JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2012. Vol. **6**(1), p. 463-466

Production of Amylase using Potato Waste Under Solid State Fermentation

N. Uma Maheswari* and M. Sherley

PG and Research Department of Microbiology,
Sengamala Thayaar Educational Trust Women's College, Mannargudi - 6141 001, India.

(Received: 20 April 2011; accepted: 02 June 2011)

Enzymes production by fermentation carried out by solid state fermentation. SSF have been established as a superior technique for the production of enzymes. The project was aimed at the production of amylase by *Aspergillus niger* and *Bacillus subtilis* using potato as substrate with the use of SSF techniques and tested for its activity. Effect of physical parameters such as concentration, temperature and pH were also assessed. *Aspergillus niger* was the maximum amylase producer (20.5 ± 0.02 IU/ml) compared to *Bacillus subtilis* using 15g of substrate concentration with an optimum pH at 4 and temperature 24ºC . Substrate tested found most suitable for enzyme production and their potential could be effectively exploited in the future for production of various commercial products.

Key words: *Bacillus subtilis, Aspergillus niger,* Amylase, Potato waste, Solid State Fermentation.

Life is an intricate meshwork involving a perfect coordination of a vast majority of chemicals reactions. Some of these reactions result in synthesizing large molecules, others in cleaving large molecules and all of them either utilize energy or liberate energy. Pressure the condition under which living cell carry on their life processes, yet in the living cells these reaction proceeds at extremely high rate. This is due to the presence of some catalysts produced and synthesized inside the body of the organism. Enzymes are biocatalysts protein in nature; they catalyze the biochemical reaction taking place in the living cell without any overall change[1].

Bacteria and fungi are mostly used organism for production of enzymes. The most widely used enzyme in the industry for starch hydrolysis is amylase. These enzymes account 65% of enzyme market in world. This enzyme catalyses the endo-cleavage of the α-1,4 glycoside linkages and the release of short oligosaccharide and limit dextrin. This enzyme is used commercially for the production of sugar syrups from starch which consist of glucose, maltose and higher oligosaccharide. It is also extensively used in starch induction. To meet the demands of these industries low cost medium is required for the production of amylase. Amylases are e.g. of hydrolase's and function in the hydrolysis of molecules. Amylases are of the most important enzymes used in biotechnology[2].

* To whom all correspondence should be addressed.
E-mail: Umasamy2004@yahoo.co.in

Although many microorganisms produce amylase but most commonly used for their industrial production are *Bacillus subtilis, Bacillus licheniformis, Aspergillus amyloliquifaciens* and *Aspergillus niger.* Among the fungal species *Aspergillus* such as *Aspergillus niger, Aspergillus awamori* and *Aspergillus oryzae* have received more attention because of their high productivity.[3] Fungal amylase can be produced using two main methods, solid state fermentation systems and submerged liquid cultivation systems. Among these two methods, solid state fermention (SSF) has gained renewed interest for the production of there enzymes in view of several economic and engineering advantages[4].

The potato is grown and consumed all over the world, and a large number of processed food industries market potato – based products. Although potato peel does not pose serious disposal and environmental problems, meaningful utilization of this nutrient – rich waste has not drawn much attention. Potato is one of the major vegetable crops[5]. It being a seasonal in a perishable crop, the gluts during peak season reduce the prices. Processing of potatoes is hence desirable .The various possibilities in potato processing are potato chips, French fries, industrial alcohol, vodka and starch[6].

The purpose of present study is to investigate the production of amylase under solid state fermentation using potato waste as a substrate by *Aspergillus niger* and *Bacillus subtilis*.

MATERIAL AND METHODS

Collection of Soil Sample

The soil samples are collected from paddy field, Thiruvarur District, Tamil Nadu, South India. Sampling was done taking all possible aseptic measures and was stored at 4ºC. The samples were processed for isolation of bacteria and fungi were screened for amylolytic potential[7].

Production media employed for the enzymes

The above mentioned fungus and bacterium was inoculated into enzyme production media such as mineral salt medium (pH4) recommended by[8] and sterilized at 121ºC for 15 minutes and inoculated with 10% fungal spore suspension and bacterial culture separately (V\W). The contents of the flasks were mixed thoroughly to ensure uniform distribution of the inoculums and left at RT.

In Solid State Fermentation three substrate are grown well and high production under control parameters. Solid State Fermentation method is effective for both small and large scale cultivation.[9]

Optimization of cultural conditions

The factors such as pH, temperature and substrate concentration affecting production of enzyme were optimized by varying parameters one at a time. The experiments were conducted in 200ml. Erlenmeyer flask containing production medium. After sterilization by autoclaving, the flasks were cooled and inoculated with culture and maintained under various optimization conditions separately such as pH (4, 5, 6, 7 & 8), temperature (24, 37, 45 & 50ºC) and substrate concentration (5, 10 & 15 gm). The culture filtrate was assayed in enzyme activity.

Enzyme Extraction[10]

The crude enzyme from the fermented material was extracted by simple contact method. For this, the fermented substrate was mixed thoroughly with distilled water containing 0.1% of Tween 80, so that the total extract volume was 100ml (i.e. if x ml of solution was added to make up a total volume of 100ml).

Enzyme Assay [11]

One ml of starch solution was added with 1ml of diluted enzyme solution and incubated at 27ºC for 15 minutes. 2ml of dinitrosalicyclic (DNS) reagent was added and kept in boiling water bath for 5 minutes, 1 ml of potassium sodium tartrate was added and allowed to cool down in running water. This solution was made up to 10 ml with sterile distilled water and absorbance was read at 560 nm.

For optimization studies, the following condition were tested such as pH (4, 5, 6, 7 & 8), temperature (24, 37, 45 & 50ºC) and the substrate concentration (5, 10 & 15g) under solid state fermentation (SSF). One unit of enzyme activity was defined as the amount of enzymes that releases 1µ mol of reducing sugar as D-Glucose per min under the assay conditions. The enzyme activity was expressed as IU/ml extra cellular protein.[12]

Statistical Analysis

The results obtained in the present investigation were subjected to statistical analysis like Mean ($\overline{X}$) and Standard Deviation (σ).[13]

RESULTS AND DISCUSSION

Basal media for *Bacillus subtilis* and *Aspergillus niger* was prepared with different potato waste and production of amylase was confirmed by Dinitro Salicylic Acid (DNS). The cost of enzyme production in submerged fermentation is high which necessitates reduction in production cost by alternative methods. The contents of synthetic media are very expensive and these contents might be replaced with more economically available agricultural by products for the reduction of cost of the medium.

The amylase producing *Bacillus subtilis* and *Aspergillus niger* were identified with the help of the zone formed in the blood agar medium. The zone is formed due to the amylolytic activity of the organisms, which cleaves protein molecules present in the blood agar medium. The results revealed the maximum amylase were noted in *Aspergillus niger* (4.054 ± 0.02 IU/ml) compared than *Bacillus subtilis* (2.836 ± 0.04 IU/ml). (Table-1) The total amount of protein present in the purified enzyme was estimated by Lowry's method. The total amount of protein in the purified enzyme was (12.06 ± 0.01 IU/ml). The maximum enzyme activity was observed at pH 4 in *Aspergillus niger* (33 ± 0.12 IU/ml) and protein content (12.5 ±0.04 IU/ml). (Table-2) The maximum enzyme activity was observed for *Aspergillus niger* at the temperature 24ºC (9.5 ± 0.15 IU/ml) and protein content (8.6 ±0.00 IU/ml). (Table-2) The maximum enzyme activity was observed at substrate concentration 15gm in *Aspergillus niger* (17.0 ± 0.02 IU/ml) and protein content (20.5 ±0.02 IU/ml). (Table-2)

Our findings were similar to[9]agro industrial residues are generally considered the best substrate for the solid state fermentation processes and use of solid state fermentation (SSF) for the production of enzymes is no exception to that a

Table 1. Assay of Amylase Activity

S.No	Organisms	Amylase productivity (IU/ml)
1	*Bacillus subtilis*	2.836 ± 0.04
2	*Aspergillus niger*	4.054 ± 0.02

Values are expressed as Mean ± Standard Deviation

Table 2. Effect of cultural condition on amylase production using potato waste by *A.niger* and *B. subtilis*.

Name of the Organisms	Substrate	Amylase Production (IU/ ml)														
		pH					Temperature (ºC)					Substrate Concentration (g)				
		4	5	6	7	8	24	37	45	50	5	10	15			
B. subtilis	Skin	2.6 ± 0.02	3.2 ± 0.03	5.6 ± 0.02	2.8 ± 0.02	3.0 ± 0.01	4.0 ± 0.02	2.0 ± 0.01	2.5 ± 0.01	6.0 ± 0.01	2.6 ± 0.01	2.8 ± 0.02	2.0 ± 0.01			
	Pulp	6.7 ± 0.01	2.6 ± 0.03	2.6 ± 0.01	5.2 ± 0.03	5.7 ± 0.02	2.0 ± 0.03	3.5 ± 0.02	1.0 ± 0.02	2.0 ± 0.02	3.0 ± 0.03	6.5 ± 0.01	3.6 ± 0.00			
	Skin + Pulp	4.2 ± 0.01	5.8 ± 0.04	6.7 ± 0.02	2.6 ± 0.02	8.2 ± 0.05	8.5 ± 0.02	1.0 ± 0.00	3.0 ±0.01	1.0 ± 0.02	2.9 ± 0.02	2.1 ± 0.00	2.5 ± 0.02			
A. niger	Skin	5.2 ± 0.03	2.6 ± 0.02	3.2 ± 0.02	1.5 ± 0.02	3.0 ± 0.02	5.0 ± 0.00	2.4 ± 0.01	2.0 ± 0.02	1.6 ± 0.03	5.0 ± 0.02	2.5 ± 0.02	6.5 ± 0.02			
	Pulp	4.2 ± 0.03	4.2 ± 0.05	8.2 ± 0.02	6.5 ± 0.03	5.7 ± 0.02	2.3 ± 0.01	3.1 ± 0.02	2.5 ± 0.03	2.7 ± 0.01	5.7 ± 0.01	3.4 ± 0.01	7.5 ± 0.04			
	Skin + Pulp	12.5 ± 0.04	8.2 ± 0.06	9.2 ± 0.01	1.5 ± 0.01	2.6 ± 0.03	8.6 ± 0.00	2.2 ± 0.00	4.5 ± 0.01	5.2 ± 0.05	12.0 ± 0.009	2.2 ± 0.05	20.5 ± 0.02			

Values are expressed as Mean ± Standard Deviation

number of such substrate have employed for the cultivation of microorganisms to host of enzyme. The substrates that have been used included wheat bran and rice bran.

The selection of a substrate for enzyme production in a SSF process depends upon several factors mainly related with cost and availability of the substrate, and thus may involve screening of several agro industrial waste. In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as anchorage for the cells. Similarly that complete amylase production was essential for efficient starch degradation.[14,15]

The optimum temperature to 4ºC was reported for *Aspergillus niger* under SSF for amylase production. Amylase production by *Aspergillus fumigatus* at 40ºC [3] and 30 to 40ºC for *Aspergillus awamori* .[16] Substrate traditionally used in solid state fermentation includes rice, wheat, millet, barely, corn and soybean. On the basis of the result of the present study, it is concluded that the utilization of potato pulp and skin as solid substrate could lead to large scale production of industrial enzyme and also contribute to safe and economic waste management in the environment, where these wastes are continuously accumulated and cause serious pollution problems.

ACKNOWLEDGMENTS

The authors acknowledge the support of the Dean Dr.V. Dhivaharan Department of Microbiology, S.T.E.T Women's College, Mannargudi, in providing necessary facilities in carrying out this work.

REFERENCES

1. Jain, J.L., Sanjay Jain. *Fundamental of Biochemistry,* 6th ed *Mc Graw - Hill company.* London. 2006; 333-336.
2. Burhan, A., Nisa, U., Gokhan, C., Ashabil, A. and Osmair, G. Enzymatic Properties of a novel themostable thermophilic alkaline and chelator resistant amylases from an alkaphilic *Bacillus* sp isolate ANT-6, *Process Biochemistry.* 2003; **38**: 1397-1403.
3. Rameh, M.V and Lonsane, J.K. Solid state fermentation for production of higher titers of thermostable amylase with two peaks for pH optima by *Bacillus licheniformis* M27. *Biotechnol.Lett.* 1989; **11**: 49-52.
4. Guzman-Maldonaldo, H. and Pandes-Lopez, 0. Amylolytic enzymes and products derived from starch. *A.review.Cri.Rev.Food sci.Nutrit.* 1995; **35**: 373-430.
5. Anonymous Director of Land Records and Agriculture, Statistical Abstract, *India pp.* 1999; 163.
6. Saha, B.C and Zeikus, J.G. Microbial glucoamylases: Biochemical and Biotechnical features. *Starch Starke*. 1989; **41**: 57-64.
7. Aneja. K. Experiments in Microbiology , Plant Pathology and Biotechnology. New Age International Publishers, New Delhi. . 2005; 269-274
8. Ragunathan, R. and Swaminathan, K. Amylase production by *Aspergillus niger* under solid state fermentation using banana fruit stalk as substrate. *J. Ecophysiol. Occup. HIth.* 2002; **2**: 21-28.
9. Pandey, A. and Soccol, C.R. New development in solid state fermentation-I, *Bioprocess and products, process Biochem.* 2002; **35**: 1153-1169.
10. Mudgett R.E. In: Manual of Industrial Microbiol (Eds.) A.L. Demain and N.A. Soloman, Americal Society for Microbiology, Washington, D.C. 1986; **11:** 66-83.
11. Miller, G.L., Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Annals. Chem.* 1959; **31**: 426-428.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 1951; **193**: 265-275.
13. Zar, J.H. Biostatistical analysis. 2nd ed . Englewood Cliffs, NJ: Prentice-Hall. 1984; **2**: 130-132.
14. Forgarty, W.M. Microbial amylases. In: *Microbial enzymes and Biotechnology,* (ed.) Applied Science Publishers. 1983; 1-92.
15. Soccol, C.R Pandey, A., Selvakumar, P.,. and Nigam, P. Solid State Fermentations for the production of industrial enzymes. *Process Biochem.* 2000**; 36**: 1153-1167.
16. Moreira, F.G., Lima, A.A., Pedrinho, S.R.F., Lenartovicz, V., Souza, C.G.M and Peralta, R.M., Production of amylase by *Aspergillus tamari. Revis. De. Microbial.* 1999; **30:**157-162.