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Study on Cellulase Production by a *Bacillus* sp. C6

AYSE DILEK OZSAHIN and OZLEM KIRAN*
Department of Biology, Faculty of Art and Sciences
Kahramanmaras Sutcu Imam University, Avsar Kampusu, Kahramanmaras, Turkey
Fax: (90)(344)2191012; Tel: (90)(344)2191316; E-mail: ozkiran@ksu.edu.tr

In this study, cellulase activities of *Bacillus* sp. strains isolated from the samples of soil, water and waste taken from nearby paper-mills in Kahramanmaras have been investigated and techniques to increase enzyme activities have been applied. By performing the intensifying of cellulase from *Bacillus* sp. strains which show the highest activity of cellulase, enzyme amounts have been investigated. In additional, interval of pH and temperature that enzyme has shown optimum activity has been found. It has been determined that cellulase enzyme performs the optimum activity at pH 10 and 40 ºC temperature. Furthermore, it has been observed that metal ions and concentration of carboxymethyl cellulose have affected enzyme activity.

Key Words: *Bacillus*, Cellulase, Temperature, pH, Metal ions, Carboxymethyl cellulose.

INTRODUCTION

Cellulases have attracted much interest because of the diversity of their applications and also for facilitating the understanding of mechanism of enzymic hydrolysis of plant carbohydrate polymers[1]. The major industrial applications of cellulases are in textile industry for bio-polishing, addition to washing powder, addition to animal feed to enhance its digestibility, pulping, extraction of olive oil, wine production, processing of fruit juices and beverage baking and in bioethanol production can be mentioned[2].

Enzymes from microbial sources generally meet industrial demands[3]. Among bacteria *Bacillus* species produce a number of extracellular polysaccharide hydrolyzing enzymes[4,5]. A fairly common observation has been that *bacilli* lack the complete cellulase system, the main activity being that of carboxymethyl cellulose which does not hydrolyze crystalline cellulose[6-8]. These include also the alkaline cellulases with potential as laundry detergent additives[9].

We have isolated cellulase producing *Bacillus* strain from paper factory in Kahramanmaras and studied the enzyme production under various culture conditions. The present study is concerned with the purification and property of the cellulase produced by *Bacillus* sp. C6.

EXPERIMENTAL

Bacillus sp. C6 was isolated from soil samples collected in Kahramanmaras, Turkey. Gram-positive spore-forming bacteria *Bacillus* sp. soil was pasteurized at 60 ºC for 0.5 h[10]. This organism was found to produce an cellulase on CMC agar plates containing CMC (carboxymethyl cellulose) 10 g, yeast extract 10 g, NaCl 10 g, Tripton 10 g and agar 15 g[11]. The organism was propagated at different temperatures (30-60 ºC) and pH values (8.0-10.5)[12,13]. Cellulase production was detected after flooding the plates with congo-red solution[14].

Enzyme production: The organism was propagated at 37 ºC for 24 h in 100 mL of medium with shaking on shaker. The supernatant of the culture after centrifugation (6000 rpm, 20 min) at 4 ºC was used to determine extracellular cellulase activity[15].

Enzyme assay: Cellulolytic activity was determined[13]. The reaction mixture contained 0.5 mL of substrate solution [2 % CMC in 0.1 M glycine NaOH buffer (pH 9)] and 0.5 mL of enzyme solution. After 0.5 h of incubation at 40 ºC, the reaction was stopped by the addition of 1 mL of dinitrosalicylic acid solution[15]. The mixture was heated at 100 ºC for 5 min and measured at 540 nm[17]. The enzyme activities were calculated using a calibration curve prepared with D-glucose as standard by following the same procedure above. One unit of activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1 mmol of D-glucose under the assay conditions.

Protein assay: The protein concentrations determined using bovine serum albumin as standard[18].

Effects of temperature and initial pH: The effect of temperature on enzyme production was determined by measuring activity at 30, 40, 50 and 60 ºC. The effect of initial pH on cellulase production was performed at pH 8.0-10.5. The buffer used was 0.1 M glycine-NaOH[10].

Effects of CMC concentration: To determine effect of substrate concentration at pH level (pH 10) that high activity was seen, the CMC in complete medium was replaced with 0.5, 0.75 and 1.0 %. Enzyme activity (U/mL/min) and total protein amount (mg) were analyzed.

Effects of metal ions: Effects of Na, Cu, Ca, Fe, Mg, Zn, Mn, Co, Ni, K (0.1, 1.0 and 10.0 mM) on bacterial growth and enzyme production were examined with the addition of these chemical to the growth medium[1,19,20]. Enzyme activity (U/mL/min) was analyzed.

RESULTS AND DISCUSSION

Enzyme production of *Bacillus* sp. C6 was analyzed at different temperatures and pH values. The production of enzyme was determined at 30, 40, 50 and 60 ºC. The optimal production was observed at 40 ºC (Fig. 1).

The maximum production was at pH 10 (Fig. 2). The experiments were repeated 3 times and mean values were used.

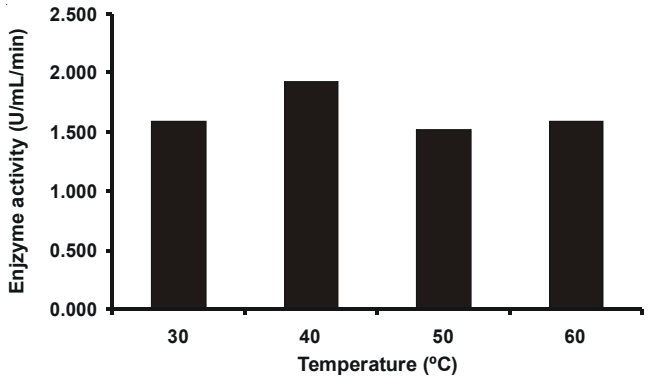

Fig. 1. Effect of temperature on cellulase production by *Bacillus* sp. C6

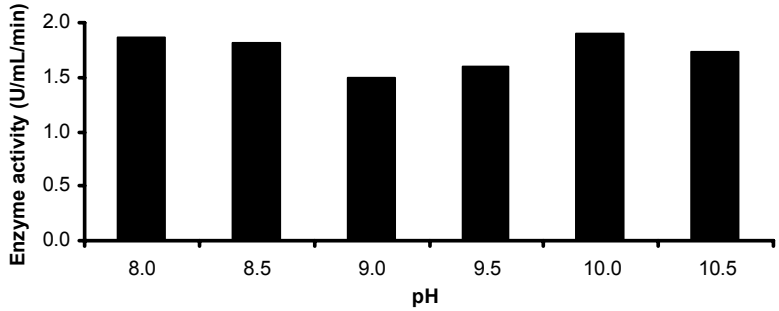

Fig. 2. Effects of initial pH on cellulase production by *Bacillus* sp. C6

Hakamada *et al.*[13] were found that enzyme synthesis of *Bacillus* sp. KSM-S237 occured between pH 9-12, with an optimum pH 9. Ito[12] reported that the maximum enzyme production of *Bacillus* sp. KSM-635 was obtained at pH 9.5. Kim *et al.*[21] were found that maximum activity of *Bacillus* sp. HSH-810 was obtained at pH 10.

Singh *et al.*[22] found that the optimum temperature of *Bacillus* brevis VS-1 strain was 37 ºC for cellulase activity. Hakamada *et al.*[13] found that enzyme synthesis and bacterial growth of *Bacillus* sp. KSM-S237 occured between 10 and 40 ºC with an optimum of 40 ºC.

Lin *et al.*[17] found that enzyme synthesis of *Bacillus* sp. TS-23 occured between 42 and 60 ºC, with optimum of 55 ºC. Robson[23] reported that *Bacillus* brevis exhibited best enzyme production at 37 ºC.

Effect of metal ions: The effects of various chemicals on cellulase production were investigated by strain C6. The results are shown in Fig. 3. The enzyme activity was strongly inhibited by Ca, Mg, K, Ni and Zn ions.

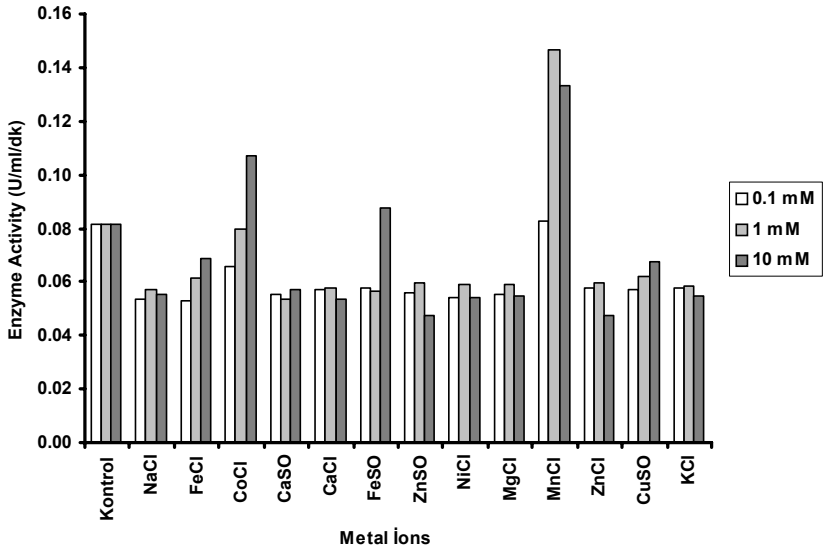

Fig. 3. Effect of metal ions on cellulase production by *Bacillus* sp. C6

Effects of metal ions were tested in 5 mM concentration sodium and potassium ions addded as chlorides slightly (10 %) inhibited the activity[24]. Ca, Mg, Zn did not significantly effect the enzyme activity, whereas Fe^{3+} slightly (15 %) activated the reaction. Cu and Mn inhibited the activity by about 30 %.

The activity was stimulated by Fe and Cu but the activity was strongly stimulated by Mn ions rather than Co ions. The stimulation by Co may be common characteristics of some *Bacillus* cellulases[6,25].

Mawadza *et al.*[1] found that (1 mM) Co stimulated the enzymatic activity (38 %) of alkaliphilic *Bacillus* sp. CH43 but most metal ions such as K, Na, Mg, Cu, Ca, Ni, Zn, Fe did not influence the activity.

Heck *et al.*[26] found that Co, Mn and β-mercaptoethanol showed stimulation on enzyme production from *Bacillus coagulans* BL-69 and inhibition with the addition of Fe, Cu, Ca, Zn, Ba, Mg and EDTA.

Similarly, 1 mM Fe and 0.1 mM Mn have been found to be a potent inhibitor (50 %) of cellulase from *Bacillus* sp. HSH-810[21].

Effects of CMC concentrations: Enzyme activity of *Bacillus* sp. C6 was analyzed at different CMC concentration. The production of enzyme was determined at 0.50, 0.75 and 1.00 %. The maximum production was observed at 1 % CMC due to substrate amount increase. The results of cellulase enzyme activity and total protein of *Bacillus* sp. C6 at different CMC concentrations are shown in Figs. 4 and 5.

Aa *et al.*[8] found that the enzyme activity of *Bacillus subtilis* CK-2 strain was increaed because of CMC concentration increase.

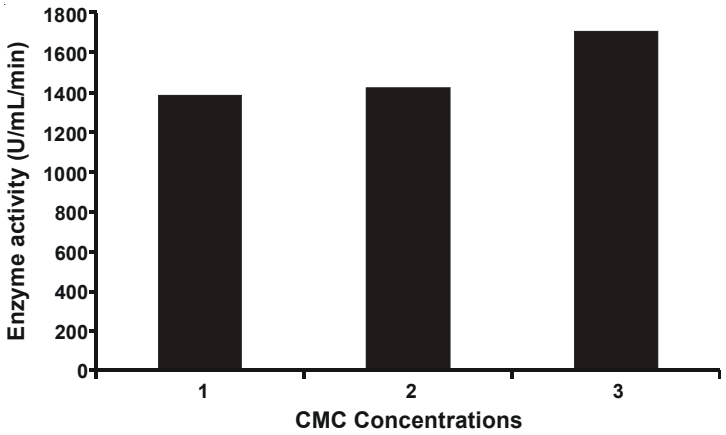

Fig. 4. Effect of CMC concentration on enzyme activity; CMC concentration levels were coded as 1, 2 and 3 for 0.50, 0.75 and 1.00 %, respectively

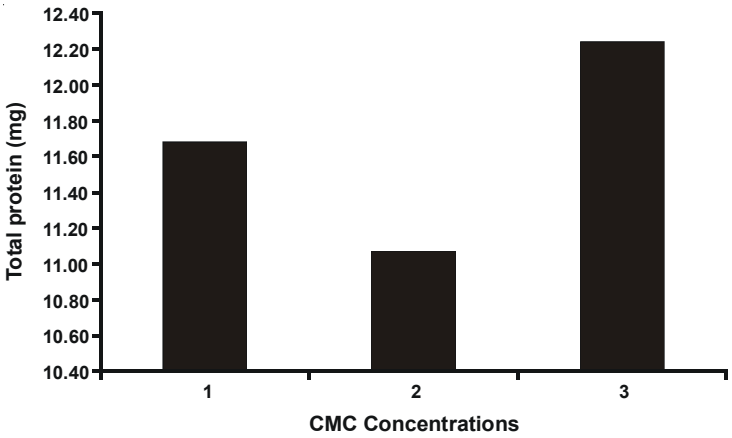

Fig. 5. Effect of CMC concentration on total protein; CMC concentration levels were coded as 1, 2 and 3 for 0.50, 0.75 and 1.00 %, respectively

Similarly, CMC concentration has been found to be augmenter of cellulase from *Ruminococcus albus*[27].

According to these results, we concluded that celluloytic *Bacillus* sp. C6 might be suitable for processing of cellulase containing wastes, paper industry, animal feed industry, laundry detergent industry and food industry under appropriate conditions. The crude cellulase was active at temperature 30-60 ºC, pH 8.0-10.5, with optima at 40 ºC and pH 10.

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