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# ISOLATION, SCREENING AND CHARACTERIZATION OF PROMISINGA-AMYLASE PRODUCING BACTERIA FROM SEWAGE ENRICHED SOIL

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**ABSTRACT:**

Among different types of enzymes obtained from microbial sources, amylases are the most widely used in industries.In the present study, bacteria were isolated from sewage soil and screened for the production of α-amylase. Among four bacterial isolates, one isolate produced maximum zone of starch hydrolysis. The bacterial isolate was identified as *Bacillus* sp. and was later used for further characterization. Maximum yield of amylase was obtained after 48h of incubation. The optimum pH for enzyme activity was found to be at pH 7 and the optimum temperature for the activity was found to be at 35 ºC.

**Keywords**: α- amylase, starch hydrolysis, cultural characterization, amylase activity, pH, temperature.

## [1]INTRODUCTION

Mystery of life is quite interesting. Life as view of science involves anabolism and catabolism. Catabolism results in the breakdown of complex molecules. This involves the enzyme. Amylases are one of the degrading enzyme which breakdown the starch into sugars [2]. All alpha amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds.[1]. Amylases are produced by variety of living organisms, ranging from bacteria to plants and humans.

Many microorganisms are able to produce amylases including *Bacillus subtilis, Lactobacillus, Escherichia, Proteus, B.licheniformis,Bacillus steriothermophilus, Bacillus megaterium, Strepotmyces sp., Pseudomonas sp. etc*. Amylase is used for various purposes such as inbrewing industry,food additive, also used in clothing and dishwasher detergents to dissolve starches. For preparation of sizing agents and removal of starch sizing from woven cloth, preparation of starch

sizing plate's liquefaction of heavy starch pastes formed during heating steps in the manufacture of corn and syrups, in bakery industryetc.,. Microorganisms are used for the industrial production due to advantages such as costeffectiveness, consistency, less time and space required for production.[3,4].Microbial amylases have replaced the chemical hydrolysis of starch in starch processing industries. These would also be useful in the pharmaceutical and fine chemical industries [13]. These increased uses have placed greater stress on increasing indigenous amylase production and search for more efficient processes [14].With the introduction of Biotechnology in today's world, the use of amylase has widened in various fields. The major advantages of using microorganisms for production of amylases are the ability to produce in bulk and ease at which it can be manipulated for desired products [15].Bacteria which can produce the amylase are widely present in nature. These bacteria can easily be screened and tested for the production of amylase.

## [2]MATERIALS AND METHODS

### [2.1]Sample collection

Soil samples near to sewage of Nagarbhavi circle Bangalore and from Kengeri and Padmashree hostel Bangalore were collected with the help of sterile spatula. Collected samples were transferred to sterile plastic bags in aseptic conditions.

### [2.2]Isolation of bacterial strains from soil sample

One gram of the above collected soil sample was weighed and mixed to 9 ml of sterile distilled water. Serial dilution was done up to $10^{-5.}$ Serial dilution of $10^{-5}$ of mixture was introduced into a sterile petri plates using the pour plate method into nutrient agar HIMEDIA fortified with 2% starch. The poured plates were incubated at 37°C for 24 hrs. [12].The bacterial isolates were further sub cultured to obtain pure culture. Pure isolates on starch agar slants were maintained at 4ºC.

### [2.3]Screening of potent amylase producing bacteria by starch hydrolysis test

The isolated pure strains were screened for the production of extracellular amylase using starch agar. Bacterial isolates were screened for amyl lytic activity by starch hydrolysis test on starch agar plate.[11] Themicrobial isolates were streaked on the starch agar plate and incubated at 37°C for 48 hours. After incubation 1% iodine solution was flooded with dropper for 30 seconds on the starch agar plate. The isolates produced clear zones of hydrolysis were considered as amylase producers and were further investigated [9].

### [2.4]Amylase production

Amylase was produced also in a complex medium containing starch 1.0%, yeast extract 0.04%, $(NH_4)_2HPO_4$ 0.4%, KCl 0.1% and $MgSO_4$7H2O 0.05%, and semi-synthetic medium containing peptone 0.4%, $(NH_4)_2HPO_4$ 0.4% and KCl 1.0%.[7]

### [2.5]Cultural characterization

The isolates were observed under the microscope to obtain the colony morphology i.e. colour, shape, size, nature ofcolony and pigmentation [2][6]

### [2.6]Enzyme assay for amylase enzyme

A suitable volume of isolated culture broth incubated for 48 hr was centrifuged at 5000 rpm for 20 min at 4°C. Supernatant was recovered. Amylase was determined by spectrophotometric method. 1.0ml of crude enzyme into a test tube and 1ml of 1% soluble starch in sodium-phosphate buffer (pH 7) was added in test tube. The test tubes were covered and incubate at 35°C for 10 minutes.[5] Then 2.0 ml DNS reagent was added in each tube to stop the reaction and kept in boiling water bath for 10 minutes.After cooling

at room temperature, final volume was made to 10ml with distilled water. And the absorbance was read at 540 nm by spectrophotometer. A unit of amylase activity was defined as the amount of amylase required to catalyze the liberation of reducing sugar equivalent to 1 mol of D glucose per minute under the assay conditions.

### [3]Characterization of α- amylase

#### [3.1]Determination of optimum pH

1% Starch was used as a substrate. Substrate solution was prepared in sodium phosphate buffer at pH 5.5,6, 6.5, 7, 7.5, 7, and 8 in different test tubes. 1 ml each of crude enzyme solution was added into buffer tubes. Then the mixture was incubated at 35°C for 10 min, reactions were terminated by adding 2 ml DNS reagent and the mixturewas incubated in boiling water for 10 min.After cooling at room temperature, final volume was made to 10ml with distilled water and the activity of enzymes was determined by taking the absorbance at 540nm.

#### [3.2]Determination of optimum temperature

1 ml of substrate was taken into six different test tubes and 1 ml of phosphate buffer pH 7 was added in each test tubes. Tubes were marked with different temperature (at 25, 35, 45, 55, 65, 75°C). 1 ml of crude enzyme solution was added in each tube. Then tubes were incubated at specific temperature for 10 minutes. Reactions were terminated by adding 2 ml DNS reagent and the mixture incubated in boiling water for 10 min .After cooling at room temperature, final volume was made to 10ml with distilled water andthe activity of enzymes were determined by taking the absorbance at 540nm.

### [4] RESULTS AND DISCUSSION

Bacterial isolates from different sources were tested for production of amylase by the starch hydrolysis test. On the basis of the area of clearance, several bacterial isolates were selected, after which one out offour isolates was selected for further evaluation.

**Table 1**: Effect of varying temperature on α-amylase activity.

| Temperature | α- amylase activity U/ml |
|---|---|
| 25°C | 5.1 |
| 35°C | 9 |
| 45°C | 7.1 |
| 55°C | 6.3 |
| 65°C | 4.2 |
| 75°C | 4 |

**Table 2**: Effect of varying pH on α- amylase activity.

| pH | α- amylase activity U/ml |
|---|---|
| **5.5** | **3.2** |
| **6** | **4.1** |
| **6.5** | **4.9** |
| **7** | **9.1** |
| **7.5** | **7.8** |
| **8** | **7.0** |

**Fig 1:** Bacillus sp. showing positive starch hydrolysis.

**[5] Cultural characterization**

Culture showed small colony, low convex elevation and was opaque with no pigmentation.Bacterial isolates were found to be gram positive and spore forming.

**[5.1]Biochemical characterization**

Culture was found to have the ability to hydrolyze Starch and gelatin, Oxidase positive, Catalase positive, Nitrate reduction positive, Urease test and VP test positive. Whereasindole and citrate test were negative.

**Table 3:** Observation of staining

| Staining | Result |
|---|---|
| **Gram staining** | Gram +ve. |
| **Endospore staining** | Spore forming. |

**Biochemical characterization**

**Table 4:** Observation of biochemical tests

| Test | Result |
|---|---|
| Starch hydrolysis | + |
| Oxidase test | + |
| Catalase test | + |
| Nitrate reduction | + |
| Gelatin hydrolysis | + |
| Indole test | - |
| VP test | + |
| Urease test | + |
| Citrate test | - |

**[5.3]Enzyme assay and Optimization**

Enzyme activity of crude enzyme was performed by using DNS reagent. And the enzyme activity observed for this strainwas found to be 9 U/ml. Among physical parameters, pH of the growth medium plays an important role by inducing morphological changes in microbes and in enzyme secretion [10].The pH change observed during the growth of microbes also affected product stability in the medium. As shown in table2the isolate was able to grow in the pH range of 5–8, but pH 7.0 was the optimum for the growth of the cultures.

Temperature also plays the significant role in the stability in enzyme activity. 35°C wasfound to be optimum temperature at which enzyme activity was found to be higher.

**Fig 2:** Effect of different pHon α amylase activity.

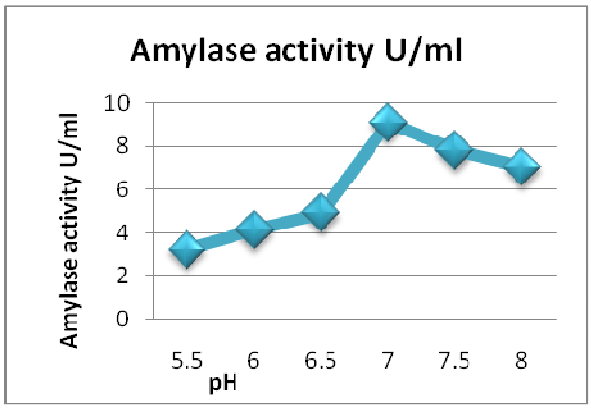
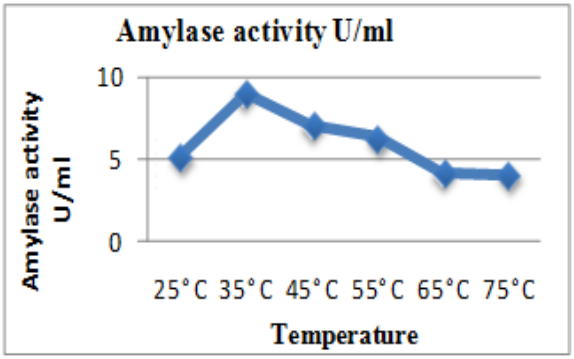



**Fig3:** Effect of different temperature on α amylase activity




**CONCLUSION**

Strain was isolated from the soil sample near sewage which has the capability to produce amylase. The nature of culture conditions,temperature and pH for the optimal production of α-amylase by the isolated bacterial strain has been developed in this study.

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## REFERENCES

[1]. Aiyer, P. V., (2005). Amylases and their application. African Journal of Biotechnology. 4(8) 1525 1529.

[2]. Dipali Parmar and Ajit Pandya.,(2012),Characterization of amylase producing bacterial isolatesBulletin of Environment, Pharmacology and Life Sciences.**;** Volume 1 [6] May: 42 –47

[3]. MK Sindhu; BK Singh; T Prased. In. Phytopathol., 1997**,** 34, 269-271.

[4]. M Rao; A Tankasale; M Ghatge; V Desphande. Microbiol. Mol. Biol. Rev., 1998**,** 62, 597–634.

[5]. Fisher, E. and I. Stein, 1961. α–amylase from human saliva. Biochem. Prep., 8: 27Sneath, P.H.A., 1994. Endospore forming gram positive rods and cocci. In: W.M. Hensyl, (ed.), Bergey's Manual of Systematic Bacteriology. pp:1104-39. Williams & Wilkins Co., Baltimore.

[6]. Quang DN, Judiet M, Rezessy S, Agoston H: Optimization of composition of media for the production of Amylolytic enzymes by Thermomyces lanuginosus ATCC 34626. Food Technol Biotechnol 2000; 38, 229-234.

[7]. Kim, T.U., G.B. Gum., J.Y. Jeong and S.C. Young, 1995. Purification and characterization of a maltotetraose forming alkaline alpha amylase from an alkalophilic Bacillus strain, GM 8901. Appl. Environ. Microbiol., 61: 3105–12

[8]. Capuccino JC, Sherman N: Microbiology- A laboratory manual. 6th edition. 2001; pp: 491-496.

[9]. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B: Microbial-amylases: A Biotechnological perspective. Process Biochem 2003; 38, 1599-1616.

[10]. Ensari,N.Y., Otludil, B. and Aytekin, M.C (2006). Effect of starch induced bacterial growth and amylase production in Bacillus subtilis. Starch, 47(8): 315-321.

[11] KR Aneja. Experiments in Microbiology Plant Pathology and Biotechnology, Fourth Edition, New Age International (P) Ltd., Publishers, New Delhi, 2003; pp 320.

[12]E Yavuz. M. Sc. thesis, İzmir Institute of Technology İzmir, Turkey, 2003.

[13] PV Aiyer. Afr. J. Biotechnol., 2005, 4(13), 1525-1529.

[14] BK Lonsane; MV Ramesh. Advances in Appl. Microbiol., 1990**,** 35: 54-56.