1995). Many bacterial and fungal species can produce xylanase. Xylanases, which are active under alkaline and thermostable conditions are widely used in paper industry to bleach craft pulp and to increase the brightness (Zamost *et al*., 1991), to improve the digestibility of animal feed and to clarify juices in food industry (Nakamura *et al*., 1995). As the xylanases produced by different microorganisms vary in physiochemical characteristics (McCarthy, 1987), it is important to optimize the procedures adopted for the purification of xylanases of different microbial sources. Usually in the purification studies more than one method is adopted. Thus the purification of thermostable alkaline xylanase (Kapilan & Arasaratnam, 2011a) produced by *Bacillus pumilus* (Kapilan & Arasaratnam, 2010) was studied.

The enzymes are purified using different techniques such as partitioning (Yang *et al*., 2008); anion exchange adsorption; hydrophobic interaction chromatography (Breccia *et al*., 1998); $(NH_4)_2SO_4$ precipitation (Breccia *et al*., 1998; Gessesse & Mamo, 1998; Bataillan *et al*., 2000); anion exchange chromatography; gel filtration and affinity chromatography (Bataillan *et al*., 2000); gel filtration and ion-exchange chromatography (Christalopoulos *et al*., 1996; Gessesse & Mamo, 1998; Cepeljnik *et al*., 2001); precipitation with Eudrgit (Gupta *et al*., 1994) and three phase portioning (Sharma & Gupta, 2002). As most of the previous studies have used more than two methods for the purification of xylanase from crude culture filtrates, experiments were conducted to find a rapid method for the purification of xylanase from *B. pumilus* (Kapilan & Arasaratnam, 2011b).

METHODS AND MATERIALS

DEAE–Sepharose (diethyl amino ethyl Sepharose fast flow) was purchased from Pharmacia, Uppsala, Sweden. All the other chemicals used were from standard sources. Xylanase was produced by solid state fermentation of *Bacillus pumillus* (Kapilan & Arasaratnam, 2010; 2011b). The spent medium was centrifuged at 3000 rpm for 30 min and the supernatant was used for purification studies.

The xylanase activity was measured in terms of reducing sugar produced by its action on xylan (Kapilan & Arasaratnam, 2010). Protein concentration was determined by Lowry's method (Lowry *et al*., 1951). One unit of xylanase activity is defined as the amount of enzyme that produces 1 µmole of reducing sugar in 1 min at pH 9.0 and 60 °C with 20 gL^{-1} of xylan.

Solid $(NH_4)_2SO_4$ was added to crude xylanase to bring different saturation values (Dawson *et al*., 1969), mixed for 2 hrs, allowed to settle, and centrifuged (8000 rpm at 4 °C, 30 min). The precipitate was dissolved

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in distilled water and dialyzed overnight against distilled water. The xylanase activity and the protein content were measured.

DEAE-Sepharose was activated with 0.1 M NaOH and HCl, and equilibrated with 0.01 M Tris buffer (pH 8.0). The precipitated and dialyzed xylanase was added and mixed (100 rpm, 30 min) at room temperature. The unbound enzyme was removed by centrifugation and the residue was re-suspended in Tris buffer (pH 8.0) to wash the unbound enzyme. The DEAE-Sepharose bound xylanase was eluted with different concentrations of sodium chloride. The bound and unbound xylanase activities and protein contents were measured.

Xylanase, which was precipitated with $(NH_4)_2SO_4$ and dialyzed overnight, was loaded to the activated and equilibrated DEAE-Sepharose (with 0.01 M Tris buffer, pH 8.0) containing column [7 × 1 cm (bed volume 5.5 mL)]. Unbound proteins were removed with 10 bed volumes of 0.01 M Tris buffer (pH 8.0) at a flow rate of 1 $mLmin^{-1}$. Bound proteins were eluted with optimized NaCl - 0.01 M Tris buffer, (pH 8.0) at a flow rate of 1 $mLmin^{-1}$. The xylanase activity and the protein contents were analyzed. The pooled purified xylanase sample was subjected to SDS acrylamide gel electrophoresis (Koseki *et al*., 1997) and molecular weight of the purified xylanase was calculated (Weber & Osborn, 1969).

RESULTS AND DISCUSSION

The culture supernatant used for the purification contained 27.9 UmL^{-1} xylanase activity and 1.5 $mgmL^{-1}$ protein. The precipitation of protein increased with the $(NH_4)_2SO_4$ saturation percentage while the enzyme activity increased up to 50 % of $(NH_4)_2SO_4$ (33.7 Umg^{-1} protein) (Table 1) and the specific activity of xylanase increased by 1.8 times.

The enzyme (2 mL) having 46.8 UmL^{-1} of xylanase activity and 1.4 $mgmL^{-1}$ protein was used for the purification. When the concentration of NaCl in Tris buffer varied, the xylanase activity and protein eluted increased up to 0.8 M NaCl (80 %) (Table 1) with the recovery of protein up to 32 %. Therefore 0.8 M NaCl was selected.

The $(NH_4)_2SO_4$ precipitated sample (2 mL - specific activity 33.2 Umg^{-1}, total protein content 4.7 mg, total xylanase activity 156.8 U) was loaded to the DEAE-Sepharose column. Fractions from 5 to 9 contained the proteins without any xylanase activity (Figure 1). The fractions from 16 to 22 (7 fractions) contained proteins with xylanase activity, fraction 18 having the highest. Pooled fraction (16 to 22) showed 18.9 UmL^{-1} xylanase activity with 0.09 $mgmL^{-1}$ protein. Thus the specific activity of xylanase was increased from 33.2 to 223.7 Umg^{-1} protein, which was 6.7 fold higher than that of the crude xylanase with 84.2 % yield (Table 2).

The distance travelled by the molecular markers and purified xylanase were measured when the purified xylanase was subjected to gel electrophoretic separation (Figure 2) and the molecular weight of the purified xylanase was estimated to be 55.4 KDa. The molecular weight of this xylanase closely resembled that of *Micrococcus* sp. AR-135 xylanase (Gessesse & Mamo, 1999) while those of *B. thermantarcticus* (Bataillan *et al*., 2000) and *B. amyloliquefaciens* (Breccia *et al*., 1997) had lower values, and that of *Bacillus* sp. strain SPS - O had a higher value (Bataillan *et al*., 2000).

Table 1: Effect of ammonium sulphate saturation percentage on the precipitation of xylanase from *Bacillus pumilus* and the effect of NaCl concentration on the elution of xylanase bound to DEAE-Sepharose

$(NH_4)_2SO_4$ (%)	Xylanase activity (UmL^{-1})	Protein ($mgmL^{-1}$)	Specific activity (Umg^{-1} protein)	NaCl (M)	Xylanase activity eluted (%)	Protein eluted (%)
10	10.0	0.54	18.7	0.0	5.0	10.0
20	19.8	0.91	21.8	0.4	60.0	28.0
30	29.4	1.02	28.9	0.8	80.0	32.0
40	33.3	1.10	30.3	1.2	80.0	35.0
50	46.8	1.39	33.7	1.6	80.0	35.5
60	45.0	1.78	25.3	2.0	80.0	36.0
70	43.8	2.65	16.6	-	-	-

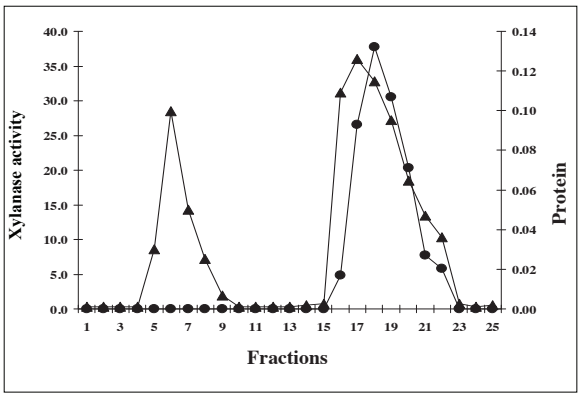

Figure 1: Separation of xylanase precipitated with 50 % ammonium sulphate and DEAE-Sepharose
(●) xylanase activity (UmL^{-1}) and (▲) protein (mg) eluted with 0.8 M NaCl - 0.01 M Tris buffer (pH 8.0) at a flow rate of 1 $mLmin^{-1}$

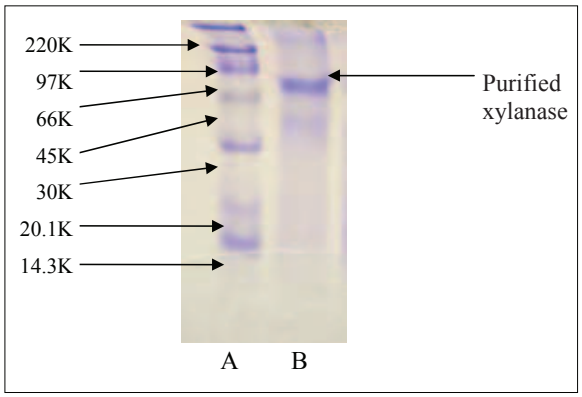

Figure 2: SDS-PAGE pattern of purified xylanase from *Bacillus pumilus*
Lane A: marker proteins; lane B: purified xylanase sample

Table 2: Purification of xylanase from *Bacillus pumilus* by precipitation with 50 % $(NH_4)_2SO_4$ saturation and DEAE-Sepharose chromatography

Purification step	Total activity (U)	Total protein (mg)	Purification fold	Recovery (%)
Crude (6 mL)	165.5	8.96	1.0	100
$(NH_4)_2SO_4$ 50 % Saturation (2 mL)	156.8	4.73	1.8	94.8
DEAE-Sepharose	132.0	0.59	6.7	84.2

CONCLUSION

The specific activity of the crude (xylanase enzyme 18.5 Umg^{-1} protein) was increased to 33.2 Umg^{-1} protein by $(NH_4)_2SO_4$ precipitation. Further purification by ion exchange chromatography using DEAE-Sepharose increased the specific activity to 223.7 Umg^{-1} protein. The molecular weight of the purified xylanase of *Bacillus pumillus* was 55.4 KDa.

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