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**OPTIMIZATION OF AMYLASE PRODUCED BY BACTERIA ISOLATED FROM
KITCHEN WASTE DUMPSITES**

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ABSTRACT

Nowadays the new potential of using microorganism as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extra cellular enzymatic activity in several microorganisms. The present study was concerned with isolation of amylase producing bacteria from kitchen waste dump site soil samples and optimization of culture conditions for amylase production. A total of 14 soil samples were collected from different areas. Based on morphological and biochemical characterization *Bacillus* sp. was isolated from 10 (71%) soil samples, out of which 8 (80%) belonged to *Bacillus subtilis* and 2 (20%) belonged to *Bacillus brevis*. *Bacillus subtilis* isolate (AP5) was used for further study for the production of amylase enzyme as it recorded the largest zone of amyolytic activity. The amylase producing ability was checked using the DNS method. Studies on crude amylase revealed that optimum temperature and pH, for enzyme activity was 35°C, 7 respectively as maximum enzyme activity was recorded on these parameters (7.2 IU/ml and 8.2 IU/ml respectively). Maximum amylase production (49.22 IU/ml) was obtained when starch was used as carbon source. Among organic and inorganic nitrogen sources trypton and ammonium nitrate gave optimum yield of 29.62 IU/ml and 56.72 IU/ml respectively. Inorganic nitrogen sources gave comparatively higher yields than organic nitrogen sources.

Keywords: *Bacillus* sp., Amylase, Kitchen waste, Optimization

INTRODUCTION

Many successful industrial fermentation processes heavily depend on enzymes from microorganisms. Amylases constitute one of the important groups of enzymes that are used in a wide range of starch industries and account for nearly 25% of the total sale of enzymes. Amylases are produced by variety of living organisms, ranging from bacteria to plants to humans. Bacteria and fungi secrete amylases to the outside for their cells to carry out extracellular digestion [1]. Although there are many microbial sources available for producing amylases, only a few such as *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquifaciens* are recognized as commercial producers [2]. There are two main types, that is α and β amylase based on their pattern of activity [3]. Amylases have wide variety of applications ranging from conversion of starch to sugar syrups to the production of cyclodextrins for the pharmaceutical industry. Amylases are also used in textile, detergent, paper and distilling industries. α -Amylase production has a wide range of applications in many industries such as baking, brewing, ethanolic production, wine and textile industries [4]. Taking into consideration the applications of amylases the present study was designed for isolation and screening of amylolytic bacteria from kitchen

waste dump soil in submerged cultures and optimization the cultural conditions for production of amylase.

MATERIALS AND METHODS

Collection of Soil Samples and Isolation of Bacteria

Fourteen different kitchen waste dump site soil samples were collected from Patiala, Sirhind, Chandigarh, Nabha, Landran, Mohali, village Tangori and Motemajra in Punjab, India. For the isolation of the starch degrading bacteria, serial dilutions (10^{-1} to 10^{-9}) of the soil sample were prepared using distilled water. It was then cultured on starch agar medium using the spread plate technique and incubated overnight at 37°C . After incubation colonies produced were observed and finally maintained on nutrient agar slants using the streak plate technique.

Plate Assay Method

The isolates were tested for amylase activity by employing zone clearing technique using starch agar medium [5]. The inoculated plates were incubated at 37°C for two days and the zone of hydrolysis of starch was detected by flooding the plates with iodine solution. The development of blue colour indicated the presence of starch, while the areas around the hydrolytic bacteria appeared clear. Strains with distinct zone of clearance were further

characterized morphologically and biochemically following Bergey's Manual of Determinative Bacteriology [6]. Strain with maximum zone of clearance was selected for further studies.

Inoculum and Fermentation Medium

Inoculum was prepared by addition of sterile distilled water into freshly grown nutrient agar slant, from this 0.5 ml of cell suspension was inoculated into 100ml of sterilized fermentation medium and incubated at 35°C for 10 hrs. The composition of the fermentation medium was (g/l) 6.0g Bacteriological peptone; 0.5g MgSO₄.7H₂O; 0.5g KCl; 1.0g starch, pH 7 [7].

Estimation of Crude Extracellular Amylase Activity

The isolate showing maximum diameter of enzyme activity was propagated in broth supplemented with 1% (w/v) starch medium at incubator shaker at 150 rpm, 37°C for 24 hrs. After incubation time resultant broth was centrifuged at 10000, rpm for 10 min. and the supernatant was collected as the source of crude enzyme. The assay was carried out by using soluble starch as substrate, few drops of 3-5, dinitro salicylic acid reagent were added and the absorbance was measured at wavelength of 540 nm. 1 unit of activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugars

equivalent to 1μmol of maltose/min under the assay conditions [8].

Optimization of Cultural Conditions

Effect of Temperature on Enzyme Activity

To determine the effect of temperature on amylase production, the submerged fermentation was carried out by varying incubation temperature of the culture from 25-50°C with an interval of 5°C in a 250mL Erlenmeyer conical flasks [7].

Effect of pH on Enzyme Activity

For determination of suitable pH range for enzyme activity, the bacterial culture was inoculated in fermentation medium prepared with different pH, ranging from 6.5-9.5 with intervals of 0.5 in 250 mL Erlenmeyer conical flasks [7].

Effect of Various Carbon and Nitrogen Sources

Carbon sources (0.04g/g dry substrate) starch, glucose, sorbitol, fructose and maltose, and organic (tryptone, yeast extract, casein and peptone) and inorganic (ammonium nitrate, ammonium chloride, potassium nitrate, urea) nitrogen sources (0.02g/g dry substrate) were supplemented as individual components to the production media to check the effect of these sources on enzyme production [9].

RESULTS AND DISCUSSION

A total number of 10 isolates were obtained from 14 different kitchen waste dump site soil

samples which showed amylolytic activity. From morphological and biochemical characteristics, isolates were identified as *Bacillus* sp., according to the description given in Bergey's Manual of Determinative Bacteriology [6]. Isolates AP1, AP2, AP3, AP4, AP5, AP6, AP7 and AP10 belongs to *Bacillus subtilis* and isolates AP8 and AP9 belonged to *Bacillus brevis*. The isolate AP5 has the highest halo (2.9 mm) and selected for further analysis (Table 1).

Effect of different temperatures on production of amylase from *Bacillus* sp. was shown in Figure 1. The maximum amylase production was obtained at 35°C with a enzymatic activity of 7.2 IU/ml. Optimum temperature was observed for the production of α -amylase from Banana stalk using *B. subtilis* was also 35°C as reported by Krishna and Chandrasekaran [10]. The enzyme stability trend as reported in the present study, agreed with the behavior of amylases from *Bacillus* sp. [11]. Sajitha *et al.* [12] also reported maximum enzyme production at 35°C. Omemu *et al.* [13] observed an increase in enzyme activity progressively with increase in temperature from 20°C which reaches maximum at 60°C. Mabrouk *et al.* [14] observed maltogenic amylase produced from acidic *Bacillus* sp. (US149) and maximum enzyme activity was obtained at 40°C.

The amylase production in the present investigation by *Bacillus* sp. isolated from kitchen waste dumpsites was found to be maximum at pH 7 (8.2 IU/ml) (Figure 2). This result of pH 7 was found similar with the results of earlier studies conducted by Vidyalakshmi *et al.* [7] and Nimkar *et al.* [15]. The optimum pH was found to be 7.5 with activity of 9.5 IU/ml [8]. The maximum enzyme activity was at a pH range from 4.8 to 9.2 for an amylase obtained for *B. licheniformis* isolated from a cassava processing waste [16]. Maximum enzyme activity was observed at pH 5 [9]. Optimum growth and enzyme production was achieved at pH 6 [17]. Utong *et al.* [18] observed the ability of *Bacillus sphericus* to produce extracellular enzyme if grown at pH 6-9 range.

The amylase activity from different sugars used as carbon source was presented in Figure 3. Enzyme production was maximum when starch was used as carbon source (49.22 IU/ml). Potato starch and corn starch were the best inducer for α - amylase production followed by maltose [19]. Highest α -amylase yield was obtained by addition of Na-citrate and sucrose for the strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens*, respectively [20]. The maximum amylase activity was observed at 2% of starch concentration in

Bacillus sp. [21].

The influence of organic nitrogen sources such as tryptone, yeast extract, peptone and inorganic nitrogen sources such as ammonium nitrate, ammonium chloride and urea on amylase production was determined (Figure 4 and 5). Tryptone was found to be good organic nitrogen source for amylase production (29.62 IU/ml). Tryptone has been reported to be the best nitrogen source for amylase production [22]. Deb *et al.* [23] (2013) also reported tryptone to be best organic nitrogen source and ammonium salts

among inorganic nitrogen sources, as best stimulators for production of amylase. Inorganic nitrogen sources gave comparatively higher yields than organic nitrogen sources. Enzyme production was maximum (56.72IU/ml) when ammonium nitrate was used as inorganic nitrogen source in the culture media in the present study. Our findings are in a good agreement with the findings of previous studies. It has been found that α -amylase production by *B. subtilis* DM-03 was maximum when ammonium chloride used as nitrogen source [24].

Table 1: Amylase Activity of Bacillus Isolates

Isolate Codes	Probable Identity	Zone of Amylase Activity (mm)
AP1	<i>Bacillus subtilis</i>	1.8
AP2	<i>Bacillus subtilis</i>	2.4
AP3	<i>Bacillus subtilis</i>	2.6
AP4	<i>Bacillus subtilis</i>	2.3
AP5	<i>Bacillus subtilis</i>	2.9
AP6	<i>Bacillus subtilis</i>	0.9
AP7	<i>Bacillus subtilis</i>	1.3
AP8	<i>Bacillus brevis.</i>	1.9
AP9	<i>Bacillus brevis.</i>	2.1
AP10	<i>Bacillus subtilis</i>	1.6

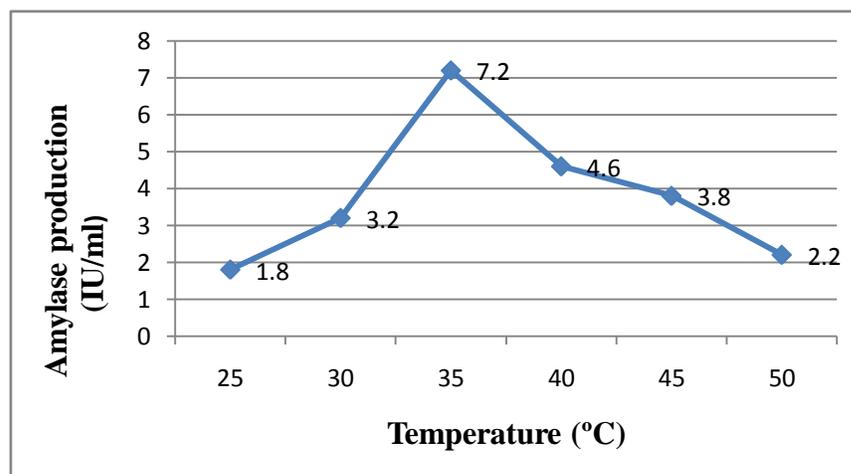


Figure 1: Effect of varying incubation temperature on amylase production

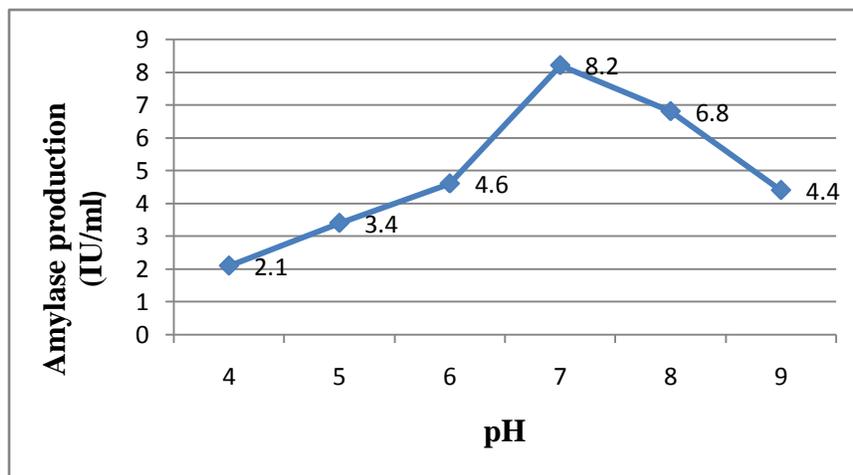


Figure 2: Effect of varying pH on amylase production

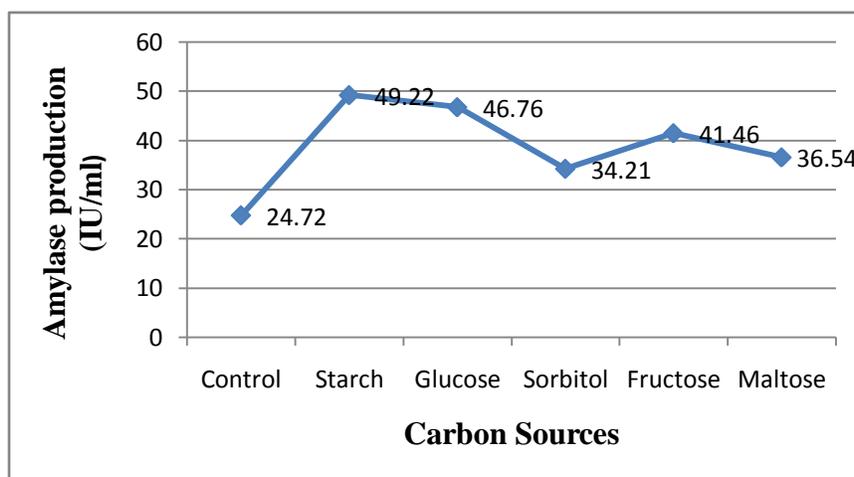


Figure 3: Effect of varying carbon sources on amylase production

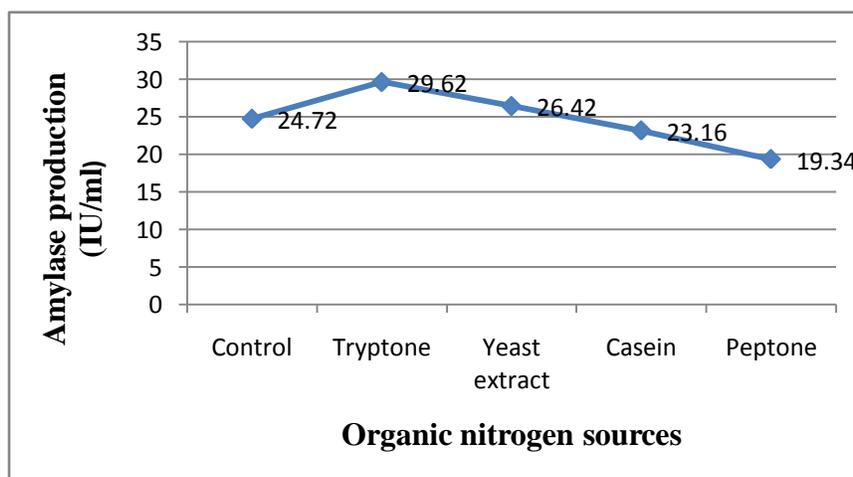


Figure 4: Effect of varying organic nitrogen sources on amylase production

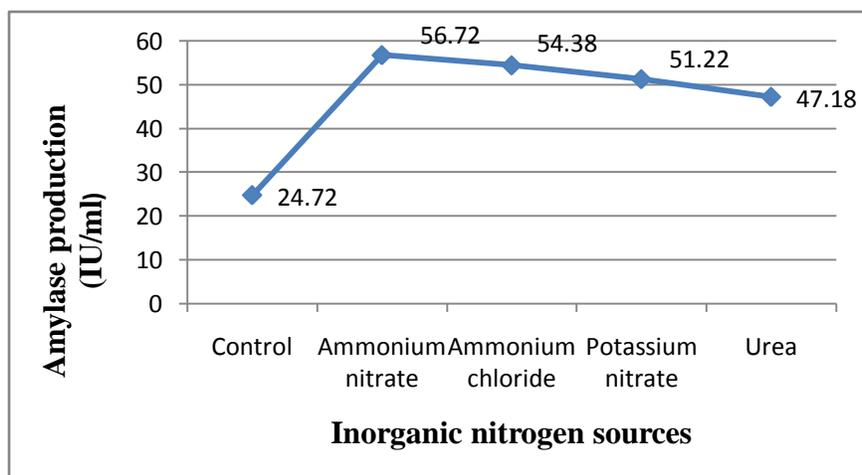


Figure 5: Effect of varying inorganic nitrogen sources on amylase production

CONCLUSION

In the present day scenario, amylases have a great commercial value in biotechnological applications. *Bacillus* sp. was isolated for amylases production. *Bacillus subtilis* AP5 isolate showed maximum amylase production and amylase production was increased by optimization of cultural conditions as medium optimization is a prime step for its commercial usage. The enzyme production by the bacterial isolate at temperature 35°C and at pH 7 was found optimum. It was revealed that starch as carbon source, tryptone and ammonium nitrate as organic and inorganic nitrogen sources gave optimum yield as compared to other carbon and nitrogen sources. It can be concluded that, *Bacillus subtilis* (AP5) can be a potential producer of amylase and simultaneously optimization of different conditions for the production of

amylase will overcome the demand of this enzyme. Due to the importance of these findings, further studies need be carried on in order to commercialize the production process.

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