Indo Global Journal of Pharmaceutical Sciences, 2015; 5(2): 149-153

INDO GLOBAL JOURNAL OF PHARMACEUTICAL SCIENCES
ISSN 2249- 1023

Study of Biosynthesis & Characterization of Microbial α-Amylase by Using Banana Peel Waste

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Keywords

Bacillus subtilis; Amylase; Banana Peels; Bradford Method, DNS Reagent.

ABSTRACT: Amylase is very essential for the conversion of starches into oligosaccharides. Alpha-amylase (1, 4-alpha-glucan-glucanohydrolases) are extracellular enzymes which hydrolyze 1, 4-Glycosidic bonds and also are endoenzymes split the substrate in the interior of the molecule. Bacillus subtilis gave maximum production of alpha amylase as compared with the other strain. The study showed that the protein content and enzyme activity are high in different samples. Soil sample was collected from sugarcane waste dumping site, Muradnagar, Ghaziabad, India. Sample were inoculated in two different media using banana peel waste as an alternative source of carbon, which is an economic and profitable due to the inherent nature of the banana waste itself, for α-amylase production by Bacillus subtilis as standard strain and by isolated culture from soil sample. The highest α-amylase enzyme production and protein content were detected in Media – II. We report that α-amylase activity was detected in the crude sample of Media – II at pH 7 and temperature 35◦C using maltose substrate. The isolated culture from soil sample shows high activity of α-amylase enzyme as compared to Bacillus subtilis standard strain. The α-amylase enzyme was used in the starch processing food, detergent, textile, paper, clinical and analytical industry.

NOTE: Full length manuscript of Sharma et al. Study of Biosynthesis & Characterization of Microbial α-Amylase by Using Banana Peel Waste . 2014; 4(3): 173.

INTRODUCTION

Enzymes are the proteins or biocatalyst synthesized by the living cells. Amylases are among the most important enzymes and play a great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market [1]. α- amylase is an enzyme which helps in the breakdown of starch to maltose [2]. α-amylase hydrolyzes the bonds between glucose repeats. Bacillus sp. are widely used for production of α-amylases and these bacteria need rich source of nutritional medium to grow, different fruit and vegetable peels usually considered a waste provide rich source of starch and nutrients for bacteria [3]. Banana peels are used as feedstock as they have some nutritional value. Banana peels are widely used for that purpose on small farms in regions where bananas are grown [4]. Banana peels are the rich source of soluble and insoluble fibres and also having antioxidant properties. Applications include paper, textile, food, fuel alcohol production, starch conversion and detergent industries.

MATERIALS & METHODS

Sampling

Sample of dry soil collected from different sites of sugarcane dumping site.

Isolation of microorganism

For the isolation of microorganism, we have to prepare the nutrient agar plate.

Identification of microorganism

We had been done many biochemical and cultural tests. Gram staining and IMViC test is done for the identification of the microbe.

Inoculum preparation

The inoculum medium was prepared by using peptone, beef extract and sodium chloride at pH 7, and sterilized in an autoclave for 1h. at 121°C. A loopful culture of Bacillus sp. raised on nutrient agar media was transferred aseptically in laminar air flow into the inoculum medium. The liquid medium was then incubated for 48 hr. at 37°C on a shaker (150rpm) for microbial growth.

Preparation of substrate

Banana peel used as substrate was obtained from fruit market and chopped into small pieces of uniform size, wash with buffer and spread on the animal tray and then oven dried at 50◦C for 48hr. After 48hr, dried pieces are separated, grind in a mixture to get in a powdered form and preserved in a polythene bags for further use.

Fermentation media

Different type of media are prepared for the liquid state fermentation

Media 1 –Glucose, Yeast extract, Peptone, Sodium chloride, Disodium hydrogen phosphate, Dihydrogen sodium phosphate, Potassium chloride, Magnesium sulphate and D.W.

Media 2 – Tryptone, Yeast extract, Sodium citrate, Ammonium nitrate, Dipotassium phosphate, Magnesium sulphate, Starch and D.W.

Liquid State Fermentation

After cooling, inoculum (1ml) was added to each flask in the LAF with the help of sterilized pipette. The flasks were then incubated at 30◦C for 48 hr without shaking in incubator. The fermentative media flasks were gently shaken after every 12 hr for uniform mixing of the substrate and microorganism.

Crude enzyme extraction

Fermented carrier was taken, after 48hr of incubation, eluted with 20 ml 0.02 M phosphate buffer, pH 7.0. It was shaken properly at 175 rpm for 10 min. The filtrate was centrifuged at 9000 rpm for 10 min. at -4◦C. The culture supernatant was used as a crude enzyme extract.

Protein Estimation

Amount of protein present in different media is estimated by the Bradford method. Absorbance was measured at 590nm.

Enzyme Assay

Enzyme concentration is estimated by the DNS method. Absorbance was measured at 540 nm. Standard curve was prepared by plotting absorbance on y-axis and maltose concentration on x-axis. The absorbance of the reaction mixture was determined at 540 nm against maltose as standard. The amylase activity was determined in IU/ml/min.

Effects of different parameters on amylase production

Effect of pH of the medium

pH of 10g (optimum) chopped banana peel was adjusted at different levels viz., 5,6,7,8,9 before inoculation and incubation for 24 hr. α-amylase exhibits max. activity at its definite pH. The pH at which the enzyme exhibits maximum activity is called its optimum pH. The pH was adjusted using the phosphate buffer.

Effect of incubation temperature

LSF media of banana peel (10gm) were inoculated (1 mL) and incubated at pH 7 under different conditions of temperature as 20◦C, 25◦C, 30◦C, 35◦C and 40◦C for 24 hours. α-amylase exhibits maximum activity at its optimum temperature.

Effect of substrate concentration

Conical flasks containing different substrate levels (10 g) were inoculated (1 mL) and incubated for 24 hours at pH 7 and 30◦c. α-amylase catalyses the hydrolysis of 1-4 glycosidic linkages and producing reducing sugars. Reducing sugars like maltose is then coupled with DNSA in alkaline medium .It produces an orange coloured complex. The intensity of the colour produced can be measured at 540nm which is directly proportional to the activity of the enzyme.

Comparative study of amylase production from soil isolates and standard strain

Protein content and enzyme activity are measured from both standard strain and soil isolates from different sites. Specific activity are also calculated.

RESULTS & DISCUSSION

Identification of microorganism

Serial dilution

This shows the isolated colonies of the specific bacteria in the petri plate.

Fig.1 Isolated colonies of microbe

Indole test

A positive result is indicated by a pink/red layer forming on top of the liquid. This shows that the strain present is the Gram positive bacteria.

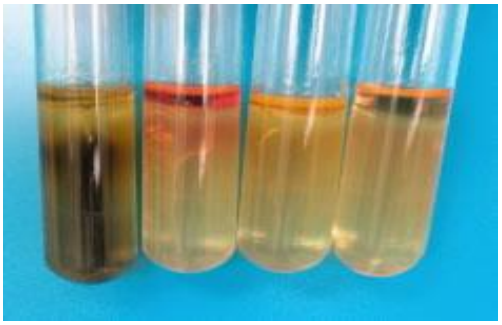


Fig.2 Indole test

Methyl red and Voges–Proskauer test

The pH indicator Methyl Red is added to one tube and a red color appears at pH's lower than 4.2, indicating a positive test. The solution remaining yellow indicates a negative test. A pinkish-red color indicates a positive test for the VP test.

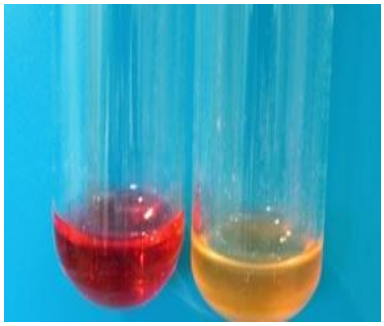


Fig.3 MR and VP test

Citrate test

The citrate agar is green before inoculation, and turns blue as a positive test indicator, meaning citrate is utilized.

Fig.4 Citrate test

Gram's staining

Pink culture shows that the strain is Gram +ve.

Catalase reactions

Bubble formation shows the presence of Bacillus strain

Fig.5 Catalase reaction

Preparation of substrate

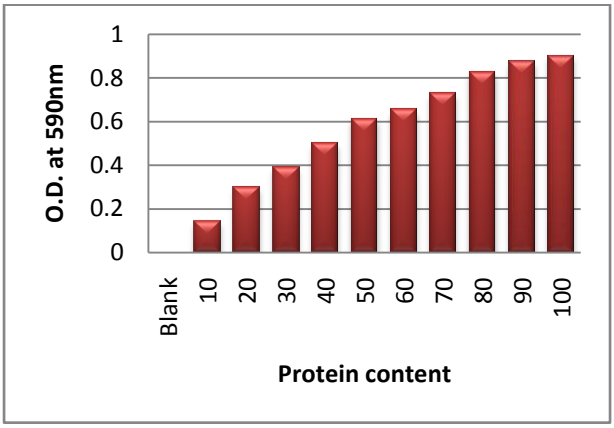
Powder form of banana peel and preserved in a polythene bag for further use.

Fig.6 Powdered Banana peel extract

Protein Estimation

Protein content present in sample is identified by using appropriate method.

Standard curve for Bradford method

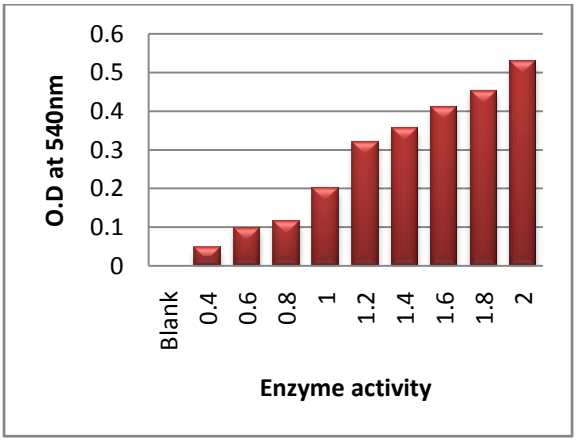


Graph 1 Protein estimation of BSA at 590nm

Enzyme Assay

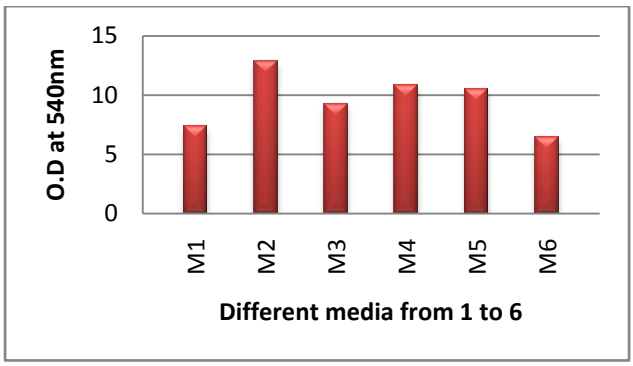
Enzyme activity checked by maltose standard using DNS method.

Maltose standard curve

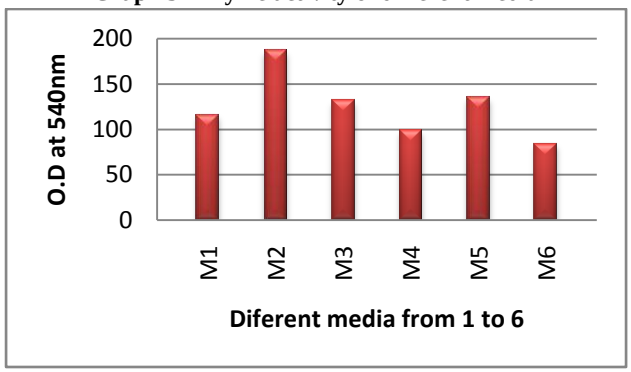


Graph 2 Enzyme activity of maltose standard at 540nm.

5. Studies of different media



Graph 3 Enzyme activity of different media

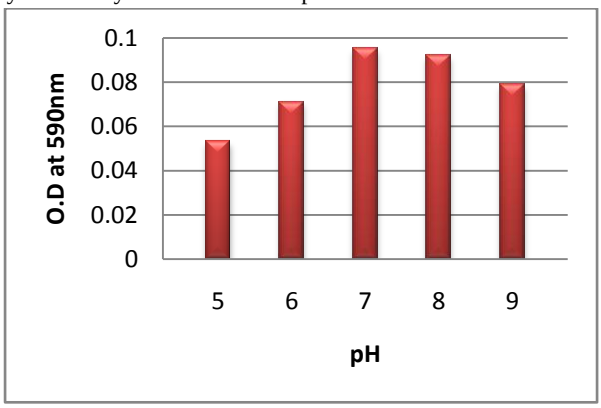


Graph 4 Specific activity of different media

Effects of different parameters on amylase production

Effect of pH

The pH of medium influences the production of amylase. The enzyme activity was maximum at pH 7.



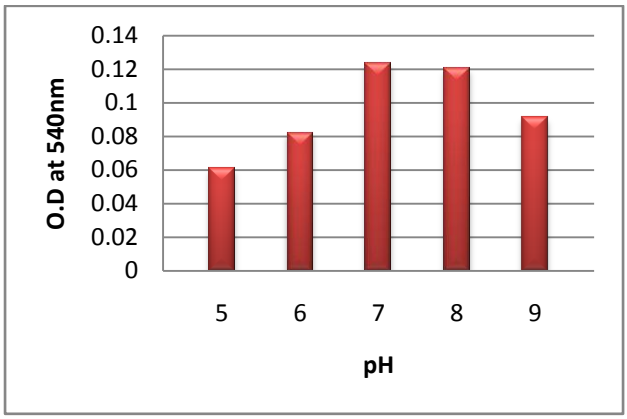
Graph 5 Protein estimation at different pH

Effect of incubation temperature

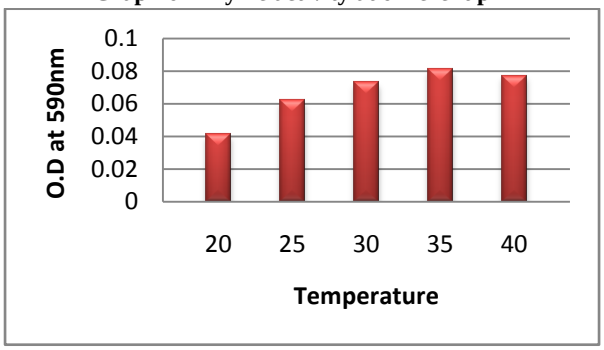
Crude enzyme was assayed at different temperatures and activity of enzyme was increased by an initial increase in temperature which was maximum at 35°C. We noted maximum activity of α-amylase produced by Bacillus subtilis at 20 to 40°C. In our results amylase showed maximum activity at 35°C.

Effect of substrate concentration

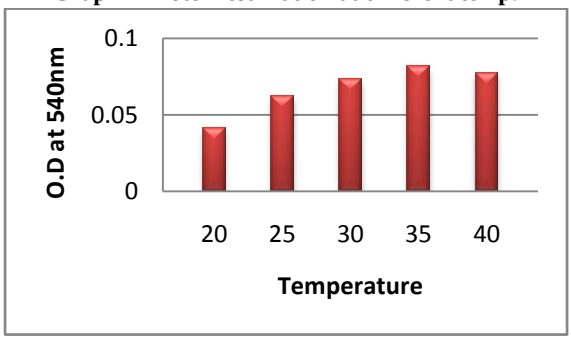
Fermentation media containing 5, 10, 15, and 20 g banana peel were sterilized, inoculated and incubated for 24 h at pH 7 and 35°C. The α-amylase activity in different substrates.



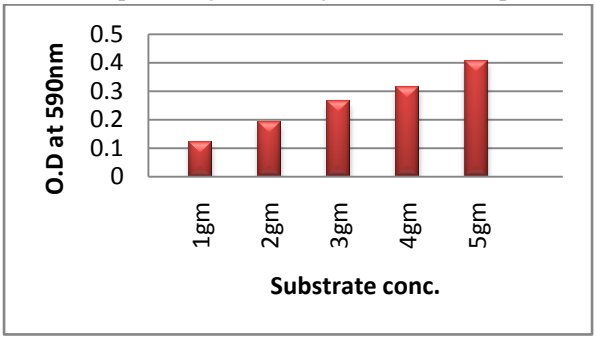
Graph 6 Enzyme activity at different pH



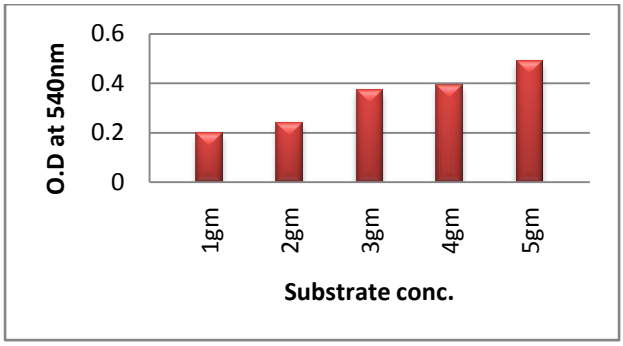
Graph 7 Protein estimation at different temp.



Graph 8 Enzyme activity at different temp.

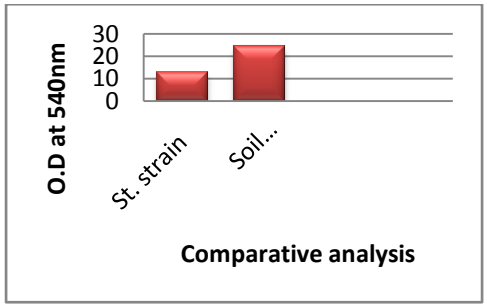


Graph 9: Protein estimation at different conc. of substrate

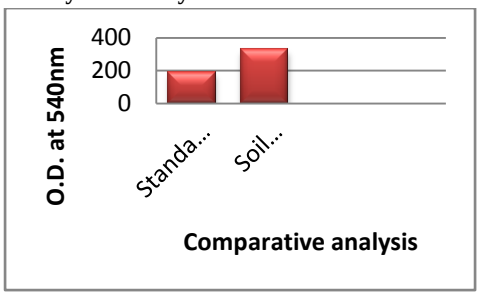


Graph 10 Enzyme activity at different conc. of substrate

Comparative study of amylase production from soil isolates and standard strain



Graph 11 Enzyme activity of isolated strain & standard strain



Graph 12 Specific activity of isolated strain & standard strain

α-amylase production using the Bacillus subtilis is well known and widely used [5]. Presently, α-amylase production by Bacillus subtilis is economically produced using LSF. Isolated strain is identified by doing IMViC test. The important parameters are substrate conc., pH and incubation temperature [6]. In order to achieve maximum α-amylase production these parameters need to be optimized. The present study revealed that the maximum α-amylase activity was observed at 50 g of banana peel in the medium. These results support the suitability of using banana waste as liqud substrate for high production of α-amylase. The study showed the maximum α-amylase activity at pH 7. It has been suggested that the metabolic activity of bacteria is very sensitive to pH level of media. These results indicate the independent nature of the temperature effect irrespective of the type of substrate used [7]. After this we had study different media. Comparative analysis had been done for soil strain and standard strain.

CONCLUSION

The present study showed that the production of α-amylase enzyme by LSF by utilization of banana waste employing Bacillus subtilis as the fermenting organism. A considerable interest can be given in using banana waste as an alternative source of carbon for α-amylase production by Bacillus subtilis which is economic and profitable due to the inherent nature of the banana waste itself. The study showed that the protein content and enzyme activity are high in different samples. Amylase by Bacillus sp. using maltose as carbon source and of different samples of soil and standard strain was recorded higher at 35◦C of pH 7. The isolated culture of soil sample showed high activity of enzyme as compared to standard strain. The enzyme was used in the starch processing food, detergent, textile, paper, clinical and analytical industry.

ACKNOWLEDGEMENT

We are thankful to ITS, Paramedical College, Muradnagar, Ghaziabad and Jaypee Institute of Information Technology University, Noida, India for providing the infrastructure facilities for this study.

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