Qatar Univ. Sci. J. (1996), 16(2): 263-267

EFFECT OF CULTURE CONDITIONS ON THE PRODUCTION OF GROWTH REGULATORS AND EXTRACELLULAR ENZYMES BY *STREPTOMYCES OLIVACEOVIRIDIS*

H.S. ALDESUQUY*, F.A. MANSOUR and H.A. HAMEDO
Botany Department, Faculty of Science, Mansoura University, Egypt.
Present address: Department of Science, Hail Teacher College, Hail, P. O. Box 1818, Kingdom of Saudi Arabia.

تأثير الظروف البيئية والتزريعيه على إنتاجية النمو والنشاط الأنزيمي بسلالة *S. olivaceoviridis*

حشمت سليمان الدسوقي و فتحي عواد منصور و هند عبد الحميد حميدو
قسم النبات – كلية العلوم – جامعة المنصورة – مصر

لقد تبين من دراسة أنسب الظروف البيئية والتزريعية لإنتاج أعلى قدر من منظمات النمو بصفة خاصة مع الأخذ في الأعتبار النشاط الأنزيمي بسلالة *S. olivaceoviridis* أن عند تنمية هذه السلالة على بيئة نشا – كازين باستخدام المزارع المهزوزة تعطي أعلى قدر من منظمات النمو والنشاط الأنزيمي ولقد إتضح أيضاً أن أنسب رقم هيدروجيني لبيئة التخمر المستخدمة للنمو وإنتاج الأنزيمات هو pH 7.0 .

وجد أن درجة الحرارة المثلي لنشاط أنزيمي الالفا أميليز والبوتينيز هي ٤٥°م، ٥٠°م على التوالي.

وجد أن أنسب تركيز لأيون الهيدروجين (pH) للنشاط الأنزيمي هو pH 4.8 بالنسبة لأنزيم الألفا أميليز، pH 7.0 بالنسبة لأنزيم البروتينيز .

أظهرت السلالة *S. olivaceoviridis* أعلى إنتاجية لمنظمات النمو والأنزيمات موضوع الدراسة عند استخدام النشا كمصدر كربوني والبيبتون كمصدر نيتروجيني .

وجد أن السلالة المختارة تعطي أعلى معدل من منظمات النمو والأنزيمات عندما يكون تركيز ثنائي فوسفات البوتاسيوم ١ جم/لتر.

وجد أن منظمات النمو والأنزيمات تتزايد بإضافة بعض العناصر الدقيقة للوسط الغذائي وقد لوحظ أن الزنك (في صورة كبريتات الزنك) هو أكثر تلك العناصر حفزا لإنتاج منظمات النمو والأنزيمات بالوسط الغذائي .

تزايدات كمية الأنزيمات ومنظمات النمو التي تنتج بواسطة الكائن زيادة ملحوظة بإضافة الفيتامينات إلى بيئة التخمر وخاصة فيتامين ب ٢ أو مستخلص الخميرة.

Key words: Culture condition, Enzymes, Growth regulating substances, *Streptomyces olivaceoviridis.*
Running title: Culture conditions effect on enzymes and hormones

ABSTRACT

It was proposed to find out the optimum environmental conditions and nutritional requirements for highest growth regulating substance and enzyme production by *Streptomyces olivaceoviridis*. The optimum α-amylase and proteinase activity was at 45°C and 50°C, respectively. The test strain appeared to prefer pH 4.8 and 7.0 for the maximum productivity of α-amylase and proteinase, respectively. The highest potentiality for α-amylase and proteinase synthesis as well as the production of growth regulating substances were attained when *S. olivaceoviridis* was cultivated on media supplemented with starch as carbon source and peptone as nitrogen source. Furthermore, the addition of K_2HPO_4 at 1.0 g/l (as phosphorus), zinc sulphate (as microelement) and yeast extract (as a vitamin source) to culture medium were found to enhance the production of growth regulating substances and enzymes by the experimental organism.

INTRODUCTION

Several investigators have pointed out the importance of culture conditions such as the chemical composition and pH of the medium, temperature and other factors in the production of enzymes [1, 2, 3 and 4].

The production of auxins, gibberellins and cytokinins is influenced by the growth and incubation time [5]. Also, culture conditions, age of cells and the presence or absence of some essential nutrients may influence greatly the secondary metabolism of microorganism [6]. The greatest amount of auxins are produced by bacteria, followed by actinomycetes. Very small amounts of gibberellin like substances are produced by bacteria and actinomycetes isolated from soil and mycorhizosphere of pine [7].

It has been authenticated by many investigators that certain microelement influence the growth and biosynthesis of different microbial products particularly enzymes [3]. Therefore, it was proposed to find out the optimum environmental and nutritional requirements for highest production of growth regulating substances and enzymes by *Streptomyces olivaceoviridis.*

MATERIALS AND METHODS

The growth regulating substances and enzymes determined as described earlier [8].

To determine the best temperature for maximum α-amylase and proteinase activity, the various enzyme preparations were subjected to temperatures of 25, 30, 35, 40, 45 50 and 60°C in an incubator for 1 hr. Subsequently the activity was determined.

To detect the suitable pH for maximum production of enzymes, the pH values of the culture medium was adjusted to 5.0, 6.7, 7.0, 7.5, 8.0 and 9.0 and the production of α-amylase and proteinase was tested at these pH values. The effect of pH value of the reaction mixture on α-amylase and proteinase activities were tested.

The suitability of various carbon and nitrogen sources by the studied strain was tested. Furthermore the effect of different microelements and vitamins was investigated.

RESULTS AND DISCUSSION

In the present investigation satisfactory enzyme productivity by *Streptomyces olivaceoviridis* was attained at 45°C and 50°C for α-amylase (20 units/ml) and proteinase (32 units/ml) respectively. These results were in good agreement with those obtained by Waksman [9]. The optimum pH values for α-amylase and proteinase production were 4.8 (30 units/ml) and 7.0 (70 units/ml) respectively. Waksman [9] stated that the optimum pH for α-amylase productivity is 6.6-6.7. Proteinase is active at a wide range of pH as shown by Mizusawa [10].

Auxins production was best on using glucose' fructose, xylose, sucrose, or starch as sole carbon source (Fig. 1). Starch followed by fructose and glucose when used as sole carbon source supported the highest amylase yield (Fig. 1). These results are in accordance with those obtained by Allam [11], working on *Thermomonospora vulgaris* and by Harhash [12] on *Fusarium moniliforme.* Starch followed by glucose, xylose, and mannitol were favourable for proteinase production (Fig. 1). In this connection, it may be mentioned that Mansour [2] stated that the most favourable carbon source for proteinase production by thermotolerans actinomycetes was starch followed by glycerol, mannitol and inositol. Starch was the best carbon source as it favoured highest biomass yield, highest sugar accumulation and highest protein biosynthesis in mycelial of *S. olivaceous* and *S. parvullus* [13].

Starch represents the best carbon source supporting the production of growth regulating substances particularly auxins and gibberellins (Fig. 1). Takaki and Dietrich [] proposed that polysacchridase activities were enhanced by GA_3. Furthermore, in corn, barley, wheat and oat grains there was a stimulation of amylolytic activity with GA_3 [15].

Peptone was the most favourable nitrogen source for α-amylase production (Fig. 2). This is in conformity with the results of Gabr and Mansour [16] and Gabr *et al.* [13]. Casein followed by peptone supported the production of high levels of auxins. This was accompanied by comparatively large amount of proteinase. On the other hand, urea and ammonium sulphate seemed to be unfavourable for proteinase production by the test organism (Fig. 2).

The results showed that the optimum concentration of dipotassium monohydrogen phosphate for growth regulating substance and enzyme production by *Streptomyces olivaceoviridis* to be 1.0 g/l. In this connection, Harhash [12] reported that production of exocellular amylase by *Fusarium moniliforme* was achieved in phosphate containing medium but no amylase was detected in phosphate deficient

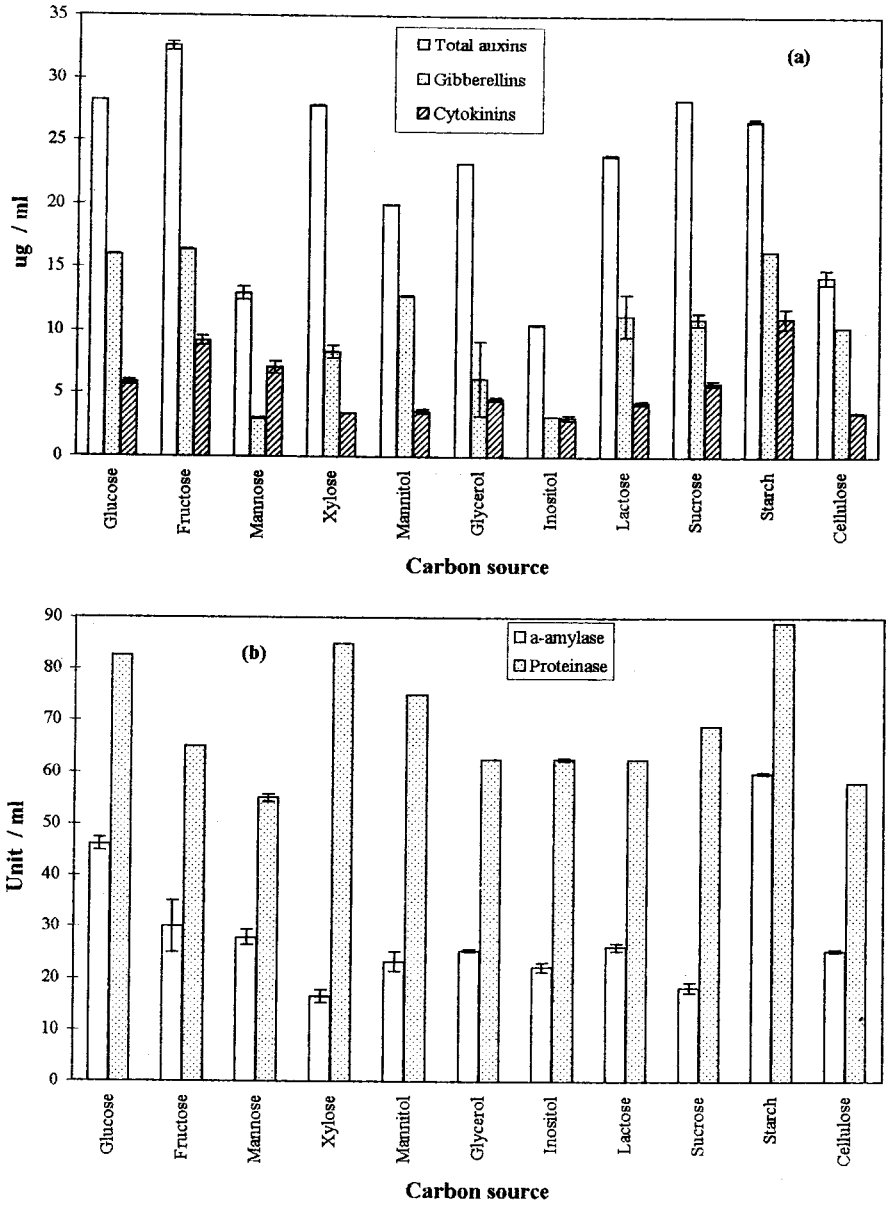

Fig. 1: Effect of different carbon sources on (a) growth regulating substances and (b) α-amylase and proteinase produced by *Streptomyces olivaceoviridis.*

medium. In the present study, the results showed that the increase in α-amylase activity was parallel to the concomitant increase in both gibberellins and cytokinins detected at the same incubation period. In support of this conclusion, Takaki and Dietrich [14] proposed that polysaccharidase activities were enhanced by GA_3. It was reported by Boothby and Wright [] that cytokinin may be substituted for gibberellins in the induction of α-amylase.

Auxins production by *S. olivaceoviridis* was enhanced by molybdenum (22 µg/ml), iron (20 µg/ml) or boron (20 µg/ml) in the medium but not by manganese or zinc. The observed auxin production by *S. olivaeoviridis* when zinc was present in the basal medium may be explained on the fact that zinc activated IAA oxidase and peroxidase which participate in auxin degradation [18, 19].

Gabr and Mansour [16] stated that Fe, Zn and Mn enhanced the biosynthesis of amylase enzyme production by *Streptomyces viridochromogenes*. On the other hand, Naguib *et al.* [20] and Gabr *et al.* [3] showed that zinc followed by nickel initiated larger activities of amylolytic activity. Moreover proteinase production was affected with different microelement i.e. nickel followed by zinc and boron. In this connection, Maceda *et al.* [21] reported that zinc and calcium ions activated proteinase production. It is obvious that, the presence of zinc in the basal medium of *S. olivaceoviridis* resulted in the marked production of gibberellins which was parallel to a progressive increase in amylolytic activity.

Auxin production was enhanced when the medium was supplemented with yeast extract or riboflavin. This was accompanied by stimulated proteinase production. Gibberellins (10 µg/ml and 7 µg/ml) was produced in media containing yeast extract or riboflavin respectively. These high levels of gibberellins were accompanied by comparatively high level of proteinase enzyme (59 units/ml and 33 units/ml respectively). This may probably be due to that

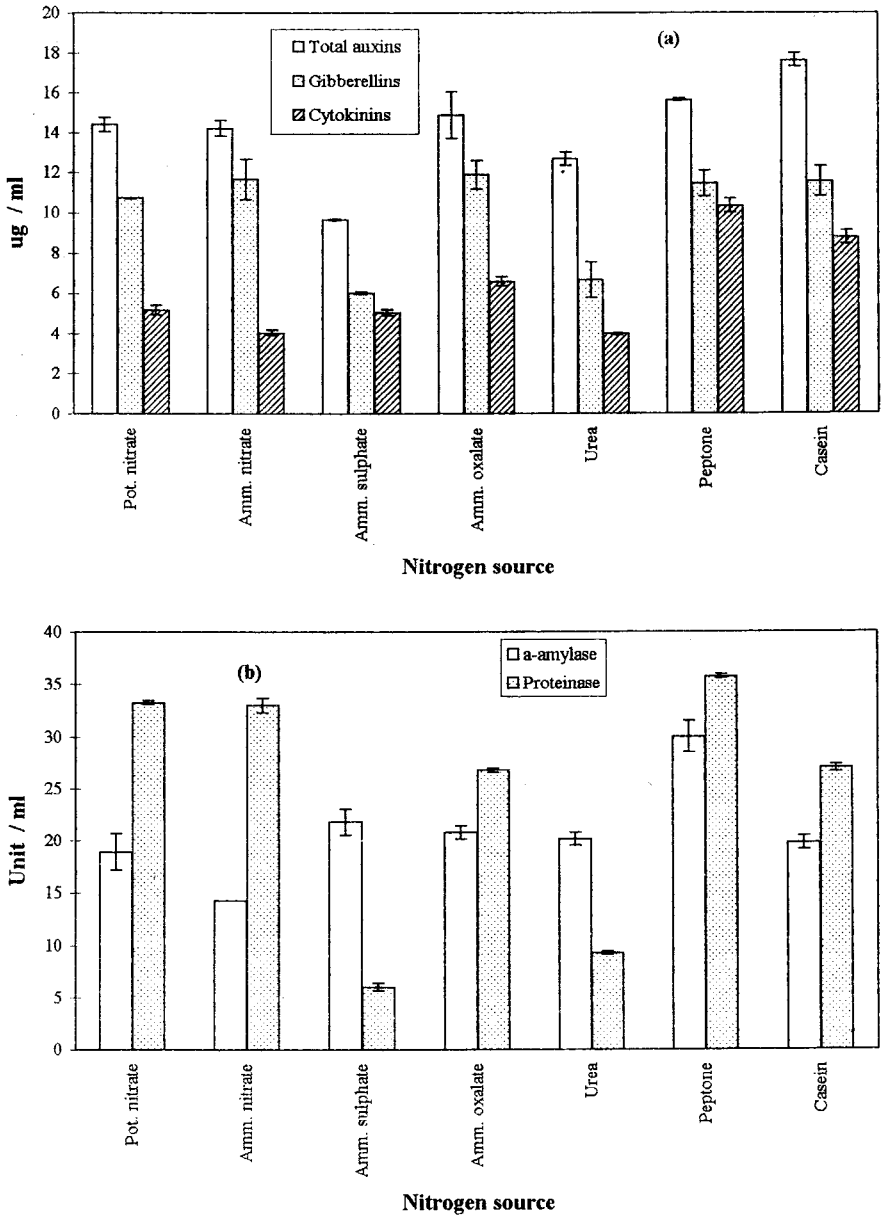

Fig. 2: Effect of different nitrogen sources on (a) growth regulating substances and (b) α-amylase and proteinase produced by *Streptomyces olivaceoviridis.*

GA_3 activates the production of many hydrolytic enzymes. Eastwood *et al.* [22] reported that GA_3 is the controlling factor that induces the synthesis of a variety of hydrolytic enzymes. Yeast extract or folic acid resulted in the production of (7 μg/ml and 8 μg/ml) cytokinins respectively.

REFERENCES

[1] Zeinat, K.M., 1983. Some factors affecting amylase production of *Streptomyces antibioticus*. Egypt, Soc. Appl. Microbiol. Proct. V. Conf. Microbiol., 1 Fermentation Microbiol.

[2] Mansour, F.A., 1985. Studies on the proeolytic enzymes of some actinomycetes. 2nd Conf. Agric. (Mansoura), 163.

[3] Gabr, M.A., Mansour, F.A. and **Mohamed, G.E.L., 1988**. Some metabolic change in *Streptomyces olivaceus* and *S. parvullus* associated with amylase and protease productions as affected by nutrient requirements IV. Effect of microelements. Egypt. J. Microbiol., 23:549.

[4] Umesh-Kumar, S., Rehana, F. and **Krishna, N., 1989**. A baiting technique for isolation of bacteria producing starch-hydrolysing. Letters in Applied Microbiology, 8:33.

[5] Gonzalez-Lopez, J., Salmeron, V., Martinez-Toledo, M.V., Ballesteros, F. and **Ramos-Cormenzana, A., 1986**. Production of auxins, gibberellins and cytokinins by *Azotobacter vinelandii* ATCC 12837 in chemically-defined media and dialysed soil media. Soil Biochemistry, 18:119.

[6] Cacciari, I., D. Pietrosanti, Y. and **Pietrosanti, W.,**

1989. Phytohormones-like substances produced by single and mixed diazotrophic cultures of *Azospirillium* and *Arthrobacter*. Plant and Soil, 115:151.

[7] **Strzelezyk, E.** and **Pokojska-Burdzieji, A., 1984**. Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus silvestris* L.). Plant and Soil, 81:185.

[8] **Mansour, F.A., Aldesuquy, H.S.** and **Hamedo, H.A., 1994**. Studies on plant growth regulators and enzymes production by some thallobacteria. Qatar Univ. Sci. J., 14:281.

[9] **Waksman, S.A., 1967**. The actinomycetes. The Ronald Press Company, New York, pp. 193.

[10] **Mizusawa, K., Ichishima, E. and Yoshida, F., 1966**. Studies on the proteolytic enzymes of thermophilic *Streptomyces*. Part II. Identifications of the organisms and some conditions of protease formation. Agr. Biol. Chem., 30:35.

[11] **Allam, A.M., Hussein, A.M.** and **Ragab, A.M., 1977**. Studies on the formation of alpha-amylase by *Thermomonosporus vulgaris*. Zentral B1. Bakteriol., 132:143.

[12] **Harhash, A.W., 1967**. Studies on amylase in *Fusarium moniforme*. Bull. Fac. Sci., Cairo University, 41:67.

[13] **Gabr, M.A., Mansour, F.A.** and **Mohamed, G.E.L., 1987**. Effect of nitrogen source on metabolic changes and enzymatic activities of two *Streptomyces* species. Egypt. J. Bot., 29-30: 151.

[14] **Takaki, M.** and **Dietrich, M.C., 1980**. Effect of GA_3 and light on polysaccharide levels and metabolism in germinating coffee seeds. J. Exp. Bot., 31:164.

[15] **Stoy, V.** and **Sundin, K., 1976**. Effect of growth regulating substances in cereal seeds germination. Res. Commun., 4:157.

[16] **Gabr, M.A.** and **Mansour, F.A., 1982**. Studies on amylase production by some *Streptomyces* isolated from Egyptian soil. The 3rd Egyptian Congress of Botany. Mansoura, 17-19 February, 1982, 1:59.

[17] **Boothby, D.** and **Wright, S.T.C., 1962**. Effect of kinetin and other plant growth regulators on starch regulation. Nature (London), 196:389.

[18] **Schnedier, E.A.** and **Wightman, F., 1974**. Metabolism of auxins in higher plants. Ann. Rev. Pl. Physiol., 25:487.

[19] **Stoneir, T.S.** and **Murthy Reddy, K.B.S., 1979**. The masking of piroxidase catalysed oxidation of IAA in *Vigna*. Phytochemistry, 18:25.

[20] **Naguib, M.I., Zeinat, K.M.** and **Mary, S., 1979**. Studies on actinomycetes of Egyptian soil. 2. Utilization of some industrial and agricultural wastes in improving the proteolytic amylolytic activities of *Streptomyces orgenteolus*. Egypt. J. Bot., 22:13.

[21] **Maceda, C.L., Vergina, E.O.** and **Agelina, L.A., 1974**. Production of proeolytic enzyme from a local strain of *Bacillus subtilis*. Philipp. J. Sci. I., 103:149.

[22] **Eastwood, D., Travener, R.J.A.** and **Laidman, D.K., 1969**. Sequential actionof cytokinin and gebberellic acid in wheat aleurone tissue. Nature (London) 221:1267.