RESEARCH PAPER | Biotechnology | Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

α-Amylase Production and Purification Using Fermented Orange Peel In Solid State Fermentation by *Aspergillus niger*

KEYWORDS: orange peel, Aspergillus niger, Ammonium sulphate precipitation, Dialysis, α-amylase .

Manoj Kumar Sindiri
M.Tech student, Department of chemical engineering, Center for biotechnology, AUCE(A), Andhra university, Visakhapatnam,

Manasa Machavarapu
M.Tech student, Department of chemical engineering, Center for biotechnology, AUCE(A), Andhra university, Visakhapatnam

Meena Vangalapati
Associate Professor, Department of chemical engineering, center for biotechnology, AUCE(A), Andhra university, Visakhapatnam

ABSTRACT *α-amylase is the most important industrial enzyme, which hydrolyze starch molecules. Solid state fermentation was carried out using orange peel as a substrate for the production α-amylase by Aspergillus niger. Substrate was fermented anaerobically for different days and then used for production of α-amylase by Aspergillus niger. The produced α-amylase showed the activity 53.72 U/ml/min for unfermented substrate and it has been increased to 1128.2 U/ml/min for 4 days fermented substrate .After purification of α-amylase the activity has been increased to 1968.8 U/ml/min for the precipitation and it was further increased to 2608.5 U/ml/min for dialysis.*

INTRODUCTION:

Alpha-amylase (EC 3.2.1.1) is an extracellular hydrolase, which cleaves internal α-1,4- glycosidic linkages in starch to produce glucose, maltose, or dextrins, and glucoamylase (EC 3.2.1.3), which cuts α-1,4- and α-1,6-glycosidic linkages to release glucose from the nonreducing ends of starch (Najafi & Kembhavi, 2005). Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry and also have potential applications in food, fermentation, and textile industries (Van der Maarel *et al*, 2002; Monga *et al*, 2011). Amylases have been most widely reported to occur in microorganisms, although they are also found in plants and animals. Microbial production of amylase is more effective than that of other sources as the technique is easy, cost effective, fast and can be modified to obtain enzymes of desired characteristics. The microbial amylases could be potentially useful in various pharmaceutical, fine-chemical industries etc (Ashwini *et al*, 2011). The main microbial sources for amylase production are *Bacillus* species (Nurmatov *et al*, 2001; Deutch, 2002)and *Asperigillus* species (Gigras *et al*, 2002; Goto *et al*, 1998). Solid state fermentation (SSF) and submerged fermentation (SMF) can be used for the production of these amylases. Solid state fermentation holds tremendous potential for the production of alpha amylase. It can be of special interest in those processes where crude fermented product may be used directly as a source of enzyme (Pandey *et al*, 2000). Selection of suitable substrates for SSF has mainly been centered around agro-industrial residues or solid biowastes due to their potential advantages for filamentous fungi, which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Ramachandran *et al*, 2004). The main objective of the present study is to utilize fermented orange peel powder for amylase production by *Aspergillus niger*.

MATERIALS AND METHODS:

Substrate:

Orange peels were collected from local market in Visakhapatnam, Andhra Pradesh. and fermented in anaerobic conditions. The fermented Orange peel is ground and stored in anaerobic conditions. The powder sample is used as substrates for amylase production.

Organism:

Fungal culture *Aspergillus niger* was maintained on potato dextrose agar (PDA) slants. The slants were grown at 30^{0}c for 5 days and stored.

Inoculum preparation:

10 mL of sterilized distilled water was added to a sporulated 4 days old PDA slant culture. An inoculums needle was used to dislodge the spore clusters under sterilized conditions and then it was shaken thoroughly to prepare homogenized spore suspension.

Preparation of Mineral salt solution:

10 g KH_2PO_4 , 2g $MgSO_4$,2g NaCl and 0.5 g $MnSO_4$ were mixed in 1 liter standard flask.

Solid state fermentation:

10 g fermented orange peel powder for five different days and 10g of non fermented orange peel powder were amended with 10 mL of mineral salt solution was taken in Six different 250 mL cotton plugged Erlenmeyer flasks, mixed homogenously and sterilized at 121°C for 15 min in an autoclave. Thereafter, the flasks material was cooled at room temperature and inoculated with 1 mL spore suspensions of *Aspergillus niger*. The flasks were then incubated at 30°C for 4 days.

Enzyme recovery:

After the specified incubation period, 50 mL of distilled water was added in each flask containing fermented mash and placed on a shaker at 200 rpm for 60 min. Afterward, the mixture was filtered and centrifuged at 10,000 rpm for 15 min at 4°C to remove the fungal spores and unwanted particles. The clear supernatant thus obtained after centrifugation was used as a source of crude enzyme (Irfan *et al*, 2012).

Amylase assay:

α-Amylase activity was determined by incubating a mixture of 0.5 mL of each enzyme source and 1 % soluble starch dissolved in 0.1 M phosphate buffer, pH=7, at 55 °C for 15 min. The reaction was stopped by adding 1 mL of 3,5-dinitrosalicylic acid, then followed by boiling for 10 min. The final volume was made up to 12 mL with distilled water and the reducing sugar released was measured at 540 nm. One unit (U) of a-amylase activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar as maltose per minute. Amylase activity was measured in U/ml/min by applying the standard formula (Hema *et al*, 2006; Madiha *et al*, 2012).

Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

$$\text{Amylase activity} = \frac{\text{Amount of sugar released} * 1000}{\text{Molecular weight of maltose} * \text{Time of incubation}}$$

Enzyme Purification:
The crude fermented broth containing amylase enzyme was subjected to the following purification steps: $(NH_4)_2SO_4$ precipitation and dialysis (Tahar *et al*, 2010).

Ammonium sulphate precipitation:
Crude enzyme that was obtained after 4 days fermentation was saturated to about 30 – 50% with ammonium sulphate ($(NH_4)_2SO_4$). The precipitate was removed by centrifugation at 10,000 rpm for 10 minutes and then ammonium sulphate was added to supernatant for 50 -60% saturation. The precipitate obtained was dissolved in 0.1M phosphate buffer at p^H 6.0 and stored.

Dialysis against phosphate buffer:
This Process is carried out to remove the excess amount of salt present in ammonium sulfate precipitate solution. 5 ml of Precipitate solution was introduced into dialysis bag and dialyzed against distilled water for 3 h, followed by dialysis against 0.1M phosphate buffer at pH 6.2 with continuous stirring at 4^{0}C. The obtained α-amylase enzyme preparation was concentrated against crystals of sucrose and kept in the refrigerator at 5°C.

RESULTS AND DISCUSSIONS:
Amylase production:
Aspergillus species are considered to be the vital sources of α-amylase production using SSF. Five Samples of orange peel was kept for fermentation for five individual days and then ground. Fermented sample was used as substrate for α-amylase production by SSF using *Aspergillus niger*. The produced α-amylase showed the activity 53.72 U/ml/min for unfermented substrate and it has been increased to 1128.2 U/ml/min for 4 days fermented substrate. Maximum α-amylase activity was shown for 4 days fermented substrate amongst six different substrates (1 unfermented substrate + 5 fermented substrates) and the activity decreased to 901.6 U/ml/min for 5 days fermented substrate.

Table 1: Amylase production by different substrate fermentation times:

Sl.no	Substrate fermentation time (days)	α-amylase **activity (U/ml/min)**
1	0 (un fermented)	53.72
2	1	152.4
3	2	232.8
4	3	781.1
5	4	1128.2
6	5	901.6

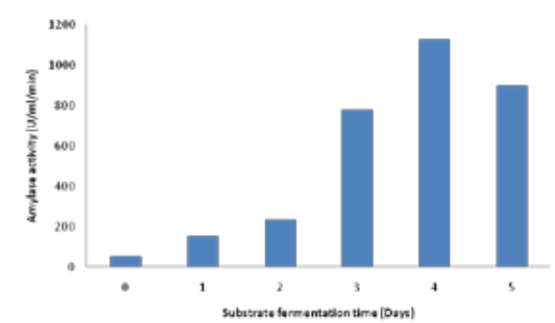

Figure 1: Amylase activity at different substrate fermentation time

Enzyme Purification:
The crude α-amylase enzyme produced from 4 days fermented substrate was subjected to the following purification steps: (NH4)2SO4 precipitation and dialysis.

The first step in purification was conventional ammonium sulphate fractionation. The fraction (50 - 60%) saturation of ammonium sulfate revealed increase in α-amylase activity from 1128.2 U/ml/min to 1968.8 U/ml/min. The precipitate solution from the first step was subjected to dialysis, the second step of purification and it is dialyzed against 0.1M phosphate buffer for 5 hours. The resulted purified sample from dialysis was showed the activity as 2608.5 U/ml/min. The α-amylase activity for the precipitation as 1968.8 U/ml/min and it was increased to 2608.5 U/ml/min for dialysis. The results were shown in Table 2 and Figure 2.

Table 2 Effect of purification steps on amylase activity

Purification step	Volume (ml)	α-amylase **activity (U/ml/min)**
Culture supernatant	50	1128.2
$((NH_4)_2SO_4$ precipitation	10	1968.8
Dialysis	10	2608.5

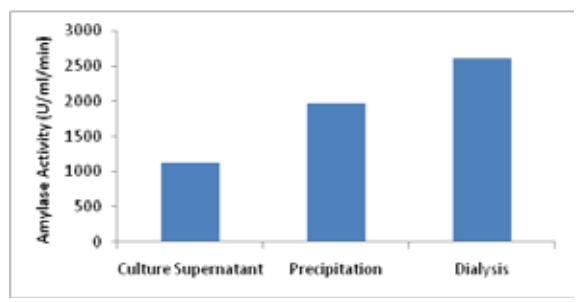

Figure 2: Amylase activity after purification

CONCLUSION:
α-amylase is one of the most important enzymes known and is of great significance having approximately 25 % of enzyme market. It has potential applications in food, pharmaceutical and fine chemical industries. The present study reports the biological production of amylase by *Aspergillus niger* using fermented orange peel as a substrate. The produced α-amylase showed the activity 53.72 U/ml/min for unfermented substrate and it has been increased to 1128.2 U/ml/min for 4 days fermented substrate .After purification of α-amylase the activity has been increased to 1968.8 U/ml/min for the precipitation and it was further increased to 2608.5 U/ml/min for dialysis. The present study also stated that solid bio wastes such as orange peel can be used as potential substrates for the production of α-amylase.

Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

REFERENCE

1. Ashwini k., Gaurav Kumar., Karthik L., Bhaskara Rao K.V. (2011), "Optimisation, production and partial purification of extracellular α-amylase from Basillus sp. marini". Archives of applied science research, 3(1), 33-42. | 2. Deutch C. E. (2002), "Characterization of salt tolerant extracellular alpha–amylase from Bacillus dipsosauri". Letters in Applied Microbiology, 35, 78–84. | 3. Gigras P., Sahai V., Gupta R. (2002), "Statistical media optimization and production of ITS alpha amylase from Aspergillus oryzae in a bioreactor". Current Microbiology, 45, 203–208. | 4. Goto C E., Barbosa E P., Kistner C L., Gandra R F., Arrias V L., Peralta R M. (1998), "Production of amylase by Aspergillus fumigates". Revista de Microbiologia, 29, 99–103. | 5. Hema Anto., Ujjval Trivedi., Kamlesh Patel. (2006), "Alpha amylase production by Bacillus cereus MTCC 1305 using solid-state fermentation". Food Technol.Bitechnol, 44(2), 241-245. | 6. Madiha Sajjad., Shahnaz Choudhry. (2012), "Effect of starch containing organic substartes on alpha amylase production in Bacillus strains". African Journal of Microbiology Research, 6(45), 7285-7291. | 7. Monga M, Goyal M, Kalra KL, Soni G. (2011), "Production and stabilization of amylases from Aspergillus niger". Mycosphere , 2(2), 129–134. | 8. Muhammad Irfan, Muhammad Nadeem, Quratualain Syed. (2012), "Media optimization for amylase production in solid state fermentation of wheat bran by fungal strains". Journal of cell and molecular biology. 10(1), 55-64. | 9. Najafi M.F., Kembhavi A. (2005), "One step puriWcation characterization of an extracellular α-amylase from marine Vibrio sp". Enzyme Microb Technology, 36, 535–539. | 10. Nurmatov S. K., Akhmedova Z. R., Rakhimov D. A. (2001), "Isolation, purification and properties of alpha amylase from Bacillus subtilis – 7A". Chemistry of Natural Compounds, 37, 364–368. | 11. Pandey A., Nigam P., Soccol C R., Soccol V, Singh D., Mohan R. (2000), "Advances in microbial amylases (review article)". Biotechnol. Appl. Biochem, 31, 135-152. | 12. Ramachandran S., Patel A.K., Nampoothiri K.M., Francis F., Nagy V., Szakacs G., Pandey A. (2004), "Coconut oil cake – A potential raw material for the production of a-amylase". Bioresour. Technol, 93, 169–174. | 13. Tahar Nouadri., Zahia Meraihi., Djekrif-Dakhmouche Shahrazed., Bennamoun Leila. (2010), "Purification and characterization of the α-amylase isolated from Penicillium camemberti PL21". African Journal of Biochemistry Research, 4(6), 155-162. | 14. Van der Maarel M.J.E.C., Van der Veen B., Uitdehaag J.C.M., Leemhuis H., Dijkhuizen L. (2002). "Properties and applications of starch-converting enzymes of the a-amylase family". J. Biotechnol, 94, 137–155.