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Screening and isolation of amylase producing *bacillus species*

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Abstract
Amylases are the starch degrading enzymes which hydrolyses the α-1,4 glycosidic linkages of the starch .Because of the numerous applications of amylases the present study was aimed at screening of the amylase producing organisms from soil samples collected from different sources. Among the different isolates obtained one of them which has a larger zone of hydrolysis was selected. The isolates were identified as *Bacillus species* by gram's staining method and further subjected for estimation of their amylase activity by performing an assay in the fermentation medium. The maximum enzyme activity was observed at an optimum temperature of 37^0C, neutral pH of 7 for an incubation period of 24hours.
Amylase is present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Foods that contain large amounts of starch are chewed because of amylase which degrades the starch into sugars to supply the body with energy. As it is a major digestive enzyme in the animals and easily produced by microorganisms the present study was aimed to screen and isolate the amylase activity by *Bacillus* species.

Keywords: amylase, *bacillus species*, enzyme and starch

Introduction

Starch is a long polymer of glucoses which are linked by glycosidic bonds. There are a class of enzymes which can degrade the starch molecules into a soluble glucose and maltose molecules. Those enzymes are called as amylases. Based on the degradation of starch there are three types of amylases α- amylase, β- amylase and γ -amylase. α - amylase reduces the viscosity of starch by breaking down the bonds and produces glucose molecules, it is the faster acting enzyme than β -amylase.It is the major digestive enzyme in animals, and its optimal pH is 6.7 to 7.0. B-amylase catalyses the hydrolysis of the second α -1, 4 glycosidic bond, cleaving off two glucose units (maltose) at a time. Its optimum pH is 4.0 to 5.0.α- amylase cleaves α (1-6) glycosidic linkages as well as the last α (1-6) glycosidic linkages and it is most active at acidic pH 3 .Plants, animals and microbes are the sources of the amylases. Bacteria and fungi secrete amylases to the outside of their cells to carry out extracellular digestion i.e. they carry absorptive mode of nutrition. Amylases are widely used in industries especially in food industries they are used for manufacturing fructose and glucose syrups, beverages, fruit juices, bread making etc. In detergent industry they are used as additives for removing tough stains. Amylases are produced by many microorganisms but for industrial production *Bacillus species* are most commonly used. Genetic engineering has been used for cloning of amylase producing strains in order to achieve desirable characteristics in the cloned host. Amylases are also used in fermentation, pharmaceuticals, saccharification, textiles, paper and distilling industries. However, with the advances of biotechnology, the application of amylases has increased in many fields such as clinical, medicinal and analytical chemistry. In molecular biology, amylase serve as an additional method of selecting for successful integration of a reporter construct in addition to antibiotic resistance. Amylase also has medical applications in the use of Pancreatic Enzyme Replacement Therapy (PERT). It is one of components into simple sugars.
The main objective of the paper deals with screening and isolation of amylase activity by Bacillus species.

Materials and methods

Sample Collection

Amylase producing organisms can be isolated from many samples. But soil is the main source of microorganisms. Soil sample of two different sources were collected. One from garden source and another from the soil receiving the kitchen waste. Soil from these sources are serial diluted up to 10^{-10} .0.1ml of dilutions from 10^{-4} to 10^{-8} was plated on starch agar medium and incubated at 37C for 24hours. The isolates were then sub cultured on the starch agar medium.

Screening for Amylase Producers

All the isolates were tested for their amylolytic activity using starch agar medium containing starch (20g), peptone(5g), beef extract (3g), agar agar (20g) and distilled water (1000g). The bacterial isolates were sub cultured on the starch agar plates.

After incubation of 24h the plates were flooded with iodine solution for 1min until the entire medium became colored in blue. Formation of clear zones around colonies in blue colored medium indicates the hydrolysis of starch. Thus the amylase producing organisms were screened. Among 8 isolates selected the zone of hydrolysis observed as 1.5cm, 2cm, 1.3cm, 1.5cm, 1.5cm, 1.3cm, 1.3cm and 1.5cm.

Quantitative Estimation of glucose by DNS method

3, 5 dinitrosalicylate (DNS) reagent react with reducing sugars in alkaline condition to give orange red color having maximum absorption at 520nm. The concentration of sugars can be determined by preparing a standard graph using a reagent blank. Dispense the standard glucose solution in aliquots from 0.1 to 1 ml in clean labeled tubes. Make up the volume to 2 ml with distilled water and add 2 ml of DNS reagent and mix well. A blank is taken without glucose but only with DNS reagent and 2 ml water. Heat the tubes in boiling water for 10 min and cool. Make up the volume to 10ml with distilled water in each tube. Read the O.D in a colorimeter at 520nm against a blank. Plot a graph by taking concentration of glucose on x-axis and O.D on y- axis.

Assay for Amylase Activity

The amylase assay was based on the reduction in blue color intensity resulting from enzyme hydrolysis of starch. The reaction was conducted in the fermentative media consists of KH_2PO_4 (0.5g), $(NH_4)_2NO_2$ (0.5g), $MgSo_4$ (0.25g), $CuSo_4$ (0.06g), $ZnSo_4$ (0.25g), sucrose (10 %), $FeSo_4$ (1.3g) and distilled water (1000ml) at pH-5. Enzyme activity can be estimated by inoculating the loop full of culture into the fermentative media and incubate for 24h. Now the 1ml of overnight culture is taken in a sterile eppendorf tubes and centrifuged at 8000rpm for 15min. The supernatant was collected for further analysis. 0.1ml of supernatant was added to 0.9ml of 0.1M phosphate buffer of pH 7.5. To this 1ml of 1% starch and incubated at 37^0c for 30min (this step was excluded for the control). To this 1ml of DNS was added and heated in boiling water bath for 15min. 7ml of water was added to this test solution to make up the volume for 10ml. This test was again diluted to 10 fold and absorbance was taken at 540nm.

Result

Of the two soil samples collected from different ecological environments were analysed producing amylase activity. The amylase producing bacteria was identified as *Bacillus species* by performing grams staining. Different types of microorganisms were obtained when observed under microscope (100X) like *Staphylococcus* .But *Bacillus species* was identified by observing its morphology (gram positive rods).

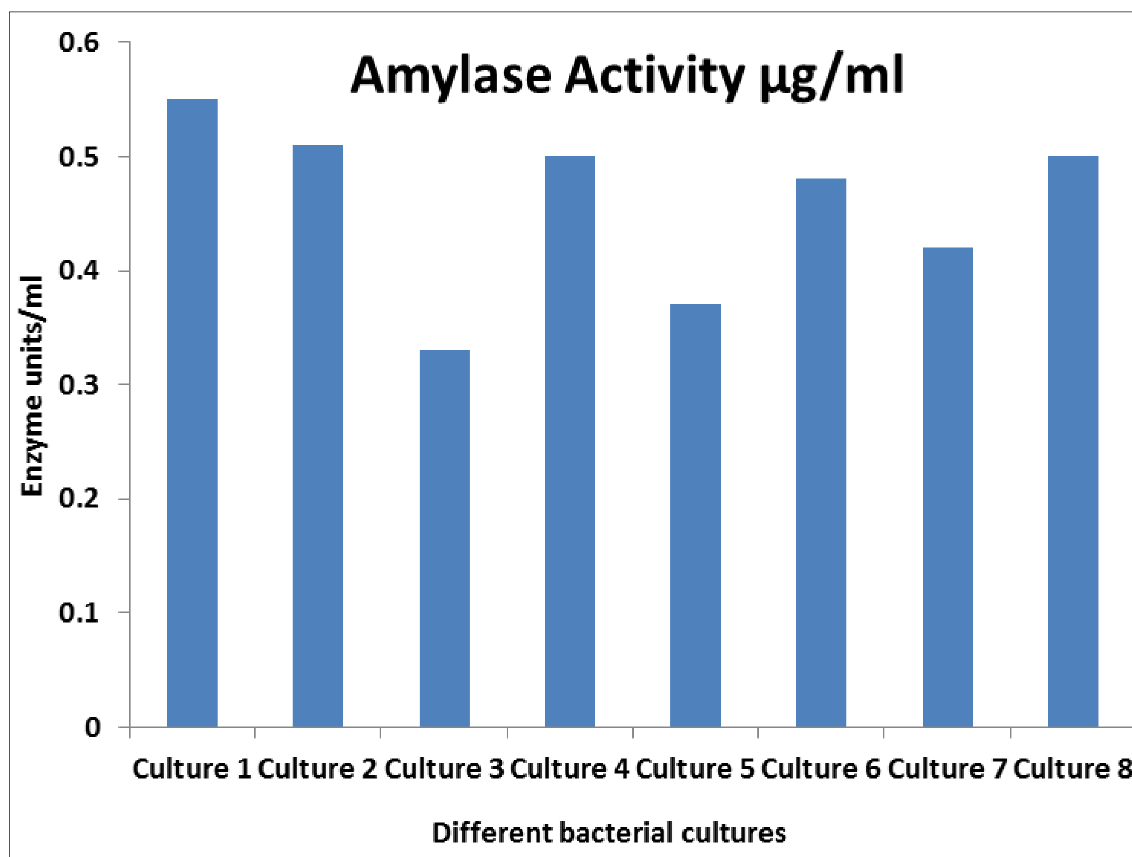

Fig 1

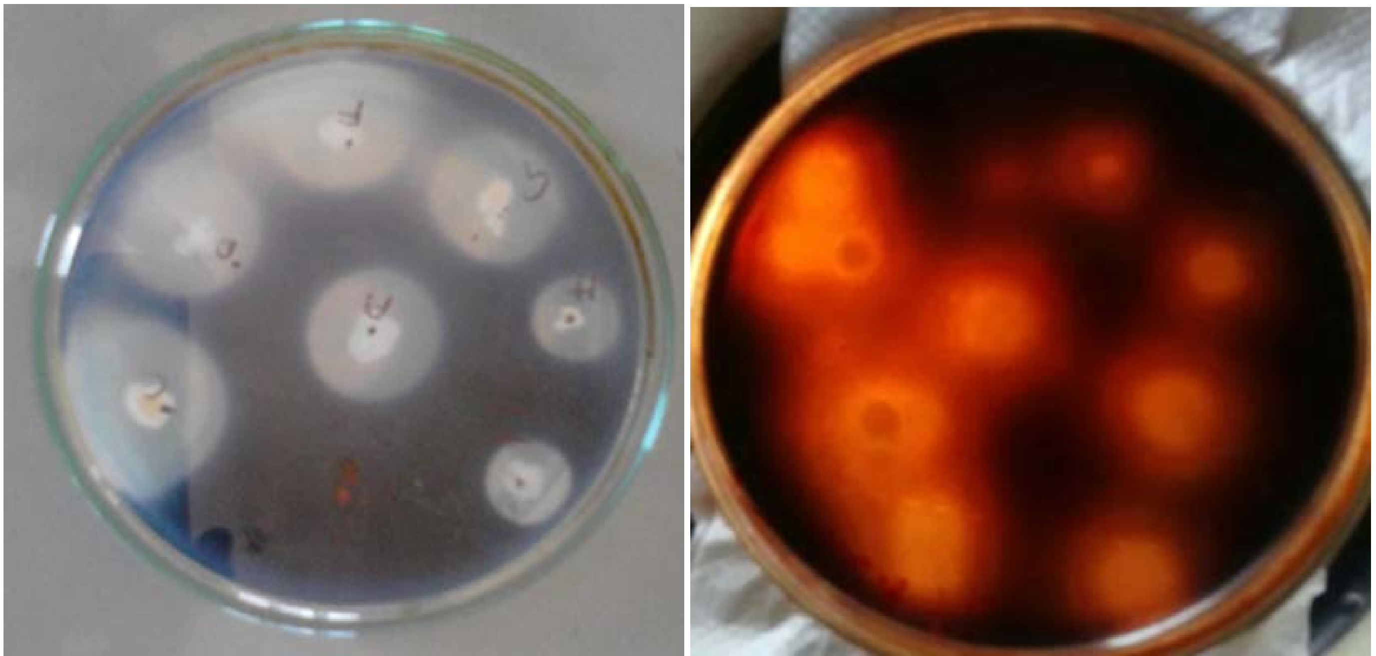

Fig 2: Starch Hydrolysis

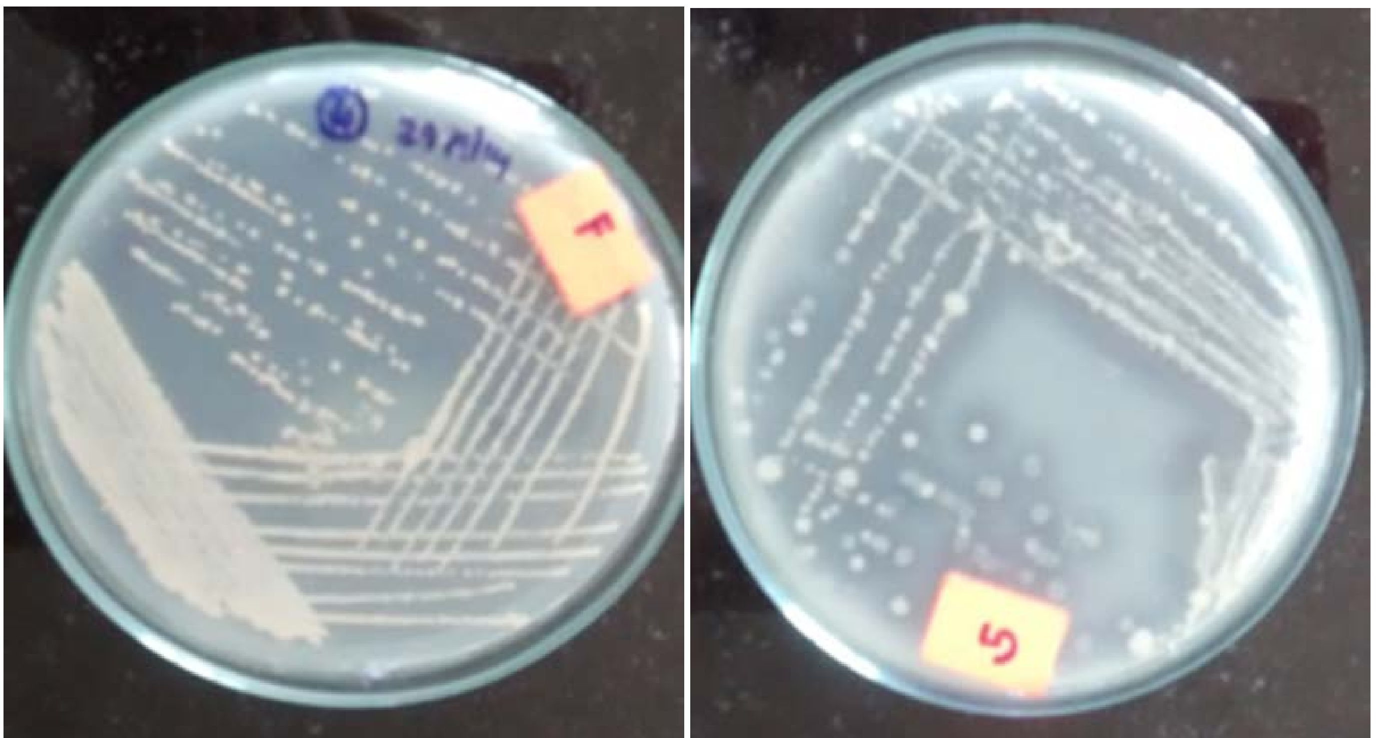

Fig 3: Subculturing of Amylase Producing *Bacillus Species*

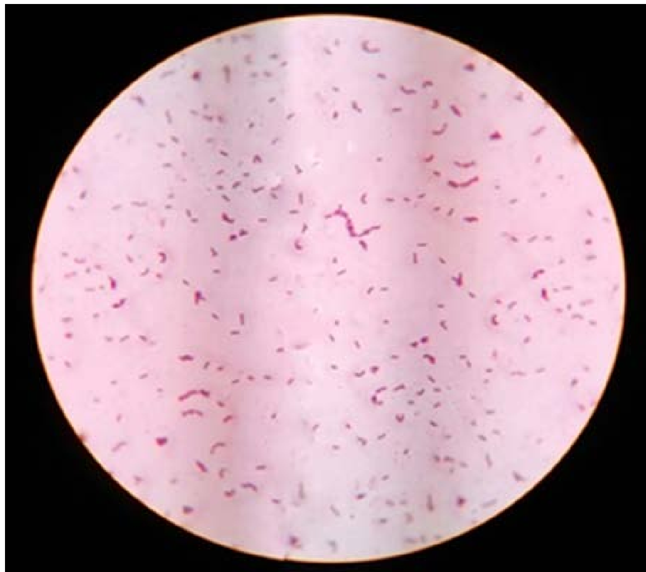

Fig 4: Amylase Producing Rod Shaped *Bacillus species*

Conclusion

These are a class of enzymes that are capable of digesting the glycosidic linkages found in starch or glycogen. The results obtained in the present study indicated *Bacillus species* isolated from different soil sources have amylase activity.

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