THE INFLUENCE OF CERTAIN NUTRITIONAL AND ENVIRONMENTAL FACTORS ON THE PRODUCTION OF AMYLASE ENZYME BY STREPTOMYCES AUREOFACIENS 77

A. M. SHATTA*
A. F. EL-HAMAHMY*
F. H. AHMED*
M.M.K. IBRAHIM*
M.A.I. ARAFA*

SUMMARY: The effect of certain nutritional and environmental factors on amylases production by the highly amylolytic Egyptian isolate Stroptomyces aureofaciens *77 was studied. The trial experiments to obtain the highest yield of amylase production resulted in modification of the recommended basal inorganic salts-starch medium. The highest yield of amylase production was obtained by increasing the soluble starch from 10g/L as a sole source of carbon up to 30 g/L and by adding corn steep liquor 20g/L as a sole source of nitrogen instead of the inorganic nitrogen source $(NH_4)_2SO_4$, and growing of the used organism in the modified medium adjusted pH 7.0 under shaken culture condition for 7 days incubation at 28°C. Optimum inoculum size was found to be 4% spore supension from 6 days old culture of the* S. aureofaciens *77.*

Key Words : Amylase, streptomyces aureofaciens.

INTRODUCTION

Amylases are well known to be the responsible group in hydrolyzation of starch to dextrins and sugars. This process is well known to be very important in the preparation of pharmaceuticals, chemicals, chocolate syrup (9), breadmaking (3), fruit juices (31), alcoholic beverages (15), sweeteners (19), detergents (4), paper and desizing of textiles (13, 21).

It is well known that the production of microbial amylases by *Streptomycetes* is affected to great extent by the medium composition, especially by the carbon and nitrogen sources (10, 18, 22,30), incubation temperature, pH values (23, 29) and aeration (9), size and age of inoculum (12, 16) and also by incubation periods (9, 25).

The present work was carried out to study certain nutritional and environmental factors affecting amylases production by Egyptian isolate *S. aureofacien* 77. In addition several local raw materials were also tested as a substratum for maximum and economic production of the enzyme.

*From Department of Botany, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

MATERIALS AND METHODS

Organism

The microorganism *Streptomyces aureofaciens* 77 was selected for its highest amylolytic activity during the screening of 177 isolates of *Streptomyces* isolated from different sources, namely, rice flour, corn flour, wheat flour, wheat bran and farmyard manure collected from local markets.

Medium used

The recommended inorganic salts-strach medium (27) for growing of *Streptomycetes* was used as a basal medium in this investigation.

Table 1: The chemical analyses of the different raw materials used.

Raw materials	Moisture %	Ash %	Total sugars %	Total nitrogen %	Protein %
Rice bran	8.45	10.80	43.14	1.79	11.20
Wheat bran	12.00	10.00	40.80	1.78	11.00
Yellow-maize meal	12.00	12.00	62.71	1.98	7.62
Dry yeast	7.00	8.50	-	7.20	45.00
Brewery yeast	86.20	1.85	-	1.36	8.52
Corn steap liquer	51.76	18.95	-	7.70	47.80

Raw materials

Wheat bran, rice bran, yellow maize meal, dry yeast, brewery yeast and corn steep liquor were separately used as a sole sources of carbon and nitrogen. Their chemical analyses are recorded in Table 1.

Microelements

Six mioroelements were tested for their effect on amylase production. Each microelement namely $MnCl_2$, $ZnSO_4$, $FeSO_4$, $CuSO_4$, and $NaMO_4$, and $HgCl_2$ was added either alone (0.001 g/L) or in combination or in standard microelements mixture ($MnCl_2$, 1.0, $ZnSO_4$ 1.0, $FeSO_4$ 1.0 mg/L) suggested by the subcommittee on Taxonomy of *Actinomycetes* of the International Committee of Bacteriological Nomenclature (11, 24).

Determination of amylase activity

The amylase activity is measured photometrically after the addition of iodine to the substrate enzyme mixture according to the method adopted by Smith and Roe (26) and Caraway (2).

RESULT AND DISCUSSION

Data in Table 2 indicated that soluble starch highly stimulated the enzyme yield (300 U/100ml) followed by dextrin 245 and Maltose 95 respectively. This is expected since the molecular weight and chain length of starch are more than the other tested chemical carbon sources, therefore, more amounts of amylase is needed for strach hydrolyzation. This result is in agreement with the results of Fairbain *et al.* (10); and Avendan and Cornejo (1).

Mean amounts of produced amylase showed gradual increase coinciding the increase in the added raw material up to 4.0% total carbohydrates in the case of wheat bran and rice bran, and 3% total carbohydrate in the case of yellow-maize meal, followed by slight decrease as the raw material increased.

Data (Table 3) show clearly that the kind of inorganic nitrogen source not clearly affected amylase production. In general ammonium nitrate was approximately more suitable than ammonium sulphate in this respect. Similarly, *S. aureofaciens* 77 utilized the different raw materials having narrow C/N ratio. Corn steep liquor proved to be the most suitable raw material for production the highest yield of amylase. This is in agreement with those obtained by others (5,6,8,9) who recorded that ammonium nitrate and corn steep liquor were the best source of nitrogen for amylase production.

The above results concluded that the highest amylase yield was obtained by using soluble starch as the sole source of carbon and by using corn steep liquor as the sole source of nitrogen. Hence, the investigators tried to find the optimum amount of each to obtain the formula for cultural medium most suitable for amylase production. The result (Table 4) show that amylase production is greatly affected by different combinations between solu-

Table 2: Effect of different pure chemicals and raw materials as a carbon sources on amylase production by *S. aureofaciens* 77.

Pure chemicals	Amylolytic Activity (U/100 ml)	Total carbohydrate %	Amylolytic activity (U/100 ml)		
			Wheat bran	Rice bran	Yellow maize meal
Soluble starch	300	1	115	90	120
		2	190	155	210
Dextrin	245	3	245	265	325
		4	280	300	300
Maltose	95	5	270	275	280

Table 3: Effect of different nitrogen sources (inorganic and raw materials) on the amylase production by *S. aureofaciens* 77.

Inorganic (N) sources	Amyloytic Activity (U/100 ml)	Total nitrogen %	Amylolytic activity (U/100 ml)		
			Brewery yeast	Dry yeast	Corn steep liquor
$(NH_4)_2SO_4$	300	0.04	125	150	185
		0.08	190	190	330
$(NH_4)NO_3$	340	0.12	280	245	380
		0.16	220	280	365
KNO_3	285	0.20	240	320	340

ble starch and corn steep liquor. The highest yield of amylase was obtained by adding 20 g/L soluble starch and 20 g/L corn steep liquor, comparing with the other combinations.

Results in Table 5 show that with the exception mercurous chloride and sodium molybdate, the addition of the different microelements resulted in different stimulatory effects on amylase production. The highest stimulatory effect was recorded with $MnCl_2$, $ZnSO_4$ and $FeSO_4$, followed by $CuSO_4$. Addition of $HgCl_2$ and $NaMO_4$, on the other hand, significantly inhibited the amylolytic activity. Application of the standard microelements mixture ($MnCl_2$, $ZnSO_4$ and $FeSO_4$, 0.001 g/L) lead to higher stimulatory effect than when the applied microelements were added together. These results confirm those recorded by Taha *et al.*, (28) whose stated that the presence of Fe^{++} plus Mg^{++} with Zn^{++} stimulate the amylase activity, they also found a positive relation between the concentration of Zn^{++} and the alpha-amylase activity.

Table 6 shows the effect of certain environmental factors on amylase production by *S. aureofaciens* 77 growing on the modified liquid medium.The enzyme production had increased gradually as the initial pH values increased from 5.0 up to 7.0. The maximum enzyme activity was obtained at pH 7.0 being 590 U/100ml. Increasing the initial pH of the medium up to pH 9.0 resulted in decreasing the amylase activity. This is in agreement with Dixon and Weeb (7) who stated that most of microbial exctracellular enzymes are produced in high yields at a growth pH somewhere near the pH for maximum enzyme activity.

Concerning the effect of incubation period, results in Table 6 show that the accumulation of amylase increased gradually as the incubation period increased. Peak of amylase production was recorded after 7 days (580 U/100 ml).

Table 4: Effect of different concentration ratios between soluble starch and cornsteep liquor on amylase production *Streptomyces aureofaciens* 77.

Con. of starch Con. of Cornsteep liquor	Amylolytic activity (U/100 ml)			
	1.0%	2.0%	3.0%	4.0%
1.0%	330	350	350	285
1.5%	380	410	425	335
2.0%	365	440	570	480
2.5%	240	350	480	420

Table 5: Effect of some microelements on amylase production by *S. aureofaciens* 77.

Microelements (mg/L)	Amylolytic activity (U/100 ml)
*Control	570
$MnCl_2$	425
$ZnSO_4$	415
$FeSO_4$	415
$HgCl_2$	50
$CuSO_4$	255
$NaMO_4$	195
Mixture	455
Without	345

* Control= Inorganic salts starch medium with adding standard microelement (mg/L) $MnCl_2$, 1.0; $ZnSO_4$, 1.0; $FeSO_4$, 1.0.

Incubation temperature 28°C was found to be the best for amylase production. This is expected, since the tested isolate is mesophilic. No growth was observed between 45-50°C for the *Streptomyces aureofaciens* 77. This is in agreement with Samia (23).

Results in Table 6 show that amylase production

Table 6: Effect of certain environmental factors on amylase production *S. aureofaciens* 77.

pH values	Amylolytic activity (U/100 ml)	Aeration	Amylolytic activity (U/100 ml)	Incubation period (days)	Amylolytic activity (U/100 ml)	Inoculum age (days)	Amylolytic activity (U/100 ml)	Incubation tempera-ture (°C)	Amylolytic activity (U/100 ml)	Inoculum Size (%)**	Amylolytic activity (U/100 ml)
5	250	shake		3	175	2	340	RT*	360	1	225
			730	4	320	4	400			2	500
6	440	culture		5	430	6	550	28	550	3	500
				6	515	8	550			4	560
7	590			7	580	10	550	35	245	5	560
				8	500	12	550			6	560
8	495	static		9	400	14	550	40	95	7	550
			395	10	290	16	550			8	560
9	360	culture		11	170	18	550	45	40	9	560
				12	80	20	550	50	-	10	560

* RT= Room temperature (around 15°C-25°C), ** % volume of medium.

increased from 395U/100ml in the case of static culture up to 730 U/100 ml in the case of shaking culture. This indicates that the shake culture method is more suitable than the static culture technique. These results confirm those recorded by Loginova *et al*., (14); and Perlman (20), who reported that aeration play an important role in the enzyme production, and the high improvement in yields may be accomplished by the use of continuous controlled aeration. Regarding the inoculum age, this factor is of importance when using mesophilic organisms due to the relatively low grow rate. The results show that the production of amylase increased gradually with the increase of age of inoculum reaching its maximum at range between 6-8 days old, after that the enzyme activity was approximately stable. With regarding to the inoculum size, it is well known that it affects the production of enzyme. It was found that 4% of the medium, volume, was the optimum economical size of inoculum. This result is in line with the results of Mahmoud *et al*., (16,17) who reported that the size of inoculum was a critical factor for amylase production by tested organisms.

REFERENCES

1. Avendano MC, Cornejo I : Formation of a raw starch-hyrolyzing-amylase by Clostridium 2021:Effect of C-sources. Biotechnol Lett, 9:123-124, 1987.

2. Caraway WT : A stable starch substrate for the determination of amylase in serum and other body fluids. Am J Clin Pathol, 32-97, 1959.

3. Cheetham PSJ : Topics in enzyme and fermentation technology 4, Ch 6, Wiley, New York, 1980.

4. Danehy JP, Wolnak B : Enzymes: Interface between technology and economics. Marcel Dekker, Basel, 1980.

5. Dawod MA : Biochemical studies on amylase and protease activity and stability during production. M Sc Thesis, Faculty of Agric, Cairo Univ, 1984.

6. De Mot R, Verachtert H : Purification and characterization of extracellular amylolytic enzymes from the yeast Filobasidium capsuligenum. Applied and Environmental Microbiology, 1474-1482, 1985.

7. Dixon M, Weeb EC : Enzymes. 3rd Edn. Longman Group Limited, London, pp 1116, 1979.

8. Dravtsatova EA : Effect of nitrogen source on amylase formation by deep cultures of A. oryzae Trudy Tesentral. Neuch - Issle-davatel Inst Spirt i Likerovoda chn Prom, 9:7-12, 1961.

9. El-Gammal SE : Production of bacteria alpha amylase and its preparation industrially. M. Sc. Thesis, Faculty of Agric, Ain Shams Univ, 1971.

10. Fairbairn DA, Friest FG, Stark JR : Extracellular amylase synthesis by S. limosus. Enzyme Microb Technol Vol 8, pp 89-92. Industrial and Applied Microbiology Vol 21, 1986.

11. Gottlieb D : Int Bull Bact Nomencl Taxon, 13:169, 1963.

12. Ibrahim MMK : Production of some enzymes by Streptomyces. Ph D Thesis Faculty of Agric, Al-Azhar Univ, 1983.

13. Lloyed NE, Lodge RE, Wynes US : Increased storage stability provided by Calcium salts US Patent; 3, 524, 1970.

14. Loginova LG, Golavacheva RC, Egarova LA : Live of microorganism under high temperatures. Ed Nauka, Moscow, 1966.

15. Macleod AM : In Brewing science (Pollock J RA ed), 1, 146-232, Academic Press, London, 1979.

16. Mahmoud SAZ, Taha SM, Attia RM : Studies of some factors affecting bacterial amylase production. J Bot UAR, 10:33-41, 1967.

17. Mahmoud SAZ, Abdel-Hafez AM, Aashoor WA, Refaat AAA, Attia RM : Some factors affecting the production of fungal amylase. Third Conference of Appl Microbiol, Cairo, 1975.

18. Masumoto S : Taxonomical studies of soil Actinomycetes in Japan. Bot Mag Vol 65, no 765-766, 71-76, 1952.

19. Peppler HJ, Periman D : microbiological Technology, 2nd edn, Chs 7-16, Academic Press, New York, 1978.

20. Perlman D : Fermentation advances. Academic Press, New York, 1969.

21. Peters RH : Textile Chemistry, 2:162-202, 1985.

22. Ryabushko TA, Vecher AS : Amylolytic activity of Actinomyces aureofaciens ISB-2201 developing under production conditions. Dokl Akad Nauk Belorussk SSR 9(5):333-336, 1965.

23. Samia AI : Production of amylase by Streptomyces. M Sc Thesis, Cairo Univ, 1969.

24. Shirling FB, Gottlieb D : Int J Syst Bact, 16:313, 1966.

25. Simpson FJ, Elizabeth Mc Coy : Amylases of five Streptomyces. Appl Microbiol, 1:228-236, 1953.

26. Smith B, Roe J : Colorimetric determination of amylase activity. J Biol Chem, 227:357, 1957.

27. Szabo IM : Microbial Communities in a Forest-Rendzina Ecosystem. Akademiai Kiado, Budapest, 1974.

28. Taha SM, Mahmoud SA, Attia RM : Factors influencing amylase production by a local strains of B. subtilis. J Bot UAR, 11:49-58, 1968.

29. Vecher AS, Ryabushko TA : Amylolytic activity of actinomycetes. Prikl Biokhim Mikrobial, 1(3):269-273, 1965.

30. Waksman SA : Studies in the metabolism of Actinomycetes. J Bact Vol 4, No 3:189-216, No 4:307-330, 1919.

31. Wiseman A : Topics in enzyme and fermentation technology, Vol 4, Wiley, New York, 1980.

Correspondence:
A. M. Shatta
Botany Department,
Faculty of Agriculture,
Al-Azhar University,
EGYPT.