CELLULASE PRODUCTION FROM ACTINOMYCETES ISOLATED FROM IRAQI SOILS: II. CELL GROWTH AND CELLULASE ACTIVITY OF *STREPTOMYCES* SP. STRAIN AT7 AT DIFFERENT TEMPERATURES

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SUMMARY: Streptomyces sp. Strain AT7 releases both carboxymethylcellulose (C_x) and Avicelase (C_1) enzymes during growth in liquid medium with maximal activities, measured as mg/ml/h, of 6.0 and 13.6 obtained at 36ºC for 2 and 9 day incubation periods respectively. C_x kept its activity at 42ºC till the end of the incubation period (18 days) while C_1 lost almost 50% of its activity at the same time. Maximum yields of cell growth (protein) obtained at 28º, 36º and 42ºC; were 0.23, 0.22 and 0.25 µg/ml respectively.

Key Words: Streptomycetes, Cellulase Activity, Carboxymethylcellulase, Avicelase.

INTRODUCTION

The hydrolysis of cellulose to soluble sugars that makes it available for feedstock in alcoholic fermentation, single cell protein, and other industrial processes (3,6,15), has stimulated research on its bioconversion processes.

The complex cellulolytic enzymes, which convert cellulose to glucose, have been shown to be an extracellular product of several microorganisms (4,9). Most work on cellulase has been focused on fungal system which produce a complex of three distinct enzymes (exo-B-1, 4 glucanase (EC 3.2.1.91); endo-B1, 4 glucanase (EC 3.2.1.4) and B-glucosidase (EC 3.2.1.21) which act sequentially to hydrolyze crystalline cellulose to glucose (14). A few reports only are found in literature about the cellulolytic activity of mesophilic and thermophilic actinomycetes and the enzyme of some thermophilic actinomycetes, which is capable of degrading cellulose, have now been studied thoroughly (5,7,13).

This paper describes cell growth and cellulolytic activity (C_X and C_1) of *Streptomyces* sp. Strain AT7 which was previously described as a strain that has a good cellulolytic activity at low and high temperatures.

MATERIALS AND METHODS

Microorganism: Streptomyces sp. Strain AT7 recently described as a cellulolytic one was used in this study. The culture was maintained on mineral salts medium (2) containing 2% carboxymethylcellulose (low viscosity, BDH) and the slant was kept at 4ºC. Fresh subcultures were prepared as required.

Cultural conditions and enzyme preparation: A spore suspension (0.2 ml) obtained by adding 10 ml of sterile distilled water to a fresh slant agar was used to inoculate Erlenmeyer flasks (500 ml) in duplicate each containing 100 ml of the mineral salts

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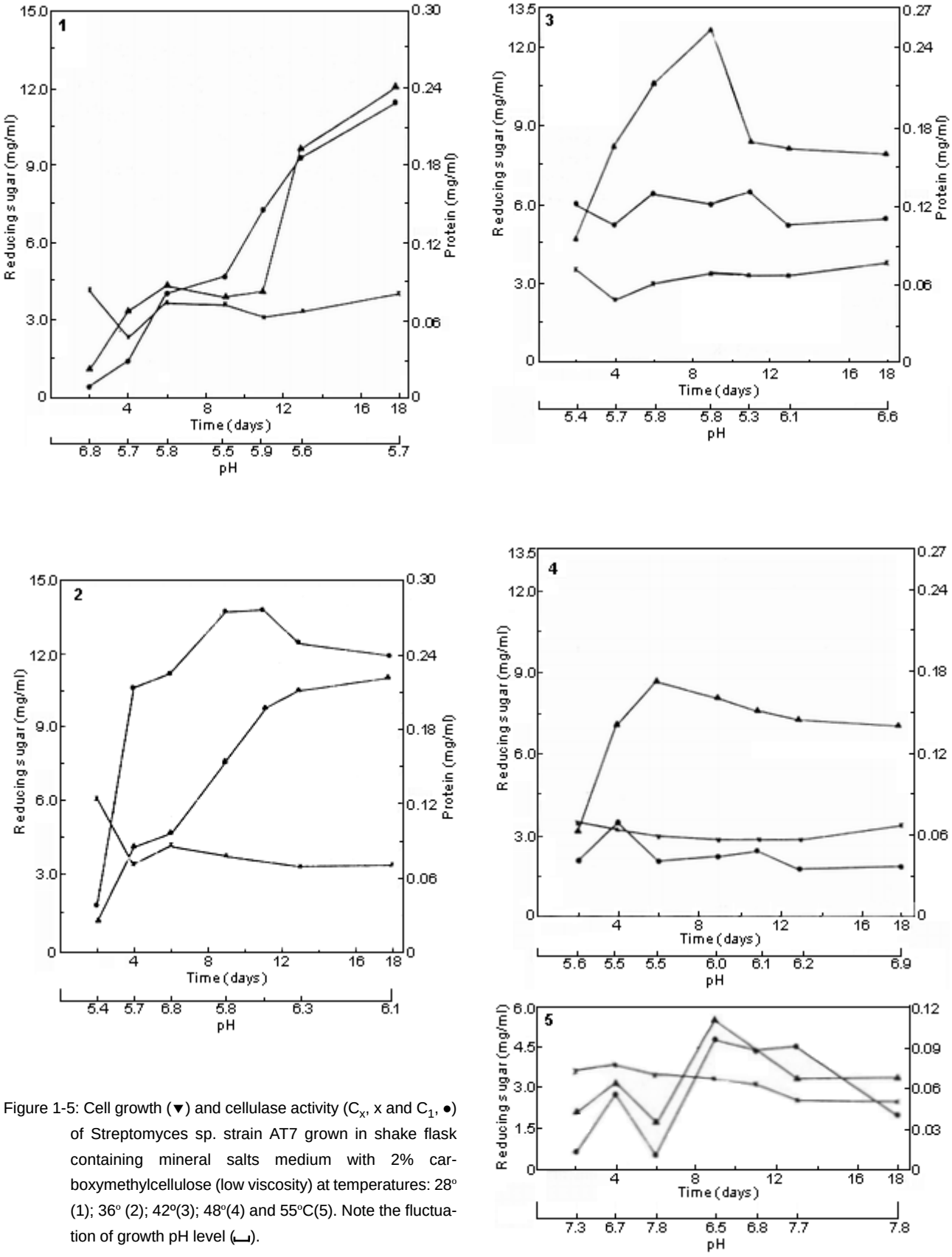

Figure 1-5: Cell growth (▼) and cellulase activity (C_x, x and C_1, ●) of Streptomyces sp. strain AT7 grown in shake flask containing mineral salts medium with 2% carboxymethylcellulose (low viscosity) at temperatures: 28° (1); 36° (2); 42°(3); 48°(4) and 55°C(5). Note the fluctuation of growth pH level (ـــ).

medium. The inoculated flasks were incubated at 28°, 36°, 42°, 48° and 55°C in an orbital shaker (Gallenkhamp) at 150 rpm for various length of time (2-18 days). No adjustment to the initial pH (7.4) in all the flasks was done. Sample of 10 ml of each flask was taken aseptically at regular intervals throughout the growth phase, centrifuged at 5000 g for 20 min at 4°C. The clear supernatant was used for enzyme measurement and its pH was recorded at each time.

Measurement of enzyme activity: Carboxymethylcellulose (C_x) and Avicelase (C_1) activities were estimated according to the method of Mandels *et al.* (10). Reducing sugars produced was estimated by 3,4 dinitrosalicylic acid (BDH) method of Miller (12), with glucose as standard.

Protein determination: Protein in culture filtrate was determined by the method of Lowry *et al.* (8) using bovine serum (BDH).

RESULTS AND DISCUSSION

The cellulase activity of crude enzyme preparation of *Streptomyces* sp. AT7 was investigated over a temperature range of 28°-55°C for various durations.

Cellulase activity of AT7 at different temperatures: The results indicate that AT7 secretes both C_x and C_1 enzymes with an active C_1. Figure 1 showed a gradual increase in C_1 activity, measured as mg reducing sugar/ml/h, during the whole incubation period with a maximum reached 11.4 mg/ml obtained after 18 days at temperature 28°C. However, at 36°C, the activity of C_1 attained its maximal values (13.6 mg/ml/h) in a relatively shorter time (9 days), Figure 2 Maximum activity of C_x enzyme was 4.1 and 6.0 mg/ml at temperatures 28°C and 36°C respectively after 2 day incubation, followed by a rapid decline to reach almost half its activity after 4 days. This declination appeared obvious by the change of the viscosity of the medium. However the activity of C_1 was 3 times more than the activity of C_x at 36°C. While the activity of C_x enzyme at 42°C followed the same pattern as that of 28°C and 36°C (Figure 3) activity. However this relation continue steadily at higher temperatures (48°C and 55°C) till the end of the incubation period. The results also showed that C_x enzyme was more stable at these high temperatures. Similar results were found by Mannig and Wood (11).

Cell growth of AT7 different temperatures: The maximum yield of cell growth of *Streptomyces* sp. AT7 (measured as soluble protein, mg/ml) was 0.23, 0.22 and 0.25 mg/ml obtained at 28°, 36° and 42°C after 18,18 and 9 days of incubation respectively. These data indicate that better growth occurred at a range of temperatures between 28°-42°C with maximum growth obtained at 42°C. No data are available on cell growth of mesophilic actinomycetes which tolerate high temperatures. Brown, *et al,* (1) in his investigation on a mesophilic fungus (*P. pinophtlum,* mutant) that had been reported to grow well at high temperatures, found that maximum growth rate of the fungus was between 35°-37°C. Although previous studies showed that strain AT7 had the ability to well on mineral salts medium at higher temperatures (48°C), less growth of AT7 was obtained in the present study at higher temperaturesk, 48° and 55°C (Figures 4 and 5), as a result of reduction in release of cellular protein.

The present data also indicate that the activity of C_1 enzyme proved to correlate better with the amount of soluble protein released than C_x enzyme although relatively smaller amounts of the enzyme were obtained. This may be due to quick substrate hydrolysis and end product inhibition, as this suppresses cellulase production (16). Best growth and cellulase activity of strain AT7 was obtained at fluctuation of pH range between 5.7-6.5 during growth period (Figures 1-5). The activity of cellulase enzyme (C_x) was found earlier to be also high when the strain was grown on solid medium with alkaline pH (unpublished data). In conclution the present study proved that strain AT7 can tolerate higher temperature and secretes both C_x and C_1 enzymes at wide range of temperatures (28°-55°C).

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