

## Isolation and Identification of Amylase Producing Bacterium from Domestic Wastewater of Buldana District (M.S.)



### Biology

**KEYWORDS :** Amylase, Wastewater, Bacillus sp., and Paenibacillus sp.

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### ABSTRACT

*Amylase is the most important industrial enzymes and having great significance in Biotechnological studies. Amylase production from bacteria is economical because the enzyme production rate is higher in bacteria as compared to other microorganism. The aim of this study was to isolate amylase producing bacteria from domestic wastewater. In this study cultural, morphological and biochemical characteristics of the bacterial isolates were studied. Total 40 bacterial cultures were isolated from collected domestic wastewater samples on nutrient agar medium. All isolates were screened for amyolytic activity by starch agar plate method. Among 40 bacterial isolates, only 8 isolates showed the best amyolytic activity. These isolates related to the genera Bacillus sp. and Paenibacillus sp.*

### Introduction:

Starch is the reserve energy store of plants and is one of the most abundant biopolymers on earth. It is a polymer of alpha glucose units connected with alpha-1, 4 and alpha -1, 6 glycosidic linkages. Starch consists of amylose which is a linear polymer of glucose and amylopectin, which is a branch chain polymer. Due to its complex structure, hydrolysis of starch requires a combination of hydrolytic enzymes to release glucose units and small oligosaccharides<sup>1</sup>.

Amylase can be simply classified in two groups. 1) Endo- acting or endo-hydrolases. E.g.  $\alpha$ -amylase: -  $\alpha$ -Amylases are extracellular enzymes which hydrolyze  $\alpha$ -1, 4-glycosidic bonds. These enzymes are endoenzymes which splitting the substrate in the interiors of the molecule. 2) Exo-amylase or exo-hydrolases e.g.,  $\beta$ - amylases, glucosidase and  $\alpha$ -glucosidase. Glucoamylases act on starch by splitting glucose units from the non reducing end<sup>2</sup>.

Microbial production of amylase is more beneficial than other sources because it is economical as well as production rate is high and can be engineered to obtain enzymes of desired characteristics. The microbial amylases could be potentially useful in various pharmaceutical, fine-chemical industries, paper industries etc. With the emergence of biotechnology, the use of amylase has widened in clinical research, medical chemistry and starch analytical chemistry. These increased uses have placed greater stress on increasing indigenous amylase production and search for more efficient processes. The major advantages of using microorganisms for production of amylases are the ability to produce in bulk and ease at which it can be manipulated for desired products<sup>3</sup>.

There are various reports on starch degrading microorganisms from different sources<sup>4</sup>. Domestic wastewater is one of the rich sources of starch degrading microorganism as it contains mostly starchy substrate. Present study is focused on the isolation and identification of amylase producing bacteria from domestic wastewater samples in Buldana District, India.

### Materials and Method:

#### Collection of samples:

Domestic wastewater samples were collected from different places in Buldana district, India. Samples were transferred to sterile bottle and immediately transported in laboratory of Shri Shivaji Science College Chikhli, District Buldana.

#### Isolation of bacterial cultures

Isolation of bacteria was performed by serial dilution and spread plate method, on Nutrient agar plates. Well isolated colonies were further transferred on nutrient agar plates and slants for morphological, cultural and biochemical characteristics. The bacterial isolates were further sub cultured on to slants and kept at 4<sup>o</sup> C for preservation.

### Screening of amylase producing bacteria by starch hydrolysis test

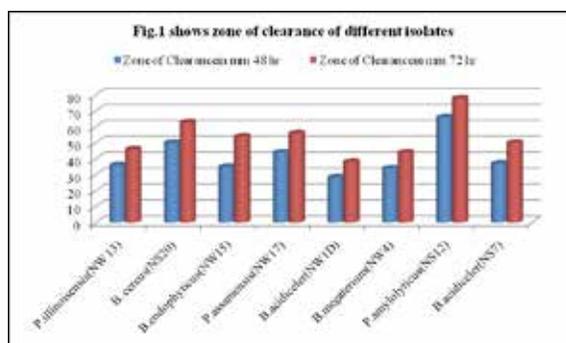
Bacterial isolates were screened for amyolytic activity by starch hydrolysis test on starch nutrient agar plate. The microbial isolates were streaked on the starch agar plate and incubated at 37°C for 48 and 72 hours. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate<sup>5</sup>. Presence of blue color around the growth indicates negative result and a clear zone of hydrolysis around the growth indicates positive result. The isolates produced clear zones of hydrolysis were considered as amylase producers and were further used for identification<sup>6,7</sup>.

### Identification of amylase producing bacteria

Bacterial strains were examined for their colony and cell morphology, motility, Gram and spore staining. Biochemical tests like catalase, Oxidase and fermentation of Dextrose, Lactose, Mannitol, Arabinose and Trehalose, Nitration reduction, H<sub>2</sub>S, Methyl Red reaction, Citrate utilization by Simmons, Indol test and VP test were performed. The isolated bacteria are characterized and identified according to Bergey's manual of determinative bacteriology<sup>8</sup>.

### Results and Discussion:

The total 40 bacterial isolates were isolated on nutrient agar media on the basis of morphological characters. All 40 isolates were streaked on starch agar medium for screening. Out of 40 isolates, only 15 isolates showed amylase production on starch agar medium. From this 15 isolates, only 8 bacterial isolates were selected which has zone of clearance above the 25 mm, at 48 hr and were used for further study. In this paper, the enzyme production was studied on the basis of zone of clearance around the colony at 48 hr and 72 hr of 8 bacterial isolates. The following graph shows zone of clearance of eight isolates at 37°C for 48 hr and 72 hr.



From the above graph it was observed that all the studied bacterial isolates show higher amylase activity at 72 hr. as compared to 48 hr. It was observed that *B. cereus* (NS20) and *Paenibacillus*

*amylolyticus* (NS12) have more amylase activity at both 48 and 72 hr. as compared with other isolates. The isolated bacteria are characterized and identified, it was found that the isolates were belong to genera *Paenibacillus sp.*, and *Bacillus sp.*, Similar results were observed by Parmar and Pandya (2012) while studying characterization of Amylase Producing Bacterial Isolates and also by Malle *et al* (2012) while studying isolation and identification of thermo stable amylase producing bacteria from Hatuasa Hotspring.

#### Conclusion:

In the present study, the bacteria was isolated from domestic waste water and identified by ABIS software system. This waste is also consisting of starchy materials and we found bacteria isolated from such places may have better potential to produce enzyme under adverse condition. On the other hand, conversion of organic waste through microbial processes decreases the amount of waste material. It leads to the decrease in water pollution.

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