JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. 4(2), p. 767-771

Optimization of Fermentation Parameters for Amylase Synthesis from *Aspergillus oryzae* through Submerged Fermentation

K.G. Siddalingeshwar[1], K.M. Sudipta[1], H. Chetan[1], G. Bhavik[1], K. Rahul[1], S. Jitendra[1], N. Rajanikanth[1], K. Sushma[1], S. Swagata[1], P. Sumana[1], K. Ranjan[1], A. Subash[1], Sourav Nath[1], Ankur Dutt[1], S.M. Mohsin[1], K.S. Dayananda[1], S.M. Gopinatha[3], A.N. Ramya[1], Hemalatha, N.B.[2] and C.P. Rajeshwari[2]

[1]Department of Studies in Microbiology, Padmshree Institute of Information Sciences, Nagarabhavi Circle, Bangalore - 72, India.
[2]Department of Biochemistry, Tumkur University, Tumkur, India.
[3]Acharya Institute of Technology, Bangalore, India.

(Received: 04 March 2010; accepted: 11 June 2010)

Studies were under taken on *Aspergillus oryzae* SCBRD11 isolated from the soil sample using potato dextrose agar and starch used as a substrate to evaluate their ability to produce amylase. The amylase producers detected by the clear zone around the colony by simple plate assay method. Among the fifteen isolates *Aspergillus oryzae* SCBRD11 is the potential strain. The amylase synthesis were increased their yield after the optimization of fermentation parameters. The optimum pH 4.5, temperature 31^{0}C and inoculum size 1.0 ml. This enzyme was growth associated.

Key words: Fermentation, Amylase, *Aspergillus oryzae*.

Alpha amylases (endo-1-4-α-D-glucan glucanohydrolase, E.C. 3.2.1.1) catalyzes the hydrolysis of α-D-(1,4) glycosidic linkages in starch or related carbohydrates releasing oligosaccharides and glucose. It has extensive commercial applications in starch liquefaction, brewing, sizing in textile industries, and paper and detergent manufacturing processes[1].

* To whom all correspondence should be addressed.
Tel.+91-9449589140; Fax:+91-80-25202429
E-mail: siddha_lingeshwar@rediffmail.com

In recent years the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms[2-4].

Several microorganisms are known to produce raw starch digesting amylase, however most of these microorganisms were more resistant to the enzymes reaction[5]. Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents[3,6].Starch degrading enzymes like amylase have received technological Significance and economic benefits. Evidence of amylase in yeast, bacteria and mould have been reported and their properties documented[3,4,7]. Among the

microorganisms, many fungi had been found to be good sources of amylolytic enzymes. Studies on fungal amylase especially in the developing countries have concentrated mainly on *Rhizopus sp* and *A. niger* probably because of the ubiquitous nature and non fastidious nutritional requirements of these organisms[8].The aim of the present studies deals with screening of amylase producers and optimization of fermentation parameters by using highest zone of clearance produced strain. The literature on screening and optimization of fermentation parameters are scanty. Therefore we made an attempt to screen and optimize amylase producing strain *Aspergillus oryzae* SCBRD[11].

MATERIAL AND METHODS

Chemicals

Starch used in the study was procured from Hi-Media Laboratories, Bombay, India, the other ingredients used for the preparation of Czapek Dox's media were also products of Hi-Media Laboratories, Bombay.

Organisms

The *Aspergillus oryzae* strains were isolated from different soils. Soils are taken from different regions from Bangalore university campus. Tentatively identified in the laboratory as described by Rapper and Fennell[9] and further the strains were identified at Agarkar research Institute (ARI), Pune.

Screening of amylase producing *A. oryzae* by plate assay

All the fifteen isolates were kept for screening of Amylase producers by plate assay method as described by Tiwari, *et.al*.[10]. *Aspergillus oryzae* was streaked on to the potato dextrose agar medium containing 1% starch. After inoculation the plates were kept for incubation for 24-48h at 30^0C.

After incubation of *Aspergillus oryzae* for 24-48h on plates containing starch, plates were flooded with 1% iodine solution for approximately 5-10 min. starch hydrolysis was evidenced by a clear zone around the fungal colony

Optimization of fermentation parameters through submerged fermentation

The production of amylase under submerged fermentation mainly depends on various factors like initial pH, temperature, inoculum size. Hence, these parameters must be optimized in order to achieve higher yields of amylase. During this optimization process, once a particular parameter was optimized, the same optimum condition of that specific parameter was employed in the subsequent studies wherein another parameter is to be optimized.

The selected *A. oryzae* SCBRD11were cultured on production medium. The production medium consist (gm/100ml) of dextrose0.1, yeast extract 0.3, KCl 0.02, NaCl, 0.01$MgCl_2$ 0.02 and starch 0.5.

Optimization of pH for amylase production

The 250 ml Erlenmeyer flasks containing 100ml of production medium were prepared by mixed with acid/alkali solution to obtain required pH. The pH was adjusted in the range of 3-6 with increments of 0.5. Thus prepared flasks were cotton plugged and autoclaved at 121^0C for 15 min. The flasks were inoculated and incubated as described by Siddalingeshwar[11].

Optimization of temperature for amylase production

The 100ml of the production medium was separately taken in 250 ml Erlenmeyer flasks and prepared for submerged fermentation as described by Siddalingeshwar[11].Thus prepared flasks were incubated at different temperatures like 28-34^0C with in increments of 1^0C.

Optimization of inoculum size for amylase production

The inoculum was prepared separately by rewiwing the 168h old culture of *A. oryzae* SCBRD11 at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25ml and then fermentation studies were carried out.

Assay of amylase

Amylase activity was determined as it is described by Okolo *et al*.[5]. The reaction mixture consist of 1.25 mL of 1 % soluble starch, 0.5 mL of 0.1 M acetate buffer (pH-5.0), and 0.25 mL of crude enzyme extract. After 10 min of incubation at 50 °C, the liberated reducing sugars (glucose equivalents) were estimated by the dinitrosalicylic acid (DNS) method. The colour developed was read at 510 nm.

International units (IU)

One unit (IU) of amylase is defined as

the amount of enzyme releasing one mol of glucose equivalent per minute under the assay conditions.

RESULTS AND DISCUSSION

All fifteen strains of *Aspergillus oryzae* produced clear zones on starch plate medium, those were selected from the soil sample. Of the fifteen strain *Aspergillus oryzae* SCBRD11 was considered to be the best and high amylase producing strain. It showed 0.8cm of cleared zone around the colony.

The data obtained in the present study on the effect of pH and temperature on submerged fermentation is shown in (Fig. 1 and Fig. 2) which reveals that the production of amylase increased with the increase in the pH of the medium up to pH 4.5, temperature 31^{0}C and thereafter the decrease of amylase was observed. The maximum production of amylase 5.210 IU was obtained at pH 4.5 and the minim um production of amylase 3.05 IU was observed at pH 3.0.

The production of amylase increased significantly with the increase in fermentation temperature from 28 - 31^{0}C and decreased above 31^{0}C. The maximum amylase production obtained at 31^{0}C was 5.28 IU and the least production was observed at 28^{0}C resulted only 2.28 IU of amylase at 72 hrs of fermentation period. Any temperature beyond the optimum range is found to have some adverse effect on the metabolic activities of the microorganisms and it is also reported by various scientists that the metabolic activities of the microbes become slow at lower or higher temperature[12].

In our study the data revealed that the pH of 4.5 was found as suitable for maximum production of amylase with *Aspergillus oryzae* SCBRD11 strain under submerged fermentation. As such our findings are in close agreement with the findings of Hayashida and Teramoto[13], Carlsen, *et al.*,[14] and Djekrif-Dakhmouche *et.al.,*[15] in Fungi of *Aspergillus* sp. such as *A. oryzae*, *A.ficuum* and *A. niger* were found to give significant yields of α-amylase at pH 5.0–6.0 in submerged fermentation .

The pH is one of the important factors that determine the growth and morphology of microorganisms as they are sensitive to the

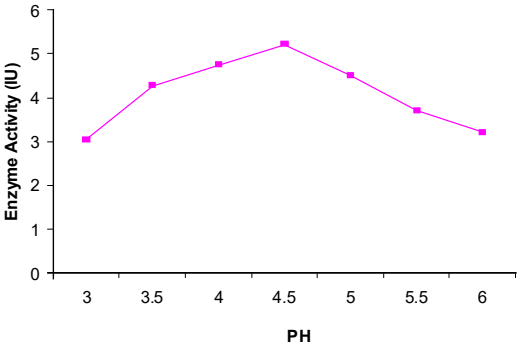

Fig. 1. Effect of pH on enzyme production

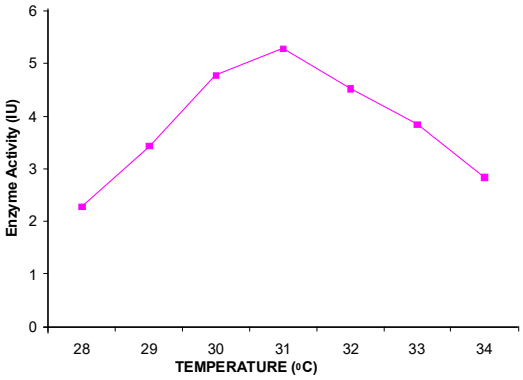

Fig. 2. Effect of Temperature on enzyme production

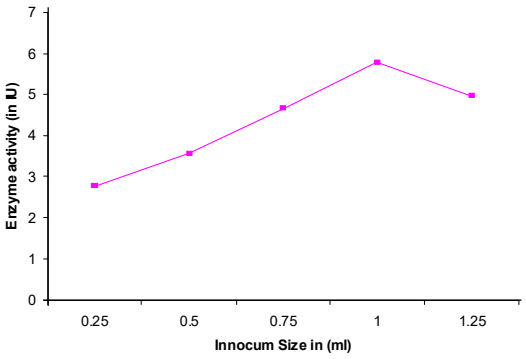

Fig. 3. Effect of inoculum size on enzyme production

concentration of hydrogen ions present in the medium. Earlier studies have revealed that fungi required slightly acidic pH and bacteria required neutral pH for optimum growth. pH is known to affect the synthesis and secretion of amylase just like its stability[16]. These variations in pH optima for amylase production may be due to the strain of the organism used, chemical composition of the substrate, fermentation system and finally the conditions under which fermentation takes place[17].

Similar reports are available on among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25-37 °C[18,19]. A raw starch degrading a-amylase was produced by *Aspergillus ficuum* at 30 °C [13].

Importance of inoculum size on microbial fermentation process is widely accepted. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml) a 0.75 ml inoculum was found to be the most suitable for high production of amylase by *Aspergillus oryzae* SCBRD11 in submerged fermentation at 72 hrs of fermentation. From Fig. 3, it is clear that the amylase production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude when enzyme productivity became maximum, thereafter no appreciable change in production of amylase with high inoculum size could be observed. The maximum enzyme activity was showed at 5.77 IU at 1.0 ml inoculum size and least enzyme activity 2.78 IU was showed at 0.25 ml of inoculum size. Gangadharan *et al.,*[20] were used 1 ml of inoculum for the production of amylase by using *Bacillus amyloliquefaciens*. Adinarayana Kunamneni *et al.,*[25] reported that 10% of inoculum is optimum for amylase production by using *Themophilic lanuginosus* through solid state fermentation.

ACKNOWLEDGEMENTS

I thank Mr.T.N. Suresh, Director for giving cooperation to carryout research work in Padmashree Institute of information sciences.

REFERENCES

1. Hewitt, C. G., Solomons, G. *I J Microbiology*., 1996; **17**: 96-99.
2. Bilinski, C.A., and Stewart G.G. Yeast Protease and Brewing. In: *Yeast biotechnology and Biocatalyst ed*. Verachert, H. and Mot, New York: Marcel Dekker. R. 1990; 147-162.
3. Akpan, I., Bankol, M.O., Adesemowo, A.M., Latunde-Dada, G.O. Production of amylase by *A.niger* in cheap solid medium using rice bran and agricultural materials. *Trop.Sci*., 1996; **39**: 77-79.
4. Buzzini, P., Martini, A. Extracellular enzymatic profiles in yeast and yeast like strain isolated from tropical environments. *Appl. Microbiol*., 2002; **93**: 1020-1025.
5. Okolo. B.N., Ezeogu, L.I., Mba, C.N. Production of raw starch digesting amylase by *A. niger* grown on native starch sources. *J. Sci. Food Agri*, 1995; **69:** 109-115.
6. Mitchell, D.A., Lonsane, B.K. In General principle of solid fermentation, *Monogram, ed* by Doelle HW, Rolz C, Publication of Oxford London 1990.
7. Adebiye, CAB., Akinyanju, J.A. Thermophilic amylase producer from soil. *Nigerian J Sci. Technol*., 1998; **11**(1): 30-38.
8. Abe, J., Bergman, F.W., Obeta, K., Hizukuri, S. Production of the raw starch degrading amylase of *Aspergillus* sp. K-27. *Appl. Microbiol biotechnology*., 1998; **27:** 447-450.
9. Rapper, K.B., Fennel, D.I. The genus *Aspergillus*, Williams and Wilkins, New York, 1965; 567-577.
10. Tiwari, K.L. Jadhava S.K., Fatima. A. Culture condition for the production of thermostable amylse by *Penicillium rugulosam. Global journal of Biotechnology and Biochemistry*., 2007; **2**(1): 21-24.
11. Siddalingeshwara, K.G. Production of tumour inhibitory L-asparaginase through solid state fermentation from *Aspergillus terreus*. *Ph.d. thesis*. Gulbarga University,Gulbarga 2005.
12. Tunga, R., Banerjee, R., Bhattacharya, B.C. Some studies on optimizatation of extraction process for protease for production in S.S.F, *Bioprocess Engineering*., 1999; **20**: 485-489.
13. Hayashida, S., Teramamoto, Y. Production and characteristic of raw-starch digesting amylase from protease negative *Aspergillus ficuum* mutant, *Appl. Environ. Microbial*., 1986; **52:** 1068-1073.

14. Carlsen, M., Spohr, A.B.,Nielsen, J., Villadsen, J. Morphology and physiology of an á-amylase producing strain of *Aspergillus oryzae* during batch cultivations, *Biotechnol. Bioeng*., 1996; **49**: 266-276.
15. Djekrif-Dakhmouche, S., Gheribi-Aoulmi, Z., Meraihi, Z., .Bennamoun, L. Application of statistical design to the optimization of culture medium for α-amylase production by *Aspergillus niger* ATCC 16404 grown on orange waste powder, *J. Food Eng*., 2005; **73**: 190-197.
16. Fogarty W M. Microbial amylase, in Microbiology and Biotechnology, Ed Fogarty. WM *Appl. Sci. Publ*., Barking, (UK) 1983: 1-92.
17. Roukas. Citric acid production from carob pod by solid state fermentation. *Enzyme Microb. Technol.*, 1994; **24**(1-2): 54-59.
18. Ramachandran, S., Patel, A.K., Nampoothri, K.M., Francis, F., Nagy, V., Szakacs, G., Pandey, A. Coconut oil cake – A potential raw material for the production of α- amylase, *Bioresour. Technol*., 2004; **93**: 169-174.
19. Francis, A., Sabu, K.M., Nampoorthi, S., Ramachandran, S., Ghosh, G. Szakacs, Pandey, A. Use of response surface methodology for optimizing process parameters for the production of α-amylase by *Aspergillus oryzae*. *Biochem . Eng. J*., 2003; **15**: 107-115.
20. Dhanya Gangadharan, Swetha Sivaramakrishnan, Kesavan Madhavan Nampoothiri, and Ashok Pandey. Solid culturing of *Bacillus amyloliquefaciens* for α-amylase production. *Food Technol. Biotechnol*. 2006; **44**(2): 269-274.
21. Adinarayana Kunamneni, Kugen Pemaul., and Suren Singh. Amylase production in solid state fermentation by the *Themophilic lanuginosus*. *Journal of Bioscience and Bioengineering*., 2005: **100**(2): 168-171.