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# Characterization of Amylase Producing Bacterial Isolates

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**ABSTRACT**
*Amylases are among the most important industrial enzymes and also have great significance in Biotechnological studies. In this study cultural, morphological, and metabolic characteristics of the bacterial isolates were studied. Total 18 bacterial cultures were isolated from collected soil samples. Among 18 bacterial isolates, 14 isolates showed the amylolytic activity. These 18 isolate was identified according to Bergey's manual of systemic Bacteriology .These isolates related to Bacillus sp. The optimum pH for the growth of all the cultures was observed at pH 7. Submerged fermentation was carried out for the production of amylase was observed in the range of 0.045-1.35 U/min/mL. The maximum activity of amylase was 1.35 (U/min/mL) after 48 hours was recorded, have great significance.*
***KEY WORDS:*** *Characterization, Amylase activity, Bacillus sp*

## INTRODUCTION

Amylases are starch degrading enzymes. They are widely distributed in microbial, plants and animals kingdoms. They degrade starch and related polymers to yield products characteristics of individual amylolytic enzymes [1]. Amylase can be simply classified in two groups [2]. **1) Endo–acting or endo–hydrolases.** E.g. α-amylase :- α-Amylases (1, 4- α-glucan- glucanohydrolases) are extracellular enzymes which hydrolyze α-1, 4-glycosidic bonds. These enzymes are endoenzymes, splitting the substrate in the interiors of the molecule.**2) Exo-amylase or exo-hyrolases** e.g., β-amylases, glucosidase and α-glucosidase. Glucoamylases (α-1, 4-glucan- glucohydrolases) act on starch by splitting glucose units from the non reducing end. β –glucosidase is usually of plant origin, but some microbial produces are also known to produce it and have greater heat resistance (>70°C) than plant β-glucosidase and the pH optimum is also higher (about pH 7.0). Many Microorganism used in α-Amylases and β-amylases production including *Bacillus subtilis, B. cereus, B. polmyxa, B. amyloliquefaciens, B. coagulans, B. subtilis, Lactobacillus, Escherichia, Proteus, B. lincheniformis, Bacillus steriothermophilu, Bacillus megaterium, Strepotmyces sp., ., Pseudomonas sp. etc..*

Amylases from plant and microbial sources have been employed as food additives. Barely amylases have been used in the brewing industry. Fungal amylases are widely used for the preparation of oriental foods, in spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production. Among bacteria, *Bacillus sp*. is widely used for thermostable α-amylase production to meet industry. Filamentous filling have been used for the production of amylases for centuries [3-9].

## MATERIALS AND METHOD

The given 18 cultures were isolated from soil of Gujarat vidyapith, sadra. To study the cultural characteristics of the bacterial isolate, the pure cultures of isolates were streaked on the sterile Nutrient agar plate. These plates were incubated in incubator (Nova make) at 37℃ for 24 h. After incubation time, colony characteristics i.e., size, shape, margin, elevation, pigments production, consistency and texture were noted. Gram staining was performed to investigate morphological properties; microscopy was carried out by compound light microscope (LABOVISION).Further confirmation of gram's reaction was carried out by 3 % KOH test and Vancomycin test. Spore staining was performed by Dorner's method. To observe the influence of pH on the growth of the organisms, organisms were allowed to grow in the nutrient broth medium having different pH (3 to 10) for the production of amylase. The amylase activity was determined the following method of an assay mixture containing, enzyme extract, starch as substrate and DNS as coupling reagent was used.

One unit of amylase activity was defined as the number of µ moles of maltose liberated by 1 mL of enzymes solution per minute. All processes were done by standard method [4,5].

## RESULTS & DISCUSSION

### Cultural and Morphological characteristics of the isolates

All 18 cultures were streaked on nutrient agar plate and after 48 h incubation time cultural characteristics were studied. As shown in all plates. Culture B, G, P, I, and Z showed small colonies, low convex elevation and semi transparent opacity with no pigmentation. All other isolates had shown big colonies with irregular and uneven margin as well raised elevation with moist consistency. No pigment production was observed in any of isolates but all the colonies were opaque. Detail results are noted in **Table No: 1.**

**Table No: 1 culture characteristics of the isolates**

| Culture No. | Characteristics | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Size** | **Shape** | **Margin** | **Elevation** | **Consistency** | **Opacity** | **Pigmentation** |
| **B** | Small | round | uneven | Low convex | Moist | Semi transparent | Nil |
| **C** | Big | round | uneven | Raised | Moist | Opaque | Nil |
| **D** | Big | Irregular | uneven | Raised | Moist | Opaque | Nil |
| **E** | Big | Irregular | uneven | raised | Moist | Opaque | Nil |
| **F** | Big | Irregular | uneven | raised | Moist | Opaque | Nil |
| **G** | Small | Round | uneven | Low convex | Moist | Semi transparent | Nil |
| **I** | Small | Round | uneven | Low convex | Moist | Semi transparent | Nil |
| **K** | Big | Round | uneven | raised | Moist | Opaque | Nil |
| **L** | Big | Round | uneven | raised | Moist | Opaque | Nil |
| **M** | Big | Round | uneven | raised | Moist | Opaque | Nil |
| **P** | Small | Round | uneven | Low convex | Moist | Semi transparent | Nil |
| **Q** | Big | Irregular | uneven | raised | moist | opaque | Nil |
| **R** | Big | Irregular | uneven | raised | Moist | Opaque | Nil |
| **V** | Big | Round | uneven | raised | Moist | Opaque | Nil |
| **W** | Big | Irregular | uneven | flat | Moist | Opaque | Nil |
| **X** | Big | Irregular | uneven | raised | Moist | Opaque | Nil |
| **Y** | Big | irregular | uneven | raised | Moist | Opaque | Nil |
| **Z** | small | round | uneven | Low convex | Moist | Semi transparent | Nil |

## Table:2 Morphological characteristics of the isolates:

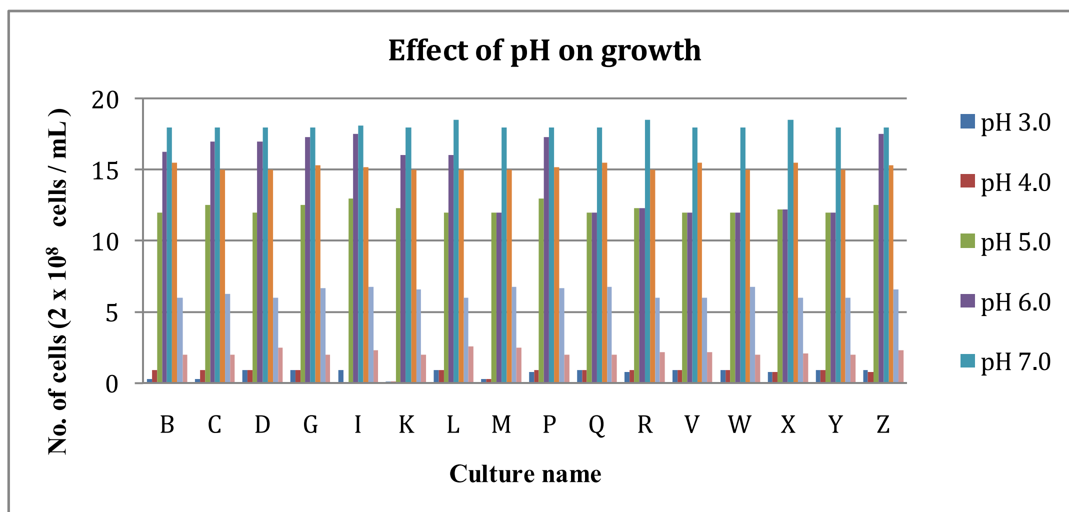
| Culture No. | Gram staining | | | | KOH test | | Vancomycin test | | | spore staining |
|---|---|---|---|---|---|---|---|---|---|---|
| | Size | Shape | Arrangement | Gram 's reaction | Observation | Result | Observation | Diameter | Result | Observation |
| B | Intermediate | rod | Single / chain | Gram negative | Gelling observed | Gram negative | Zone of inhibition not observed | - | Gram negative | Spores not observed |
| C | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 15mm | Gram positive | Spores observed |
| D | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 18mm | Gram positive | Spores observed |
| E | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 18mm | Gram positive | Spores observed |
| F | Small | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 16mm | Gram positive | Spores observed |
| G | Intermediate | rod | Single / chain | Gram negative | Gelling not observed | Gram negative | Zone of inhibition not observed | - | Gram negative | Spores not observed |
| I | Intermediate | rod | Single / chain | Gram negative | Gelling not observed | Gram negative | Zone of inhibition not observed | - | Gram negative | Spores not observed |
| K | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 17mm | Gram positive | Spores observed |
| L | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 14mm | Gram positive | Spores observed |
| M | Big | rod | Single / chain | Gram | Gelling | Gram | Zone of inhibition | 18mm | Gram positive | Spores |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | positive | observed | Positive | observed | | | observed |
| P | Intermediate | rod | Single / chain | Gram negative | Gelling not observed | Gram negative | Zone of inhibition not observed | - | Gram negative | Spores not observed |
| Q | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 18mm | Gram positive | Spores observed |
| R | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 21mm | Gram positive | Spores observed |
| V | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 17mm | Gram positive | Spores observed |
| W | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 17mm | Gram positive | Spores observed |
| X | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 17mm | Gram positive | Spores observed |
| Y | Big | rod | Single / chain | Gram positive | Gelling observed | Gram Positive | Zone of inhibition observed | 16mm | Gram positive | Spores observed |
| Z | Intermediate | rod | Single / chain | Gram negative | Gelling not observed | Gram negative | Zone of inhibition not observed | - | Gram negative | Spores not observed |

As shown in **Table No: 2.** All 18 isolates were studied for their morphological characterization observed under light microscope, all of them were in rod shape and arranged in chain or singly. The cultures B, G, I, P and Z were found gram's negative while other isolates were gram's positive. For further confirmation of Gram reaction was done with 3% KOH test and Vancomycin test, as shown cultures B, G, I, P and Z showed no gel formation and were not inhibited by Vancomycin which support the gram's staining results. As shown cultures B, G, I, P and Z were non spore formers while other isolates were spore formers.

### Effect of pH on growth of the isolates

As shown in **Graph 1** all the isolates were able to grow in the pH range of 3–10, but pH 7.0 was the optimum for the growth of all the cultures. All the organisms could grow well in the pH range of 5–8 but above or below this range no. of cells density markedly decreases with all the cultures. Effect of pH was studied on the growth of amylolytic organisms, all the isolated were able to grow in the pH range of 3-10, for all the isolates most suitable pH was 7.0, following research support our results.




### Determination amylase activities in submerged fermentation

As described in **Table No: 3** all the cultures were studied for amylase production in 2% starch containing broth and amylase assay was carried out after 24 and 48 h of incubation time, which clearly show that after 24h culture P and Q has given maximum 0.65 and 0.50 U/min/mL amylase production whereas after 48h culture I was able to produce 1.35 U/ min/mL amylase.

**Table: 3 Amylase activities after 24 and 48 h**

| Source | Culture No. | Incubation time | Amylase Activity ( U/min/mL) | Incubation time | Amylase activity(U/min/mL) |
|---|---|---|---|---|---|
| | **B** | ↑ | 0.12 | ↑ | 0.28 |
| 2% starch broth | **C** | | 0.39 | | 0.86 |
| | **D** | | **0.42** | | **0.96** |
| | **E** | | 0.07 | | 0.16 |
| | **F** | 24 h | 0.13 | | 0.28 |
| | **G** | | 0.02 | | 0.045 |
| | **I** | | **0.42** | 48 h | **1.35** |
| | **K** | | 0.09 | | 0.16 |
| | **L** | 24 h | 0.09 | | 0.21 |
| | **M** | | 0.08 | 48 h | 0.20 |
| | **P** | | **0.65** | | **0.9** |
| 2 % starch broth | **Q** | ↓ | **0.50** | ↓ | **0.94** |
| | **R** | | 0.14 | | 0.28 |
| | **V** | | 0.41 | | 0.90 |
| | **W** | | 0.38 | | 0.75 |
| | **X** | | 0.32 | | 0.70 |
| | **Y** | | 0.33 | | 0.74 |
| | **Z** | | 0.24 | | 0.50 |

## CONCLUSION

The main objective of our study was to characterize the 18 amylase producing cultures and to check their ability enzyme production especially amylase. Morphologically all the isolates were single or in chain arrangement and actively motile during microscopic examination, except cultures B, G, P, I and Z all others were Gram positive, rod shaped, spore former organisms, Culture B, G, P, I and Z were Gram negative or Gram variable isolates. All the isolates were able to grow in a wide range of pH (3-10), but optimum pH for the growth for the all isolates was near neutral (7.0). Submerged fermentation was carried out for the production of amylase. Culture I was found to be most promising culture for production of amylase. This was a preliminary study for characterization of amylase producing isolates.
D, I, P and Q cultures saws many high amylase activity under both condition i.e 24 h and 48 h incubation, as compare to other cultures. They shows amylase production significantly high in 48 h i.e nearly double activity in culture I the activity increase from 0.42 to 1.35 (U/min/mL) is nearly 3 times higher at 48 h then 24 h. This may be due to positive adaptation and faster growth as compare to D, P and Q. Such types of organisms should be used in industries to get faster and higher yield.

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