ORIGINAL RESEARCH PAPER

Biotechnology

ISOLATION, SCREENING AND CHARACTERIZATION OF AMYLASE PRODUCING BACTERIA FROM SOIL

KEY WORDS: Amylase, Starch hydrolysis, IMViC, Enzyme assay, Protein assay

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ABSTRACT

In the present investigation, the amylase producing bacteria were isolated from soil samples collected from 3 different places such as kitchen waste, bakery waste and vegetable waste. Primary screening of amylase producing bacteria were done using starch agar plate. The isolated bacteria were characterized through morphological characterization and biochemical tests. Enzyme assay for two high enzyme producing strains showed that the strain A3 gives highest enzyme activity i.e., 35±1.8 IU/ml/min after 48 hrs. of incubation. Two isolates with high enzyme index were also analyzed for total protein by Lowry's method. Among the two bacterial isolates, A3 showed highest protein content i.e., 0.85mg/ml. This study has shown that these 2 bacterial strains are able to synthesize amylase that is evidenced in the hydrolysis of starch which is very important in biotechnology. For further studies, the strain A3 with highest enzyme activity has been selected for optimization, characterization and purification of enzyme.

INTRODUCTION

Microorganisms enjoy cosmopolitan distribution. They have been found to thrive in conditions of mild to extreme environmental stress. In addition to being widely distributed, they play major roles in the maintenance of proper function of their immediate environment. Enzymes are biological molecules, act as catalyst to support almost all of the chemical reactions required to sustain life1. Isolation and purification of such enzymes incur high costs and their recovery for subsequent reuse is also difficult. In addition to that they are more prone to substrate or product inhibition and their products may leads to allergic reactions2. These factors limit the uses of such enzymes.

Microbial enzymes have a lot of applications in medicine and industries owing to their stability, catalytic activity, ease of production and optimization than plant and animal enzymes. They are used in food, textile, agriculture, pharmaceuticals and other industries because of their reduced processing time, low energy input, cost effectiveness, non-toxic and eco-friendly characteristics.

Microbial enzymes are also used to degrade the toxic compounds present in industrial and domestic wastes3. The global market for industrial enzymes was expected to develop at a compound annual growth rate of approximately 7% over the period from 2015 to 2020 to reach nearly $6.2 billion (2015, Industrial enzyme market).

Amylases are starch degrading enzymes which can be obtained from plants, animals and microbes. Starch degrading enzymes are widely distributed in bacteria and fungi. Amylases are extensively used in processed food industry. α-amylases are widely used in baking industry.

It also plays a major role in textile industry and paper industry. Surface of paper is normally coated with starch to make it smooth and strong, to improve writing quality of the paper. The present study also focused on the isolation of amylase producing bacteria from soil. It is also having very importance in day-to-day life.

MATERIALS AND METHODS

The bacteria used in the present study were isolated from the soil samples collected from the top soil of kitchen waste (S1), vegetable waste (S2) and bakery waste (S3). Sterile polythene bags were used to collect the soil samples and transported to the laboratory for further analysis.

Isolation and Primary screening of amylase producing bacteria Isolation of bacteria was done by serial dilution of soil sample. Total bacteria present per gram of soil sample was calculated as:

$$\text{Number of colony forming units (CFU) per gram of soil} = \frac{1}{\text{Dilution factor}} \times \text{Number of colonies}$$

Primary screening of all the selected isolates were done to determine their amylase production using starch agar medium. Briefly, each bacterial isolate was streaked on to starch agar plates and incubated at 37°C for 3 days. After incubation, the plates were spread with iodine solution (0.3% iodine and 1% KI). Then the iodine solution was discarded from the plates after 10 min. Based on the clear zone formation around the bacterial growth, amylase producing bacterial strains were identified and recorded. Enzymatic index was calculated as:

$$\text{Enzyme index} = \frac{\text{(Diameter of zone of degradation)}}{\text{(Diameter of colony)}}$$

Morphological and biochemical characterization of bacteria

Isolated bacterial strains were characterized through morphological characterization by Gram staining and through several biochemical tests such as IMViC test (Indole test, Methyl red test, Voges Proskauer test and Citrate utilization test).

Measurement of amylase activity

Amylase activity was determined by DNS method5. 100 ml of L.B broth with 8% (w/v) inoculum was incubated for 48 hours with continuous agitation (150 rpm). Then it was centrifuged at 4000 rpm for 15 min. Cell were discarded and supernatant was used as crude enzyme for enzyme assay5. The reaction mixture contained 0.2ml of crude enzyme and 0.8ml of 100mM phosphate buffer (pH 7) containing 1% (w/v) of soluble starch. The mixture was incubated at 10min at 80°C and the reaction was stopped by adding 2ml of DNSA (3,5-dinitro-salicylic acid). The contents were boiled exactly for 5min in water bath and cooled for 20-25min after which, 1ml of 40% Rochelle salt was added. Finally, the colour developed was read at 540nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that releases 1μ mol of reducing sugar as maltose per minute under the assay conditions.

$$\text{Activity of enzyme (IU/ml/min)} = \frac{\text{(mg of maltose X 1000)}}{\text{(volume of enzyme taken X incubation time)}}$$

Total protein estimation

The protein content of the enzyme preparations was estimated by Lowry's method using Bovine serum albumin as standard. 1 mg/ml stock solution is prepared and from that stock solution various dilutions ranging from 0.1 mg/ml -1.0 mg/ml were prepared and standard plot was performed.

0.2ml of solution was taken in different test tubes from each dilution and then 2 ml of alkaline copper sulphate reagent was added and the mixture was incubated for 10 minutes at room temperature. Then kept for 30 minutes incubation after adding 0.2 ml of Folin-Ciocalteau reagent. Absorbance was read at 595 nm in an UV Vis Spectrophotometer after incubation.

Data analysis

Data were analyzed by using a statistical software package (SPSS for Windows, ver. 17, 2012). Treatment means were compared by one way analysis (ANOVA) and the Duncans New Multiple range test were applied to the result at 0.05 level of significance ($p < 0.05$).

RESULTS AND DISCUSSION

The present study focused on the isolation of amylase producing bacteria from soil. The presence of amylase producing microorganism from the soil agrees with earlier reports that the soil is known to be a reservoir of amylase producers6. The total bacterial population (CFU/ml) was observed at 10(-4) dilution for all the tested soil samples. The population was high (2 x 105 CFU/ml) in the soil sample collected from kitchen waste (S1), followed by soil sample of bakery waste (S2) (1.5 x 105 CFU/ml) and finally with a soil sample (0.63 x 105 CFU/ml) of vegetable waste (S3). The details of soil samples are described in the table 1.

Table – 1 The Details Of The Soil Samples Collected From Different Locations.

Soil Samples	Location	Number of CFU/ml
S1	Kitchen waste	2 x 105
S2	Bakery waste	1.5 x 105
S3	Vegetable waste	0.63 x 105

Primary screening for amylase production

Primary screening of the isolated bacterial colonies (A1, A2 & A3) was done individually for their ability to produce amylase on the starch casein agar plates. Among the 3 bacterial isolates, only 2 isolates (A1 & A3) were found to be the producers of amylase in starch agar (Fig.1). Zone of hydrolysis was measured and enzyme index was calculated for each colony (Table 2). Two isolates showing high values include A1 (1.7) and A3 (3) as shown in Table 2 and Figure 1. Similar to the result of the present study, it has been reported that the use of starch agar medium and iodine solution for the detection of amylase producing bacteria7.

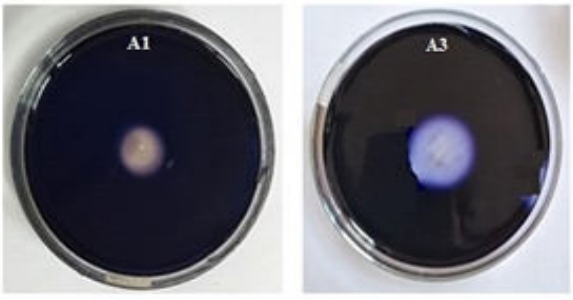

Figure 1: The zone formation around the colony due to the hydrolysis of starch by amylolytic enzymes (A1 & A3)

Table – 2 Amylase Production By Bacterial Isolates

Isolate No	Diameter of colony (mm)	Diameter of Zone of degradation(mm)	Enzyme index
A1	10	17	1.7
A3	10	30	3

Morphological characterization of bacteria

It was done by Gram staining. The results of colony morphological study of isolated bacteria presented in Table 3. Morphological characteristic revealed that the isolated bacterial strains (A1 & A3) on the agar medium were rod shaped and gram positive.

Table – 3 Colony Morphology Of Isolated Soil Bacteria

Colony Features	Bacterial Strains	
	A1	A3
Colour	White	white
Shape	Rod	Rod
Margin	Filamentous	Regular
Elevation	Flat	Flat
Surface	Smooth	Smooth
Pigments	Nil	Nil

Biochemical characterization of bacteria

The tubes inoculated with bacterial strains A1 showed the positive reaction for IMViC test. While the bacterial strain A3 showed negative reaction for the same (Fig.2).

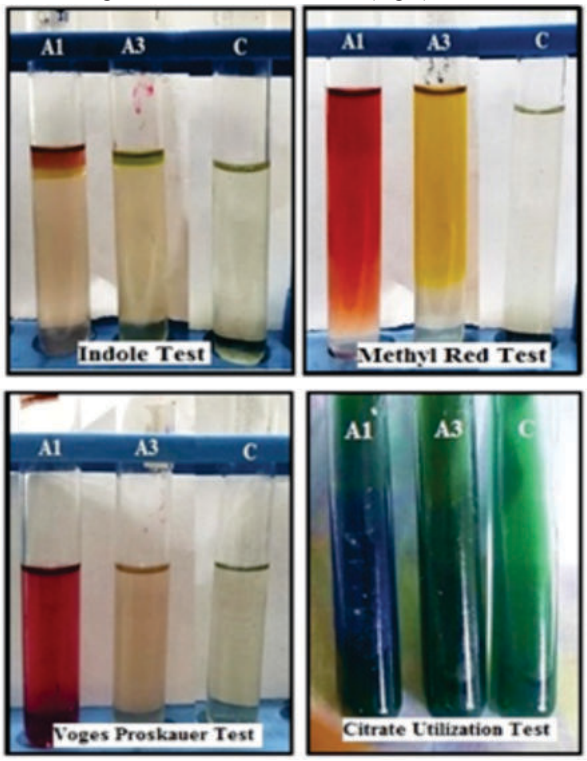

Figure 2: IMViC test [A1, A3 & C (uninoculated)]

Enzyme activity

Four isolates with high enzyme index were also analyzed for enzyme activity. Enzyme activity was determined by DNS method by reference to a standard calibration curve of maltose (Fig.3). It was found that 48 hrs was the effective incubation period for maximum amylase activity.

Among the two bacterial isolates, A3 showed highest enzyme activity i.e., 35±1.8 IU/ml/min and it was significantly different ($p < 0.05$) with the enzyme activity of bacterial strain A1 (3.1±1.45 IU/ml/min) (Fig.3). The present study is also in conformity with other report8.

Total protein estimation

Total protein, as determined by Lowry's method by reference to a standard calibration curve of bovine serum albumin. Effect of incubation period on total protein production showed that 48 hours was the optimum duration for maximum protein activity for all the two colonies.

Among the two bacterial isolates, A3 showed highest protein content i.e., 0.85mg/ml and it was significantly different ($p < 0.05$) with the protein content of bacterial strain A1 (0.27mg/ml) (Fig.4).

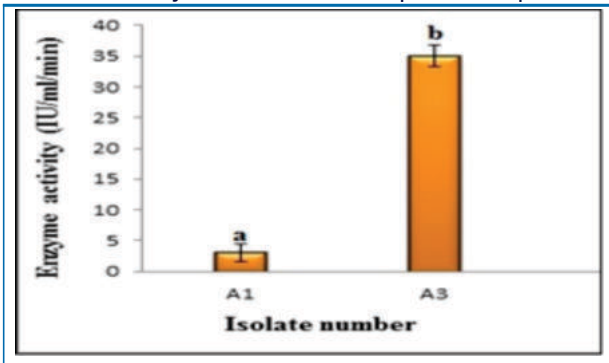

Figure 3: Enzyme activity of bacterial isolates

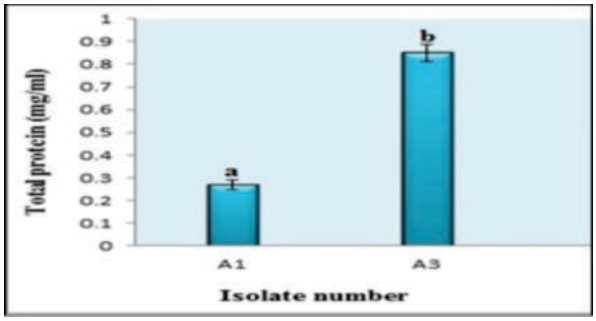

Figure 4: Total protein of bacterial isolates

CONCLUSIONS

Present study highlighted the ability of soil microbes to produce industrially important enzyme capable of starch degradation. Amylase enzyme activity was determined by DNS method and it was concluded that 2 out of 3 bacterial colonies were potent and showed their maximum enzyme activity after 48 hrs of incubation. This study has shown that these 2 bacterial strains are able to synthesize amylase that is evidenced in the hydrolysis of starch which is very important in biotechnology and that the amylase production by these bacteria may in future, be used in different industrial sectors as well as other research areas.

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