International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 1, Issue 6, pp: 268-282, 2016 **http://www.ijzab.com**
https://doi.org/10.5281/zenodo.1310819
Research Article

AMYLASE ACTIVITY OF *BACILLUS AMYLOLIQUEFACIENS* AND *ASPERGILLUS NIGER* FROM AGRO INDUSTRIAL WASTES BY SOLID STATE FERMENTATION

C. Elaiyaraja[1*], V. Senthil[2], M. Ayyavoo[2] and S. Ramu[3]

[1] Department of Zoology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode-637 205, Tamil Nadu, India
[2]Department of Zoology, A.V.V.M. Sri Pushpam College, Poondi-613 502, Thanjavur, Tamil Nadu, India
[3]Department of Zoology, M.R. Govt. College, Mannargudi-614 001, Tamil Nadu, India

Article History: Received 11th October 2016; 15th November 2016; Published 31st December 2016

ABSTRACT

The solid state fermentation (SSF) has been reputable as a superior technique for the enzyme production. The present study targeted on the amylase production from *Bacillus amyloliquefaciens and Aspergillus niger* by using different agro industrial waste like sago waste, rice bran and wheat bran as substrate with the use of SSF techniques and tested for its activity. The effect of different parameters such as time, pH and temperature were also tested. *B. amyloliquefaciens and A. niger* was identified as the best producers of amylase at pH 7 and maximum production was in rice bran and wheat bran. Substrates tested for amylase production could be effectively exploited in the future production of various commercial products.

Keywords: Solid state fermentation, Amylase, Wheat bran, Sago waste, Rice bran.

INTRODUCTION

A large number of manufacturing processes in the industrial, food technological and environmental areas take advantage of enzymes in mammoth quantities for production of product from the microorganisms. Microbes is produced a variety of enzymes which act as biocatalyst for various biochemical reactions and also formation of fermentation products. Enzymes are biocatalysts protein in nature and react in the living cell without any overall change (Jain and Sanjay Jain, 2006). Enzymatic degradation of starch yields glucose, maltose and other low molecular weight sugars and these products serve as important substances for food and pharmaceuticals industries (Khire and Pant, 1992). Nowadays using microorganism as industrially relevant enzymes has stimulated interest in exploration of extra cellular enzymatic activities (Bilinski and Stewart, 1995).

Amylases are a group of enzymes and it has been found in microorganisms like bacteria (Murakami *et al.*, 2008; Mukherjee *et al.*, 2009) and fungi (Kathiresan and Manivannan, 2006; Gouda and Elbahloul, 2008). It is a starch degrading enzyme and commercially produced for industrial processes such as starch-glucose industry, textile industry and brewing industry (Goyal *et al.*, 2005). Sources of amylases in yeast, bacteria and molds have been reported and their properties have been described (Buzzini and Martini, 2002). On commercial basis, amylase of fungal origin is found to be more stable than the bacterial origin. However, many attempts have been made to optimize culture conditions and suitable strains of fungi (Abu *et al.*, 2005). Amylase enzymes are employed in starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar constituents (Suganthi *et al.*, 2011). They are great deal of attention because of their perceived technological significance and economic benefit. Amylases are hydrolytic enzymes, which are extensive in nature, being found in animals, microorganisms and plants (Octavia *et al.*, 2000). Hence the present study is to attempt the amylase production from the *Bacillus amyloliquefaciens and Aspergillus niger* under solid state fermentation by using agro industrial wastes (sago waste, rice bran and wheat cake) collected from the Namakkal, Tamil Nadu, India.

*Corresponding author: Dr. C. Elaiyaraja, Assistant Professor, Department of Zoology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode, Email: kcesraj@gmail.com, Mobile: +91 8015107949

MATERIALS AND METHODS

Sample collection

The industrial wastes such as sago waste, rice bran and wheat bran were collected from the sago industries located in Namakkal, Tamilnadu, India. The bacterial culture *Bacillus amyloliquefaciens* (MTCC 1270) *and Aspergillus niger* (MTCC282) were obtained from the Microbial Type culture collection and Gene bank (MTCC), Chandigarh, India and maintained in Nutrient Agar slants (NA) for bacterial culture and Potato Dextrose Agar (PDA) for fungal culture at 4°C and sub cultured periodically.

Solid State Fermentation

The *B. amyloliquefaciens* (MTCC 1270) *and A. niger* (MTCC282) were subjected to solid state fermentation in different substrates like rice bran, sago waste and wheat bran, which was used as solid substrates for SSF. Five gram of each bran was weighed and hydrated with 5 ml of basal salt solution $(NH_2)_2SO_4$; 2g, KH_2 PO_4; 1g, $MgSO_4$ $7H_2O$; 0.5g, $ZnSO_4$; 0.1g per liter and adjusted with moisture content from 43-81 %. 1 % was inoculated after sterilization.

Effect of time, temperature and pH

The effect of incubation time on enzyme production was investigated by checking the enzyme activity on 12, 24, 36 and 48 hours of incubation in the different substrates at room temperature. The broth culture suspension of microbes was transferred to the production media with different pH range such as 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 and incubated at three different temperature such as 30, 37 and 39°C for *B.amyloliquefaciens* and 18, 20 and 22°C for *A. niger* in a rotary shaker. After 24 hours of incubation, about 10 ml of the production media centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for amylase estimation. The estimation of amylase activity was followed by the method proposed by Manning and Campbell (1961).

RESULTS AND DISCUSSION

The selection of appropriate solid substrate is a significant factor, so that the selection of agricultural byproducts is necessary steps for the study of amylase production. We were preferred four agriculture byproducts to find out suitable substrate for optimum production of α-amylase. The influence of time on amylase production was analyzed by incubating the reaction mixture from 12 to 48 hrs of intervals. At the optimum incubation time of 24 h, *B. amyloliquefaciens* was produced 9.9 U/mg amylase from sago waste, 9.46 U/mg from wheat bran and 8.25U/mg from rice bran (Figure 1A). In case of the influence of time on culture (4^{th} to 7^{th} day of incubation), the highest amylase production of 9.7 U/mg was in sago waste, 9.58 U/mg in wheat bran and 8.82 U/mg in rice bran at the 5^{th} day of incubation in *A. niger* (Figure 2A). Sonjoy *et al.* (1995) reported that short incubation period offers potential inexpensive production of enzyme. The present observation also proved that the incubation period varied with enzyme production. Similarly, the mycelial growth on starch reached a maximum after five days and maximum amylase activity was produced after two days of cultivation (Ely Nahas and Mirela Waldermarin, 2002). The decreased activity in the later phase of growth was probably due to catabolite repression by glucose released from starch hydrolysis, in agreement with the results reported in *Humicola grisea* and *H. Brevis*, but different from *Papulasporia thermofilia* in which the highest amylase activity was observed during the period of fungus autolysis and reported the highest production of amylase enzyme at five days of incubation period at 30°C (Gupta *et al.*, 2008).

The bacterium (*B. amyloliquefaciens*) was incubated at pH of 4.0 to 9.0 and temperature of 30°C to 39°C. Similarly the fungi (*A. niger*) was incubated at pH 4.0 to 9.0 and temperature of 18°C to 22°C. The temperature was maintained by using rotary shaker. The enzymes were extracted and the specific activity of the amylase was recorded (Figures 1 and 2). The maximum amylase production by *B. amyloliquefaciens* was observed at 9.8 U/mg in the sago waste substrate at pH 7.0 (37°C) (Figure 1C). The highest amylase activity was recorded as 9.84 U/mg in sago waste, 9.76 U/mg in sago waste and 9.69 U/mg in wheat bran at pH 7.0 (37°C, 39°C and 30°C) respectively (Figure 1B to D). *B. amyloliquefaciens* has widely been reported to produce amylase enzymes (Abante *et al*., 1999), but so far it has not been reported to produce amylase enzyme by using agro industrial waste. Likewise, Chessa *et al* (1999) reported that the optimum pH value for maximum α-amylase activity was at pH 7.5. Chakraborty *et al.* (2000) concluded that the purified α-amylase showed a wide range of pH tolerance and maximum activity was observed at 7.0. Moreover, Malhotra *et al* (2002) concluded that the purified α-amylase showed a maximum activity at the optimum pH value of 8.0. While the temperature below or above 35 ºC exhibited lower production of amylase. Other investigators were reported that, the optimum temperature for maximum purified α-amylase activity was 30 ºC (Strumeyer and Fisher, 1982). The same finding was reported by El-Safey (1994), who indicated that, the purified MM-α-amylase displayed maximal activity at 30 ºC corresponding to 50 ºC for purified RH-α-amylase. It gave a good performance in the amylase production in three substrates (sago waste, rice bran and wheat bran) with different pH and temperature by using rotary shaker.

The enzyme activity of *A. niger* MTCC282 was observed as highest (9.48 U/mg) activity in wheat bran at pH 7.0 (20°C) (Figure 2B). Followed to this, wheat bran at pH 7.0 (18°C) showed enzyme activity of 9.27 U/mg and maximum activity was observed in sago waste (9.7 U/mg) at pH 7.0 (20°C) than other substrates (Figures 2B and C). The maximum yield of amylase was recorded 6.97 U/mg at pH 8.0 (25°C) of incubation in sago waste than other substrate (Figure 2D). There was noticeable increase in the yield till pH 8.0 and then there was a very low activity at pH 9.0 in all the substrates used in SSF. Wheat bran was found to be the best substrate for α- amylase production by

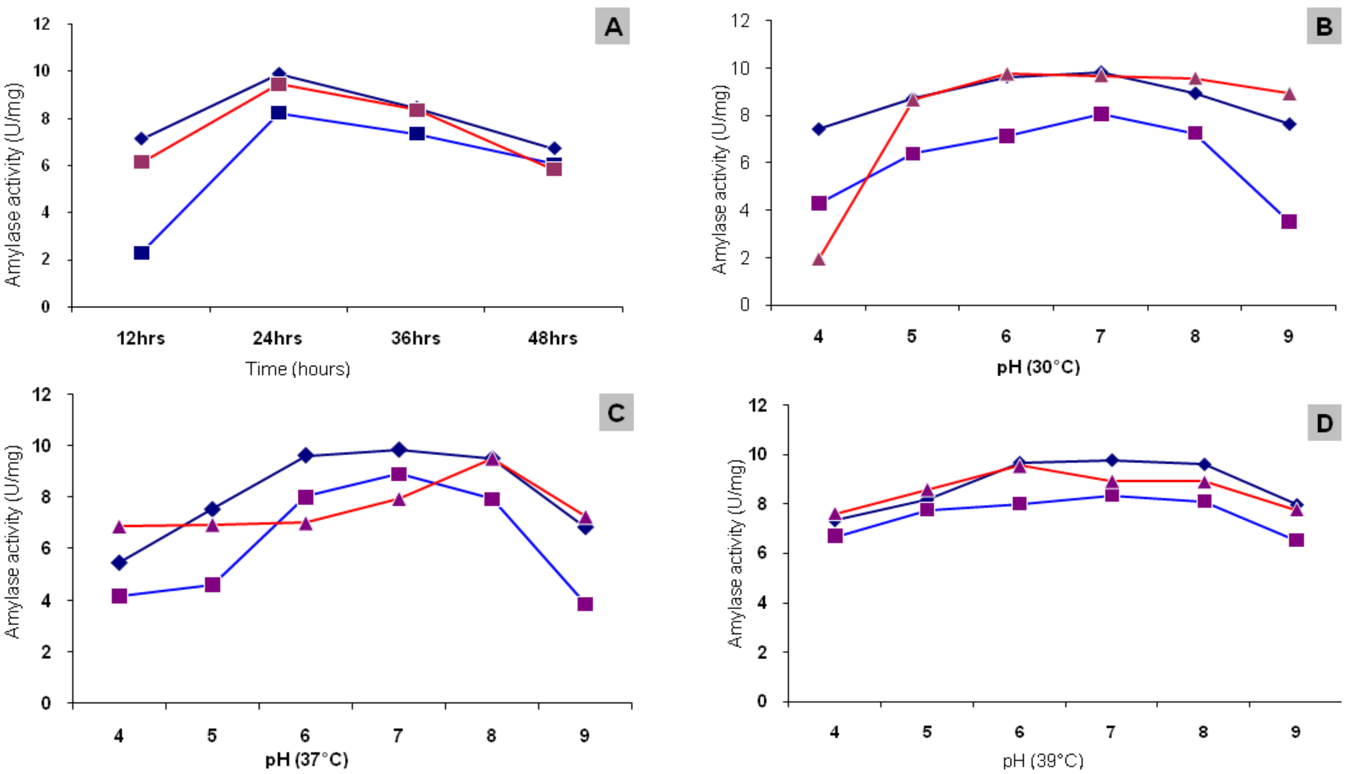

Figure 1. Specific activity (U/mg) of the amylase enzyme by *Bacillus amyloliquefaciens* in different substrate at different parameters.

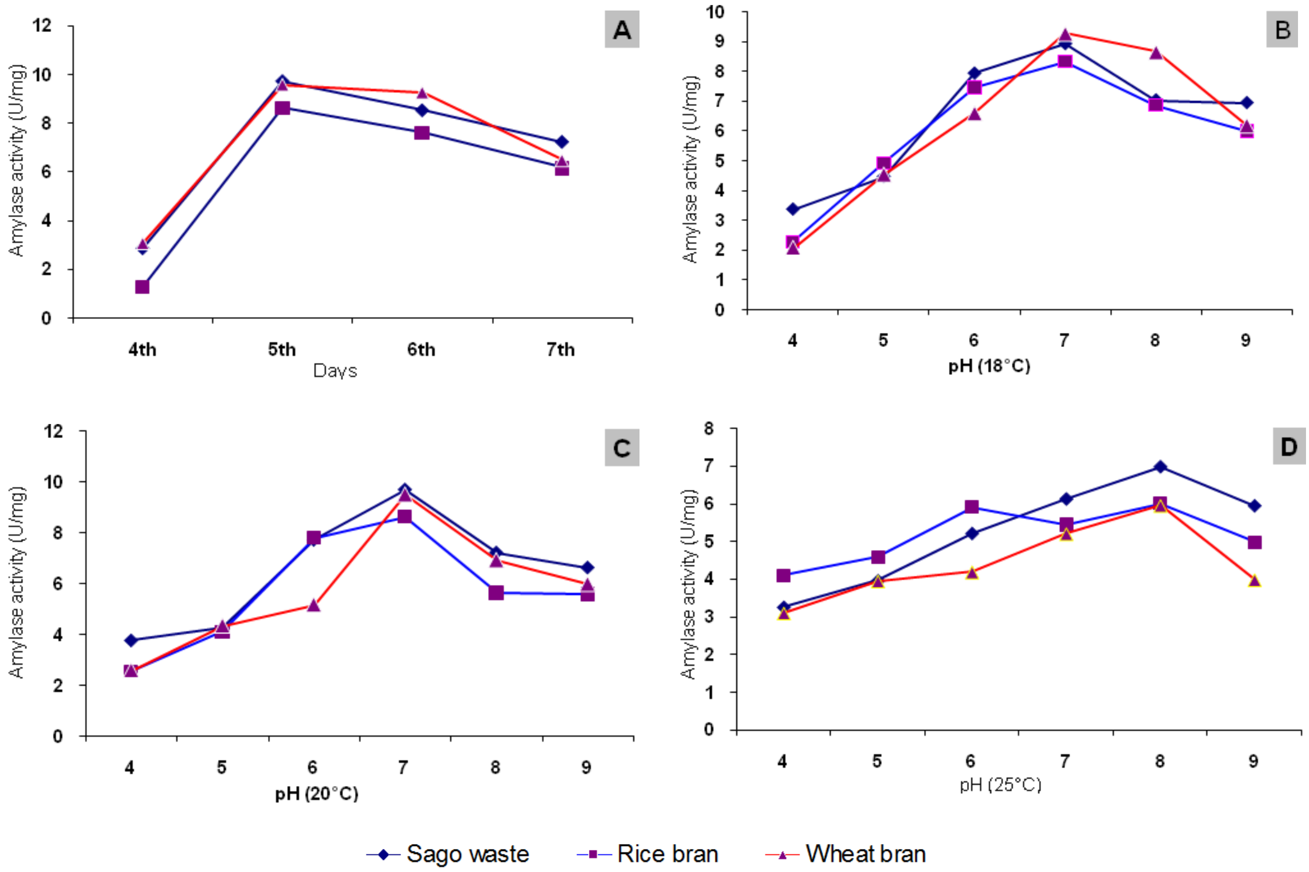

Figure 2. Specific activity (U/mg) of the amylase enzyme by *Aspergillus niger* in different substrate at different parameters.

a thermophilic fungus *Humicola lanuginose* (Singh *et al.*, 2009). In contrary to our results, Varalakshmi *et al.* (2009) reported the maximum enzyme activity of 75 U/mg of protein at pH 9.5. Previous studies reported that acidic pH optima for amylases from *A. niger* (Gupta *et al.,* 2008; Hernandez *et al.,* 2006; Mitidieri *et al.,* 2006). The great yield temperatures of amylase were between 30-37°C (Suganthi *et al.*, 2011). However the optimum temperature for enzyme production was reported at 30°C (Alva *et al.*, 2007). Previously 30°C and 45°C were reported as optimum temperature for amylase production by *Aspergillus flavus* and *Myceliophthora thermophilia* (Nandakumar *et al*., 1999). In the present observation, the effect of pH on the activity specifies that the amylase is active in the pH range of 6.0-8.0. This suggests that the enzyme would be useful in course of action that requires wide variety of pH amend from neutral to some extent to alkaline range. Similarly multiple pH optima were observed for amylolytic activities in the crude amylase preparation in various literatures (Yamasaki *et al.,* 1997; Bergmann *et al*., 1998). In the present observation, different time, pH and temperature of incubation gave capable results for the production of amylase by *B. amyloliquefaciens* (MTCC 1270) and *A. niger* (MTCC282) with rice bran and wheat bran. The results have shown the potential of a new approach of amylase production from microbes by using some agro-industrial wastes that are abundant in Namakkal district of Tamil Nadu. The strain showed improved amylase activities in all the substrates.

CONCLUSION

Thus from these results we conclude that the obtaining microbial isolates which have the ability to use different agro-industrial wastes as very despicable and available substrates for obtaining bacterial and fungal amylases. All the solid substrates rice bran (RB), wheat bran and sago waste (SW) can be used for supported production of amylase using *B.amyloliquefaciens and A. niger* under solid state fermentation system. Sago waste has been enhanced to other solid substrates for the production of amylase from *B. amyloliquefaciens and A. niger* under solid state fermentation. The maximum production of amylase (9.84 U/mg and 9.7 U/mg) was achieved by utilizing sago waste as the substrate in pH 7.0 at 37°C and 20°C from the *B. amyloliquefaciens and A. niger* respectively by regulate the temperature by rotary shaker. Thus the strain of *B. amyloliquefaciens* (MTCC 1270) and *A. niger* (MTCC282) was found to be the best producer of amylase. An interesting scope for further research would be to use the optimized medium for the synthesis of amylase and the investigation of enzyme kinetics for determination of industrially accessibility. The strain is maintained in our laboratory for further analysis regarding industrial applications.

ACKNOWLEDGEMENT

The authors thank the principal of A.V.V.M. Sri Pushpam College, Thanjavur and Chairman and Secretary, Vivekanandha Educational Institutions for the facilities provided and also special thanks to Dr. K. Ravi, Director, Biogeneic institute of Biotechnology, Namakkal for the kind provision of amylase activity analysis.

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