PRODUCTION OF AMYLASE FROM *BACILLUS* SP. AY3 USING FRUIT PEELS AS SUBSTRATE

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Abstract

In the present study, amylase producing *Bacillus* sp. AY3 was isolated from the soil samples. Various fruit peels and starch were used in media as substrates separately and the production of amylase was optimized. *Bacillus* sp. AY3 produced extracellular amylase by using the fruit peels. Marked production of amylase was achieved by 5% banana, melon and grape fruit peels at 37˚C. These waste thus provided the cheap source for amylase production which would be better and more economical for industrial processes.

Introduction

Fruits and vegetables wastes are the major source of environmental pollution (Garg and Ashfaque, 2010). Agriculture wastes are the source of starch containing substrates and contain necessary carbon and nitrogen source for bacterial metabolism. These wastes can be utilized by adding as a substrate of amylase in culture medium. Various agriculture wastes such as millet starch, potato and wheat bran are widely used for the production of amylase (Sajjad and Choudary, 2012).

Amylases are the enzymes that act as a biocatalyst for the conversion of starch into valuable products. It has been produced from bacterial, fungal, plant and animal sources from which bacterial amylases have gianed the priority due to their stabilty at high temperatures (Amutha and Priya, 2011).

Bacterial amylases have been used in food industry in the improvement of fermentation process, shelflife and softness of the baked product. It has been widely used for the production of sugar syrups (Souza and Magalhaes, 2010). These enzymes also have great applications in paper industry as they act as best paper sizing agent. Process of bioconversion of starch into ethanol and several other organic compounds required the utilization of amylase in liquefaction and saccharification process (Sivaramakrishnan *et al.*, 2006; van der Marrel *et al.*, 2002).

Materials and Methods

Isolation of microorganism and culture conditions: Soil samples from various juice centers dump sites were collected in sterile bottles and stored at 4˚C till use. Soil samples were inoculated in Luria-Bertani starch broth (10g/L NaCl, 10g/L Tryptone, 5g/L yeast extract and 10g/L starch). pH was maintained at 7.0. Samples were incubated for 42 hours at 37˚C.

Screening of amylase production: Samples were diluted and spread on Luria-Bertani agar plates containing 1% (w/v) soluble starch. Plates were incubated at 37˚C for 24 hours. After flooding the iodine solution on plates *Bacillus* sp. AY3 was selected on the basis of maximum zone of starch hydrolysis among all isolates.

Utilization of fruit peels by *Bacillus* sp. AY3 for amylase production: Fruits and vegetables peels were dried at 80˚C. Luria-Bertani medium was prepared by adding 1% (w/v) of all substrates including starch, banana peel, grape fruit peel, melon peel and potato peel separately. *Bacillus* sp. AY3 was inoculated and incubated at 37˚C and 50˚C for 72 hours. Aliquots were taken after every 24 hours and enzyme units were determined as described by Bernfeld (1955). One unit of enzyme was defined as the amount of enzyme that liberates 1µmol of reducing sugar from the substrate in 1 minute at 37˚C.

Effect of peel concentration on amylase production: Luria-Bertai medium was prepared by adding various concentration of banana, melon and grape fruit peel (0.5, 1, 5 and10%). *Bacillus* sp. AY3 was inoculated and incubated at 37˚C for 48 hours. Enzyme activity was determined as described by Bernfeld (1955).

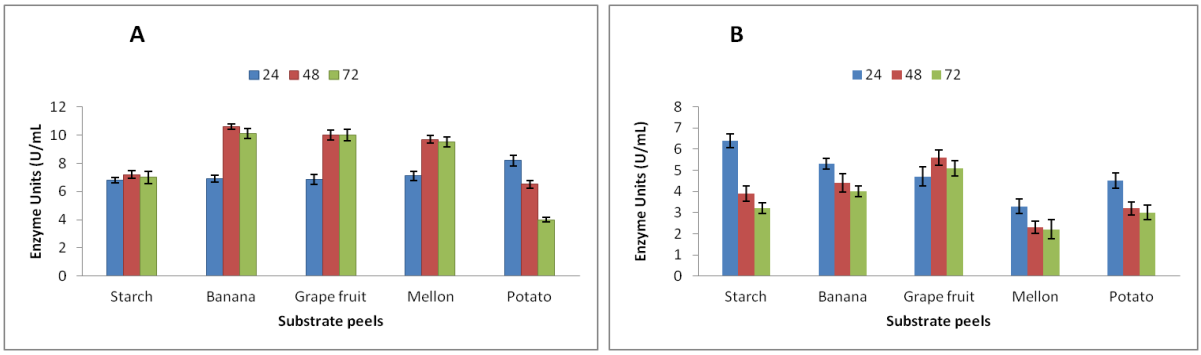

Fig. 1. Effect of fruit peels on amylase production at different temperatures (A) 37^{O}C (B) 50^{O}C.

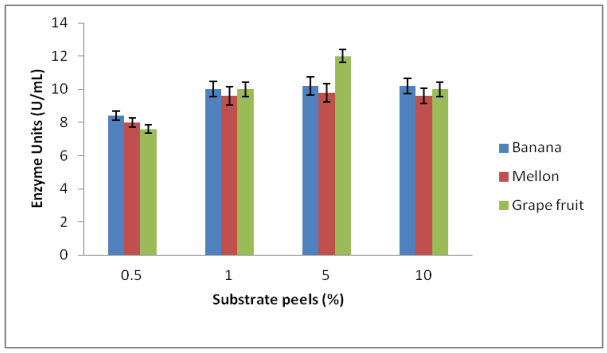

Fig. 2. Effect of various concentrations of fruit peels on amylase activity at 37°C.

Results and Discussion

Bacillus sp. AY3 is a gram positive, rod shaped and spore former bacterium isolated from the soil around fruit peel dump site. It showed the maximum zone of starch hydrolysis therefore selected for this study.

Among various fruits and vegetables peels tested banana, grape fruit and melon peels were found to be the best substrate for extracellular amylase production from *Bacillus* sp. AY3. Figure 1A provides the information that at 37˚C initially after 24 hours all substrates tested showed significant amylase activity but after 48 hours of incubation banana, grape fruit and melon peels yielded 10.16, 10.0 and 9.7 U/mL amylase activities respectively. These fruit peels contain high concentration of carbohydrates therefore can serve as a good substrate for amylase production (Krishna *et al.*, 2011). Similarly results of the present study shows that these fruit peels serve as better substrate source as compared to research grade available soluble starch.

The effect of temperature change on enzyme production was also determined. It was found that 37˚C was the suitable temperature as compared to 50˚C for the growth of *Bacillus* sp. AY3 in the presence of these substrates (Fig 1B).

Various concentrations of banana, grape fruit and melon peels were also tested for the production of amylase. It was found that maximum amylolytic activity was obtained when 5% of grape fruit peels were incorporated in the fermentation medium as substrate (Fig. 2). Banana peels has been used for the production of amylase in solid state fermentation (Paul and Sumathy, 2013; Unakal *et al.*, 2012). It has also been reported the increased production of amylase was achieved by the combined use of banana and potato peels (Jadhav *et al.*, 2013).

It may be concluded that fruits wastes could be utilized for the production of amylases which might be beneficial for various starch processing industries.

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