EXTRACELLULAR AMYLASE SYNTHESIS BY ASPERGILLUS FLAVUS AND PENICILLIUM PURPURESCENCE

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SUMMARY: The potentiality of the two mold fungi A. flavus and P. purpurescence to produce extracellular amylase when grown on starch-containing medium as a sole carbon source in shaked flasks was tested. The maximal amylase productivity was achieved after 7 days incubation at 45°C. An initial pH value of 7 was found to be the optimum for amylolytic activity. Best activity was achieved upon using 2% (v/v) inoculum with 50 ml medium in 250 ml culture flask The greatest yield of amylolytic activity was obtained when starch or glycogen were used as carbon source. A starch concentration of 15% (w/v) favoured the highest enzyme activity, Ca^{+2} and Na^{+} ions showed stimulatory effect on enzyme productivity.

Key Words: Amylase, Aspergillus flavus, Penicillum purpurescence.

INTRODUCTION

Amylolytic enzymes of mesophilic fungi, especially *Aspergillus terreus, A. corneus, A. oryzae, Penicillium expansum, Fusarium moniliform* and *Phoma sorqhina* are well described (2,5,16). Some analyses produced by thermophilic fungi were studied (1,6).

The present investigation was undertaken to study the factors affecting growth and amylase production by the two mold fungi *Aspergillus flavus* and *Penicillium purpurescence*.

MATERIALS AND METHODS

Microorganisms

The two mould fungi used in this study were isolated from El-Alameen crude oild sludge (11) and identified by the staff of the Commonwealth Mycological Institute at Kew, Surrey, England. These were *Aspergillus flavus* and *Penicillium purpurescence*. The cultures were maintained on glucose peptone slopes.

Cultivation

The organisms were allowed to grow in 50 ml portions of the basal medium dispensed in 250 ml Erlenmeyer flasks. The basal medium had the following composition (g/1.): $NaNO_3$, 3.0: $MgSO_4$. $7H_2O$, 0.5; KCI, 0.5: KH_2PO_4, 1.0; $FeSO_4$. $7H_2O$, 0.01; $CaCl_2$, 0.1 and starch, 15.0. The reaction of the medium was adjusted to an initial pH 7.0 before sterilization.

All nutritive solutions were sterilized by autoclaving for 15 min at a pressure of 15 Ib/in^2 to raise the temperature to 121°C. The sterilized media were inoculated with 2% (v/v) spore suspension at age 24-48h. The inoculated media were agitated on a rotary shaker (200 shakes/min; amplitude 7 cm) at 45 ± 2°C for 7 days.

Enzyme recovery and assay

The culture broth was centrifuged at 8000 g for 10 min at 4°C and the supernatant was used for enzyme assay.

The amylase activity was assayed according to the method of Morgan and priest (8). The reducing sugar thus produced was estimated according to Meyer *et al.* (9). One unit of amylase activity was defined as the amount of enzyme that produced 1μg glucose equivalent per min under the experimental conditions (14).

Protein assay

The extracellular protein was assayed according to modified Loury's (10).

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Table 1 : Growth and amylase production by *A. flavus* and *P. purpurescence* as affected by different incubation temperatures.

Temperature °C	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular proteinmg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
20	161	0.09	0.71	129	0.15	0.6
30	540	0.53	4.50	598	0.59	3.3
40	630	0.71	5.10	601	0.61	3.7
45	721	0.77	5.90	616	0.64	4.0
50	250	0.21	4.60	210	0.42	3.8
55	138	0.05	3.00	101	0.11	3.1
60	90	0.02	2.30	60	0.08	2.1

Table 2 : Growth and amylase production by *A. flavus* and *P. purpurescence* in the basal medium adjusted to different pH values.

Initial pH	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
3	420	0.47	4.05	320	0.43	2.90
4	460	0.49	4.12	397	0.51	3.00
5	492	0.52	4.21	450	0.59	3.17
6	560	0.59	4.63	610	0.61	3.38
7	721	0.77	5.91	616	0.64	3.99
8	698	0.52	4.20	511	0.54	3.37
9	609	0.48	3.76	501	0.49	3.01
10	504	0.33	3.04	420	0.42	2.84

Determination of fungal biomass

The biomass of the fungal cells expressed as dry weight was determined after centrifugation and washing with a subsequent evaluation of the weight of dry mycelia.

RESULTS AND DISCUSSION

Effect of incubation temperature

The growth of the two experimental organisms and their amylase productivity at different temperatures were estimated (Table 1). Significant growth was attained at 30°C and reached maximum level at 45°C. Amylase activity was proportional to the cell growth, reaching its maximal value at 45°C.

Effect of the initial pH

The tested fungi were grown at pH values in the range of 3-11 to determine their effect on amylase production. Results given in Table 2 reveal that the degree of enzyme production is highest at the initial pH 7.0. It reduced with the decrease or increase in this pH value. Growth yield

Table 3 : Growth and amylase production by *A. flavus* and *P. purpurescence* as affected with the volume of the cultures medium.

Volume of the medium (ml)	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
20	201	0.43	2.55	198	0.06	0.04
30	589	0.48	3.37	530	0.32	1.91
40	690	0.55	4.13	602	0.54	3.32
50	721	0.77	5.91	616	0.64	3.99
75	701	0.56	7.42	597	0.49	2.97
100	230	0.32	2.92	340	0.23	1.68

followed the same pattern for both organisms reaching maximum values at pH 7.0. Minoda *et al.* (7) reported that *A. awamoi* var. Fumeus produces maximum ∝-amylase production by *Aspergillus niger* strains NRRL 337 and 330.

Effect of the volume of culture medium

As clearly shown on Table 3, dispensing 50 ml aliquots of the basal medium was the most favourable for best amylolitic activity as well as for high production of extra cellular protein.

Effect of size of inoculum

The results represented in Table 4 show that no dramatic change occurs upon using 2,3 or 4% inoculum. Thus a 2% (v/v) was applied for further experimentation.

Effect of incubation period

The growth of *A. flavus* and *P. purpurescence* and their amylolytic activities were estimated during a fermentation period which extended to 8 days. The results (Table 5) show that the biosynthesis of extracellular enzyme increased almost linearly until the stationary phase of

Table 4 : Growth and amylase production by *A. flavus* and *P. purpurescence* as affected with the size of inoculum.

Size of inovulum (v/v) %	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
1	413	2.51	0.31	311	1.53	0.21
2	728	5.99	0.77	615	3.94	0.64
3	725	5.82	0.76	615	3.92	0.64
4	720	5.63	0.76	611	3.73	0.63

Table 5 : Growth and amylase production by *A. flavus* and *P. purpurescence* as affected by incubation period.

Incubation period (days)	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
1	150	0.21	0.60	120	0.08	5.00
2	248	0.38	1.25	230	0.17	9.70
3	410	0.40	1.81	401	0.21	1.18
4	609	0.45	3.20	594	0.24	1.17
5	685	0.58	4.13	598	0.62	3.43
6	708	0.60	4.61	601	0.63	3.71
7	722	0.75	5.64	616	0.64	3.94
8	630	0.49	4.26	570	0.49	3.03

Table 6 : Growth and amylase production by *A. flavus* and *P. purpurescence* grown on different carbon sources.

Carbon source	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
Glucose	620	0.57	3.79	501	0.51	3.09
Fructose	322	0.51	2.62	450	0.54	2.54
Sucrose	410	0.55	4.22	410	0.42	3.37
Rhamnose	295	0.50	2.56	311	0.57	2.28
Mannose	224	0.42	2.42	302	0.55	2.12
Ribose	199	0.41	2.35	298	0.54	2.05
Arabinose	173	0.40	2.32	294	0.51	2.03
Stach	721	0.77	5.91	616	0.64	3.99
Glycogen	731	0.80	6.12	620	0.69	4.12

Table 7 : Growth and amylase production by *A. flavus* and *P. purpurescence* as affected with different levels of starch.

Starch level (g/l)	*A. flavus*			*P. purpurescence*		
	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
5	212	0.46	3.10	210	0.12	0.71
10	531	0.56	4.16	514	0.24	1.75
15	721	0.77	5.98	616	0.64	3.99
20	710	0.61	4.85	598	0.60	3.34
25	630	0.48	3.01	430	0.49	2.99
30	410	0.31	2.24	390	0.36	2.07

Table 8 : Effect of metal ions added to the culture medium on amylase production by *A. flavus* and *P. purpurescence.*

Metal ion	Concentration mg/ml	*A. flavus*			*P. purpurescence*		
		Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}	Dry wt. mg/100 ml	Extracellular protein mg ml^{-1}	Amylase activity unit ml^{-1}
Control	-	710	0.67	5.91	616	0.64	3.99
Cu^{2+}	0.25	722	0.91	6.02	650	0.60	4.91
	0.50	716	0.74	5.51	620	0.58	4.28
	1.00	610	0.48	3.82	520	0.46	1.89
	2.00	208	0.39	1.69	203	0.12	0.67
Mn^{2+}	0.25	730	1.30	6.21	700	0.75	5.96
	0.50	670	0.61	4.83	619	0.65	4.01
	1.00	668	0.61	4.73	585	0.60	3.43
	2.00	668	0.61	4.73	402	0.42	2.40
Zn^{2+}	0.25	690	0.70	5.19	591	0.60	3.30
	0.50	670	0.68	4.97	567	0.38	2.33
	1.00	610	0.48	3.82	520	0.25	1.89
	2.00	212	0.39	1.73	201	0.12	0.67
Co^{2+}	0.25	668	0.60	4.73	586	0.60	3.44
	0.50	208	0.39	1.68	230	0.15	0.87
	1.00	104	0.20	1.01	188	0.08	0.50
	2.00	104	0.20	1.01	88	0.01	0.10
Ca^{2+}	0.25	730	1.30	6.21	701	0.75	5.97
	0.50	740	1.50	7.08	675	0.75	5.43
	1.00	740	1.50	7.08	670	0.75	5.43
	2.00	712	0.70	5.37	623	0.58	4.28
Na^{+}	0.25	740	1.50	7.08	708	0.76	6.01
	0.50	740	1.50	7.08	689	0.76	5.70
	1.00	720	0.77	5.94	685	0.76	5.70
	2.00	720	0.77	5.94	685	0.76	5.70

growth was attained. Growth, extracellular protein as well as amylase activity reached maximal values after 7 days incubation for both organisms. Other organisms showed that profuse growth did not necessarily yield high activities of extracellular enzymes (3, 12, 15).

Effect of different carbon sources

The two experimental fungi were able to grow and produce amylolytic activity on all the carbon sources tested (Table 6). Significant growth and relatively high yields of activity were found with glycogen, starch and glucose. The

greatest yield of activity was found with glycogen as a C source. The effect of substrates on the growth and enzyme production have previously been reported for other organisms (4, 6, 20).

Effect of carbon level

The results in Table 7 indicate clearly that the biomass yields for both organisms increase with the increase of the starch concentration, reaching the highest value at the 15% level. As far as the amylolytic activity is concerned, it followed the same pattern of growth where it steadily increase and ultimately reaches its maximum value at the 15% level.

Effect of metal ions

The results in Table 8 indicate that Ca^{+2} and Na^{+} ions are the most stimulating among all the metallic ions tested. In most of the purified amylases the Ca^{+2} remains in quite high amounts (13). The maximum accumulation this enzyme is thus due to the presence of excess Ca^{+2} ions in the medium which may accelerate extra biosynthesis. Ca^{+2} ions activation of crystalline extracellular $\propto$-amylases has been demonstrated with different organisms (17, 18, 21).

Zn^{+2} and Co^{+2} metal ions showed inhibitory effect. This is probably due to the inhibitory effect of these ions in the system (14).

REFERENCES

1. Adams PR: Mycopathologia 76:97, 1981.

2. Belloc A, Palla JC, Verrier J: Ann Technol Agric 23 (2): 141, 1974.

3. Dekker RFH, Richards GN: Carbohydr Res 42:107, 1975.

4. Fogarty WM, Word OP: Biochem Soc Trans 1:260-262, 1973.

5. Kvesitadze GI, Kokonash Vili GN: Dokl Akad Nauk SSS R 217(2):470, 1974.

6. Jensen B, Olsen J, Allermann K: Biotechnology letters 9(3):313, 1987.

7. Minoda Y, Mogi K, Nakamura J: Nippon Noger-Kagaku kaishi 29:115, 1955.

8. Morgan FJ, Priest FG: J Appl Bacteriol 50:107, 1981.

9. Meyer KH, Noelting G, Bernfeld P: Helv Chim Acta 31:103, 1974.

10. Ohnishi TS, Barr JK: J Anal Biochem 86:193, 1978.

11. Omar SH, Olama ZA: Commun Sci Devel Res 12:1, 1985.

12. Richards GN, Shambe T: Carbohydr Res 491:371-381, 1976.

13. Saito N: Arch Biochem Biophys 55:296, 1973.

14. Sen S, Chakrabarty: J Ferment Technol 62(5):407-413, 1984.

15. Shambe T: Carbohydr Res 110:176, 1982.

16. Shambe T, Ejembi O: Enzyme Microb Technol 9(5): 308, 1987.

17. Stein EA, Fischer EH: Biochem Biophys Acta 39:287, 1960.

18. Stein EA, Junge JM, Fischer EH: J Biol Chem 235:371, 1960.

19. Tsuchiya HM, Corman J, Koepsell HJ: Gereal Chem 27:322, 1950.

20. Wilson RW: Can J Microbiol 16:629, 1970.

21. Yamamoto T, Fukumoto J : Bull Agric Chem Soc Japan 24:16, 1960.

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