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CHARACTERIZATION AND ANALYSES OF STABILITY OF BACTERIAL XYLANASE

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ABSTRACT

Xylanases, including endo-β-D-1,4-xylanase (EC 3.2.1.8) and β-D-xylosidase (EC 3.2.1.37), are complex enzyme that hydrolyze xylan to xylo-oligosacharide and xylose. The xylan degrading enzyme can be produced by aerobic or anaerobic mesophilic or thermophilic microbe. The objectives of this study were to characterize and analyze the stability of xylanase obtained from xylanolytic bacterial isolate.

Xylanolytic isolates were isolated from Crabs by using Hungate method and screened for the highest enzymatic activity. The xylanolytic bacterial isolate were then cultivated for xylanase production. To produce xylanases, the isolate was grown in a medium using oat spelt xylan as substrate under anaerobic condition at 35°C and pH 11 for 7 days. The culture supernatant was separated by centrifuging and used as the source of xylanase. The supernatant obtained was concentrated by the addition with ammonium sulphate at levels of saturation of 70%. Concentrated supernatant was then dialyzed against 10 mM acetate buffer. Then the enzyme obtained was purified using ion-exchange (DEAE-cellulose) chromatography. Purified enzyme was characterized its optimum activity under different pH buffer condition (4 - 8), temperature (30 - 60°C) and substrate concentration (xylan).

The results showed that the purified enzyme had the highest activity at pH 4.5 (0.962 U/mg) and temperature 50°C (0.846 U/mg). The value of K_m and V_{max} were 83.33 mg ml^{-1} and 69.93 U mg^{-1} respectively. It was also found that xylanase isolated from xylanolytic bacteria having stability at pH 4-7.

Key words : characterization, stability, bacterial xylanase.

INTRODUCTION

A wide variety of microorganisms are known to produce xylan-degrading enzymes. In recent years, important applications for xylanases in different industrial processes have been found. Potential applications of xylanase in biotechnology include biopulping wood, pulp bleaching, treating animal feed to increase digestibility, processing food to increase clarification, and converting lignocellulosic substances into feedstocks and fuels (7). Because xylan is soluble at alkaline pH, xylanases active and stable at alkaline pH and high temperature could be very important for such applications (2).

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Xylan, a major component of plant cell wall hemicellulose, is composed of a backbone of β-1,4-linked xylose units which are substituted with arabinose and acetate residues (9, 14), also contain glucoronic acid, and/or mannose substitutes (10, 13).

Xylan-degrading enzymes are produced by a wide variety of microorganisms (2), including aerobic and anaerobic mesophiles and thermophiles (10). Many organisms show hemicellulose activities, and one species can produce more than one enzyme. These enzymes include endo-β-1,4-xylanases (1,4-β-D-xylan xylanhydrolase, EC 3.2.1.8), β-xylosidase (1,4-β-D-xylan xylohydrolase, EC 3.2.1.37) (14), and enzymes which cleave side chain sugars from the xylan backbone, such as α-arabinofuranosidases (EC 3.2.1.55) and acetyl esterases (EC 3.1.1.6) (9, 12). In general terms, the xylanases attack internal 1,4-β-bond xylosidic linkages on the backbone of xylan (4), and the β-xylosidases release xylosyl residues by endwise attack of xylooligosaccharaides (6, 14). Although many xylanases are known to release xylose during the hydrolysis of xylan or xylooligosacchrides, xylobiose activity has only been reported in β-xylosidases (14).

We are interested in understanding the stability of xylanase on pH. In this study, we have characterized the purified extracellular xylanolytic enzymes of bacterial xylanase isolated from crabs.

MATERIALS AND METHODS

Bacterial isolate, culture condition, and media. For production of the extracellular xylanolytic , bacterial isolate was grown in the anaerobically medium (11). Whole medium (250 ml), adjusted to pH 11 (3) and containing 2% xylan (oat spelts [Sigma]) as a carbon source, was sterilized at 120°C for 20 min, cooled down to 35°C, and inoculated by adding 25 ml of the bacterium culture. Incubation was carried out at 35 °C under anaerobic conditions (3). After 7 days, incubation was discontinued to obtain a crude enzyme by centrifuging the culture fluid at 10.000 x *g* for 15 min.

Purification of xylanases. The crude enzyme was concentrated by the addition with ammonium sulphate at levels of saturation of 70% , and dialyzed with dialysis tubing (22 kDa-molecular weight-cutoff membranes). Dialysis of concentrates was performed against 10 mM sodium-acetate buffer (pH 6.0), and fed into a DEAE-cellulose column (20 by 1 cm) previously equilibrated with the same buffer, we eluted the column with a linear gradient of 50 mM to 500 mM sodium acetate buffer (pH 6.0). This concentrate was used as the enzyme source to carry out the characterization of enzymes reported in the study.

Assay of enzyme activity. Xylanase activity was assayed by measuring the amount of reducing sugar liberated from xylan. Briefly, assays containing 0.4 ml of 50 mM sodium acetate buffer (pH 6.0) with 0.2 ml of 4% soluble oat spelt xylan, and 0.2 ml of enzyme preparation were incubated at 50°C for 120 min (10), after which the amount of reducing sugar was detected by the Nelson-Somogyi method with D-xylose as the standard (8). Substrate and enzyme controls were always used. All assays were performed in duplicate. One unit of activity is defined as the amount of xylanase needed to liberate 1 μmol of D-xylose per min under these assay condition (10).

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Measurement of protein concentration. The protein concentration of the enzyme preparation was measured by Lowry method (8), with bovine serum albumin as the standard.

Measurement of enzyme properties. The effect of temperature on the reaction was assessed by incubating the reaction mixtures at different temperatures in the range from 30 to 60°C. For the pH effect study, the sodium acetate buffer was used. pH was adjusted to the desired values in the range from 4 to 8 by adding necessary volumes of 0.1 N HCl or 0.1 N NaOH

The effect of substrate concentration on xylanase activity was studied by measuring the activity in mixtures containing 0.2 ml of enzyme and soluble oat spelt xylan to a final concentration ranging from 10 to 60 mg ml^{-1} in 50 mM sodium acetate buffer (pH 4.5). Incubation were performed at 50°C for 20 min.

To analyze the effect of pH on xylanase stability, the pH of this solution was adjusted using 50 mM sodium acetate buffers for pH 4 to 8, after which it was incubated for 1 h at 50°C. The residual xylanase activity was assayed at pH 6.

RESULTS AND DISCUSSION

Effect of temperature and pH on xylanase activity. Maximum xylanase activity occurred at 50°C (Fig. 1). Most xylanase known to date are optimally active at or below 50°C and at acidic or neutral pH range (2). The activity of the enzyme quite stable in the temperature range from 35 to 45°C, when the temperature was increased at 55°C the enzyme lost 70% of its activity. Incubation at higher temperatures rapidly inactivated the enzyme (1).

The effect of pH on xylanse activity is shown in Fig. 2a. The properties of the purified enzyme indicated that the xylanase activity remained considerable in the alkaline pH range. Xylanase exhibited an activity maximum in the pH range from 4.0 to 6.5 at 50°C, and showed an optimum activity in the pH 4.5.

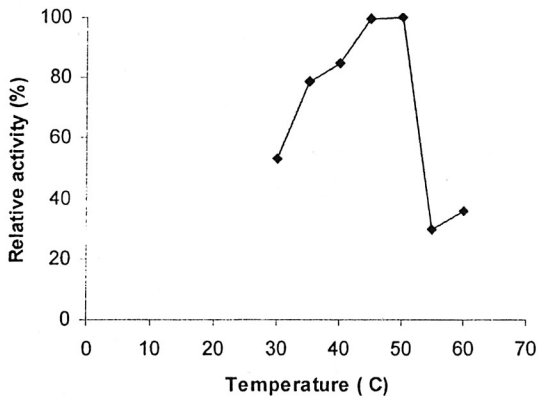

Fig. l. Effects of temperature on the activity of xylanase

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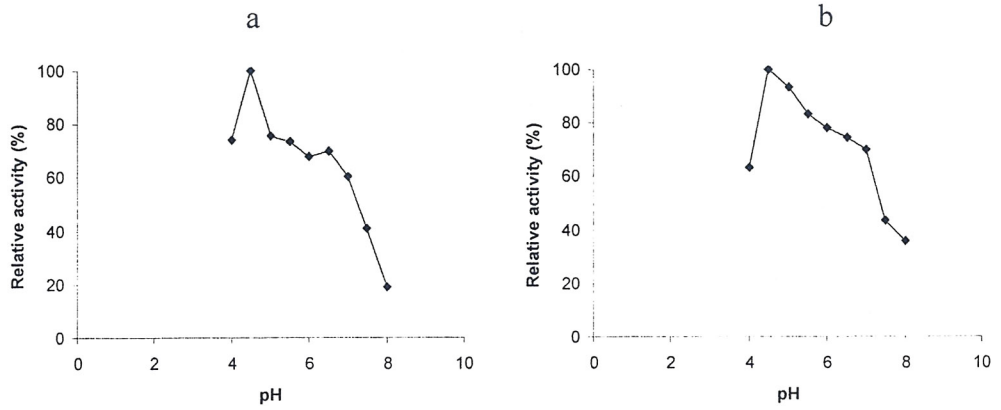

Fig. 1. Effects of pH on the (a) Activity and (b) Stability of xylanase

Regarding the effects of pH on xylanase stability (Fig. 2b), this xylanase retained its activity during 1 h in the pH 4.5, and only lost 7% and 17% of activity after 1 h at 5 and 5.5 respectively. The enzyme was stable in pH 4 to 7, and it retained 63 to 78% activity after 1 h of incubation in that pH. The majority of xylanase reported to date are optimally active in the acidic or neutral pH range. From the application point of view, xylanase active and stable in the alkaline pH range and at elevated temperature are very important. Most alkaliphilic and alkalotolerant microorganisms produce xylanases optimally active around neutrality (5). The xylanase from other alkaliphilic microorganisms are stable at pH up to 12 (9), it retained full activity in the pH range of 5 to 11 (2).

Effect of substrate concentration on xylanase activity. The data of the effect of substrate concentration (xylan) on the activity of xylanase were plotted according to the Lineweaver-Burk plot, and got the regression y=1.1753x + 0.0143. Michaelis-Menten parameters derived from the above plot. The substrate concentrations dependence of the xylanase showed a decrease in the rates at substrate concentrations higher than 40 mg ml^{-1}; therefore, the above kinetic data for this activity only consider the value corresponding to substrate concentrations below the inhibitory concentration in this case. The K_m and V_{max} value determined for the xylanase on oat spelts xylan were 83.33 mg ml^{-1} and 69.93 U mg^{-1} respectively.

REFERENCES

Blanco, A., T. Vidal, J.F. Colom, and F.I.J. Pastor. 1995. Purification and Properties of Xylanase A from Alkali-Tolerant *Bacillus* sp. Strain BP-23. *Appl. and Environ. Microbiol.* Vol. 61 (12) : 4468-4470.

Gessesse, A. 1998. Purification and Properties of Two Thermostable Alkaline Xylanases from an Alkaliphilic *Bacillus* sp. *Appl. and Environ. Microbiol.* Vol. 64 (9) : 3533-3535.

Hanim, C. 2003. Growth Optimalization of Xylanolytic Bacterial from Crab (*Eriocheir sinensis*). Bulletin of Animal Sci., 27 (4) : 168-176.

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Iefuji, H., M. Chino, M. Kato and Y. Iimura. 1996. Acid Xylanase from Khamir Cryptococcus sp.S-2 : Purification, Characterization, Cloning, and Sequencing. *Biosci. Biotech. Biochem.*, 60 (8) : 1331-1338.

Kang, M.K., P.J. Maeng, and Y.H. Rhee. 1996. Purification and Characterization of Two Xylanases from Alkaliphilic *Cephalosporium* sp. Strain RYM-202. *Appl. and Environ. Microbiol.* Vol. 62 : 3480-3482.

La Granga, D. C., I. S. Pretorius and W. H. Van Zyl. 1996. Expression of a *Trichoderma reesei* - xilanase Gene (XYN 2) in *Saccharomyces cerevisiae*. *Appl. and Environ. Microbiol.* Vol. 62 (3) : 1036 - 1044.

Li, X.L., Z.Q. Zhang, J.F.D. Dean, K.E.L. Eriksson and L.G. Ljungdahl. 1993. Purification and Characterization of a New Xylanase (APX-II) from the Fungus *Aureobasidium pullulans* Y-2311-1. *Appl. and Environ. Microbiol.* Vol. 59 (10) : 3212-3218.

Plummer, D. T. 1971. *An Introduction to Practical Biochemistry*. Tata Mc Graw Hill Publ. Co. Ltd. Bombay New Delhi. Pg : 156 - 157.

Ratanakhanokchai, K., K.L. Kyu and M. Tanticharoen. 1999. Purification and Properties of a Xylan-Binding Endoxylanase from Alkaliphilic *Bacillus* sp. Strain K-1. *Appl. and Environ. Microbiol.* Vol. 65 (2) : 694-697.

Ruiz - Arribas, A. J. M. Fernandaz - Abalos, P. Sanchez, A. L. Garda and R. I. Santamaria. 1995. Overproduction, Purification, and Biochemical Characterization of a Xylanase (Xys 1) from Streptomyces halstedii JM 8. *Appl. and Environ. Microbiol.* Vol. 61 (6) : 2414 - 2419.

Skinner, F.A. 1971. Isolation of Soil Clostridia. In : *Isolation of Anaerobes*. The Society for Applied Bacteriology Technical Series No. 5. Academic Press, London, New York.

Sunna, A. and G. Antranikian. 1997. Xylanolitic Enzymes from Fungi and Bacteria. Graham G. Stewart and Inge Russell (Eds.). *Crit. Rev. Biotech.* Vol. 17 (1) : 39 - 67.

Ward, O. P. and Moo - Young. 1989. Enzymatic Degradation of Cell Wall and related Plant Polysaccharides. Graham G. Stewart and Inge Russell (Eds.). *Crit. Rev. Biotech.* Vol. 8 (4) : 237 - 265.

Wong, K.K.Y., L.U.L. Tan and J.N. Saddler. 1988. Multiplicity of β-1,4-Xylanase in Microorganisms : Function and Application. *Microbiol. Rev.* Vol. 52 (3) : 305-317.