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Research Article

# Isolation and characterisation of potential amylase producing strain from the agriculture waste.

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**ABSTRACT**

Four amylase producing bacteria were isolated from soil samples from agriculture waste. Further one efficient amylase producing Gram negative rod, motile bacteria was selected from the isolates and designated as AW1, which was used for the production and characterization of amylase. The morphological, microscopic and biochemical characterization of selected isolate was performed. Identification of selected amylase producer was carried out by using Bergey's Manual of Systemic Bacteriology Volume -1 and it was identified as *Pseudomonas mallei*. It showed optimum growth on Nutrient agar medium at pH 7 and temperature 30°C. AW1 have produced 50µg/ml and it was determined by DNSA assay method.

**Key Words:** soil sample, morphological and biochemical characterizations, Amylase producer.

## INTRODUCTION

Amylase is a digestive enzyme that aids in the breakdown of carbohydrates by breaking the Bonds between sugar molecules in polysaccharides through a hydrolysis reaction. It can be found in animals, plants, and bacteria[1].

There are three different types of Amylase i.e. alpha-amylase, beta-amylase, and gamma-amylase, which are differing in how they hydrolyze the polysaccharide bonds. Animal tissue does not contain beta or gamma amylases in their body instead of that they contain alpha-amylase, which is an important enzyme in digestive and metabolic processes[2].

The important function of Amylase is to catalyses the breakdown of starch into sugars. After breakdown of long-chain carbohydrates by $\alpha$-Amylase, product yielding is maltose or maltose, glucose, and "limit dextrin" from amylose and amylopectin respectively. Wide spectrum of organisms produces amylase which are biochemical phenotypes and significantly differ in parameters like pH and temperature optima as well as metal ion requirements[3]. Variety of industries like detergent industry, food, fermentation, textile to paper industries, as well as in alcohol production and brewing industry efficiently utilizes amylases to carry out processing of substrate. To satisfy the industrial demands we have searched several fields and search on efficient amylase producers.

## MATERIALS AND METHODOLOGY

### 1) Isolation and Screening

The soil sample was collected in pre-sterilized cotton bags from agriculture waste. Soil sample were diluted and soil aliquots were spread on Nutrient Agar Plates (pH-7).Plates were incubated at 30°C for 24 hours. Isolated Bacterial colonies were cultivated on Nutrient Agar slants[8,10].

Selected isolate suspension on Nutrient Agar plates amended with 1% Starch and after incubation of 24 hours plates were flooded with Iodine solution to observe Amylase production. Efficient Amylase Producers was further identified by observing its morphological and biochemical characters[9]. Among

four selected isolates, the most efficient, high yield strain was further used for production of amylase.

**2) Production, Extraction and Partial Purification**

The Amylase was produced in fermentation media containing NaCl 0.04%, peptone 0.20%, yeast extract 0.1% and starch 1%. pH was adjusted to 7 with 0.1 N NaOH. The media was sterilized by autoclaving at 121°C and 15 lbs pressure for 15 min. After cooling, the media was inoculated with 1% of the selected bacterial isolates and incubated at 30°C for 24 hours. The fermented liquor was centrifuged at 6000 rpm for 30 min and supernatant was used as the source of extracellular enzyme. The Amylase activity in the supernatant was determined by the spectrophotometric DNSA Assay method at 540 nm[11,12].

**3) Enzyme Assay**

Determination of Amylase activity was carried out by measuring the release of reducing sugar from soluble starch. The reaction mixture contained starch and partial purified enzyme. The amount of reducing sugar released in the mixture was determined by the addition of 3 ml of 3, 5- dinitrosalicylic acid method followed by boiling for 5 min to develop color. For standard curve D-glucose was used and absorbance of the mixture was measured at 540 nm (Fig 1). Enzyme activity was calculate as the amount of enzyme releasing reducing sugar equivalent to 1 µmol glucose per minute under the assay condition, which give one unit of Enzyme[1,12-14].

## RESULT

Four Gram negative rods were isolated on nutrient agar plates. These were designated as AW 1, AW 2, AW 3, and AW4. All isolates were motile. Out of four isolates all were Catalase positive, two showed positive results towards citrate utilization , two isolate showed positive result for amylase, three isolate were urease positive results and all isolate showed negative result for cellulase, pectinase and laccase.

Carbohydrate metabolism for all isolate were also studied in which all isolates showed positive result for glucose, maltose, sucrose, and fructose while only two showed positive result for ribose and xylose whereas lactose utilization by all isolates were negative.

Isolate AW 1 showed luxuriant growth besides largest zone of hydrolysis on starch agar plates, therefore selected as amylase producer for further study. On the basis of morphological and biochemical characterization isolate AW 1 was identified as *Pseudomonas mallei* by using Berguye's manual of systemic bacteriology Vol 1. The production and purification of amylase by this isolate AW 1 was carried out. AW 1 has produced 50µg/ml amylase and it was determined by DNSA assay method. The enzyme was active at neutral pH and 30 $^{o}$C temperature.

## CONCLUSION

In conclusion, Amylase producing bacteria was isolated and identified as *Pseudomonas mallei* from vicinity of agriculture waste. After production, partially purified 50µg/ml amylase was obtained by above strain and it may be commercialized after optimizing the favorable conditions for enzyme production.

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**Table 1**
**Morphological Characters**

| Characters | Observed Character of selected isolate | | | |
|---|---|---|---|---|
| | AW1 | AW2 | AW3 | AW4 |
| **Size** | 1mm | 2mm | 1mm | 0.5mm |
| **Shape** | Circular | Irregular | Circular | Circular |
| **Colour** | Faint yellow | White | white | White |
| **Margin** | Entire | Undulate | Entire | Entire |
| **Surface** | Smooth | Smooth | Smooth | Smooth |
| **Elevation** | Convex | Flat | Convex | Raised |
| **Consistency** | Mucoid | Sticky | Non sticky | Sticky |
| **Opacity** | Opaque | Transparent | Opaque | Opaque |
| **Grams Nature** | Negative | Negative | Negative | Negative |
| **Motility** | Motile | Motile | Motile | Motile |

**Table 2**
**Biochemical characters of Isolate.**

| Test/isolate | AW1 | AW2 | AW3 | AW4 |
|---|---|---|---|---|
| **Catalase** | + | + | + | + |
| **Indole production** | – | + | – | – |
| **Methyl red** | + | + | + | – |
| **VP** | – | _ | – | + |
| **Citrate utilization** | + | _ | – | + |
| **Asculin Hydrolysis** | – | _ | – | – |
| **Nitrate Reduction** | – | _ | – | – |
| **Enzyme profile** | | | | |
| **Amylase** | + | + | – | – |
| **Urease** | + | _ | + | + |
| **Lipase** | – | + | – | – |
| **Cellulase** | – | _ | – | – |
| **Pectinase** | – | _ | – | – |
| **Laccase** | – | _ | – | – |
| **Sugar profile** | | | | |
| **Glucose** | + | + | + | + |
| **Ribose** | + | + | - | – |
| **Maltose** | + | + | + | + |
| **Lactose** | – | _ | – | – |
| **Sucrose** | + | + | + | + |
| **Xylose** | + | - | – | + |
| **Fructose** | + | + | + | + |

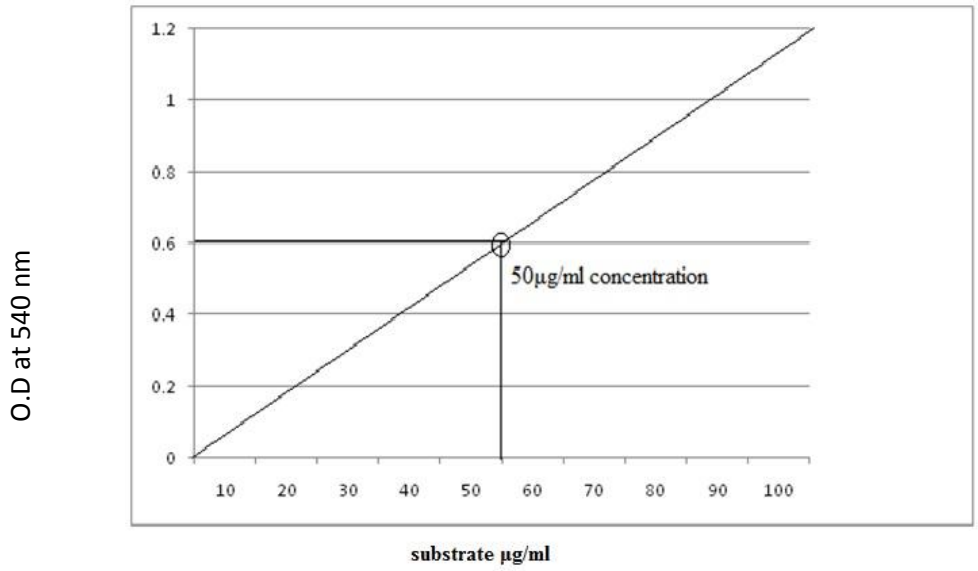


**Fig 1**
**Spectrophotometric analysis of amylase by DNSA assay method**

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