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

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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 Elbahoul, 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.

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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 (NH₄)₂SO₄; 2g, KH₂PO₄; 1g, MgSO₄·7H₂O; 0.5g, ZnSO₄; 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 sterilization. The estimation of amylase activity was 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 (4th to 7th 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 5th 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 Waldemarini, 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 Humincola 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 (Abane 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 °C exhibited lower production of amylase. Other investigators were reported that, the optimum temperature for maximum purified α-amylase activity was 30 °C (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 °C corresponding to 50 °C 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 7.0 (20°C) than other substrates (Figures 2B and C). The maximum yield of amylase was recorded 6.97 U/mg at pH 7.0 (20°C) than other substrates.
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.
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REFERENCES


