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Purification and characterization of a xylanase produced by *Streptomyces* sp. during growth in wheat bran as carbon source

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ABSTRACT

In the present work a xylanase produced by a bacterium isolated from Brazilian Cerrado soil and identified as Streptomyces sp. was purified and biochemically characterized. Crude enzyme obtained by filtration and centrifugation of liquid culture containing wheat bran as carbon source was used as enzyme source. One Xylanase was purified to 6.0-fold with 1.1 % yield using Sepharose ANX and Sepharose CM chromatographic columns. The purified enzyme showed as a single band on SDS-PAGE and xylan zymography with an apparent molecular mass of 33 kDa. Optimum pH was detected in pH 5,0 and maximum activity was obtained at 50 °C. The purified xylanase retained 80% of its activity after incubation at 50 °C for two hours. The kinetics parameters K_m and V_{max} was determined using xylan oat spelt as substrate and were 3.5 µmol ml^{-1} and 0.5955 µmol min^{-1}, respectively.

Keywords: *Streptomyces sp*, wheat bran, xylanase, purification, characterization.

INTRODUCTION

Cellulose, hemicellulose and lignin are the main constituents of plant cell walls and among them; hemicellulose is the second most abundant in nature, accounting 25-35% of plant cell wall biomass (Kumar et al., 2008). Hemicellulose is mainly constituted by xylan a heteropolysaccharide which contains D-xylose, D-mannose, D-glucose, L-arabinose, D-galactose, D-glucuronic acid, and D-galacturonic acid as main subunits (Soni and Kango 2013). Xylan complete hydrolysis requires a variety of cooperatively acting enzymes or multiple xylanases as: endoxylanase (endo-1,4-β-xylanase, E.C. 3.2.1.8), β-xylosidase (xylan 1,4-β-xylosidase, E.C. 3.2.1.37), α-glucuronidase (α- glucosiduronase, E.C. 3.2.1.139), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C. 3.2.1.55), and acetylxylan esterase (E.C. 3.1.1.72). All these enzymes act cooperatively to convert xylan into its constituent sugars (Beg et al., 2001; Bastawde 1992). The use of agro-industrial wastes as raw material in biotechnological processes has gained interest since they are abundant, cheap, and environmentally friendly and represent high potential for production of microbial xylanases for applications in various industrial fields, including the pulp and paper industries, bioethanol production and feed industry (Soni and Kango 2013).

Members of the genus *Streptomyces* are able to produce and secrete a variety of enzymes including those involved in the degradation of cellulose, hemicellulose, lignin and many other enzymes of industrial importance (Singh et al., 2015). The present work describes

the production (wheat bran as carbon source), purification and biochemical characterization of a xylanase from the *Streptomyces* sp. isolated from Brazilian Cerrado soil.

MATERIALS AND METHODS

Microorganism and enzyme production: A new *Streptomyces* sp. was isolated from Cerrado soil of the central region of Brazil and cultivated under submerged fermentation containing 0.5% (w/v) wheat bran, according Brito-Cunha et al., 2013.

Enzyme assay and protein estimation: Xylanase activity was assayed by measuring reducing sugars release according to the method of Miller, 1959 with slight modifications and using xylan. Protein concentration was determined by the method of Bradford, using bovine serum albumin as standard.

Enzyme purification: The crude enzyme was concentrated by precipitation with 60% (w/v) ammonium sulfate and dialyzed against buffer A (50 mM Tris-HCl, pH 8.0). The protein sample was loaded onto an ANX-Fast Flow column (1 cm x 5 cm) and was eluted with a linear gradient containing 0.1-1 M NaCl in buffer A. The fractions with xylanase activity were dialyzed against 50 mM sodium acetate pH 4.0 (buffer B) for 12 h. Then the dialyzed sample was loaded onto a CM-Sepharose Fast Flow column (1 cm x 5 cm). The bound proteins were then eluted from the column using a linear gradient of 0.1-1 M NaCl in buffer B. Fractions containing xylanase activity were pooled, dialyzed extensively against water, and stored at -20 °C.

SDS-PAGE and Zymogram: SDS-PAGE electrophoresis was used to determine protein purity and the molecular mass of the purified enzyme under denaturing conditions using a 12% (w/v) polyacrylamide gel. The protein was silver stained as described by Blum et al., 1987). For zymogram analysis, the sample was analyzed by PAGE electrophoresis containing 12 % (w/v) polyacrylamide and oat spelt xylan 0.1 % (w/v), according Ninawe et al., 2008.

Enzyme characterization: The effect of pH on the enzyme activity was determined by varying the pH of the reaction mixtures using 50 mM buffer (pH 4 –10). The effect of temperature on the enzymatic activity was determined at pH 5, in the range of 30–80 °C. Thermostability was determined by pre-incubation of purified enzyme at 40, 50 and 60 °C for 8 h. The residual xylanase activity was represented as the percentage of activity with respect to the activity of the enzyme without heat treatment. The kinetics parameters were estimated using the purified xylanase at concentrations of oat spelt xylan ranging from 2.5 to 5.0 $mg.ml^{-1}$. Activities were determined by the standard procedure and kinetics parameters (K_m and $V_{máx}$) were calculated from Linewever–Burk plot of initial velocity data obtained under the standard conditions.

RESULTS AND DISCUSSION

The result of each step of enzyme purification is shown in Table 1. The crude enzyme was precipitated with 60% saturation of ammonium sulfate and then further purified on anion exchange and cation exchange chromatography, respectively. It was purified 6.0-fold with an overall yield of 1.1% and specific activity of 60.9 $U.mg^{-1}$. The low yield may be due to a loss of enzyme during the purification procedures because only fractions with strong xylanase activity (0.6 IU), were recovered in order to increase the purity of the enzyme.

Table 1. Summary of the purification steps of the xylanase produced by *Streptomyces* sp.

Step	Total protein (mg)	Total activity (IU)	Specific activity ($U.mg^{-1}$)	Purification (fold)	Yield (%)
Crude enzyme	38.25	386.1	10.1	1	100
$(NH_4)_2SO_4$ precipitation	11.73	249.51	11.48	1.1	64.6
ANX-Sepharose	1.86	51.8	27.85	2.8	13.4
CM-Sepharose	0.069	4.2	60.87	6	1.1

The purified xylanase appeared to be homogenous by analysis on SDS-PAGE as shown in Fig. 1. The enzyme molecular mass was estimated to be approximately 33 kDa, based on its mobility and calculated by comparison with standard calibration proteins and is well within the average range of molecular mass for xylanases i.e. 11–85 kDa (Beg et al., 2001). Similar to our result, a xylanase molecular weight of 30 kDa produced by *Streptomyces* sp. 7b was reported by Bajaj and Singh 2010.

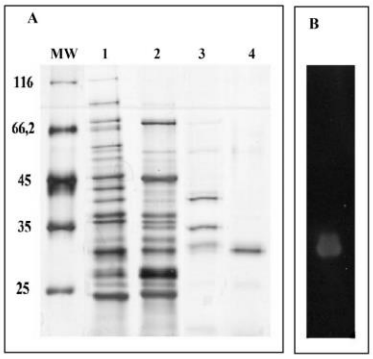

Fig. 1 A) SDS-PAGE analysis of xylanase purification steps (MW: molecular mass marker proteins; lane 1: crude enzyme; lane 2: $(NH_4)_2SO_4$ precipitation; lane 3: after ANX-FF column (pH 8.0 Tris–HCl); lane 4: after CM-FF column (pH 4.0 acetate buffer). B) Activity of the purified xylanase.

Optimum pH was detected at pH 5.0, which is in agreement to those described for *Streptomyces rameus* (Li et al., 2010), *Streptomyces sp* (Deesukon et al., 2012) and *Streptomyces cyaneus* (Ninawe et al., 2008). In addition, xylanase optimum temperature was found to be 50°C at pH 5. This values is in according to optimum temperatures described for xylanases produced by *Streptomyces sp*. PC22 (Chungool et al., 2008) and *Streptomyces thermocarboxydus* (Chi et al., 2013). The purified xylanase retained its activity for at least 60

min when pre incubated at 40 °C and 50 °C, after 120 min a loss of 20% was observed. The values of K_m and $V_{máx}$ determined using Lineweaver-Bunk plot and xylan oat spelt as a substrate were 3.5 µmol ml^{-1} and 0.5955 µmol min^{-1}, respectively.

CONCLUSIONS

A xylanase from *Streptomyces* sp. was purified approximately 6.0 fold with an overall yield of 1.1 % and specific activity of 60.87 $U.mg^{-1}$ with optimum pH at 5.0 and temperature at 50°C. It is a single peptide chain with a molecular weight of 33 kDa. Further experiments are in progress to evaluate its potential application in industrial processes.

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