Delta J.Sci.(11)(3)1987 , 1153-1167

BIOSYNTHESIS OF AMYLASE BY Streptomyces lipmani

BY

Mostafa A. El-Sayed * , Abd El-Raheem R. El-Shanshoury *,
Maher Abou El-Hawa **, and Mohamed F. Aly ** .
* Botany Department, Faculty of Science,
Tanta University, A.R. Egypt.
** Botany Department, Faculty of Science,
Zagazig University, A.R. Egypt.

ABSTRACT

A locally isolated strain of streptomyces lipmani was found to produce considerable amounts of the enzyme amylase. Optimal amounts of amylase production were obtained after 6 days of incubation at 30° C under static culture conditions in a medium containing 0.3% starch as the only source of carbon, 0.25% potassium nitrate, 0.2% dipotassium hydrogen phosphate, 14 µg / L riboflavin and 0.00008% calcium chloride. The pH value was adjusted at 7.5. One such enzyme, amylase, has been purified to molecular homogeneity by a sequence involving gel filtration on sephadex G.200 and biogel P150. The purified enzyme was able to hydrolyze extensively 0.4% starch as well as glycogen and dextran. The optimum activity of the enzyme was obtained at pH 7.0 and temperature of 37°C after 60 minutes. Calcium and strontium ions increased the activity of the produced amylase.

Delta J.Sci.(11)(3)1987

Biosynthesis of amylase

INTRODUCTION

Recent reports on the production, purification and characterization of microbial amylases [1-5] stimulated the authors to search for the isolation of a local Streptomyces spp which can produce appreciable amounts of amylase. In this investigation, the production of amylase by a locally isolated strain of S. lipmani was studied. Furthermore, the conditions under which optimal amounts of amylase can be obtained. The enzyme was also purified and its properties were described.

MATERIALS AND METHODS

Isolation

The soil samples which were collected from different localities as well as from the Nile banks of Cairo were treated following the method adopted by Tsao et al. [6] and plated in a medium composed of (G/L) : starch, 20.0 ; KNO_3, 2.0 ; K_2HPO_4, 1.0 ; $MgSO_4 . 7H_2O$, 0.5 ; NaCl, 0.5;Ca CO_3, 3.0, Fe $SO_4 . 7H_2O$, 0.01 ; agar, 20.0 and trace salt solution (Cu $SO_4 . 5H_2O$, 0.64 gm ; Fe $SO_4 . 7H_2O$, 0.1 gm ; $MnCl_2$ $4H_2O$, 0.79 gm ; Zn $SO_4 . 7H_2O$, 0.15 gm; distilled water 100.0 ml), 1 ml at pH 7.0. The plates were incubated at 30°C for 7 days and flooded with Gram's iodine solution. The clear zones around the colonies in the blue medium indicated the production of amylase enzyme.

Delta J.Sci.(11)(3)1987

Measurement of the amylolytic activity

For enzyme production, 2 ml inoculum of 48 h old culture were transferred to 48 ml of the same liquid medium incubated at 30°C for the appropriate times. The amylolytic activity was measured by the Bernfeld method [7]. The Streptomyces strain which was able to produce the highest value of amylase in liquid culture was selected for further study.

Purificatian of amylase

The enzyme produced by the experimental organism on the follwing medium (G/L) : starch, 30; KNO_3, 0.2;K_2HPO_4, 2.0; riboflavin, 0.14; at pH 7.5, incubated for 6 days at 30°C , was purified using the method cited in Aly [8].

RESULTS

The local streptomyces which was proved to be the most active producer of amylase was subjected to various schemes of identifications. It was identified as Streptomyces lipmani and used as the experimental organism in the current work.

A series of experiments were carried out aiming at studying the optimal physical and nutritional conditions at which the maximal value of amylase was obtained. The data of these experiments are presented in Figs 1-2, and resulted in the follwing : The pH of the medium was 7.5 ,

the incubation period was 6 days at 30°C. The nitrogen source was 0.25% potassium nitrate, the carbon source was 3% starch, the phosphorus salt was 2% potassium dibasic phosphate & riboflavin was 0.2% . Calcuim carbonate was proved . unimportant for the production of amylase enzyme.

When the purified enzyme was incubated at various temperatures, it showed a temperature optimum at 37°C (Fig 3).

Different pH values were examined on the amylase activity by using citrate and phosphate buffers. The enzyme was active at pH 7. Higher and lower pH values were deleterious (Fig. 3).

The purified enzyme was adjusted at pH 7.0 and incubated at 30°C for various periods of time . The purified enzyme recorded its maximal activity after 60 min and the activity declined afterwards (Fig 3).

The produced and purified amylase was able to hydrolyze starch (0.4%), glycogen as well as dextran at 30°C and pH 7 after 30 minutes incubation (Table 1).

Metalic ions under investigation were incubated with the reaction mixture for 30 minutes at pH 7.0, and the

activity of the enzyme was determined as described before. The activities of the enzyme indicated that Ca^{++} and strontium stimulated the enzyme activity. Ni^{++} had no effect on amylase activity. Hg^{++} and Cu^{++} had little deleterious effect on .S.lipmani amylolytic activity. The rest of metalic ions repressed the activity of the purified enzyme (Table 2).

DISCUSSION

Among a number of actinomycetes isolated from local habitats, one was proved to be the most active in producing the enzyme amylase. This organism was subjected to different studies and comparisons with various schemes and was finally identified as S. lipmani [9-15].

Since the amylolytic activity of the experimental organism was found to be high, various environmental factors and cultural characteristics were investigated in a trial to improve the value of biosynthesized amylase.It was indicated that the maximum amylase production was obtained in a medium containing 0.25% potassium nitrate, 0.3% starch, 0.2% dibasic potassium phosphate, 0.2% calcium chloride and 14 µg/L riboflavin in a pH 7.5 at 30°C for 6 days. The optimal physical conditions were similar to the findings of Sinha and Chandra [5], Srivastova et al. [16] and Gabr and Mansour [17], they reported that maximun

amylase biosynthesis was attained after 6 days, at 30°C, optimum pH being 7.5.

Dipotassium hydrogen phosphate proved to be the best phosphorus source for amylase production compared to the others. However, 0.2% dipotassium hydrogen phosphate stimulated amylase production. The promoting effect of dipotassium hydrogen phosphate on amylase activity may be attributed to the effect of potassium ions rather than its phosphorus content. Since disodium hydrogen phosphate did not exhibit such effect.

The nitrogen source necessary for the production of amylase seems to be versatile among the actinomycetes. Strain variation plays a role in that respect. In the current work 0.25% potassium nitrate is the most convenient. However, Sinha and Chandra [5] recommended ammonium acetate as a sole source of nitrogen for amylase production. Feniksova [18] selected ammonium sulfate as the sole source of nitrogen for amylase production by Aspergillus terreus.

Starch (0.3%) was the best among the tested sources of carbon. This source of carbon was found to be the most convenient by many investigators [2,16,19,20,21].

As a result of a series of experiments, the purified

enzyme exhibited its maximal activities when incubated with starch at 0.4% at 37°C for 60 min at pH 7.0. These results are parallel with those obtained by Baldwin [22].

The highest enzyme activity was also recorded with dextran, glycogen or starch especially at levels 0.4%. Strontium and calcium increased the enzyme activity by 6 and 9% respectively when supplemented individually to the reaction mixture. Nickel played no role in the enzyme activity while the other tested micronutrients exhibited various inhibitory effects according to the element used. These results were found to be in accordance with many investigators [23,24].

REFERENCES

1- Abd El-Malik, Y., El- Leithy, M.A. and Ibrahim, S.A. (1973). Production of amylases by streptomyces isolated from Egyptian soils. Zentral Bl. Bakteriol., 128 : 304.

2- Fogarty, W.M. and Edward, B. J. (1983). Production and purification of amylase from Bacillus subtilis. J. Chem. Technol. Biotechnol., 33 B (3) : 145 - 154.

3- Obi, S.K.C. and Obibo, F.J.C. (1984). Purification and characterization of thermostable actinomycetes B- amylase. Appl. Environ. Micro-

biol. 47(3) : 571 - 575.

4- Shinke, R. Kunimi, K., and Nishira, H. (1975). Isolation and characterization of B-amylase producing microorganisms. J. Ferment. Technol. 53:687-692.

5- Sinha, S., and Chandra, L. (1981) . Isolation of amylase-producing Streptomycete from soil and its characterization. ZbL. Bakt. II. Abt. 136 : 209 - 214.

6- Tsao, P.H., Leben, C., and Keih, G.W. (1960). An enrichment method for isolating actinomycetes that produce diffusable antifungal antibiotics. Phytopathol. 50 : 88 - 89.

7- Bernfeld, P. (1955). Amylase, alpha and beta. In the Methods in Enzymology vol.1, pp. 149 - 150. Academic Press. Inc. New York.

8- Aly, M. F. E. (1985). Studies on some actinomycete isolates. Ph. D. Thesis. Fac. Sci. Zagazig Univ. Egypt.

9- Bergey'S (1974). Manual of Determinative Bacteriology, 8th Ed. The Williams and Wikins Co., Baltimore.

10- Nonomura, H. (1974). Key for classification and identification of 458 species of Streptomyces included in ISP. J. Ferment. Technol. 52 (5) : 78 - 92.

11- Shirling, E. B., and Gottlieb, D. (1968). Cooperative description of type cultures of streptomyces from first study. Int.J. Syst. Bacteriol: 18(2): 169 - 189.

12- Shirling, E. B., and Gottlieb, D. (1969). Cooperative descriptions of type cultures of Streptomyces : IV. Species descriptions from the second, third and fourth studies. Int .J.Syst. Bacteriol. 19 (4) : 39 - 512.

13- Shirling, E. B., and Gottlieb, D. (1972) . Cooperative V. descriptions of type strains of Streptomyces. Additional descriptions. Int .J. Syst. Bacteriol. 22 : 265 - 394.

14- Szabo, I. M., Marton, M. Buti, I., and Fernadez, C. (1975). A diagnostic key for the identification of species of Streptomyces and Streptoverticillium included in the International Streptomyces Project. Acta Botanica. Acad. Scient. Hung. 21 : 387 - 418.

15- Waksman S. A. (1961). The Actinomycetes, Vol. II. Classification, identification and description of genera and species. The Williams and Wilkins, Baltimore.

16- Srivastova, R. A. K., Nigam, J. N., and Pilliai, K.R. (1981). Effect of certain carbohydrates, temperature and pH on the biosynthesis of amylase enzyme. Indian J. Microbiol. 21 (4) : 291 - 298.

17- Gabr, M. A., and Mansour, F. A. (1982). Studies on amylase production by Streptomyces isolated

from Egyptian soil. The 3 rd Egyptian Congress of Botany, Mansoura, I : 59 - 81.

18- Feniksova , R. V. (1957) . Physiology of nutrition of Aspergillus oryza in relation to the formation of active amylase by this fungus. Proc. Int. Symp. Enzyme, Chem. (Tokyo and Kyoto) 2 : 482.

19- ALLam , A. M., and Khalil, N. A. (1972). Amylase of Aspergillus niger. Egypt. J. Chem., 15 (2) : 157 - 165.

20- El-Manawaty. H. K., Salem, F. A. and El-Saadany, R.M.A. (1984). A high yield of amylase from Aspergillus niger by the effect of gamma irradiation. Dep. of Food Science, Fac. Agric., Zagazig Univ., Egypt.

21- Kassim, E. A. (1983). Induction of bacteria α -amylase from Bacillus subtilis as influenced by certain carbon sources. Egypt. J. Microbiol. 18 (1-2) : 141 - 149.

22- Baldwin E. (1967) Dynamic Aspects of Biochemsistry, 15 th ed. Cambridge, University Press.

23- Tajima, M. I., Urabe, K. V., and Okade, H. (1976). In Biochemistry of thermophly. S. M. Fridman and Edm (ed.) 1978, PP. 233. Academic Press, London, New York.

24- Yatani, K. (1976). In: Enzymes and proteins from thermophilic microorganisms (H. Zaber ed.) PP. 91. Birkhanser Verlag Base.

Delta J.Sci.(11)(3)1987

Table (1): Effect of different substrate on the activity of the purified amylase produced by S. lipmani.

Substrate	Enzyme activity mg/100 ml/h.
Starch	155.0
Glycogen	110.0
Dextrane	105.0

Talbe (2): Effect of different metalic ions (cations and anions) on the activity of the purified amylase activity produced by S. lipmani.

Metalic ions	In form of	Enzyme mg/100 ml/h.
Control	---	160
Ca	$CaCl_2$	175
Sr	$SrSo_4$	170
Ni	$NiCl_2.6H_2O$	160
Zn	$ZnCl_2$	145
Hg	$HgCl_2$	140
Cu	$CuSO_4.5H_2O$	125
Ar	Sod. arsenate	110
Mn	$MnSO_4.H_2O$	100
Sn	$SnCl_4.5H_2O$	90
CN	KCN	75
Mo	$Na_2MoO_4.2H_2O$	55
Co	$CoCl_2.6H_2O$	50
Li	$LiSO_4.H_2O$	40
Wo	$Na_2WO_4.2H_2O$	25

Delta J.Sci.(11)(3)1987

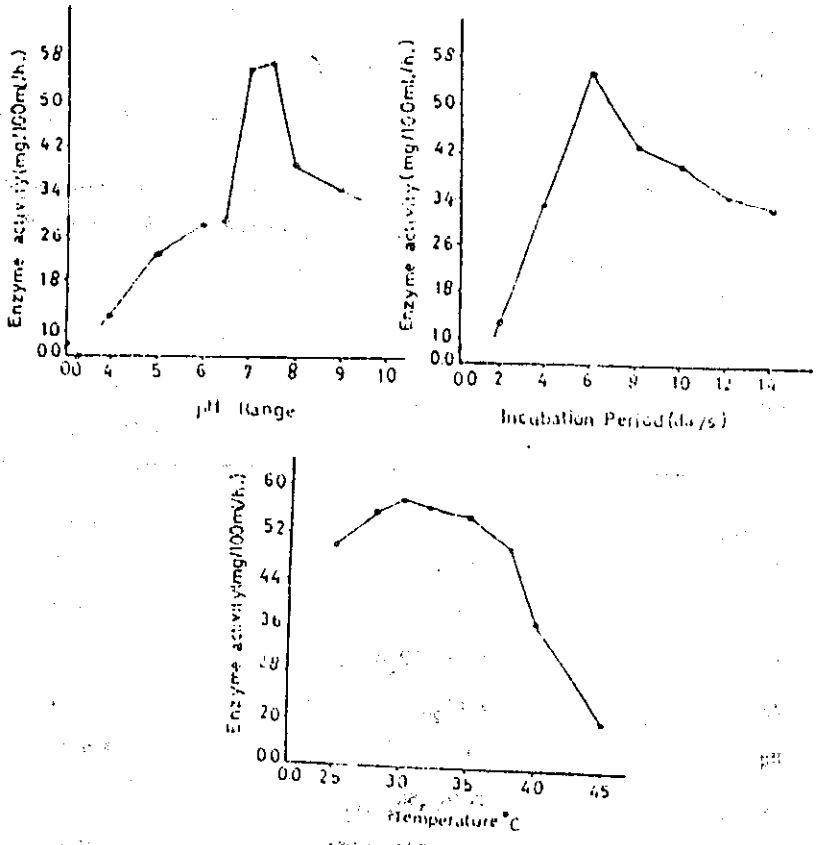
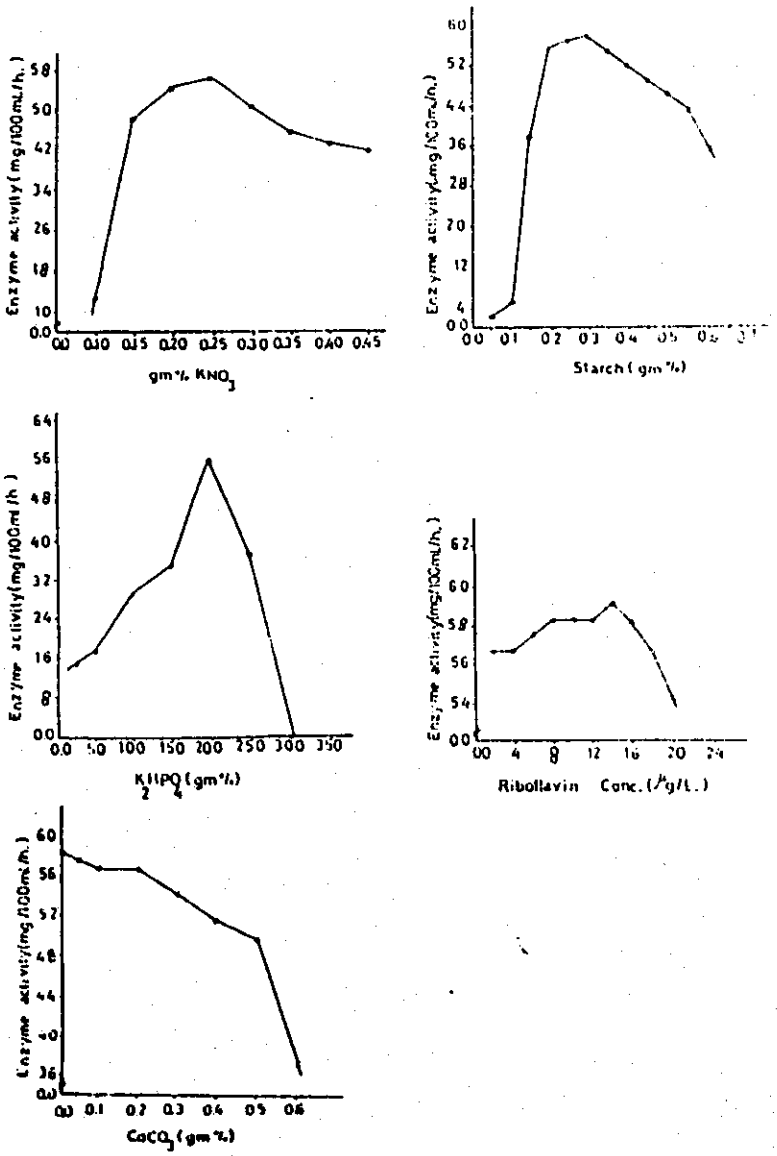


Fig (1) Effect of pH, incubation Period and temperature on amylase Production by S Lipmani

Delta J.Sci.(11)(3)1987



Fig(2) Effect of potassium nitrate, starch, dipotassium hydrogen phosphate, riboflavin and calcium carbonate on amylase production by S. Lipmani

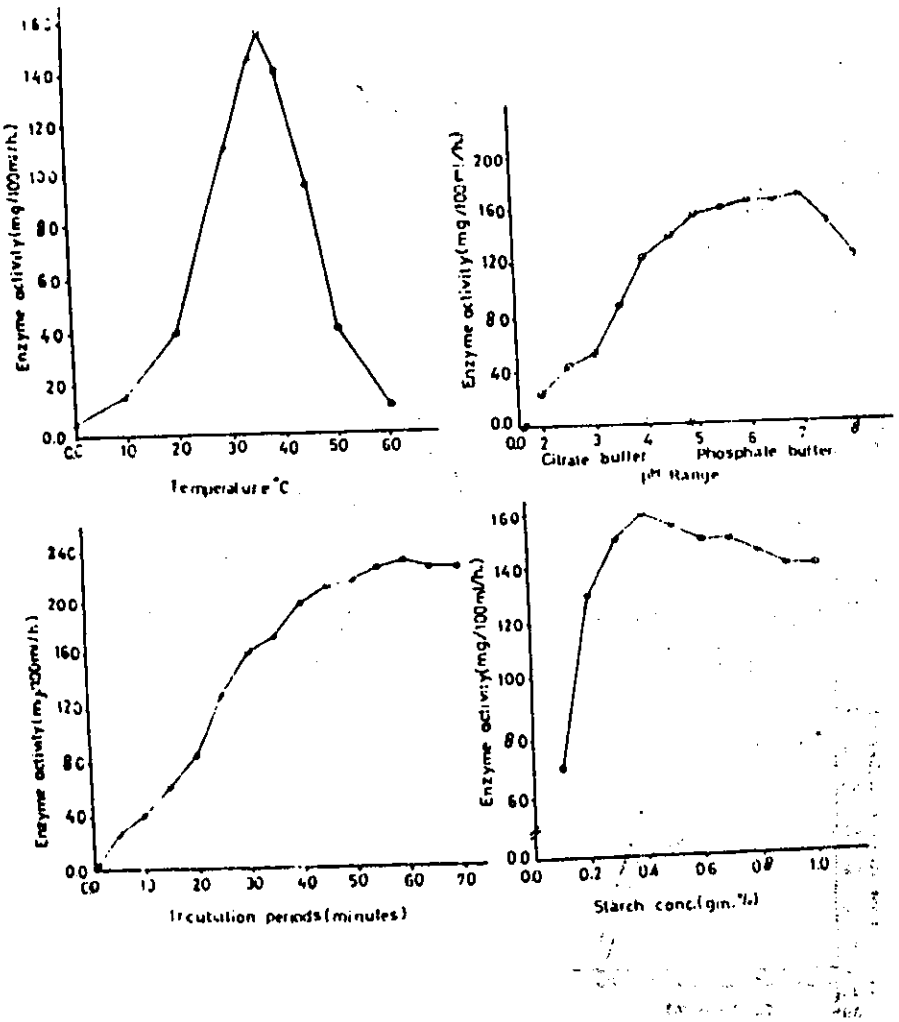


Fig (3) Effect of temperature, pH, incubation period and starch on the activity of the purified amylase.

انتاج انزيم الاميلير بواسطة استربتوميسس لبمانى

* مصطفى أحمد السيد *عبد الرحيم رمضان الشنشورى ** ماهر أبو الهوى
** ومحمد فاروق على
* قسم النبات ـ كلية العلوم ـ جامعة طنطا ـ جمهورية مصر العربية
** قسم النبات ـ كليه العلوم ـ جامعة الزقازيق ـ جمهورية مصر العربية

تم عزل سلاله محليه من استربتوميسس لبمانى ووجد ان لها القدره على انتاج انزيم الاميليز بكميات أكبر من الكائنات الاختباريه الاخرى.

وجد ان الظروف المثلى لانتاج الانزيم بعد فتره تحضين سته ايام عند درجه ٣٠ م فى مزارع ثابته فى وسط غذائى يحتوى على ٣,٠٪ نشا كمصدر وحيد للكربون ، ٠,٢٥٪ نترات البوتاسيوم ، ٠,٢٪ فوسفات البوتاسيوم ثنائى القاعده ، ١٤ ميكروجرام / لتر ريبوفلافين و ٠,٠٠٠٨٪ كلوريد الكالسيوم وذلك بعد ضبط تركيز الاس الايدروجينى عند ٧,٥.

تم تنقيه الانزيم بواسطة عامود من عجينه السيفادكس ج ٢٠٠ ، والبيوجيل ١٥٠ب . ووجد ان الانزيم النقى له المقدره على تحليل كلا من النشا والجليكوجين والدكسترات عند تركيز ٠,٤٪.

ولقد ثبت ان افضل ايون هيدروجينى للانزيم النقى هو ٧ ، وان افضل درجه حراره لنشاط الانزيم عند درجه ٣٧ م لمده ٦٠ دقيقه.

وثبت ايضا ان اضافه عنصرى الكالسيوم والاسترنشيوم زادتا من نشاط الانزيم النقى.