

**Original Article**

**ANALYSING THE BIOCATALYTIC ASPECT OF AMYLASE FROM PLANT AND MICROBIAL SOURCES FOR INDUSTRIAL APPLICATION**

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**ABSTRACT**

**Objective:** Biocatalysts have a wide role in industries and amylases are one of the best enzymes used in various industries as they stay stable physically and chemically even under environmental or physical stress. Extraction of amylase from plant sources like chickpea and green pea and also the microbial source of *Bacillus subtilis*.

**Methods:** Enzymatic activity was determined by enzyme assay and purified through the following steps such as salt precipitation, dialysis, Ion-exchange chromatography and characterized using SDS PAGE.

**Results:** Enzymatic activity of amylase produced by *bacillus subtilis* was determined the highest (4600 U/ml) activity and molecular weight 48.9kDa was recorded.

**Conclusion:** Amylase is a highly important enzyme that breaks down the starch molecules into simple sugars. It has potential in a wide range of industrial processes such as food, fermentation and pharmaceutical industries. All the sources used in this study proves that the bacteria have the highest concentration of enzyme in the form of protein.

**Keywords:** Amylase, Industrial application, Enzymatic activity, Sources of amylase, Biocatalytic aspect

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**INTRODUCTION**

Biocatalysts have been used since long, owing to their wide applications in both clinical and industrial fields. Several enzymes have been found to be of great importance in food and pharmaceutical industries besides their other applications. The industrial enzyme market is huge and the challenge is to look for better sources which are cost-effective and easy to process for industrial use. The rapid development in the application of enzymes in industries over the past 4 decades was the motivation for our study. Enzymes found in nature have been used since ancient times in the production of food products, such as cheese manufacturing of products such as leather, lignin etc. Though the use of recombinant gene technology has improved manufacturing processes and enabled the commercialization of enzymes that could previously not be produced; the search for better sources and ways to utilize the enzyme for industrial use is always there. Furthermore introducing protein engineering and directed evolution has revolutionized the development of industrial enzymes. Overall, the estimated value of the worldwide use of industrial enzymes has grown from \$1 billion in 1995 to \$1.5 billion in 2000 [1].

Amylases and celluloses used in industries such as the starch, textile, detergent and baking industries, represent the second largest group. The technical industries, dominated by the detergent, starch, textile and fuel alcohol industries, account for the major consumption of industrial enzymes. In this study, we tried to analyze the catalytic activity of the amylase enzyme from plant and microbial sources to assess its industrial application. Amylase is a hydrolase enzyme. It breaks down the starch into simple sugars. It acts on  $\alpha$ -1,4-glycosidic bonds. As diastase, amylase was the first enzyme to be discovered and isolated [2, 3]. There are many sources of amylase such as plants and the microbial sources, besides human isoenzymic forms. Several studies have indicated industrial benefits from microbial sources more than plant sources [3].

In the present study, amylase sources were from chickpea, green gram and bacillus. Chickpea and Greengram are good plant sources of amylase. The amylase present in them helps in the breakdown of starch molecules. It is observed that 50% of starch content is

reduced during the germination phase in seeds as amylase breaks down the starch molecules into simple sugars [4]. *Bacillus subtilis* also known as the hay bacillus or grass *bacillus*, is a Gram-positive bacterium; is an extremely common bacterium. It is found in soil, water, air, and decomposing plant matter. They can form hard protective endospore and be impervious to extreme temperatures, chemicals, environmental factors, even some types of radiation. This makes them an excellent choice for use in the industrial process [5].

**MATERIALS AND METHODS**

**Sample collection and reagents**

Chickpea and Green Gram were procured from the local market and soil sample was collected from the garden. All reagents were purchased from hi-media and Sigma Aldrich.

**Bacterial culture**

Five gram of each sample chickpea, green gram and soil were suspended in 25 ml of Saline Water (0.89%). Sample suspensions were diluted ( $10^{-7}$ ) with saline water and 100  $\mu$ l of bacterial suspension was spreaded on Nutrient Agar media plate and incubated at 37 °C for 24 h. Bacterial colonies were isolated and grown in Nutrient Agar media. Bacteria were characterized by biochemical screening as described below.

**Gram staining**

Gram staining was performed for all isolated colonies according. A smear of bacterial cells was prepared. The smear was flooded with crystal violet solution for one minute. Smear was washed with distilled water followed by adding mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and washed with water. Finally, safranin was used as a counterstain for 60-80 sec. and washed with water. Cells were then examined under the microscope.

**Starch hydrolysis test**

A single colony was streaked on Starch agar media and the bacteria inoculated plates incubated at 37 °C for 24 h. After incubation, flood

the surface of the plates with an iodine solution with a dropper for 30 seconds. Pour off the excess iodine. Cleared zone around of bacterial growth was examined.

#### Voges-proskauer test

Isolated single colony was inoculated in MRVP Broth media and incubated at 37 °C for 24 h. After incubation added 12 drops of VP I reagent and 3 drops of VP II reagent. Mixed well and examined the result.

#### Simmons citrate agar test

Isolated single colony was streaked in Simmons Citrate Agar media and incubated at 37 °C for 24 h and examined the result. Green color media turns into blue color which was shown a positive result.

#### Production of enzyme

##### Production from green gram and chickpea

5 gms of chickpea and green gram were added in two different beakers containing 50 ml of buffer solution. Sealed the cap and stored Beaker in the refrigerator for about 24 h. After 24 h chickpea and green gram grinded separately with the help of mortar and pestle. Filtered the homogenized suspension by filter paper and centrifuged at 6000 rpm for 10 min. the supernatant was collected and EDTA and sodium benzoate were added.

##### Production from *Bacillus subtilis*

The amylase production was performed in 250 ml conical flasks containing 50 ml medium with the following composition-soluble starch-5g/l, yeast extract-5 g/l,  $(\text{NH}_4)_2\text{SO}_4$ -2.5 g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.2 g/l,  $\text{KH}_2\text{PO}_4$ -3 g/l, and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ -0.25 g/l incubated at 50 °C under shaking conditions (200rpm) and inoculated with 24 h ours old bacterial culture. After the cultivation time, the culture broths were centrifuged at 10,000 rpm for 10 min and the supernatant was collected for enzyme screening.

#### Qualitative screening of $\alpha$ -amylase enzyme activity

Screening of  $\alpha$ -amylase was performed using well-cut assay with slightly modifications<sup>6</sup>. The starch agar plates were prepared. After agar solidification, around 8mm diameter of the well was cut out aseptically using cork borer. The well was filled with 100  $\mu$ l of culture filtrate, incubated overnight at 37 °C, flooded with 1% of iodine solution, then the hydrolytic zone around the well (clear zone) is measured. Sterile water used as a negative control.

#### $\alpha$ -Amylase enzyme activity

Enzymatic activity of  $\alpha$ -amylase was performed according to Sigma Aldrich Assay.

#### Purification of enzyme

##### Ammonium sulphate precipitation

Ammonium Sulphate precipitation was used for the purification step. 10 ml of the supernatants of Green Gram, Chickpea and *Bacillus subtilis* supernatant were taken in a beaker and kept over ice bags to maintain the temperature and 44% Ammonium Sulphate was added pinch by pinch. The resulting precipitate was removed by precipitation. Centrifuged the sample at 10000 rpm for 10 min. Collect pellets and dissolved in phosphate buffer.

#### Dialysis

5 ml of ammonium sulphate containing samples were kept in the dialysis bags separately using a micropipette. The bags were sealed properly. Commence dialysis against water for at least 1 h. Ensure that the dialysate is gently stirring throughout the procedure. After completion of dialysis, dry the outside of the bag using tissue and take the sample out using a micropipette. Measured the volume of the sample after dialysis.

#### Ion exchange chromatography

The dialyzed samples were applied to an anion-exchange chromatographic (diethylaminoethyl cellulose) column equilibrated with 25 mM Tris-HCl buffer pH 8. The bound enzyme was eluted at flow rate 1 ml/min by using a linear gradient from 0-100% of 1M NaCl in 25 mM Tris-HCl buffer pH 8. Fractions containing  $\alpha$ -amylase activity were determined.

#### Protein determination

Protein concentration was determined by the method of Lowry<sup>7</sup>. Bovine serum albumin was used as a standard.

#### SDS-polyacrylamide gel electrophoresis

SDS-PAGE was performed described by Laemmli<sup>8</sup> and the protein visualized by staining solution following standard procedure. A low molecular range protein standard (Spectra Multicolor Broad Range Protein Ladder, Fermentas) was used as the marker.

## RESULTS AND DISCUSSION

#### Isolation and characterization $\alpha$ -amylase producing bacteria

The bacterial isolate that gave positive for  $\alpha$  amylase was conformed as *Bacillus subtilis*. Different staining and biochemical tests gave as Gram-positive rod bacteria. Different biochemical tests such as starch hydrolysis, Voges-Proskauer Test and Simmons Citrate Agar Tests were shown a positive result. All biochemical tests were followed by Bergey's manual of determinative bacteriology.



Fig. 1: Gram's staining

#### Enzyme activity

The enzymatic activity for the  $\alpha$ -amylase enzyme extracted and purified from Chickpea, Green Gram and *B. subtilis* were showed in table 1. It was indicated that during purification steps the total  $\alpha$ -amylase activity increased. In comparison among chickpea, green gram and *Bacillus subtilis* produced an enzyme, bacterial produces enzyme recorded highest enzyme activity compare to chickpea and green gram.

Table 1: Enzymatic activity of  $\alpha$  amylase extracted from a different source

Sample name	Crude enzyme U/ml	Salt ppt U/ml	Dialyzed U/ml	Purified U/ml
Chickpea	560	1600	2880	3360
Green Gram	760	1200	1360	1560
<i>Bacillus subtilis</i>	1000	1800	4000	4600

Table 2: Protein concentration of  $\alpha$  amylase

Sample	Protein concentration ( $\mu$ g/ml)
Chickpea	261.11
Green Gram	284.84
<i>Bacillus subtilis</i>	335.71

### Protein estimation

Protein concentration of purified samples was presented in table. BSA used as standard and protein concentration was determined by BSA standard curve. Bacterial produced enzyme was measured highest protein concentration as compared to chickpea and green gram.

### Characterization of purified $\alpha$ amylase

Character determination of the purified  $\alpha$ -amylase enzyme using SDS-PAGE electrophoresis with coomassie brilliant blue staining and it measured not only for determining the molecular mass of the purified enzyme but even a method to specify the enzyme purity. The molecular mass of the bacterial produced purified  $\alpha$ -amylase enzyme was estimated at about 48.9 kDa. Whereas chickpea was estimated 25.947kDa and green gram was estimated about 35 kDa.



Fig. 2: SDS PAGE 1. Marker, 2. Bacterial Enzyme, 3. Chickpea, 4. Green Gram

### DISCUSSION

The results and observations of our study showed that *Bacillus subtilis* was the source of amylase with the highest enzymatic activity among the sources chosen for the study. Many Industries utilized these bacteria to produce high amounts of amylase, as they are a source which are easy to process with less compliance and high cost-effectiveness. Though the growth of micro-organism is quite rapid, the virulence of the bacteria and contamination caused by it should be administered frequently.

Chickpea and green grams were also found to be a good source for amylase activity, but for industrial use, they would not serve to be very cost-effective. Human salivary amylase under certain pathological conditions is not enough and there are situations where there is a need to take amylase through diet. In these situations plant sources containing the amylase come into picture where they play a prominent role and thus chickpea and green gram serve the purpose.

### CONCLUSION

Alpha-Amylase enzyme was isolated from chickpea, green gram and *Bacillus subtilis*. *Bacillus* was highly efficient in producing alpha-amylase enzyme and therefore has a high potential for industrial applications. Submerged fermentation was used for large scale production. The present study had its limitations; further study with more micro-organism is the need of the time to explore the potential sources of amylase for industrial applications.

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Nil

### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

### CONFLICT OF INTERESTS

Declare none

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