ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology, Volume 1 Number 2-4: 61-66, 2002
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Isolation and Screening of Fungi for the Biosynthesis of Alpha Amylase

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Abstract: This study is concerned with the isolation and screening of mould cultures for the biosynthesis of alpha amylase. Forty mould culture were isolated from soil and tested for the production of alpha amylase. Of all the culture tested, the *Saccharomyces cerevisiae*-20 and *Aspergillus niger*-34 gave maximum production of alpha amylase. These strains were tested for the screening of culture media and M1 containing yeast extract, peptone, soluble starch ammonium sulphate, $CaCl_2$, $MgSO_4$, $FeSO_4$ in 100 ml of phosphate buffer gave maximum production of alpha amylase by *Saccharomyces cerevisiae*. However, *Aspergillus niger*-34 gave insignificant result. Thus, *Saccharomyces cerevisiae* -20 was selected for the production of alpha amylase. The production of alpha amylase was reached optimum 72 h after inoculation at pH 5.5.

Key words: Alpha amylase, *Aspergillus niger*, fermentation, isolation, *Saccharomyces*

Introduction

Alpha amylase an extracellular enzyme, degraded α-1-4 linkage of starch. This enzyme is extensively used in paper, food, pharmaceutical and detergent industries. Different microorganisms have been extensively used for the biosynthesis of alpha amylase (Fogarty and Kelly, 1980; Nigam and Sing 1995; Haq *et al.*, 2002). The amylase of fungal origin was found to be most stable than the bacterial enzyme (Duochuan *et al.*, 1997). Therefore, it is worthwhile to isolate a potent strain of fungi and search for more efficient processes. Selection of suitable fermentation medium is very essential for the growth of microorganism as well as for the production of alpha amylase. The production of alpha amylase by mould has been greatly affected by the addition of different carbon and nitrogen sources. (Dubey *et al.*, 2000). The carbon sources affected not only the mode of amylase formation but also the velocity with which the carbohydrates are metabolized (Prescott and Dunn's, 1987). Both organic and inorganic nitrogen sources are essential for the production of cell mass as well as for enzyme formation (Pedersen and Nielsen, 2000). The objective of this study was the selection of suitable fungal strain and optimization of cultural conditions for the production of alpha amylase.

Materials and Methods

Isolation of organism: The different mould cultures were isolated from soil by serial dilution method (Clark *et al.*, 1958). One gram of the soil sample was dissolved in 100 ml of sterilized distilled water.

The soil suspension was diluted up to 10^{-3}-10^{-3} times and 0.5 ml of the diluted suspension was then transferred to petri plates containing malt extract starch agar medium. The petri plates were placed at 30°C for 3-4 days. The young colonies of mould cultures were aseptically picked up and transferred to potato dextrose agar slants. The slants were then incubated at 30°C for 3-5 days for maximum growth. The slants were then stored at 5°C in the refrigerator.

Inoculum preparation: The cell suspension was used as inoculum in case of yeast while conidial or spore suspension was prepared in case of filamentous fungi. The suspension was prepared in the sterilized monoxal O.T. (Di-Octyl ester of sulpho succinic acid). . Each ml of cells suspension contained 2.6×10^6 cells.

Fermentation technique: Fifty ml of the fermentation medium was transferred to each of 250 ml of cotton plugged conical flask. The flasks were sterilized in the autoclave at 15lb/inch^2 pressure (121°C) for 15 min and cooled at room temperature. One ml of inoculum was aseptically transferred to each flask. The flasks were then placed in the rotary shaking incubator (200 rpm) at 30 °C for 72 h. After 72h the fermented broth was centrifuged at 7000 rpm for 30 min. The cell free supernatant was used for the estimation of alpha amylase.

Table 1: Fermentation media (M)

Composition	M1 (g l^{-1})	M2 (g l^{-1})	M3 (g l^{-1})	M4 (g l^{-1})	M5 (g l^{-1})	M6 (g l^{-1})
Yeast extract	30	30.0	-	30	-	-
Peptone	20	-	20	20	-	-
Starch	10.0	10.0	10.0	-	10.0	-
$(NH_4)_2SO_4$	2.0	2.0	2.0	2.0	2.0	2.0
$CaCl_2$	2.0	2.0	2.0	2.0	2.0	2.0
$MgSO_4$	0.5	0.5	0.5	0.5	0.5	0.5
$FeSO_4$	0.1	0.11	0.1	0.1	0.1	0.1
Phosphate buffer	1000	1000	1000	1000	1000	1000

Enzyme assay: Alpha amylase estimation was carried out according to the method of Rick and Stegbauer (1974). The enzyme solution at pH 7.5 was incubated at 40°C using 1% soluble starch solution. The reducing sugars were measured by adding 3,5-dinitro salicylic acid reagent, boiling for 5 min, cooling and measuring the O.D at 540 nm in the spectrophotometer (Model CECIL CE7200 Aquaris UK) against maltose as standard. One unit of activity is equivalent to that amount of enzymes, which in 30 minutes liberates reducing group from 1% Lintner's soluble starch corresponding to 1 mg maltose hydrate. Treatment effects were compared by the method of Snedecor and Cochrane (1980). Post Hoc Multiple Comparison tests were applied under one-way ANOVA. Significance has been presented in the form of probability ($p < 0.05$) values.

Results and Discussion

Isolation and selection of suitable organism is very necessary for maximum production of alpha amylase. Forty mould cultures were isolated from soils of different areas of Lahore by observing clear zone of hydrolysis of starch in the petri plates. These cultures were tested for alpha amylase production in 250 ml conical flask. Of all the cultures tested, the culture No. 20 and 34 gave maximum production of alpha amylase. These strains were identified according to Onion *et al.* (1987). The culture No. 20 was found to be *Saccharomyces cerevisiae* and assigned the code as *Sacchromyces cerevisiae* GCB-20 (Table 2). However, the strain No. 34 was identified as *Aspergillus niger* and assigned the code as *Aspergillus niger* GCB-34. Both the strains were used as organisms for further studies.

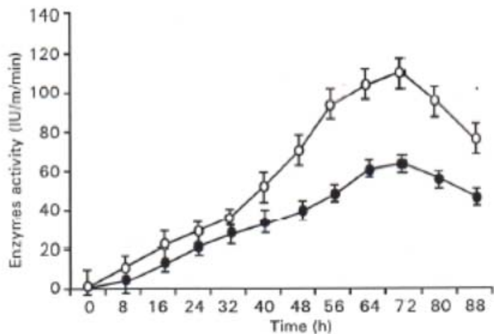

Fig. 1: Comparison between mould strains for the rate of alpha amylase fermentation

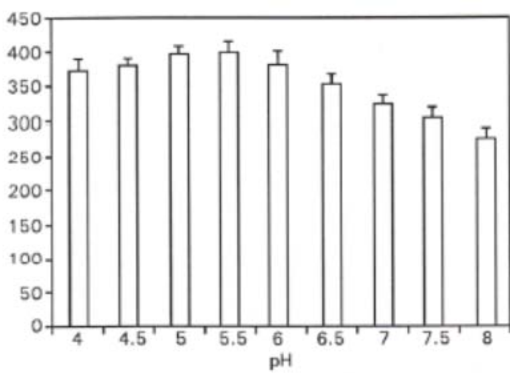

Fig. 2: Effect of pH on the production of alpha amylase by Saccharomyces cerevisiae GCB-20.

Table 2: Screening of mould cultures for the production of alpha amylase

No of strains	Enzyme activity (IU/ml/min)	No of strains	Enzyme activity (IU/ml/min)
1	34±1	21	34±2
2	28±2	22	33±1
3	32±3	23	31±4
4	44±1	24	20±2
5	31±4	25	40±1
6	38±4	26	48±3
7	36±2	27	15±4
8	41±3	28	12±2
9	39±1	29	34±4
10	50±4	30	28±4
11	34±1	31	20±1
12	26±1	32	5±1
13	43±1	33	35±2
14	48±3	34	51±1
15	42±2	35	43±3
16	39±2	36	41±1
17	34±1	37	39±1
18	27±3	38	14±2
19	40±1	39	16±3
20	52±3	40	46±2

Table 3: Screening of culture media for the production of alpha amylase

Culture media	*Saccharomyces cerevisiae*	*Aspergillus niger*
M1	100±6	50±3
M2	75±3	17±4
M3	96±4	18±3
M4	48±8	24±4
M5	87±3	41±9
M6	47±2	24±2

Each value is an average of three parallel replicates. ± indicated standard deviation from mean value.

Suitable fermentation media plays a very critical role in the production of enzymes. Six different media were tested for the production of alpha amylase by both the strains (Table 3). Of all the media evaluated M1 medium containing (g l^{-1}) yeast extract, peptone, soluble starch ammonium sulphate, $CaCl_2$, $MgSO_4$, $FeSO_4$ in 100 ml of phosphate buffer gave maximum production of alpha

amylase by *Saccharomyces cerevisiae* GCB-20. Both organic and inorganic nitrogen source was fond to be essential for the production of alpha amylase. The yeast extract and peptone was acted as organic nitrogen source while the ammonium sulphate was acted as inorganic nitrogen source. However, the other medium gave insignificant results. It might be due to these media lacking any of the component that was essential for the growth as well as for the production of alpha amylase. The *Aspergillus niger* GCB-34 gave insufficient production of alpha amylase in all the medium.

The comparison between *Saccharomyces cerevisiae* GCB-20 and *Aspergillus niger* GCB-34 for time course of alpha amylase fermentation was carried out (Fig. 1). The production of alpha amylase was increased with increase in the incubation temperature and found maximum 72 h after inoculation by both the culture. However, *Saccharomyces cerevisiae* gave almost 2 folds more enzyme than the *Aspergillus niger*. Further increase in the incubation period resulted decrease in the production of alpha amylase by both the culture. It might be due to the fact that after 72 h, the production of other by-product and depletion of the nutrients. These by-products inhibited the growth of fungi and hence enzyme formation (Duochuan, 1997).

The enzyme is very sensitive to pH. Therefore, the selection of optimum pH is very essential for the production of alpha amylase (McMahon *et al.*, 1999). In this study, the effect of initial pH of fermentation medium was studied (Fig. 2). The fermentation medium was carried out at different pH (4.0-8.0). The production of alpha amylase was found to be the best at pH 5.5. Further increase in pH resulted decreased in the production of alpha amylase by the mould culture. The production was greatly inhibited at alkaline pH (8.0). However, the acidic pH (5.5) was selected for the production of alpha amylase.

It was concluded that both organic and inorganic nutrients were required for the optimum growth of organism as well as for the production of alpha amylase. Acidic pH was required for the optimum production of alpha amylase as the *Saccharomyces cerevisiae* was used as organism.

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