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Production of Xylanase by a *Bacillus* sp. 2B5 Isolated from Paper Mills in Kahramanmaras, Turkey

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In this study, xylanase activity of *Bacillus* sp. strains, isolated from the samples of soil and waste taken from nearby paper-mills in Kahramanmaras. Enzyme synthesis occured at 20-55 °C. There was a slight variation in xylanase synthesis within the pH 4.0 to10.5. It has been determined that xylanase enzyme performs the optimum activity at pH 7.0 and 42 °C temperature.

Key Words: Xylanase, *Bacillus* sp., Paper Mills, Kahramanmaras.

INTRODUCTION

In recent years, an interesting in xylanolytic enzymes from microbial sources have been developed. Their role in hydrolysis of xylan is of significance in a variety of well documented industrial applications. Xylan is the second most abundant biopolymer after cellulase and the major hemicellulosic polysaccharide found in plant cell walls. It is a heteropolymer with a backbone of β-1,4-D-xylopyranosyl residue and branches of neutral or uronic monosaccharides and oligosaccharides[1]. In recent years increasing attention has been given to the study of xylan-degrading enzymes because of their potential application in different industrial processes.

One area of application is in the pulp and paper industry where xylanase can be used for the bleaching of kraft pulps[2,3]. The use of xylanase prior to the normal bleaching operation has been shown to significantly reduce the amount of chlorinated organic compounds formed during the bleaching process[2], thus reducing the risk of environmental pollution. Since the kraft process of pulp and paper making is carried out at alkaline pH and high temperature, the use of alkaline xylanases with higher temperature optima is considered to be advantageous. Alkaline xylanases will also find a number of other applications. For example, because of the high solubility of xylan at alkaline pH, alkaline xylanases may have good potential for the hydrolysis of hemicellulosic wastes to fermentable sugars[4].

In the present study the production of a xylanase by an alkaliphilic *Bacillus* sp. isolated from in Kahramanmaras and the properties of the enzyme are reported.

EXPERIMENTAL

Organism and culture conditions: *Bacillus* sp. 2B5 was isolated from soil and waste paper collected in Kahramanmaras. Gram positive spore-forming bacteria *Bacillus* sp. soil was pasteurized at 60 °C for 0.5 h[5]. A loop of the sample was streaked onto xylan containing nutrient agar plates. After 48 h incubation at 37 °C, individual colonies were transferred to fresh xylan-containing nutrient agar plates and incubated as above. Xylanase-producing strains were selected by flooding the plates with 0.1 % aqueous Congo red for 15 min followed by repeated washing with 1 mol L^{-1} NaCl. All colonies showing a clear zone on agar plates were further screened by growing them in liquid medium and assaying enzyme activity from the cell-free culture supernatant fluid[6].

Enzyme production: The growth medium used for xylanase production was composed of (g L^{-1}) oat spelt xylan, 5; peptone, 5; yeast extract, 1; K_2HPO_4, 1; $MgSO_4.7H_2O$, 0.2; CaCl, 0.1 and Na_2CO_3, 10. Sodium carbonate was autoclaved separately and added to the rest of medium after cooling. The organism was propagated at 37 °C for 2 d in 100 mL of medium with shaking on a shaker. The supernatant of the culture after centrifugation (6000 rpm, 20 min) at 4 °C was used to determine extracellular xylanase activity[4].

Determination of enzyme activity: Xylanase activity was assayed by measuring the release of reducing sugar from oat spelts xylan following the dinitrosalicylic acid (DNS) method[7]. To 0.9 mL of substrate in 50 mmol L^{-1} glycine-NaOH buffer, pH 9, 100 µL of appropriately diluted enzyme was added and incubated at 50 °C. After 10 min, 1.5 mL of dinitrosalicylic acid solution was added to the reaction mixture and boiled for 5 min. Absorbance was measured at 540 nm against a reagent blank. One unit of xylanase activity was defined as the amount of enzyme that released 1 µmol reducing sugar equivalent to xylanase perm in under the above assay conditions[4].

Effect of pH and temperature on xylanase activity: Xylanase activity was measured at different pH values under assay condition with oat spelt xylan as substrate. The activity of the enzymes at pH 4-10.5 was assayed using 50 mM citrat buffer (pH 4-6), fosfat buffer (pH 6- 8.5) and glycine-NaOH buffer (pH 8.5-10.5)[4]. At various temperatures (22, 37, 42, 50 °C) at pH 7-9, the enzyme activities were also assayed.

RESULTS AND DISCUSSION

Enzyme production of *Bacillus* sp. 2B5 was analyzed at different pH values and temperatures. The bacteria were grown at the temperature 22, 37, 42, 50 °C. The production of enzyme was determined at 22, 37, 42 and 50 °C. The optimal production was observed at 42 °C (Fig. 1). The basal oat spelt xylan medium was prepared in the pH range from 4.0 to 10.5. The maximum production was at pH 7 (Fig. 1). The experiments were repeated 3 times and mean values were used.

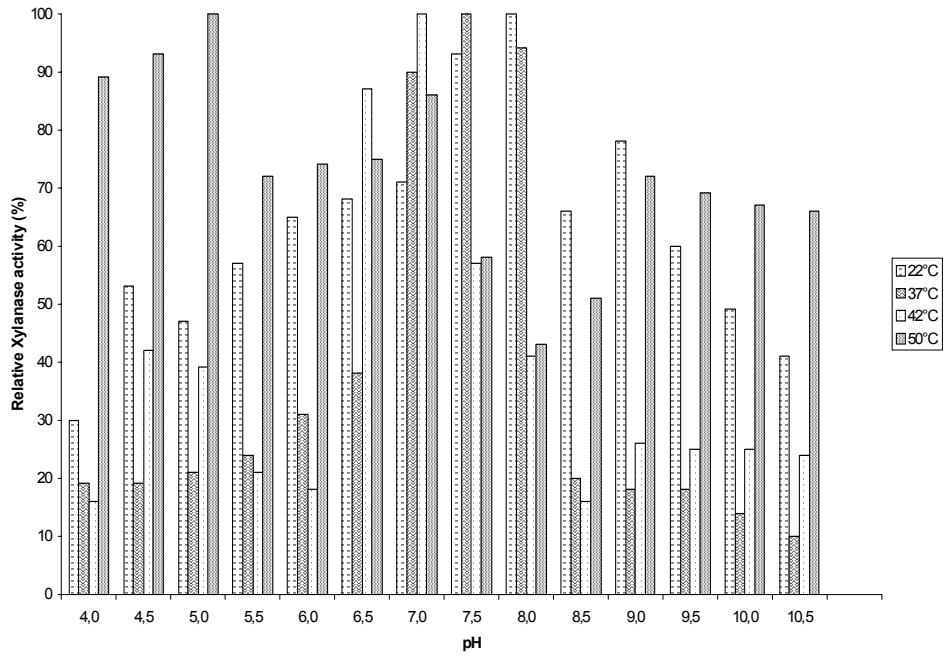

Fig. 1. Effect of temperature and pH on xylanase production by *Bacillus* sp. 2B5

The pH range at which xylanase T-6 is active was determined in tree different buffers covering the range between pH 4.5 and 10.5. The enzyme was most active in the neutral pH range[8], between pH 6.5 and 7.0.

Most xylanases known today are active at acidic[9,10] or neutral pHs[11-14]. Recently, however, several alkaline tolerant xylanases were characterized[8].

Rtanakhanokchai *et al.*[15] found that optimum enzyme activity at pH 5.5. Botaillen *et al.*[16] found that xylanase was active over 9 pH range of 5.0 to 9.0, with an optimum at pH 6.0 to 7.0. Dhillon *et al.*[17] found that the optimum pH for xylanase was 6-7. Qureshi *et al.*[18] found that in *Bacillus subtilis* (pBA7) the optimal xylanase activity was at pH 7.0.

Most xylanases known so far have their optimum pH around neutrality. Even xylanases produced by most alkaliphiles reported to date have their optimum pH around neutrality[4]. According to these results, it is concluded that xynoloytic *Bacillus* sp. 2B5 might by suitable for processing of xylanase containing astes, paper industry and animal feed industry under appropriate conditions.

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