

Research Article



Screening of Potential α -Amylase Producing *Bacillus* VIT-SS2 from Vellore Rice Mill Using Culinary Vegetables

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ABSTRACT

This study was performed to screen for an α -amylase producing isolate from soil. The enriched soil samples were collected from various rice mills surrounding places around the town of Vellore. Microorganisms were isolated using baiting plate method using various fruits and vegetable pieces like carrot, tomato, potato, pumpkin and cabbage. After 24 hour incubation, the plates were observed and three colonies were isolated and sub-cultured by streaking. The amylase activity was checked by spread plate method using 1% starch in LB agar. The colony that was showing high amylase activity was then subjected to Gram staining and spore staining. The microbial growth was tested at various temperatures and pH to specify its growth limitation. The growth curve of this microorganism was plotted as a function of OD at various time intervals, checked by a colorimeter at 600nm. Finally, the biochemical testing was done for further characterization of the amylase producing microorganism.

Keywords: α -amylase, Baiting-plate, In-reached soil, Starch, Zone formation.

INTRODUCTION

Starch is a polysaccharide which is a common source of energy produced in plants. It is formed by the polymerization of glucose monomers linked together by glycosidic bonds. Starch is composed of a small fraction of linear-amylose and a large fraction of highly branched amylopectin. Starch is classified into three types based on the concentrations of amylose and amylopectin, namely high amylose, normal and waxy starch.¹ Starch when hydrolyzed, has wide applications in the fields of pharmaceutical, beverage, detergent and textile industries.

The chemical hydrolysis of starch has various disadvantages like it requires corrosion-resistant vessels, high temperature, gives low glucose yields, requires low pH and results in the formation of bitter tasting and unwanted coloured compounds. Since the hydrolyzed starch has wide application and chemical hydrolysis method has so many disadvantages, newer methods of starch hydrolysis need to be explored. Hence, the enzymatic method of starch hydrolysis is in high demand.²

Amylases function to hydrolyze glycosidic linkages and they belongs to three families (GHs)³: GH 13 (α -amylases)^{4,5}, GH 14 (β -amylases)⁶ and GH 15 (glucoamylases).⁷ This division is based on different catalytic activity, structural difference, amino-acid sequence and reaction mechanism.

Alpha amylase is unable to function without the presence of calcium. It acts on starch to yield glucose and "limit dextrin" from amylopectin, maltotriose and maltose from amylose. The rate of hydrolyzing activity of α -amylase is higher than β amylase.

Alpha amylase has a wide functional range on pH scale 2-12 but, Alpha amylase mostly functions at pH7.⁸ Extracellular endoenzymes amylases hydrolyses starch to produce smaller polymers of glucose and dextrans. In industrial enzyme market this class of enzymes has 25% occupancy. Amylase are not only used in pharmaceutical as a digestive aid, preparation of digestive juices, cakes, fruit juices, starch syrups but it has a vast application in sugar, textile, paper, brewing, baking and in distilling industries.

Every living being animal, plant or microorganisms needs α -amylase. Microorganisms can easily multiply and can produce enzyme in bulk. Microorganisms are very economical and easy to produce.

Today in the era of biotechnology, world economy needs to shift towards more sustainable and eco friendly reagents. Enzymes are most common reagent catalysts which provide required results without compromising quality and purity of the product. α -amylase is one of the most, preparation of digestive juices, cakes, fruit juices, starch syrups important aspect of sugar, textile, paper, brewing, baking and in distilling industries. My research intends to shed light on the production of alpha amylase from bacterial sources.

MATERIALS AND METHODS

Sample collection

The samples were collected from Vellore and Katpadi zone. The temperature was 35°C. The soil samples were collected from various rice mills. The samples were taken from just below the mills where grain wastes generally fall. Five vegetable samples were collected from the nearby market – cabbage, carrot, potato, pumpkin, tomato. The vegetable were cut to 0.1g in small pieces.⁹



Isolation of microorganism

The vegetables were washed 10 times with tap water, and then five times with distilled water. They were cut into small pieces and their surfaces were sterilized in 70% v/v ethanol. They were illuminated under UV for 30 minutes. 4g of Luria Bertini agar and 1.5g of agar powder was prepared in 100ml of distilled water, and then autoclaved at 121°C for 20 minutes. The media was cooled to 45°C and then poured into 6 sterilized plates and then 0.1g soil sample was added to it. The vegetables were baited on the semi-solidified media. The plates were incubated at 38°C for 24 hours, after which the growth was observed. Each type of colony from all the plate was sub-cultured on Luria Bertini agar plates. The plates were kept in incubator at 38°C. After 24 hours, the streaks were observed.

α -Amylase test

The pure colonies were obtained after sub-culturing. This was followed by test for production of α -amylase. Three plates with Luria Bertini agar media were prepared. After the media in the plates were cooled to room temperature, the identified colonies were spread on them. The plates were incubated at 38°C for 48 hours. 0.5ml of iodine was poured on the plates and left for 10 minutes to check of formation of clear zone.

Characterization and staining

The colonies producing amylase were identified and microbes were gram stained. The amylase producing microbe was smeared and heat fixed on a clean glass slide. The slide was flooded with crystal violet for 1 minute, followed by Grams iodine for 1 minute, then decolouriser for 30 seconds and last safranin for 1 minute. It was viewed under 10X, 40X and finally 100X oil immersion lens in a light microscope.

Growth curve

100ml Luria Bertini production media (broth) was prepared in Erlenmeyer flask. The flask containing media was autoclaved at 121°C for 20 minutes. Then it was cooled to 45°C It was inoculated with potential amylase producing microbe identified by α -Amylase test. The inoculation was done with a separate colony, taking the bacteria from one of the corner of the colony. Similarly, a sterilized media was prepared to serve as negative control. The colorimeter, set at 600nm was subjected to take reading for growth curve. The optical density reading was noted a graph was plotted. The microbial growth was observed at different temperature and pH as well.

Biochemical tests

To identify the microbial strain, biochemical testing was done on hi-media kb002 kit. The media was inoculated in 10ml Luria Bertini and after 24 hours of incubation at 38°C, it was inoculated in the biochemical testing kit. The kit contained tests for Citrate utilization, Lysine utilization, Ornithine utilization, Urease detection,

Phenylalanine de-amination, Nitrate reduction, Hydrogen peroxide production, Carbohydrate Utilization Test (with five different sugars - Glucose, Adonitol, Lactose, Arabinose, Sorbitol). After 48 hours, the colour change was observed in wells.

RESULTS AND DISCUSSION

Isolation of microorganism

After 24 hours of incubation the baiting plates were observed in it. The highest number of colonies was observed in plate containing potato as bait. There were twelve colonies in carrot and 8 colonies in cabbage. There was fungal growth in tomato baited plated and no growth in plate containing pumpkin as a bait.



Figure 1: Colonies in baiting plates

There were mainly three types of colonies observed in all the plates. Around potato there was colourless, shiny colony. Another was white branched colony, and the last one was patches like colony at the entire plate.



Figure 2: The sub culturing

All the three types of microorganisms were sub-cultured. The sub culturing of the above mentioned microorganisms brought us the pure strain.

α -Amylase test

The alpha amylase test was done with iodine to identify the clear zone formation. The colony surrounding the potato was identified to produce active amylase which digested the starch around it. Hence, as the iodine was poured on the plates, there were clear zones. The light zones specify the starch degradation which means amylase production by the micro-organism.

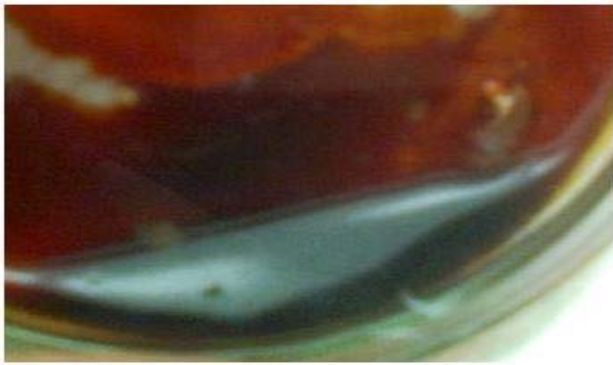


Figure 3: Clear zone formation

Characterization and staining

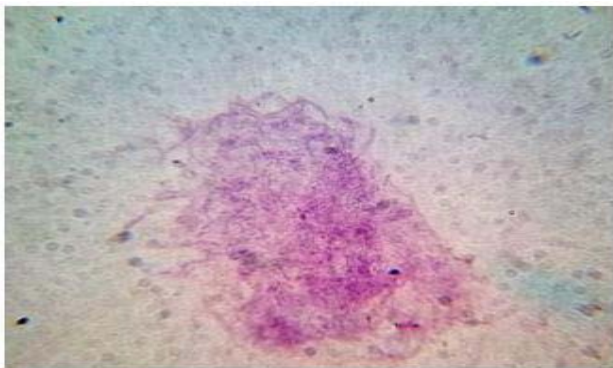
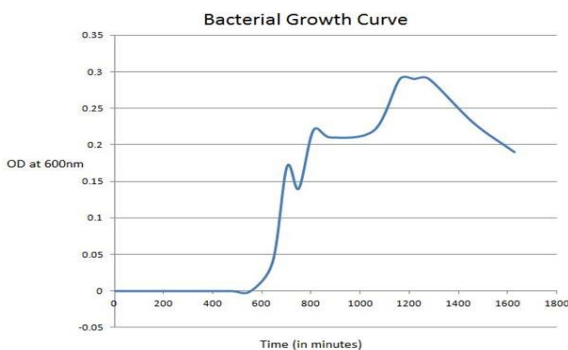


Figure 4: Gram positive

The stained slide was observed under light microscope. The bacteria were identified and they were violet which means the microbes were gram positive. The result gram positive specifies many characteristics of microorganism. It specifies it has cytoplasmic lipid membrane, thick peptidoglycan layer, lipoteichoic acids (teichoic acids and lipids are present) which is a chelating agent and serves certain type of adherence and the rigid cell wall formed by peptidoglycan.⁹

Growth curve

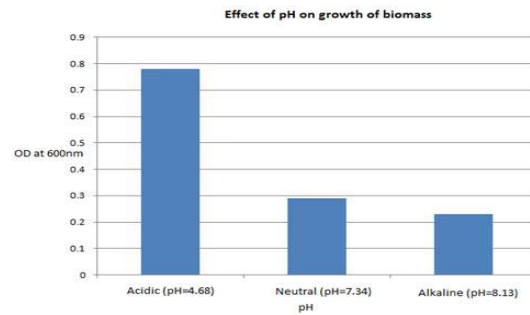


Graph 1: The Standard growth curve

Biochemical testing

Citrate utilization(-), Lysine utilization(-), Ornithine utilization(+), Urease detection(+), Phenylalanine deamination(-), Nitrate reduction(-), Hydrogen peroxide production(-), Carbohydrate Utilization Test (with five

different sugars – Glucose(+), Adonitol(+), Lactose(-), Arabinose(+), Sorbitol(+).



Graph 2: Different pH effect at 38°C

The maximum growth was obtained at 38°C, at pH 4.68, followed by neutral at pH 7.34 and least microbial growth at alkaline pH 8.13.

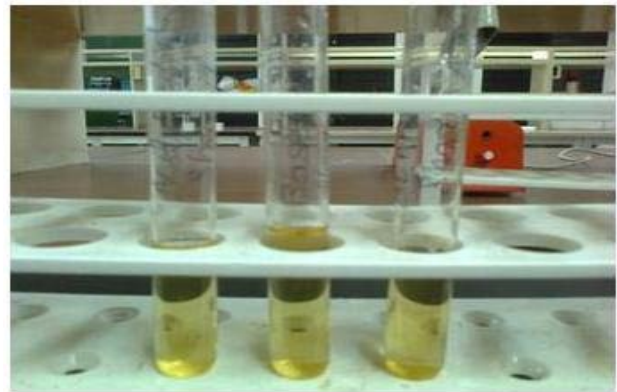


Figure 5: No growth in media at 0°C and at 45°C

At 0°C at there was no growth at any of the pH (acidic, neutral and alkaline). Furthermore, even at 45°C, there were no growth in acidic and neutral test-tubes containing Luria Bertini media broth, but the test-tube containing alkaline Luria Bertini media broth showed a little growth of 0.1 OD at 600nm.



Figure 6: Biochemical test result

CONCLUSION

In the starch processing industry alpha amylase is the most employed enzyme to produce starch hydrolysate. Potential alpha-amylase producing bacteria were screened from Vellore culinary vegetables and further we

are proceeding for sequencing. The best environmental condition concluded for the production of alpha amylase is at pH 4.68 and temperature 38°C.

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