ISOLATION, SCREENING AND CHARACTERIZATION OF AMYLASE PRODUCING BACTERIA FROM SOIL OF POTATO DUMP SITES FROM DIFFERENT REGIONS OF MADHYA PRADESH

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Abstract: Amylases are one of the most widely used enzymes in industries such as food, fermentation, starch processing, textile and paper. In the present study, bacteria were isolated from potato dump sites of different regions of Madhya Pradesh, screened for the production of amylase and their optimum growth conditions were determined. A total of 18 bacterial colonies were isolated from the different soil samples. Five bacterial isolates, displayed zones of clearance in starch hydrolysis test. The isolate, which showed maximum amylase activity on quantitation, was selected. Characteristic studies and microscopic observation of the strain indicates that it belongs to the genus *Bacillus* and will be later used for further characterization at molecular level. The optimum pH for enzyme activity was found to be at pH 7.0 and the optimum temperature for maximum amylase activity was observed at 40ºC.

Keywords: Activity, Amylase, Characterization.

Introduction: Among various industrial enzymes, amylases are of great significance in the biotechnology industries with widespread application in food, fermentation, textile and paper [1]. They are degrading enzymes which breakdown the starch and give various low molecular weight products like glucose, maltose, maltotriose and dextrins [2]. Amylases are acquired from several origins like plant, animal, fungus and bacteria. Amongst these, microorganisms are mainly used for the industrial production due to advantages such as bulk production capacity, ease of manipulation and thermostability [3], [4]. Many bacteria are used for the production of amylases including *Bacillus sp., Lactobacillus, Escherichia, Strepotmyces sp., Pseudomonas sp. etc*. There is a need to isolate and characterize more potential strains for large scale production of amylase at industrial level.

In the present work, isolation, screening and characterization of amylase producing bacteria from the soil samples collected from different potato dump sites has been reported. Production conditions like pH, temperature, etc. were optimized to achieve high amylase production and enhanced activity.

Materials and Methods:

Sample collection: Soil samples were collected from different potato dump sites of surrounding regions of Rau, Indore and Dhar districts of Madhya Pradesh, with the help of sterile spatula. The samples were transferred to sterile plastic bags in aseptic conditions.

Isolation of Amylase Producing microorganisms: One gram of the collected soil sample was added to 9 ml of sterile distilled water. Serial dilution was done up to 10^{-5} and spread plated onto nutrient agar enriched with 1% starch. Then the plates were incubated at 37°C for overnight.

Screening for Amylase Activity (Starch Iodine Test): Bacterial cultures were screened for production of amylase by starch hydrolysis test on starch agar plate [5]. The pure isolated colonies were streaked on starch agar plates with starch as the only carbon source. After incubation at 37ºC for 24 hrs., the plates were flooded with Gram's iodine (Gram's iodine- 1 g iodine crystals added to 2 g potassium iodide solution, and 100 ml of water, stored at room temperature) to produce a deep blue colored starch-iodine complex. A zone of clearance forms, with disappearance of blue color, which is the basis of the detection and screening of a amylase producing strain. The amylase producers displaying maximum diameter of zone of clearance, were further quantitated [6]. The pure cultures in starch- nutrient agar slants were kept at 4° C.

Amylase production medium: A loopfull of bacterial culture was transferred from starch-nutrient agar slants to starch- nutrient broth at pH 7 for activation and incubated in a shaker at 37°C at 120 rpm for 24 h. Fermentation medium contained soluble starch (10 g/L) peptone (5 g/L), $(NH_4)_2 SO_4$ (2 g/L), KH_2PO_4, (2g/L), K_2HPO_4 , (2 g/L), $MgCl_2$, (0.01 g/L).The fermentation medium was inoculated with the activated culture (20% v/v) and incubated in shaker at 37° C for 24 hrs. After this, the culture medium was centrifuged at 10000 rpm for 15 min to obtain the supernatant, which was used for enzyme assay.

Enzyme assay: Amylase activity was assayed as described by Bernfeld et al. [7] with some modifications. Briefly, 1.5 ml of 1% starch in 2 ml, 0.1M phosphate buffer (pH 6.5) and 0.5ml of diluted enzyme were incubated for 15 min at 37° C. The reaction was halted by adding 1ml of dinitrosalicylic acid (DNS) reagent and kept in a boiling water bath

for 10 min and diluted with 8 ml of distilled water. The absorbance was measured at 540 nm against blank prepared as above without incubation. One unit of α-amylase activity was defined as the amount of enzyme that liberates 1µmole of reducing sugar (maltose equivalents) per minute under the assay conditions [8]. All experiments were carried out in triplicate.

Characterization of Amylase:

Morphological and Biochemical characterization of the isolated strain: Gram staining was performed to know whether the isolate was Gram positive or negative. The isolates were observed microscopically to obtain the colony morphology i.e. color, shape, size, nature of colony and pigmentation [9]. The tests for biochemical characterization were performed.

Determination of optimum pH: 1% Starch was used as a substrate. Substrate solution was prepared in phosphate buffered saline (PBS) at pH 6, 6.5, 7, 7.5 and 8 in different test tubes. 0.5 ml each of diluted crude enzyme solution was added into all the tubes. Then the mixture was incubated at room temperature for 15 min, reactions were stopped by adding 1 ml DNS reagent and then the tubes were kept in boiling water bath (100°C)for 10 min. After cooling at room temperature, final volume was made to 12 ml with distill water and the activity of enzyme was determined spectrophotometrically at 540 nm.

Determination of optimum temperature: 1.5 ml of 1% starch was taken into six different test tubes and 2 ml of phosphate buffer saline was added in each test tube. Tubes were marked with different temperature (at 30, 35, 40, 45, 50, 55°C). 0.5 ml of diluted enzyme solution was added in each tube. Then tubes were incubated at specific temperature for 10 minutes. Reactions were terminated by adding 1 ml DNS reagent and the tubes were incubated in boiling water bath (100°C) for 10 min. After cooling at room temperature, final volume was made to 12 ml with distill water and the activity of enzyme was determined by taking the absorbance at 540 nm.

Results and Discussion:

Isolation and selection of α-amylase producing bacteria: Bacteria isolated from starch rich materials may have better potential to produce enzyme under adverse conditions [10]. Microorganisms that produce amylases could be isolated from places such as soil around mills, processing factories as well as flour markets [11]. During the study, amylase producing bacterial strains was isolated from the soil of potato dump sites. A total of 18 bacterial strains were isolated. Among which 5 strains gave zone of clearance with iodine solution on starch hydrolysis test. These were further selected and quantified (Table I). The isolate showing maximum absorbance (Fig. 1) was further optimized and characterized and found to belong to the genus *Bacillus*. Among physical parameters, pH of the growth medium plays an important role by inducing morphological changes in microbes and in enzyme secretion. Most of the amylase producing bacterial strains revealed a pH range between 6.0 and 7.0 for normal growth and enzyme activity [6]. As shown in table IV the isolate was able to grow in the pH range of 6–8, but pH 7.0 was optimum for the growth of the culture. Temperature also has an important contribution in the stability in enzyme activity. 40°C was found to be optimum temperature at which enzyme activity was found to be higher (Table V).

Fig 1: Isolate APIB-2 showing positive starch hydrolysis test

Table I: Quantitation of all the isolates (OD at 540 nm)	
Isolates	**Absorbance (540 nm)**
APIA5	0.363
APIC7	0.388
APIB2	0.486
APIA9	0.452
APIC10	0.342

Table II: Microscopic characteristics of the strain APIB2	
Form	Irregular
Elevation	Flat
Size	Medium
Margin	Regular
Opacity	Translucent
Colour	Pale
Surface	Moist, shiny
Gram staining	(+)ve
Shape	Rod
Motility	Motile

Table III: Biochemical and Cultural characteristics of the strain APIB2:	
Starch hydrolysis	(+) ve
Indole production	(-) ve
Voges-Proskaeur test	(-) ve
Catalase production	(+) ve
Citrate production	(+) ve
Oxidase production	(+) ve
Urease test	(-) ve
Lactose fermentation	(+) ve
Nitrate reduction test	(+) ve

Table IV: Effect of varying pH on amylase activity of isolate APIB2:	
pH	Amylase activity (U/ml)
6	6.72
6.5	8.26
7.0	9.19
7.5	7.58
8.0	7.24

Table V: Effect of varying temperature on amylase activity of isolate APIB2:	
Temperature	Amylase activity (U/ml)
30	6.32
35	7.83
40	9.36
45	8.98
50	7.95
55	7.66

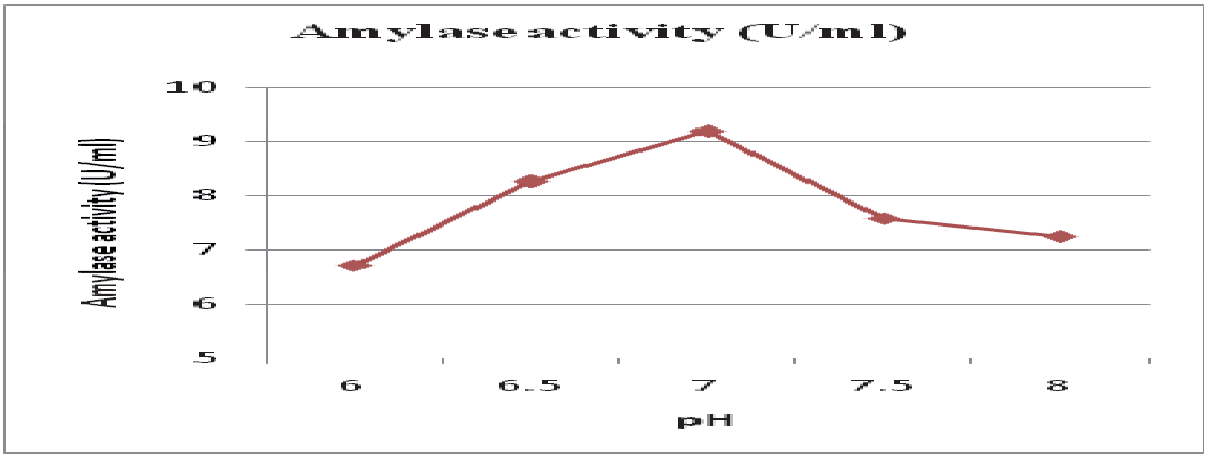

Fig 2: Effect of different pH on amylase activity

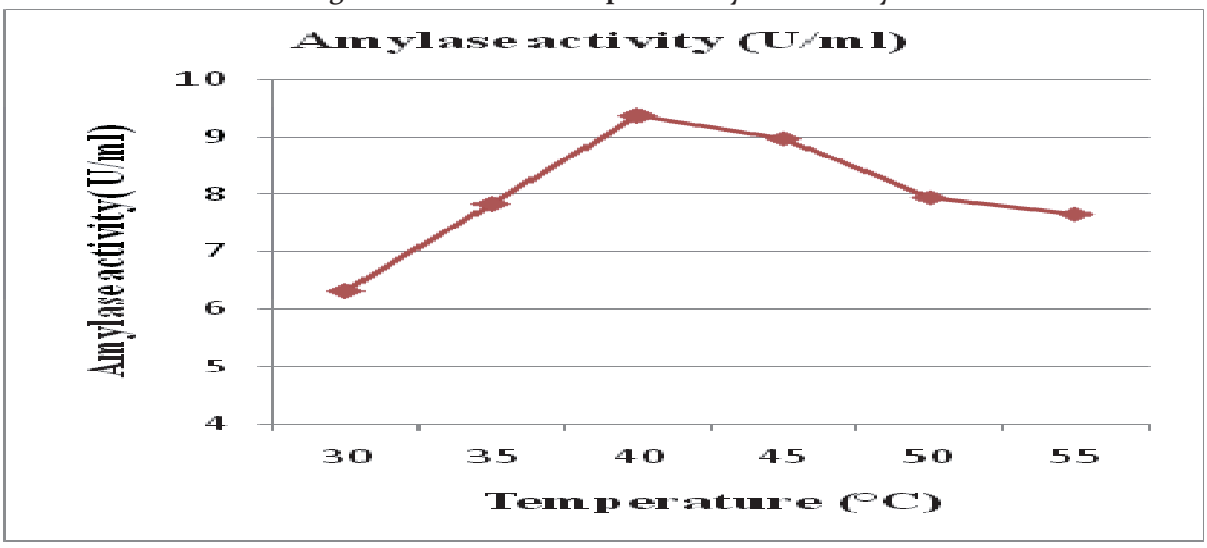

Fig 3: Effectof different temperature on amylase activity

Conclusion: A *bacillus* strain was isolated from the soil sample of potato dump site which has the capability to produce amylase. The nature of culture conditions, temperature and pH for the optimal production of amylase by the isolated bacterial strain has been developed in this study.

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References:

1. Pandey, P. Nigam, C. R. Soccol, V. T. Soccol, D. Singh, R. Mohan, "Advances in microbial amylases,"Biotechnol. Appl. Biochem. vol. 31, pp. 135-152, 2000.
2. P. V. Aiyer, "Amylases and their application,"Afr J Biotechnol. vol. 8, pp. 1525-1529, 2005.
3. M. K. Sindhu, B. K. Singh, T. Prased, In. Phytopathol., vol. 34, pp. 269-271, 1997.
4. Burhan, U. Nisa,C. Gökhan, C. Ömer, A.Ashabil, G. Osman, "Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic*Bacillus* Sp. isolate ANT-6," Process Biochem. vol. 38, pp. 1397-1403, 2003.
5. J. F. Shaw, F. P. Lin, S. C. Chen, and H. C. Chen, "Purification and properties of an extracellular α-amylase from *Thermus*sp.," Botanical Bulletin of Academia Sinica. vol. 36, pp. 195-200, 1995.
6. R. Gupta, P. Gigras, H. Mohapatra, V. K. Goswami, B. Chauhan, "Microbial α-amylases: a biotechnological perspective," Process Biochem. vol. 38, pp. 1599-1616, 2003.
7. P. Bernfeld, "Amylase α and β," in Methods in Enzymology, Colowick SP, Kaplan NO, ed., Academic Press Inc, New York 1: 1955, pp. 149-158.
8. G. L. Miller, " Use of dinitrosalicyIic acid reagent for determination of reducing sugar," Analytical Chemistry. vol. 3, pp. 426-428, 1959.
9. D. Parmar, A. Pandya, "Characterization of amylase producing bacterial isolates," Bulletin of Environment Pharmacology and Life Sciences.vol.6, pp. 42 –47, 2012.
10. S. Mishra, N. Behera, "Amylase activity of starch degrading bacteria is isolated from soil receiving kitchen wastes,"Afr J Biotechnol.vol. 7, pp. 3326–3331, 2008.
11. T. Fossi, F. Taveaand, T. Ndjonenkeu, "Production and partial characterization of a themostable amylase from ascomycetes yeast strain isolated from starchy soils," Afr. J. Biotechnol. vol. 4, pp. 14–18, 2005.

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