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Isolation of Amylase Producing Bacteria from Decayed Brinjal - *Solanum Melongena*

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ABSTRACT

Amylases have potential application in a wide number of industrial processes such as food, fermentation and pharmaceutical industries. The production of amylase is essential for conversion of starches into oligosaccharides. A large number of microbial α-amylases have applications in different industrial sectors such as food, textile, paper and detergent industries. In the present study a mature semi decayed brinjal (*Solanum melongena*) was collected for bacterial isolation. Eight bacterial isolates were isolated and screened for amylase production, One isolate (B6) displaying the highest activity was selected for further study. The enzyme was purified by ion exchange chromatography and its specific activity was found to be 0.83 U/ml/min. The determination of the α-amylase activity was performed by FT-IR analysis

Keywords: α-Amylases, enzyme production, bacterial amylase, starch and FT-IR analysis.

1. INTRODUCTION

Microbial enzymes are widely used in industrial processes due to their low cost, large productivity, chemical stability, environmental protection, plasticity and vast availability [1, 2]. Bacillus species such as *Bacillus subtilis, Bacillus amyloliquefaciens* and *Bacillus licheniformis* are used as bacterial workhorses in industrial microbial cultivations for the production of a variety of enzymes as well as fine biochemical for decades. Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25-30% of the world enzyme market [3, 4]. New amylases could be potentially useful in the pharmaceutical and fine chemical industries of enzymes with suitable properties could be identified [5].

The amylases can be derived from several sources, such as plants, animals and micro-organisms. Because of their short growth period, the enzymes from microbial sources generally meet industrial demands [6, 7]. Growth conditions and nutrients promote high yields of microbial amylases. However, carbon sources such as dextrin, fructose, glucose, lactose, maltose and starch are very expensive for commercial production of these enzymes [8, 9]. At present, *Bacillus, Aspergillus, Rhizopus* and *rhizobial i*solates specified are considered to be the most important sources of industrial amylases [10]. Nevertheless, various other sources of microbial amylases are been investigated in the world. The present study aimed to isolate amylase producing bacteria from the decayed brinjal.

2. MATERIALS AND METHODS

Sample

A mature semi decayed Brinjal (*Solanum melongena*) was taken from house, kept in a sterile polyethylene bags. It was transferred to Laboratory for bacterial isolation. Also, the sample was preserved in the refrigerator for further use.

Isolation of bacteria

Bacteria were isolated from the decayed brinjal sample by serial dilution agar platting method. About 1gm of sample was cut out using sterile knife, weighed and suspended in 100 ml of sterile distilled water. Then incubated in an orbital shaker incubator at 28^0C with shaking at 200 rpm for 30 min. Mixtures were allowed to settle, and serial dilution were done up to 10^{-5} using sterile distilled water and agitated with the vortex at maximum speed. An aliquot of 0.1 ml of each dilution from 10^{-2} to 10^{-5} was taken and spread evenly over the surface of Nutrient agar medium. The inoculated plates were incubated at room temperature for 24-48 hours.

Isolation of pure culture

After incubation, morphologically different bacterial isolates were selected and sub cultured in nutrient agar plates. Repeated streaking on nutrient agar plates led to pure colonies. Then the pure strains were maintained in nutrient agar slants for further use.

Screening test for amylase

All the bacterial isolates were screened for amylolytic activity by starch hydrolysis test on starch agar plate. The microbial isolates were streaked on the starch agar plate and incubated at 37°C for 48 hours. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate. Presence of blue colour 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 investigated [11, 12].

Characterization and identification of potential strain

Morphological, cultural and physiological characterizations were studied followed by [13]

Production of amylase enzyme

After enzyme screening, one potential amylase positive strain was selected for enzyme production quantitatively. Amylase enzyme was produced in batch culture method in 250ml of Erlenmeyer flask with 50ml of production medium containing 1% starch as a substrate. A test tube with 2ml of production medium, previously inoculated with bacterial strain was used as seed inoculums for enzyme production. The flask was incubated at 37°C for 48 hours in a shaking condition.

Extraction of crude enzyme

After incubation of production medium, the bacterial cells were removed by centrifugation (10000 rpm for 10 min) and the supernatant was collected into a fresh tube, used as a crude enzyme for assay.

Assay of enzyme activity

The assay was carried out by DNS method using soluble starch as substrate, few drops of DNS (3-5, Dinitro salicylic acid) reagent were added and the absorbance was measured at wavelength of 540 nm. Enzyme activity was defined as the micro mole of product released by 1ml of enzyme extract in 1 minute [11].

Estimation of protein content

Total protein content was estimated using the protocol of [14].

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

In this present investigation, ATR model of FTIR Spectrophotometer (Bruker Co.) was used for the analysis of crude enzyme. The spectrum (400-4000 nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

3. RESULTS AND DISCUSSION

Isolation and Screening of Fungi Isolates

Eight fungal colonies were isolated from the collected water samples, the entire isolates were screened for amylolytic activity on starch agar plates. In this present investigation, four fungal isolates showed positive for amylase production on starch agar and one isolate was selected for further study based on the size of clear zone on starch agar.

Identification of Fungi

The selected fungal isolate was identified by studying cultural and morphological characteristics include color, texture, pigmentation and colony morphology (Table 1) and was identified as *Bacillus sp*.

Table 1: Characterization and identification of potential strain

Sl. No.	Characterization	Result
1	Gram's reaction	+
2	Morphology	Rods
3	Catalase test	+
4	Oxidase test	+
5	Spore stain	-
6	Indole test	-
7	Methyl red test	+
8	Voges proskauer test	-
9	Citrate test	-
10	TSI	Acid

11	Protease activity	-
12	Arabinose	+
13	Glucose	-
14	Galactose	-
15	Dextrose	-
16	Lactose	-

Probable identity	***Bacillus sp***

Enzyme Activity and Protein Content

Amylase activity was assayed quantitatively by DNS method using starch as substrate. In this study the enzyme showed 0.83 U/ml /min of quantity. Protein concentration of amylase enzyme was assayed by Lowry's method using BSA as standard. In this study the enzyme showed 1.8mg/ml of protein. (Table 2).

Table 2: Enzyme Activity and Protein Content of Amylase

Enzyme Suspension	Total Protein (mg/ml)	Enzyme Activity (U/ml)
Purified	1.8mg/ml	0.83 U/ml

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

Figure 1, shows the FT-IR spectra for starch solution after complete hydrolysis. FTIR analysis showed 11 functional groups/ions from 400-4000nm of spectra. Among these, 3 were found in high peaks. The action of α-amylase upon starch causes hydrolytic cleavages at random α-1,4 glucan bonds at the inner part of the starch chains[15]

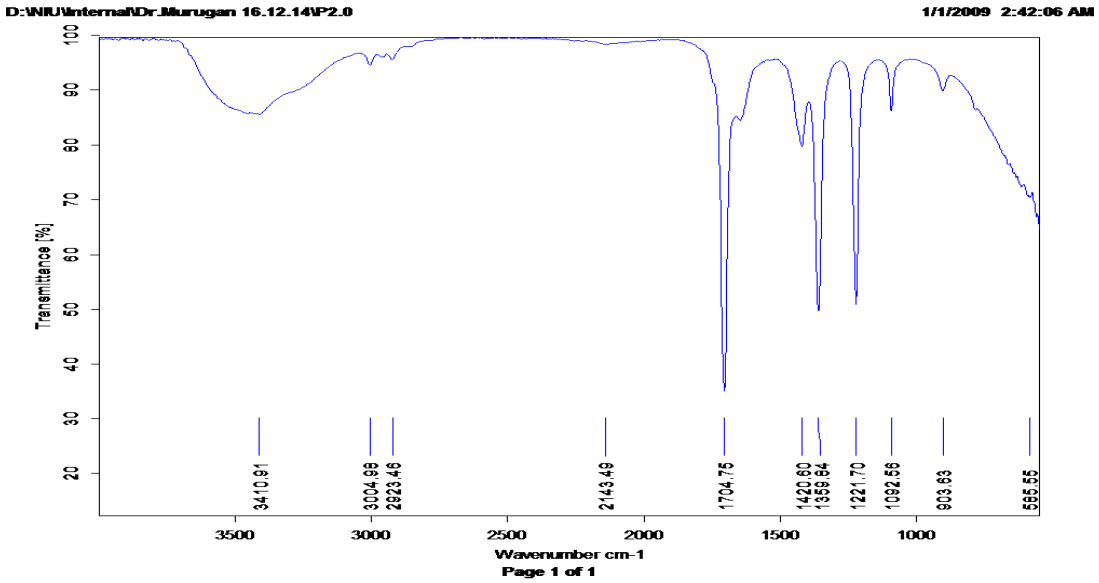

Figure 1: FT-IR analysis of starch after enzymatic hydrolysis

A variety of bacteria are reported to produce organic acids such as α-amylase, oxalic, succinic and malic acid. Among them, α-amylase production using the *Bacillus subtilis* is well known and widely used by industries producing food, beverages, chemicals and pharmaceutical products [16]. The α-amylase production by *Bacillus subtilis* is influenced by number of fermentation parameters. There are significant variations in fermentation environments reported in the previous studies for α-amylase production by *Bacillus subtilis*. For achieving high production of α-amylase, it is essential that the study of influence of physical and chemical environments on α-amylase production [17]. Presently, α-amylase production by *Bacillus subtilis* is economically produced using solid state fermentation. However, the global demand for α- amylase is growing faster than its production, implying that more economical processes are required to supplement or replace the present processes [18, 19].

4. CONCLUSION

It can be concluded that, amylase enzyme can be produced for industrial purposes from *Bacillus sp*. FT-IR spectroscopy was successfully applied for the determination of α- amylase activity in aqueous solutions. Also, the bacteria *Bacillus sp* produced amylase enzyme with good enzymatic activity and concentration. Hence, α-amylase from *Bacillus sp* got potential to be used in various industries where utilization of starch is concerned.

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