# Partial purification and characterization of xylanase from *Streptomyces mexicanus* 901

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## Abstract

Currently, the demand of thermotolerant xylanase has been increasing because of its potential applications in industrial process. Thermostable xylanase was produced by *Streptomyces mexicanus* 901 using corncob as carbon source at 50°C. Crude xylanase was partially purified by ultrafiltration and gelfiltration chromatography using Shephadex-G100 column. The result found that xylanase was purified 6.41 folds with specific activity of 55.43 U/mg. Partial purified xylanase exhibited optimum activity at pH 5.0 and 50°C. Stability was high at pH range 3.0-6.0 and 30-50°C. Xylanase activity was stimulated by 10 mM $Mn^{2+}$ and was inhibited by 10 mM EDTA. The inhibitory effect of EDTA suggests that metals are needed for the enzymatic reaction.

**Keywords:** xylanase, purification, *Streptomyces mexicanus*

## Introduction

Xylan is one of the major polymeric constituents of hemicelluloses in plant cell walls and is the second most prevalent biomass after cellulose in nature. It is a heteropolysaccharide that contains a backbone of β-1,4-linked xylopyranose units with substituent groups of acetyl, arabinosyl and glucuronosyl residues (Bastawde, 1992). In nature, the completed hydrolysis of xylan requires the synergistic action of different xylanolytic enzymes, including endoxylanase, β-xylosidase and accessory enzymes such as α-arabinofuranosidase, acetyl esterase and α-glucuronidase (Sunna and Antranikian, 1997). Among these, endoxylanase (β-1,4-xylan xylohydrolase; EC 3.2.1.8) is particularly important to catalyze the hydrolysis of long-chain xylan into short xylooligosaccharides, which are further hydrolyzed to xylose by β-xylosidases (β-D-xyloside xylohydrolase; EC 3.2.1.37) (Collins *et al.*, 2005).

Xylanases have significant applications in a wide range of industrial processes; such as baking industry (Butt *et al.*, 2008). Xylanases can improve the bread volume, crumb structure and reduce stickiness, in feed industry (Gilbert and Hezlewood, 1993; Panwar *et al.*, 2014). Additionally, xylanases are used as feed additives for enhancement of feed nutritional value. In textile industry, it was utilized for desizing of cotton and micropoly fabrics (Battan *et al.*, 2007). Meanwhile, in pulp and paper industries, xylanases were applied to use for prebleaching of pulps, improving the pulp fibrillation and water retention, reduction of beating times in virgin pulps, restoration of bonding and increasing freeness in recycled fibers and selective removal of xylans from dissolving pulps(Saleem *et al.*, 2009).

Microorganisms are the most common source of industrial enzymes due to their broad biochemical diversity and the feasibility of large scale production (Nagar *et al.*, 2012). In addition, the low cost of xylanase production from microorganisms is interested in commercial scale. Although many microorganisms including bacteria, fungi, yeast and especially actinomycetes (Sunna and Antranikian, 1997) have been reported for xylanase production but only a few of them could produced xylanase with especial characteristics such as acidic-stable and thermostable. *Streptomyces mexicanus* 901 was thermophilic actinomycetes isolated from waste water in paper industry. The previous study found that *S. mexicanus* 901 produced high levels of xylanases activity (59.47 U/ml) using cheap agricultural waste (2.0% corn cob) as carbon source (Khaibuddee *et al.*, 2014).

Hence, the objective of the present study was to purify and characterize the extra-cellular xylanase from *S. mexicanus* 901 isolated from waste water in paper industry.

## Materials and Methods

Microorganism

*Streptomyces mexicanus* 901 in the present study was isolated from waste water in paper industry as described by Sirisuntornsakul *et al.* (2013).

Xylanase production

For xylanase production, ISP2 medium was used and prepared as follows (g/l): 4.0 g of yeast extract, 10.0 g of malt extract, 4.0 g of glucose and supplemented with 20 g of corn cob as carbon source. The initial pH of ISP2 medium was adjusted to 7.0 with 50 mM NaOH. For the preparation of seed culture, *S.mexicanus* 901 was grown in ISP2 medium at 50°C for 24 h. Five percent of seed culture was used to inoculate 50 ml of ISP2 medium in 250 ml Erlenmeyer flasks. The cultures were incubated at 50°C for 5 days under shaking at 250 rpm. The enzyme was harvested by centrifugation at 10,000 rpm for 20 min. The supernatant was analyzed for xylanase activity.

Xylanase assay

Xylanase activity was assayed by the developed method of Saleem *et al.* (2009) using 1.0% of birchwood xylan in 50mM phosphate buffer (pH 7.0) as substrate. The reaction mixture containing 0.1 ml of enzyme solution and 0.1 ml of substrate was incubated at 50°C for 30 min. The enzyme reaction was stopped by adding 0.2 ml of DNS reagent and by boiling the reaction mixture for 5 min. After cooling on ice, the reducing sugar released was measured at 540 nm (Miller, 1959). Xylose was used as a standard for the xylanase activity test. One unit of xylanase activity was defined as the amount of enzyme to release 1 µmol of xylose per one minute.

Protein assay

Protein concentration was determined according to the Lowry method (1951) using bovine serum albumin as the standard.

Partial purification of xylanase

The supernatant was concentrated by using ultrafiltration with 10 kDa molecular weight cut-off of cellulose membrane. The concentrated xylanase was loaded onto Sephadex G-100 column (2.0 cm×50 cm) and eluted with 50mM citrate buffer, pH 5.0 at flow rate of 0.5 ml/min. The elution fraction (2 ml each) were collected by fraction collector and measured the proteins content at 280 nm. Xylanase activity in each fraction was measured using DNS method (Miller, 1959) and protein concentration was measured using Lowry method (1951). The highly active xylanase fractions were pooled and used for further studies. The purity of xylanase was checked by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (1970), using 4 % (w/v) stacking and 12 % (w/v) resolving gel. After electrophoresis, the proteins were stained with Coomassie Brilliant Blue.

Characterization of partial purification xylanase

Effect of pH on xylanase activity and stability

The effect of pH on xylanase activity was determined by measuring the xylanase activity in pH range from 3.0 to 12.0 at 50°C for 30 min. The various buffers used for determination of xylanase activity were 50mM citrate buffer (pH 3.0-5.0), 50 mM phosphate buffer (pH 6.0-8.0) and 50 mM glycine-NaOH buffer (pH 9.0-12). The pH stability was determined by incubating xylanase at different pH in range of 3.0-12.0 for 24 h at 4°C. After treatments, residual enzyme activities were determined at pH 5.0 by xylanase assay as described in 2.3.

Effect of temperature on xylanase activity and stability

The optimum temperature for xylanase activity was determined by incubating the xylanase with beechwood xylan in optimal pH buffer at different temperatures varying from 30–80°C for 30 min. The liberated reducing sugars were determined by DNS method (Miller, 1959). Xylanase was incubated in different temperatures (30–80°C) for 1 h to determine thermostability. After the treated xylanase were cooled on ice, the residual xylanase activities were measured by xylanase assay as described in 2.3.

Effect of metal ions and chelating agent on xylanase activity

The effect of various metal ions and chelating agent on xylanase activity were determined by xylanase assay as described in 2.3. The metal ions and chelating agent used in this study were $Na^{+}$, $K^{+}$, $Mg^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Fe^{2+}$, $Ca^{2+}$ and EDTA. The final concentration of metal ions and chelating agent used were 10 mM. The residual activity of each sample was compared with control containing no metal ion in the reaction mixture.

**Result and discussion**

Xylanase purification

The xylanase production by *S. mexicanus* 901 in ISP2 medium supplemented with corn cob was reached the maximum level (41.17 U/ml) at days 5th of cultivation. The crude xylanase was purified by ultrafiltration method followed by gel filtration chromatography. A summary of purification procedures was presented in Table 1. The crude xylanase had a total activity of 41,173.70 U and specific activity of 8.65 U/mg. After the crude extract was concentrated by ultrafiltration with 10 kDa cut-off cellulose membrane, the small molecules of protein in crude xylanase were removed. Thus, the specific activity was increased to be 26.89 U/mg while the purified concentrated xylanase was greater to be 3.11 folds. The concentrated xylanase was loaded onto Sephadex G-100 column for gel filtration chromatography and a single peak protein with xylanase activity was detected (Figure 1). After the final purification step, xylanase was purified to 6.41 folds with specific activity of 55.43 U/mg and 18.11% activity recovery. There have been reported about purification of xylanase produced from various microorganisms. Liao *et al.* (2014) reported that the purification of xylanase up to 6.4 folds with 6.9% recovery obtained from *Penicillium oxalicum* using both types of ion exchange chromatography followed by gel filtration. Ximenes *et al.* (1999) purified xylanase from *Acrophialophora nainiana* and obtained around 5.62 folds and 7.14% recovery by using ultrafiltration and gel filtration chromatography.

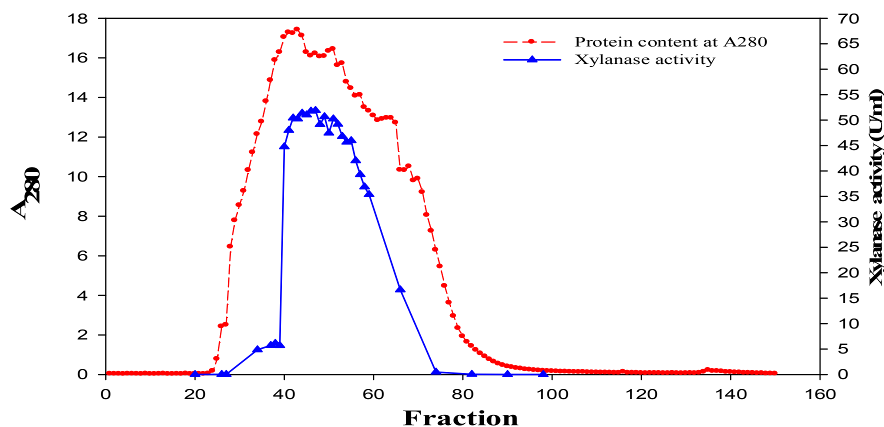


**Figure 1** Elution profile of protein and xylanase on Sephadex-G100 column

**Table 1** Purification of xylanase from *S. mexicanus* 901

| Purification step | Volume (ml) | Total protein (mg) | Total activity (U) | Specific activity (U/mg protein) | Purification (fold) | Recovery (%) |
|---|---|---|---|---|---|---|
| Crude xylanase | 1,000 | 4,757.80 | 41,173.70 | 8.65 | 1.00 | 100 |
| Ultrafiltration | 20 | 313.87 | 8,438.36 | 26.89 | 3.11 | 20.50 |
| Sephadex G-100 | 168 | 134.52 | 7,456.84 | 55.43 | 6.41 | 18.11 |

The purity of xylanase was checked by SDS-PAGE. The result found that the number of band of partial purified xylanase on gel after Coomassie Blue staining less than band of crude xylanase (Figure 2). A similar to the molecular mass of xylanases from *S. rameus* L2001 was 21 kDa (Li *et al.*, 2010), *S. actuosus* A-151 showed molecular masses 20 kDa (Wang *et al.*, 2003) and the molecular mass of xylanase $X_{IB}$ and $X_{IB}$ form *Streptomyces* sp. EC10 were 21 and 22 kDa, respectively (Lumba and Penninckx, 1992).

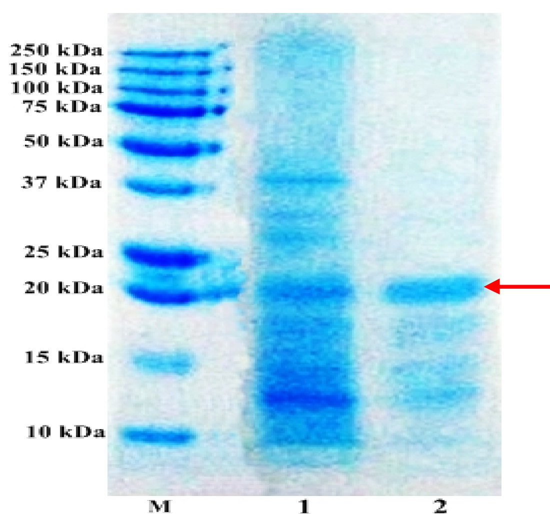


**Figure 2** SDS-PAGE analysis of purified xylanase at each purification step from *S. mexicanus* 901. Lane M: the protein markers, lane 1: crude xylanase, lane 2: partial purified xylanase

Effect of pH on xylanase activity and stability

Enzyme activity is markedly affected by pH because the substrate binding and the enzyme catalysis are often dependent on charge distribution of both substrate and particularly enzyme molecules (Li *et al.*, 2010). The effect of pH on the partial purified xylanase was determined within the pH range of 3.0-9.0. Xylanase activity remained more than 70% relative activity at pH range of 3.0-6.0. However, the optimum pH for partial purified xylanase activity was observed at pH 5.0 whereas the partial purified xylanase was low activity at alkaline condition, which was only 49% relative activity at pH 8.0 and 7.7% relative activity at pH 12.0 (Figure 3(a)). This was similar to the xylanase obtained from *Streptomyces* sp. 3137, which showed the high activity in the pH range of 5.0-6.0 (Marui *et al.*, 1985). The other reports showed that the optimal pH of xylanase found in *S. olivaceoviridis* A1, *S. thermocyaneociolaceus* and *S. rameus* L2001 were 5.2, 5.0 and 5.3 respectively (Zhang *et al.*, 2003; Shin *et al.*, 2009; Li *et al.*, 2010).

The stability of the enzyme was determined by incubating at 4°C for 24 h in different pHs. The result showed that the partial purified xylanase was stable over the range of pH 3.0-6.0 and retained more than 80% of initial activity. The xylanase activity decreased rapidly under alkaline conditions while the xylanase activity retained about 41% of its initial activity at pH 12.0 (Figure 3(b)). The xylanase activity decreases at alkaline condition due to charge in active site of enzyme molecules changes which there is effect on enzyme-substrate reactions (Pal and Khanum, 2011). Taking advantage of its acidophilic and elevated acidic stability, this enzyme has potential applications in the various industries desiring a low pH condition.

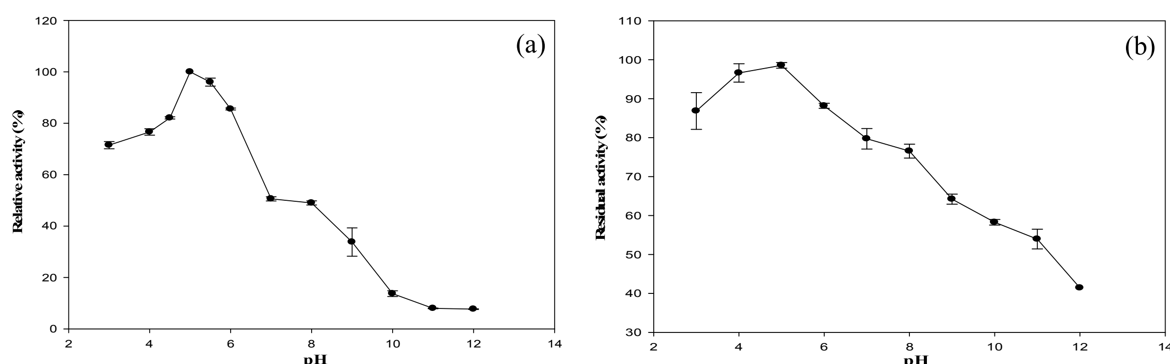


**Figure 3** Effect of pH on (a) xylanase activity and (b) stability

Effect of temperature on xylanase activity

The partially purified xylanase was active in the broad range of temperatures 30-70°C, and optimum temperature was at 50°C with xylanase activity of 92.71 U/ml. The enzyme exhibited more than 80% relative activity in the temperature range from 40 to 60°C. The xylanase activity was dropped at temperature above 70°C and only 0.29% of relative activity was detected at 80°C (Figure 4(a)). The optimum temperature of this xylanase was similar to the xylanases from *Streptomyces* spp. SKK1-8 (Meryandini *et al.*, 2006), *Streptomyces* sp. 7b (Bajaj and Singh, 2010) and *S. thermocarboxydus* subsp. MW8 (Chi *et al.*, 2013).

The thermal stability of partial purified xylanase was measured by incubating the enzyme at various temperatures varied from 30 to 80°C for 1 h. The partial purified xylanase was stable at 30-50°C and retained more than 90% of initial activity. However, the xylanase activity decreased when temperature increased and no xylanase activity was detected at 80°C (Figure 4(b)). Thus, its thermal stability is an attractive feature for industrial applications.

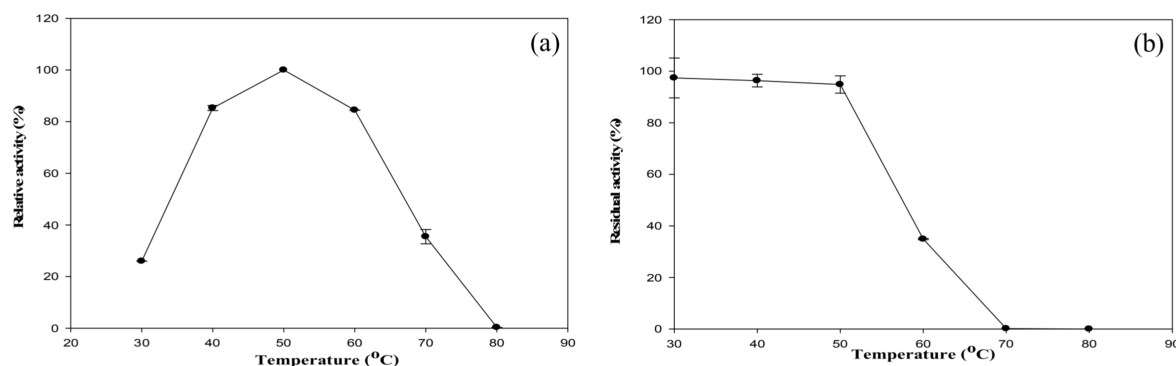


**Figure 4** Effect of temperature on (a) xylanase activity and (b) stability

Effect of metal ions and chelating agent on xylanase activity

The effects of different metal ions and chelating agent on partial purified xylanase were tested by assaying xylanase activity in the presence of different metal ions or chelating agent. The xylanase activity was not affected by $Na^{+}$, $Ca^{2+}$, $K^{+}$, $Mg^{2+}$ and $Fe^{2+}$ similar to the xylanase from *Streptomyces* sp. CS624 (Mander *et al*., 2014), *J. denitrificans* (Nawel *et al*., 2011) and *F. proliferatum* (Saha, 2002). Meanwhile, the xylanase activity was stimulated by $Mn^{2+}$. A similar xylanase from *J. denitrificans* has also been reported to enhance the xylanase activity in the presence of $Mn^{2+}$ (Nawel *et al*., 2011). It could be supposed that $Mn^{2+}$ exerts its effect by interacting with some amino acid residues involved in the active site, which causes a change in conformation leading to higher activity whereas other metal ions did not influence the active site (Nakamura *et al*., 1993; Nawel *et al*., 2011). However, its activity was inhibited by EDTA which the inhibition of xylanase activity in the presence of EDTA. It was suggested that the metals are needed for the enzymatic reaction. The results was similar to reports of xylanase from *Streptomyces* sp. CS624 (Mander *et al*., 2014) and *S. matensis* DW67 (Yan *et al*., 2009).

**Table 2** Effect of metal ions and chelating agent on xylanase activity.

| Metal ions/Chelating agent | Xylanase activity (U/ml) | Relative activity (%) |
|---|---|---|
| Control | 92.71±1.89 | 100.00[a] |
| $CaCl_2$ | 86.28±1.23 | 93.10[a] |
| NaCl | 88.02±1.39 | 94.97[a] |
| KCl | 86.86±1.39 | 93.72[a] |
| $MnSO_4$ | 117.40±1.89 | 126.67[b] |
| $MgSO_4$ | 89.01±0.98 | 96.03[a] |
| $FeSO_4$ | 88.19±1.48 | 95.16[a] |
| EDTA | 81.01±3.61 | 87.43[c] |

*Note: Mean values in the same column followed by a different letter represent significant differences ($P < 0.05$).

**Conclusion**

Crude xylanase from *S. mexicanus* 901 was purified by ultrafiltration followed by gel filtration chromatography. After the final purification step, xylanase was purified greater to be 6.41 folds with specific activity of 55.43 U/mg and 18.11% activity recovery. The optimal pH and temperature for partial purification xylanase were 5.0 and 50°C, respectively. The maximum xylanase stability was found in the pH range of 3.0–6.0 and the temperature at 30–50°C. The xylanase activity was stimulated by $Mn^{2+}$ and was inhibited by EDTA. The inhibitory effect of EDTA suggests that metals are needed for the enzymatic reaction.

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