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Production of Amylase from Marine Fungus Using Spoiled Banana Fruit as a Substrate

KEYWORDS: Amylase, Aspergillus oryzae, Penicillium sp. Optimization

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ABSTRACT *Solid state fermentation was carried out using banana powder as a substrate for the production of amylase through marine fungus. Totally twenty different kinds of fungal species were isolated from marine sediments, and screened for amylase enzyme production. Among them two species, such as Aspergillus oryzae and Penicillium sp. were selected based on their high level of amylase activity. Banana (fruit) powder was used as a substrate for high yield of amylase production during very short period. Mineral salt medium with different carbon sources (sucrose, glucose, starch) were used for the optimization of enzyme production. The starch supplementation showed high level of enzyme activity than glucose and sucrose. The growth and amylase production of A. oryzae and Penicillium sp on banana fruit substrate with different carbon sources, incubation time, pH and temperature were studied. The optimum conditions for amylase production were determined. The enzyme activity was marginally better in Aspergillus oryzae than Penicillium sp.*

INTRODUCTION:

Amylase is one of the most important enzymes and of vast significance in present day biotechnology and having approximately 25% of the enzyme market. Amylases are employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents. To convene the demand of these enzymes in industries, a low cost medium is vital for the fermentation of amylase. The Solid State Fermentation (SSF) process offers a better opportunity for the biosynthesis of high cost products with the help of a low cost medium. Agro industrial residues are generally considered as the best substrate; and the uses of agricultural wastes make SSF an attractive another method (Ellaiah et al., 2002).The spectrum of amylase applications has expanded into many other fields such as clinical, medicinal and analytical chemistry and other applications. The amylases can be derived from several sources, such as plants, animals and microorganisms. Amylases derived from fungi (Wang et al., 2001) are the most important and are great significant in industrial sector because of their short growth period, the enzymes from microbial sources generally meet industrial demands.

Banana is grown extensively in tropical and sub tropical countries, particularly in India the productions more. Banana easily spoiled than compared to other fruits. Thus, the present study was to carry out banana as a substrate for amylase production. Therefore, supplements of sugars in the form of diluents should be provided to enhance the microbial growth and enzyme productivity. In present research work, attempt has been made to optimize the cultural conditions for the amylase production by Aspergillus oryzae and Penicillium sp using banana as a raw material.

MATERIALS AND METHODS:

Isolation of Fungi:

Marine Sediments were collected at a depth within 10-15cm from the surface of the soil. Then, 10 fold serial dilutions of the samples were prepared. lml of serially diluted sample were plated in the modified potato Dextrose Agar medium and incubated at 37 °C for 24-48 hrs. The pure culture of fungi were maintained in agar slants and stored for further studies.

Primary screening for amylase production

The fungus strains were streaked on starch-agar media and incubated at 37°C for 48 hrs and then were flooded with Gram's Iodine. It was allowed to stand for few minutes and then it was poured off. Clear distinct zones have appeared around the colonies which produce amylase.

Preparation of Substrate

Spoiled Banana fruit was used as a substrate for amylase production. It was obtained from fruit market and chopped into small pieces of uniform sizes and dried at 70°C for 24 hours. The dried slices were ground and stored in sterile polyethylene bags at room temperature until use.

Preparation of inoculum

The fresh over night culture was used as an inoculum for the growth and the production of enzyme studies. A volume of 50 ml of Potato Dextrose broth was taken in a 250-ml Erlenmeyer flask was inoculated with a loop full of fungal culture and kept at 37°C in a rotary shaker.

Amylase Production on Solid State Fermentation

The experiment was conducted in 250 ml Erlenmeyer flask which contain 5 g of substrate (Banana) powdered with the mineral salt medium recommended by Ramesh and Lonsane (1989) and sterilized at 121°C for 15 minutes and inoculated with 1ml of fungus culture suspension (V/W). The contents of the flasks were mixed thoroughly to ensure uniform distribution of the inoculums of Aspergillus oryze and Penicillium sp separately and left at room temperature. After incubation the contents were filtered through Whatman no.l filter and the mycelia dried to constant weight at 60° C and weighed. The culture filtrate was centrifuged at 10,000 rpm (400 C) for 15minutes and the supernatant was used for amylase assay. Amylase activity was assayed following the method of Bertrand et al. (2004) using 3, 5 - dinitrosalicyclic acid method (DNSA).

Optimization of amylase production

Effect of Carbon source (Sugars):

To investigate the effects of various carbon sources on amylase production, fungus was grown in Mineral salt media containing banana powder with glucose, sucrose and starch as a carbon sources.

Effect of incubation time

The optimal time for enzyme (amylase) activity was determined by assaying activity of the enzyme at different incubation time 24, 48, 72 and 96hrs. Samples were taken at regular intervals and analyzed for amylase activity.

Effect of pH

The optimum pH for enzyme (amylase) activity was determined by running the assay activity between pH ranges of (5.5, 6, 6.5, 7 and 7.5) acidic to alkaline. The enzyme activity for each pH was determined using the method of Bertrand et al. (2004).

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Effect of temperature
The optimal temperature for amylase activity was determined by assaying activity of the enzyme at different temperature of 25, 30, 35, 40 and 45oC.

RESULTS AND DISCUSSION

In the present investigation, totally twenty different kinds of marine fungus were isolated from marine sediments collected in east coast of India. Among them two species, such as Aspergillus oryzae and Penicillium sp. were produced high level of amylase and selected for further studies. Amylase production was also reported from various fungus such as Penicillium griseofulvum (Ertan et al., 2005) Aspergillus sp (Nguyen et al., 2005). Amylase production was enhanced both submerged and solid state fermentation using Aspergillus sp was reported (Moreira et al., 2001). Solid state fermentation was carried out using banana peel as a substrate for the production of amylase by Penicillium sp was studied (Vijayarahavan et al., 2011). In the present study banana used as a substrate for the production of amylase by inoculating the marine derived fungi.

The optimized condition for amylase production in solid state fermentation by A.oryzae and Penicillium sp were analyzed using different parameters such as carbon source, incubation time, pH and temperature. Among the banana fruit with different carbon sources, starch supplementation was exhibited better response with regard to biomass and enzyme production (250 U/mg of A.oryzae and 220 U/mg of Penicillium sp) than glucose and sucrose. The incubation period 96 hrs was the best possible duration for highest amylase enzyme activity (180 U/mg of A.oryzae and 150 U/mg of Penicillium sp). In the effect of pH, A.oryzae and Penicilium sp were able to produce high levels of biomass and enzyme activity at pH 6.5 (155 U/mg of A.oryzae and 135 U/mg of Penicillium sp). Both isolates were able to produce high levels of enzyme activity at 350C and it was the favorable temperature for maximum amylase production (135 U/mg of A.oryzae and 130 U/mg of Penicillium sp) (Fig: 1). Ragunathan and Swaminathan, (2002) were reported that under SSF amylase produced in 400C and 96hrs for incubation time of amylase production by Aspergillus. These studies proved that the temperature range between 35 and 400C was suitable for enzyme production in SSF method.

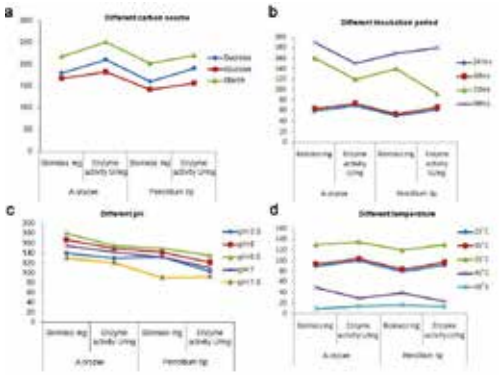

Fig.1 Optimization of Amylase production under solid state Fermentation

CONCLUSION

From the present investigation, it was concluded that within a short period the banana powder as a substrate used for high amount of amylase production and also contribute to safe economic waste management in the environment.

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