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Isolation of Alkaline α-Amylase Producing Paenibacillus Illinoisensis from Lonar Lake.

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ABSTRACT

Enzymes from microbial sources were generally meet industrial demands. The bacterial isolate is screened for α-amylase production on starch agar. The effect of different parameters such as pH, temperature, incubation time, Carbon and Nitrogen sources were studied on the production of alkaline α-amylase. The optimum productions alkaline α - amylase were seen at pH 10.0, 40^0C and 72 hrs incubation times, in the 1% dextrose and Starch as a Carbon source and 0.5% Peptone Nitrogen source. The amylase activity from the bacteria is comparable with the activity of amylases from other organisms. Hence amylase would have a potential application in the food and pharmaceutical industry.

Keywords: Metallo-enzymes, α-amylases, Carbon and Nitrogen sources.

I. INTRODUCTION

Thousands of enzymes are found in living cells where they act as catalysts for the thousands of chemical reactions which occur. In addition to making life possible, many enzymes have numerous applications that affect our daily lives in other ways such as food processing, clinical diagnoses, sewage treatment, and the textile industry (Miller, 1992). The α- amylases (EC 3.2.1.1) are calcium metallo-enzymes, completely unable to function in the absence of calcium. Amylases are among the most important enzymes and are of great significance in present day biotechnology. Enzymes from microbial sources were generally meet industrial demands (Waghode and Garode, 2013; 2014).

The advantages of using thermostable amylases in industrial processes including the decreased risk of contamination, cost of external cooling and increased diffusion rate (Lin et al, 1998). Amylases with broad pH range have potential applications for Starch saccharification in Starch, textile industries and ingredient in detergents for automatic dish washers and laundries (Kim et al, 1995). The industrially important Bacillus strains, which are extensively used to produce α-amylase enzyme by Bacillus amyloliquefaciens, Bacillus licheniformis (Fogarty and Kelly, 1980),

The objectives of the study are to isolate of alkaline α-amylases producing Paenibacillus illinoisensis from the Lonar crater of Buldana district and study the effect of different parameters such as pH, temperature, incubation time, Carbon and Nitrogen sources on the production of alkaline α-amylases.

II. MATERIALS AND METHOD

Water samples are collected from Lonar lake and analyzed for isolation of bacteria. Bacteria are isolated on the nutrient agar which directly prepared in Lonar lake water. The standard Hi- Medias are used for the works. The bacterial isolate is screened for α-amylase production on starch agar with pH 10.5. The isolate of bacteria is characterized and identified according to Bergey's manual of determinative bacteriology (Holt, et al., 1994; Olajuyigbe et al., 2005). The Starch agar plates having pH-10.5 were inoculated on centre of the petriplates and incubated the plates at 37^0C for 24 hrs. After incubation, zone of hydrolysis of Starch on Starch agar were observed by flooding with surface of agar with iodine solutions. The objectives of the study are to

isolate of alkaline α-amylases producing Paenibacillus illinoisensis. The effect of different parameters such as pH, temperature, incubation time, Carbon and Nitrogen sources were studied on the production of alkaline α-amylase.

III. RESULT AND DISCUSSION

Bacterial flora was isolated from Lonar lake water samples. The Starch agar plates having pH-10.5 were inoculated on centre of the petriplates and incubated the plates at 37^0C for 24 hrs. After incubation, zone of hydrolysis of Starch on Starch agar were observed by flooding with surface of agar with iodine solutions. The zone of hydrolysis of Starch was measured at 48 hrs and 72 hrs for each isolates of bacteria which was shown in table 2.

Table 2. Screening of maximum yield of alkaline α- amylase producing Microorganism

Sr. No.	Isolate	Source	Amylase Production on Starch Agar (pH-10.5)		
			24 Hrs (mm)	48 Hrs (mm)	72 Hrs (mm)
1	Paenibacillus illinoisensis	Water	21	38	66

Production of alkaline α - amylase enzyme from *Paenibacillus illinoisensis* was done by using the different parameters such as pH, temperature, incubation time, Carbon and Nitrogen sources. The different pH such as 8.0, 9.0, 10.0 and 11.0 were used for the enzyme production. The optimum pH was found 10.0 where α - amylase enzyme formation as 4.30 mg. The different temperature such as 20, 30, 40 and 50^0C were used for the enzyme production. The optimum enzyme production was seen at temperature 40^0C where α - amylase formation as 4.55 mg. The effect of different incubation time such as 42, 48, 72 and 96 hrs was used for α - amylase enzyme production. The optimum enzyme production was seen at 72 hrs incubation where optimum α - amylase activity was 4.65 mg which shown in figure 1.

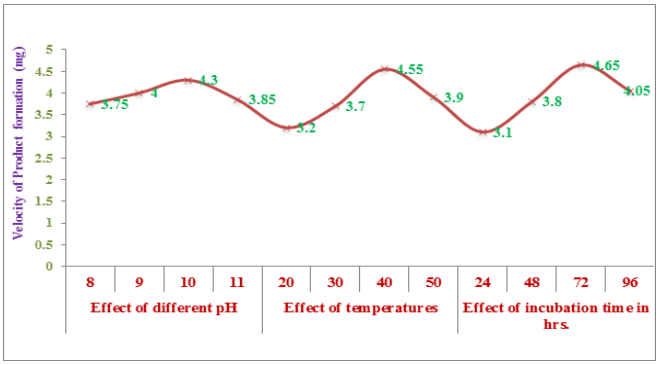

Figure 1. Effect of diferent parameters on enzyme α-amylase Production from Paenibacillus illinoisensis.

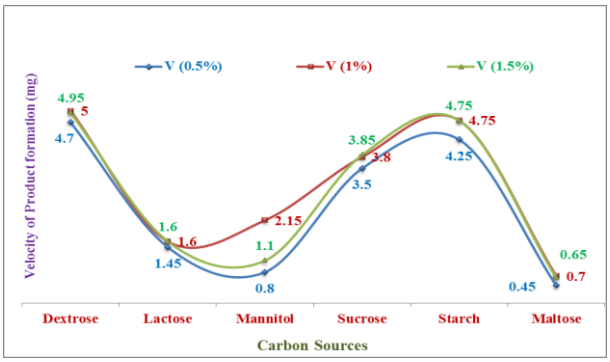

Figure 2. Effect of Carbon sources on enzyme α-amylase Production from Paenibacillus illinoisensis.

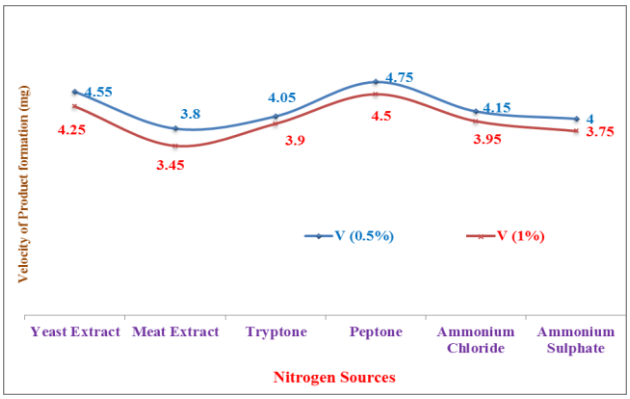

Figure 3. Effect of Nitrogen sources on enzyme α-amylase production from Paenibacillus illinoisensis

The different six type of Carbon sources such as Dextrose, Lactose, Mannitol, Sucrose, Starch and Maltose were used for the production of alkaline α - amylase enzyme. In the 0.5%, 1.0% and 1.5% of

Dextrose were shown maximum velocity of α - amylase enzyme formation as 4.7, 5.00 and 4.95 mg respectively which were shown in figure 2. The different six types of Nitrogen sources such as organic Nitrogen sources Yeast Extract, Meat Extract, Tryptone, Peptone and inorganic Nitrogen sources Ammonium Chloride and Ammonium Sulphate were used for the production of enzyme. These Nitrogen sources were used in different concentration such as 0.5% and 1.0% and 1.0% Starch was used as control Carbon source for the production of enzyme which was shown in figure 3. Maximum velocity of α - amylase enzyme production was seen at 0.5% Peptone as Nitrogen source which was 4.75 mg.

The maxiumim production of alkaline α-amylase was seen in the 1% Dextrose and Starch in the used different Carbon sources and 0.5 % Peptone as a Nitrogen source which were used for optimization of enzyme productions from the Paenibacillus illinoisensis. The production medium was inoculated with bacteria and incubated for 72 hours at 40^0C temperature. After 72 hours incubation, cells were separated by centrifugation at 5000g for 15 min. The optimum productions alkaline α - amylase were seen at pH 10.0, 40^0C and 72 hrs incubation times, in the 1% dextrose and Starch as a Carbon source and 0.5% Peptone Nitrogen source.

Similar findings of Mukesh Kumar et al., in (2012) effect of different Carbon and Nitrogen sources were studied in order to determine the optimum conditions for amylase production by Bacillus species. Waghode and Garode (2014) found that the nature and amount of Carbon and Nitrogen source used for the growth of the Bacillus subtilis strain. Peptone and Starch in media were promoted α - amylase productivity. Mrudula and Kokila (2010) was used different Carbon and Nitrogen sources such as Glucose, Peptone and calcium chloride, respectively enhanced production of enzyme α- amylase. Deb et al., in (2013) reported that studies on crude amylase revealed that optimum pH, temperature and reaction time of enzyme activity was 6.5, 40^0C and 40 minutes respectively.

IV. CONCLUSION

The nature and relative concentration of carbon, nitrogen sources and other parameters are important in production of amylase. Paenibacillus illinoisensis is a potential producer of extracellular α- amylase which could find applications in industry and biotechnology. The enzyme is produced presently under optimization. The amylase activity from the bacteria is comparable with the activity of amylases from other organisms. Hence amylase would have a potential application in the food and pharmaceutical industry.

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