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Full Length Research Paper

Isolation and optimization of amylase producing bacteria and actinomycetes from soil samples of Maraki and Tewedros campus, University of Gondar, North West Ethiopia

Gebreselema Gebreyohannes

Department of Biology, College of Natural and Computational Sciences, University of Gondar, P. O. Box 196, Gondar, Ethiopia.

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The objective of the present study was to isolate, identify and optimize potential amylase producing bacteria and actinomycetes from soil samples. The soil samples were collected from Maraki and Tewedros campus, University of Gondar. Isolation was done by serial dilution and spread plate method. Primary screening of amylolytic activity of the isolates was performed by starch agar plate method. The submerged state fermentation method followed for the production of amylase by the optimization of temperature, pH, fermentation time and substrate concentration. From the soil samples, 18 isolates were identified and subjected to primary screening for amylolytic activity. Of which, five isolates were observed with maximum amylolytic activity during the primary screening. During the submerged state fermentation, maximum amylase activity was observed at 48 h and then declined. The optimum temperature observed for maximum amylase activity of *Bacillus* was 40°C and *Streptomyces* at 37°C. The highest amylase activity was observed at neutral pH and 4% of starch concentration. The colony morphology, Gram reaction, biochemical tests and Bergey's manual of determinative bacteriology confirm the promising isolates belong to the genus *Bacillus* and *Streptomyces.* This preliminary study could provide base line information for the discovery of novel microbes from the natural resources for the production of amylase which will be used for multipurpose.

Key words: Amylase, isolation, optimization, submerged state fermentation.

INTRODUCTION

Amylase is an enzyme obtained from the microbes has been used by many industries as a source for production of foods and beverages. With the utilization of microorganisms it is possible to produce large scale and also easily manipulated for desired products (Sumrin et al., 2011). In general, enzymes produced from fungal and bacterial sources have many applications in industries (Aiyer, 2005). In addition, recent advancement in

*Corresponding author. E-mail: ggebreselema@yahoo.com.

biotechnological tool, utilization of amylase has widened in clinical research, medical chemistry and starch analytical chemistry. Earlier literatures highlighted that bacterial strains from the genus *Bacillus*, *Pseudomonas* and *Clostridium* and from the genus *Streptomyces* have been used to synthesize amylase (Kafilzadeh et al., 2012; Oyeleke et al., 2010). Multi-potential application and demand pave the way for increasing indigenous amylase production and searching for more efficient processes (Hmidet et al., 2009). In Ethiopia, there is no much work done in this area of research. The country has several undisturbed natural soil habitat. In this research work soil samples were collected from various locations of Maraki and Tewodros campus since these areas are abundant in plant biodiversity and soil types. Therefore, to contribute new knowledge in scientific world amylase producing microorganisms were isolated, identified, optimized and reported for the first time from this study area.

MATERIALS AND METHODS

Sample collection

Soil sample were collected from five locations in Maraki and Tewedros campus, University of Gondar, Ethiopia. The study area is located in the latitude and longitude of 12° 35' 21" N / 37° 26' 39" E. From the selected area, 100 g of top soil samples was collected after careful removal of debris in the collection site. The soil samples were collected by using a sterile spatula, kept in the polyethylene bag and transported to the microbiology laboratory for further analysis. Stock soil samples were stored at 4°C in a refrigerator for subsequent analysis.

Screening of potential amylase producing bacteria and actinomycetes by using starch hydrolysis test

Ten gram of soil sample was suspended in 90 mL of sterile saline water in a conical flask and mixed by vortex mixer. From this 10 mL of the diluted suspension was transferred into three conical flasks containing 90 mL of sterile saline water serially. From each conical flasks, 0.1 mL was transferred into starch agar plates (meat extract 3 g/L; peptic digest of animal tissue 5 g/L; soluble starch 2 g/L; agar 15 g/L; pH 7.2 ± 0.1) in triplicate. Then, the sample was distributed evenly by using L-shaped glass rod and incubated at 37ºC for 24 h. After incubation period, colonies were further sub-cultured on the respective medium to obtain pure isolates and maintained at 4°C in a refrigerator for further investigation. The isolates were screened for amylolytic activity by streaking on the starch agar plates and incubated at 37°C for 24 h. Iodine solution was flooded on the starch agar plates for 30 s after 24 h incubation. Presence of clear zone around the growth of isolates were considered as amylase producers and sub-cultured on starch agar slants for further investigation.

Amylase production by using submerged state fermentation

A mineral broth medium (peptone 6 g/L; $MgSO_4$ 0.5 g/L; KCl 0.5 g/L and starch 1 g/L) was prepared. From the broth medium, 90 mL was transferred into 150 mL capacity Erlenmeyer flasks and sterilized at 121°C for 15 min. A loopful of inoculum was transferred into five test tubes having a 10 mL of sterile nutrient broth. The test tubes were incubated at 37°C for 24 h until the visible turbidity and density becomes equal to 0.5 McFarland standards ($1x10^8$ CFU/mL). Then after, 2 mL suspension of the isolates was taken from overnight cultures of test tube and inoculated into 90 mL of flasks and incubated in a water bath by adjusting the temperature at 25, 30, 35, 37, and 40°C for 24, 48, 72 and 96 h under the rotary shaker by the speed of 150 rpm. Finally, the fermented culture was poured into centrifuge tubes, spin for 20 min at 5000 rpm and extracted by decantation method (Aiyer, 2005).

Effect of substrate concentration and fermentation time

The effect of substrate concentration was determined by using different concentrations of starch (1.0, 2.0, 3.0, 4.0 and 5.0%) in the amylase production medium. The effect of fermentation time was also determined by incubating the amylase production medium at different fermentation time (24, 48, 72 and 96 h).

Effect of pH and temperature

The effect of pH and temperature on amylase activity was confirmed by adjusting the pH value of the fermentation medium at 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and the temperature at 25, 30, 35, 37 and 40°C respectively.

Characterization and identification of amylase producing isolates

Cultural, morphological and biochemical characterization of isolates

The isolates were observed macroscopically and microscopically to characterize the colony morphology and gram reaction respectively. In addition, isolates were also characterized biochemically by different biochemical tests such as Simmons citrate, Urease, Methyl Red/Voges Proskauer (MR/VP) and Starch hydrolysis tests.

RESULTS AND DISCUSSION

Isolation and primary screening of amylase producing bacteria and actinomycetes

A total of 18 isolates were obtained from the collected soil samples and coded as Sp1-Sp18 based on their maximum clear zone respectively. Of the 18 isolates, five isolates showed higher clear zone after flooding with iodine solution (Table 1) and this result agreed with the report of Hmidet et al. (2009) and only a few selected strains of bacteria from soil sample were obtained. According to Bergey's Manual of Determinative Bacteriology, isolates were grouped into two genera; namely, genus *Bacillus* (Sp1, Sp3, Sp4 and Sp5) and genus *Streptomyces* (Sp2). As per the primary screening of the isolates, these two genera could be potential candidates for several industrial applications which were agreed by the report of Ashwini et al. (2011).

The five isolates were characterized by cultural and microscopic methods to differentiate their respective genera. Most of the isolates have shown a regular form,

Table 1. Isolates and their clear zone on starch agar plates during primary screening.

Isolates	Clear zone (mm)	Isolates	Clear zone (mm)
Sp1*	22 ± 0.20	Sp10	8 ± 0.01
Sp2*	20 ± 0.50	Sp11	7 ± 0.90
Sp3*	19 ± 0.20	Sp12	6 ± 0.55
Sp4*	18 ± 0.60	Sp13	6 ± 0.32
Sp5*	18 ± 0.10	Sp14	5 ± 0.45
SP6	11 ± 0.40	Sp15	5 ± 0.31
Sp7	10 ± 0.70	Sp16	5 ± 0.56
SP8	10 ± 0.90	Sp17	4 ± 0.11
Sp9	10 ± 1.00	Sp18	3 ± 0.22

Mean ± Standard deviation of triplicate determination for primary screening, *Isolates selected for amylase production.

Table 2. Cultural and microscopic characteristics of the five isolates.

Characteristics	Isolates				
	Sp1	Sp2	Sp3	Sp4	Sp5
Form	Regular	Irregular	Regular	Regular	Regular
Color	Creamy	Rough whitish	Creamy	Creamy	Creamy
Gram staining	Positive	Positive	Positive	Positive	Positive
Shape	Rod	Filamentous	Rod	Rod	Rod

Table 3. Biochemical characteristics of isolates.

Biochemical characters	Isolates				
	Sp1	Sp2	Sp3	Sp4	Sp5
Starch hydrolysis test	+	+	+	+	+
Urease test	+	+	+	+	+
Simon's Citrate test	+	-	-	-	+
Methyl Red/Voges Proskauer test	+/+	+/+	+/+	+/+	+ /+
Indole test	+	+	+	+	+

+ Positive, - Negative.

creamy color and rod shape of colony morphology (Table 2).

Biochemical characterization

Based on the biochemical tests, all the isolates showed positive results of starch hydrolysis, Urease, MR/VP and indole tests (Table 3).

Effect of fermentation time on amylase activity

All the isolates were showed maximum amylase activity at 48 h of submerged fermentation time and then declined (Figure 1). Similar findings were also observed on *Bacillus subtilis* and *Bacillus* sp. DLB9 (Shyam et al., 2013). The reason for amylase activity decrement after 48 h might be due to the suppression and accumulation of other by-products in the fermentation medium and also depletion of nutrients as reported by other studies (Haq et al., 2010).

Effect of starch concentration on amylase activity

In general, amylase activity was increased with the increment of starch concentration from 1 to 4%. In this study, highest amylase activity was observed at 4% starch concentration (Figure 2). If the starch concentration

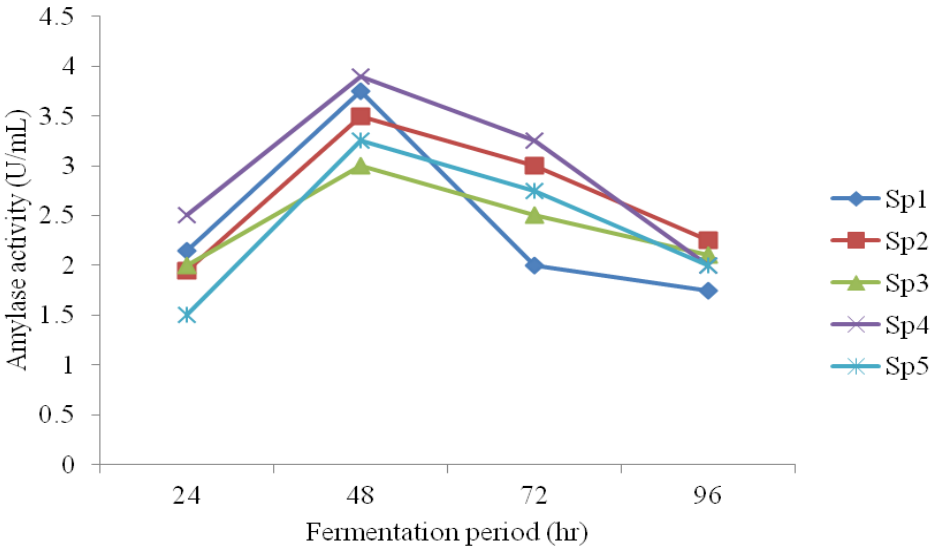

Figure 1. Effect of fermentation time on amylase activity.

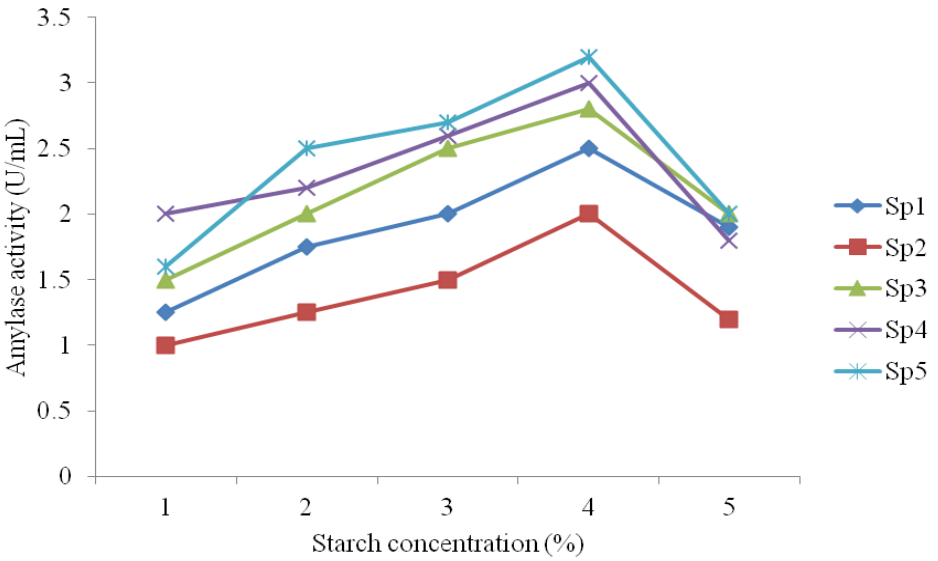

Figure 2. Effect of starch concentration on amylase activity.

goes beyond 4% amylase activity was declined. This might be associated with metabolizing capacity of the isolates within the short period of time when the starch concentration was increased. The present findings are in corroborating with the report of earlier findings on amylase activity obtained from *Bacillus* species (Oyeleke and Oduwole, 2009).

Effect of pH on amylase activity

In this study, highest amylase activity was observed at neutral pH. These results was also in agreement with the previous report for amylase activity of *Bacillus* strains such as *B. thermooleovorans* NP54, *B. coagulans*, *B. licheniformis*, and *B. subtilis* JS-2004 within the range of 6-7 pH (Gupta et al., 2010; Mendu et al., 2005; Adeyanju et al., 2007; Mrudula and Kokila, 2009). This implies that capability of the amylase activity within the neutral pH might be due to the fact that the isolates were inactive in the acidic or alkali medium. Different microorganisms have different optimum pH; if any variation on pH value results in poor microbial growth and amylase activity (Lonsane and Ramesh, 2009; Pandey et al., 2000).

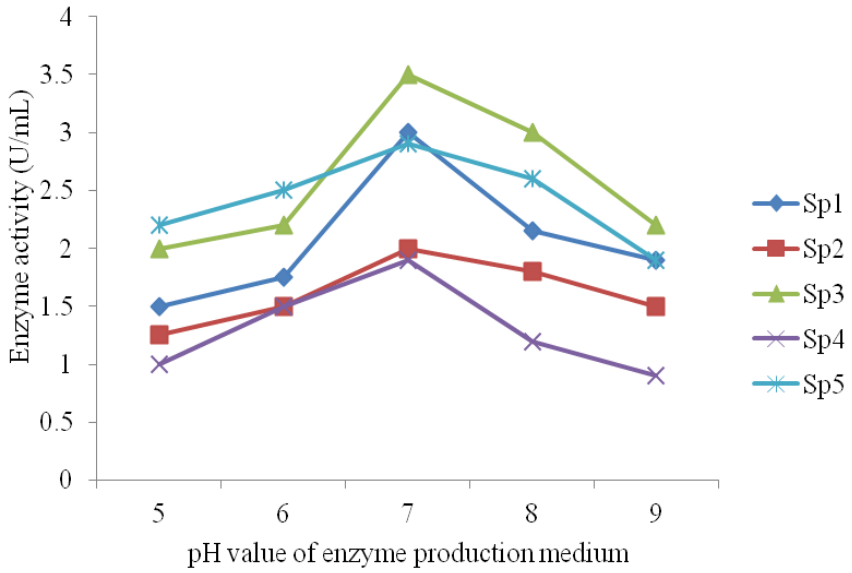

Figure 3. Effect of pH on amylase activity of the five isolates.

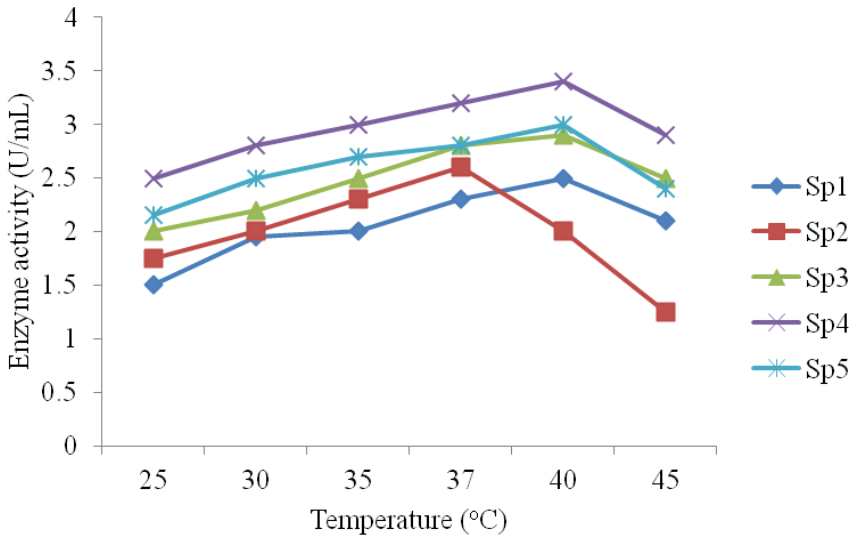

Figure 4. Effect of temperature on amylase activity.

(Figure 3).

Effect of temperature on amylase activity

Temperature is one of the environmental factors for amylase production which is usually varied from one organism to another. For example, *Bacillus amyloliquefaciens, B. subtilis, B. licheniformis* and *B. stearothermophilus* are some of the commonly used *Bacillus* sp. reported to produce amylase at 37-60°C (Asgher et al., 2007). In the present study, maximum amylase activity was observed at 40°C (Figure 4). The result also showed a positive correlation between the amylase activity and the incubation temperature up to 40°C, followed by a gradual decrease. At higher temperature, bacterial growth gets suppressed and consequently amylase activity was also inhibited (Oyeleke and Oduwole, 2009). The isolates grown well and revealed high amylase activity in the temperature ranged from 35 to 40°C. However, maximum amylase activity of *Bacillus* was 40°C and *Streptomyces* at 37°C. Mishra and Behera (2008) also reported that most of the bacterial isolates were produced and showed amylase

activity at elevated temperature in particular amylase activity of *Bacillus* species at the range of 40-45°C.

Conclusion

Based on the present findings, it is concluded that the soil is a potential source for amylase producing microorganisms, which could be exploited for the production of important industrial amylase. The results also showed that there was appreciable high amylase production from the isolates under optimized conditions of fermentation time, temperature, pH and starch concentration. *Bacillus* was found to be most frequently occurring amylolytic bacteria followed by *Streptomyces*.

Conflict of interests

The author declared that there is no conflict of interest.

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