INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Effect of C: N sources on the activity of Alkaline α-Amylase from *B. horikhoshi*

Sopan M. Waghode, A.M. Garode.

P.G. Department of Microbiology,

Shri Shivaji Science & Arts College, Chikhli, Dist. Buldana, India.

ABSTRACT

Enzymes from microbial sources generally meet industrial demands. Alkaline α-amylase is used in the starch, textile industries and ingredient in detergents. Collected water samples from Lonar crater are analyzed for isolation of bacteria. Bacteria are isolated on the nutrient agar which directly prepared in Lonar lake water. An isolated bacterium was identified on the basis of cultural, morphological and biochemical characterization. The results are found that the *B. horikhoshi* is produced efficient zone of starch hydrolysis on the starch agar by synthesizing the alkaline α -amylase. Alpha amylase enzyme was produced by using different six carbon and six nitrogen sources. Enzyme alkaline α amylase assay was done by DNSA method. The optimum alkaline amylase production was observed in the 1% starch as a carbon source and 0.5% peptone and 0.5% meat extracts as a nitrogen source.

Keywords: Alkaline α-amylase, *B. horikhoshi*, Lonar Crater and DNSA method.

INTRODUCTION

The enzyme α-amylase have been derived from several fungi, yeasts, bacteria and actinomycetes. The source enzymes from fungal and bacteria have dominated applications in industrial sectors[1]. Almost all species of the genus *Bacillus* synthesize α-amylase, thus this genus has the potential to dominate the enzyme industry[2]. The α-amylases enzymes are used in textile and garments, paper industries, starch liquefaction, food, adhesive, sugar production and pharmaceuticals and starch processing in the food industry[3,4].

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. The spectrum of amylase application was widened in many other fields, such as clinical, medical and analytical chemistry, separate applications in starch saccharafication, textile industry, and the food, brewing and distilling industries[1,5].

Alkaline amylases that have optimum pH values higher than 8.0 have potential applications for hydrolyzing starch under high pH conditions in the starch and textile industries and as an ingredient in detergents for automatic dishwashers and laundries[6-8]. Alkaline amylases also retain activity at the pH at which detergents function[9]. This work reports the influence of media composition on alkaline α-amylase production from *Bacillus subtilis* CB-18 isolated from the soil[10].

The objectives of the study are to isolate of alkaline α-amylases producing *Bacillus horikhoshi* from the Lonar crater of Buldana district. Also study the effect of different carbon and organic and inorganic nitrogen sources on the activity of alkaline α-amylases.

MATERIALS AND METHOD

Water samples are collected from Lonar crater 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 activity by amylase assay on starch

agar with pH 10.5. The isolate of bacteria is characterized and identified according to Bergey's manual of determinative bacteriology[11,12].

Effect of Carbon and Nitrogen sources on alkaline α –amylase production:

Certain carbon and nitrogen sources of the growth medium were used in this investigation. However, different carbon sources of growth were used in 1% concentration such as Starch, Maltose, Lactose, Dextrose, Sucrose, and Mannitol. Different the nitrogen sources were used in 0.5% concentration as inorganic nitrogen sources such as Ammonium chloride, Ammonium sulfate and organic nitrogen sources such as Peptone, Tryptone, Meat extract and Yeast extract. By using different sources of carbon and nitrogen, the optimum productions of alkaline α-amylase production were studied from *Bacillus horikhoshi*. The isolate is grown in basal media on laboratory scale and cells are removed by centrifugation, the supernatant is used as crude enzyme preparation[13]. The routine enzyme assay is used for alkaline amylase activity involved measuring the reducing sugars resulting from the hydrolysis of soluble starch. The Di-Nitro-Salicyclic acid (DNSA) reagent method is used for assay[14].

RESULT AND DISCUSSION

Water samples are collected from Lonar crater and analyzed for isolation of bacteria. *Bacillus horikhoshi* is identified on the basis of cultural, morphological and biochemical characterization. The results are found that the *Bacillus horikhoshi* is produced efficient zone of starch hydrolysis on the starch agar by flooding the plates with iodine. The production of the alkaline α –amylase is shown Figure 1.

The optimum production of alkaline α - amylase production from *B. horikhoshi* was seen at 72 h at 37^0C. The zone of hydrolysis of alkaline α - amylase was maximum shown 65 mm on starch agar. Akcan *et al*., was shown to produce extracellular α-amylase from bacterial strain Bacillus subtilis RSKK96 and optimum enzyme synthesis occurred at 72 h with an optimum of 37°C[15]. Effects of various carbon and nitrogen on α-amylase production were examined. Alpha amylase enzyme production was done by using different six carbon and six nitrogen sources. Then assay the activity of alkaline α amylase by DNSA method. The optimum alkaline amylase production is observed in the 1% dextrose and 0.5% starch (control) as a carbon source and 0.5% peptone (control) and 0.5% meat extracts as a nitrogen source which shown Figure 2. Similar finding of Mrudula and Kokila is used different carbon, nitrogen, glucose, peptone and calcium chloride, respectively enhanced production of enzyme α amylase[16]. Waghode and Garode observed that the results are found that the *Bacillus licheniformis* is produced efficient zone of starch hydrolysis on the starch agar by producing the enzyme alkaline α –amylase[18]. The optimum α - amylase was produced by using different carbon and nitrogen sources. Then assay of the activity of alkaline α amylase performed by DNSA method. The optimum alkaline amylase production was observed in the 1% starch as a carbon source and 0.5% peptone and 0.5% meat extracts as a nitrogen source.

Bhutto and Umar reported that maximum production of α -amylase was obtained on 0.5% Dextrose as carbon source[17]. The α-amylase production was also optimized by using different nitrogen sources such as peptone (control), tryptone, yeast extract, corn steep liquor, casein hydrolyzed, casein soluble, urea, sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride and ammonium sulphate and the maximum production of α -amylase were found in the presence of 1.5% peptone.

CONCLUSION

The culture conditions and media components were optimized for better production of both the enzymes. The nature and relative concentration of carbon and nitrogen sources are important in formation of amylase. The lower levels of nitrogen are enhanced for the enzyme production and excess nitrogen is equally detrimental causing enzyme inhibition. The results obtained in this study show that there is appreciable high production. *B. horikhoshi* is a potential producer of extracellular α- amylase which could find applications in industry and biotechnology. The enzyme thus is produced presently under optimization. The amylase activity from the bacteria is comparable with the activity of maltohexaose producing amylases from other organisms. Hence amylase would have a potential application in the food and pharmaceutical industry for the production of maltohexaose.

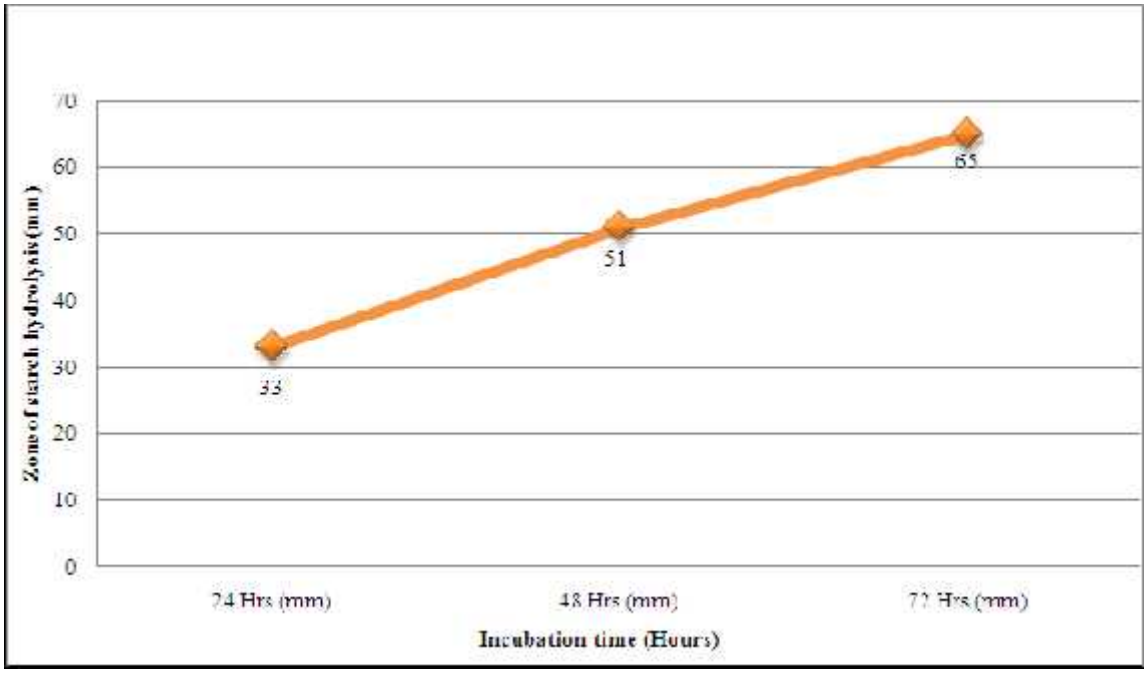

Fig. 1
isolation of maximum yield of alkaline α- amylase enzyme producing *B. horikhoshi*

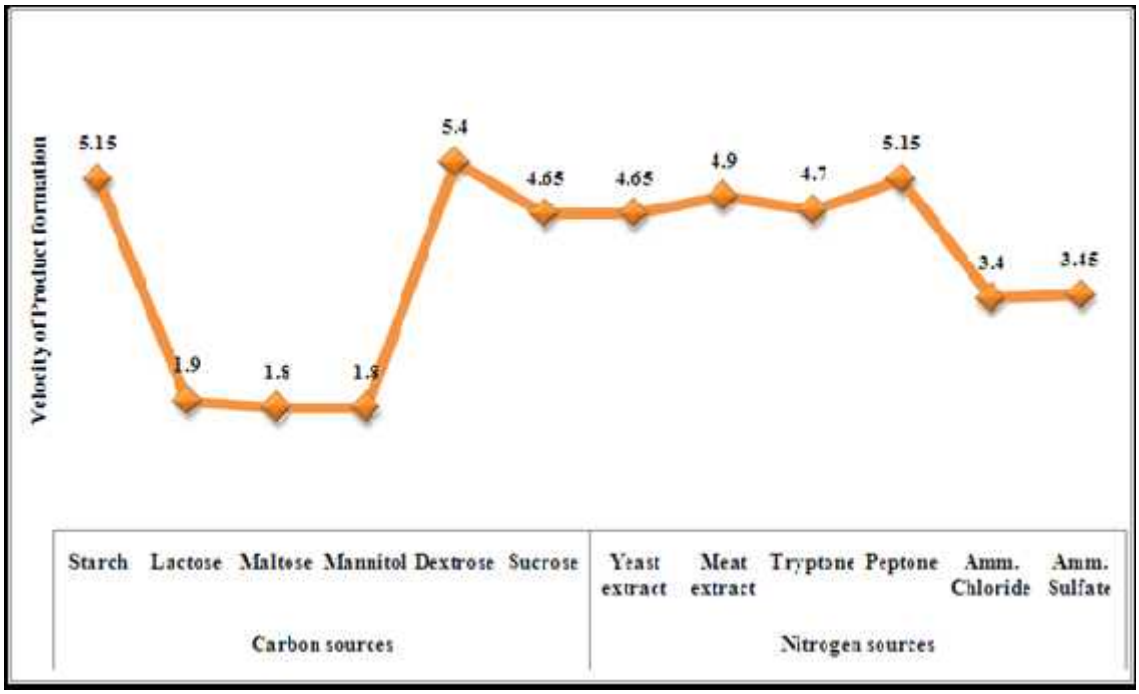

Fig. 2
Effect of C: N sources on production of alkaline α- amylase from *B. horikhoshi*

REFERENCES

1. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D and Mohan R. Advances in microbial amylases. Biotechnol. Applied Biochem. 2000;, **31(2)**: 135-152.
2. Pretorius IS, Koch MJ, Britz HJ, Potgieter HJ and Lategan PM. Numerical taxanomy of amylase producing Bacillus species. J. Appl. Bacteriol, 1986; **60(4)**: 351-360.
3. Bajpai P and Bajpai PK. High temperature alkaline α-amylase from Bacillus licheniformis TCRDC-B13. Biotechnol Bioeng., 1989; **33(1)**: 72-78.
4. Liebl W, Meike Ballschmiter and Ole Futterer. Identification and Characterization of a Novel Intracellular Alkaline α -Amylase from the Hyperthermophilic Bacterium Thermotoga maritima MSB8. Applied and Environmental Microbiology. 2006;72(**3**): 2206–2211.
5. Ozlem K, Ugur Comlekcioglu and Burhan Arikan. Effects of Carbon Sources and Various Chemicals on the Production of a Novel Amylase from a Thermophilic Bacillus sp. K-12. Turk. J. Biol, 2005; **29**: 99-103.
6. Grant WD, and Horikoshi K. Microbiology of extreme environments and its potential for biotechnology. In M. S. Dacosta, J. C. Duarte, and R. A. D. Williams (ed.), Elsevier Science Publishers Ltd., Essex, England. Alkaliphiles, (1989), p. 346–366.
7. Nakai, R., T. Sato, and K. Okamoto. Manufacture of alkaline amylase with Streptomyces. Japanese Kokai Koho patent. 1986; **86,** 209,588.
8. Ozaki, A., and A. Tanaka. Heat-stable alkaline amylase from Bacillus. Japanese Kokai Koho patent, 1990, **9**: 049, 584.
9. Ito S, Kobayashi T, Ara K, Ozaki K, Kawai S and Hatada Y. Alkaline detergent enzymes from alkaliphiles: enzymatic properties, genetics and structures. Extremophiles.1998; 2(**3**): 185-190.
10. Ogbonnaya N. and Odiase A. Influence of media composition on the production of alkaline α-amylase from Bacillus subtilis CB-18. Acta Sci. Pol., Technol. Aliment, 2012; 11(**3**): 231-238.
11. Olajuyigbe, Folasade M and Joshuao Ajele. Production dynamics of extracellular protease from Bacillus species. African. J. Biotechnol. 2005; 4(**8**):776-779.
12. Holt. G.J., Noel Krieg R., Peter Sneath H.A., James Stanley and Williams T. Bergy's manual of determinative bacteriology, ninth edition, (1994), 559-561.
13. McTigue MA, Kelly CT, Doyle EM, and Fogarty WM. The alkaline amylase of the alkalophilic Bacillus sp. IMD 370. Enzyme Microb. Technol.1995; **17:** 570-573.
14. Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., (1959), **31:** 426–428.
15. Akcan, Nurullah. High Level Production of Extracellular α-Amylase from Bacillus licheniformis ATCC 12759 in Submerged Fermentation. Romanian Biotechnological Letters. 2011; 16(**6**): 6833-6840.
16. Mrudula, S. and R. Kokila. Production of Thermostable a-amylase by Bacillus cereus MK in solid state fermentation: Partial purification and characterization of the enzyme. The Internet Journal of Microbiology, (2010), 8(**1**): 1-16.
17. Bhutto M. Aqeel and Dahot M. Umar. Effect of alternative carbon and nitrogen sources on production of alpha-amylase by Bacillus megaterium. World Applied Sciences Journal 8 (Special Issue of Biotechnology & Genetic Engineering): (2010), 85-90.
18. Waghode SM and AM Garode. Effect of different C: N sources on the activity of alkaline α-amylase from Bacillus licheniformis. Int. J. Bioassays, (2013), 2 (**7**), 946-948.