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EFFECT OF DIFFERENT C: N SOURCES ON THE ACTIVITY OF ALKALINE α-AMYLASE FROM BACILLUS LICHENIFORMIS

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Abstract: Amylases are among the most important enzymes and are of great significance in present day biotechnology. Alkaline amylases are used in the starch, textile industries and as an ingredient in detergents. Water samples are collected from Lonar lake of Buldana district (M.S.). The collected water samples are analyzed for isolation of bacteria on the nutrient agar which directly prepared in Lonar lake water. Bacterial isolate is identified on the basis of cultural, morphological and biochemical characterization. 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. 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.

Keywords: Lonar Lake, *Bacillus licheniformis*, α –amylase, starch and peptone.

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; CAS# 9014-71-5, alternative names: 1,4-α-D-glucan glucanohydrolase; glycogenase) are calcium metallo-enzymes, completely unable to function in the absence of calcium. Industrial applications of these microorganisms have been investigated extensively and some of their enzymes such as alkaline amylases have been put to use on an industrial scale (Horikoshi, 1999; Aygan *et al*., 2008).

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 (Grant and Horikoshi, 1989; Nakai *et al*., 1986; Ozaki and Tanaka, 1990). Alkaline amylases also retain activity at the pH at which detergents function (Ito *et al*., 1998). This work reports the influence of media composition on alkaline amylase production from *Bacillus subtilis* CB-18 isolated from the soil (Ogbonnaya and Odiase, 2012).

The demand for α-amylase for use in laundry and automatic dishwashing detergents has also been growing for several years (Upadek and Kottwitz, 1997). However, most of the *Bacillus* liquefying amylases, such as the enzymes from *Bacillus amyloliquefaciens* (BAA) and *Bacillus stearothermophilus* (BSA) (Manning and Campbell, 1961), including BLA (Saito, 1973), have pH optima of between 5 and 7.5 (Yamamoto, 1988). Since Horikoshi (1971) first reported an alkaline amylase from alkaliphilic *Bacillus* sp. strain A-40-2, many alkaline amylases have been found in cultures of, for example, *Bacillus* sp. strain NRRL B-3881 (Ozaki and Tanaka, 1990), *Bacillus* sp. strain H-167 (Hayashi *et al*., 1988), *Bacillus alcalothermophilus* A3-8 (Boyer and Ingle, 1972), and *Bacillus* sp. strain GM8901 (Kim *et al*., 1995).

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

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 work. 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 (Holt, *et al*., 1994; Olajuyigbe *et al*., 2005).

Effect of C: N sources on alkaline α –amylase production:

Certain carbon and nitrogen sources of the growth medium were used in this investigation. However,

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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 licheniformis*. The isolate is grown in basal media on laboratory scale and cells are removed by centrifugation, the supernatant is used as crude enzyme preparation (McTigue *et al*., 1995). 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 (Miller, 1959).

RESULT AND DISCUSSION

Water samples are collected from Lonar lake of Buldana district (M.S.). The collected water samples are analyzed for isolation of bacteria on the nutrient agar which directly prepared in Lonar lake water. Bacterial isolate is identified on the basis of cultural, morphological and biochemical characterization. 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. The production of the alkaline α –amylase is shown fig. 1.

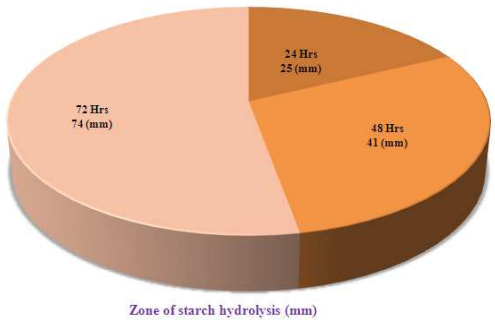

Fig. 1: Isolation of maximum yield of alkaline α-amylase enzyme producing *B. Licheniformis*

The optimum production of alkaline α - amylase production from *B. horikhoshi* was seen at 72 h at 37°C. The zone of hydrolysis of alkaline α - amylase was maximum shown 65 mm on starch agar. Bhutto and Umar (2010) reported that maximum production of α - amylase was obtained on 0.5% Dextrose and 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 sulfate and the maximum production of α - amylase were found in the presence of 1.5% peptone which shown fig. 2.

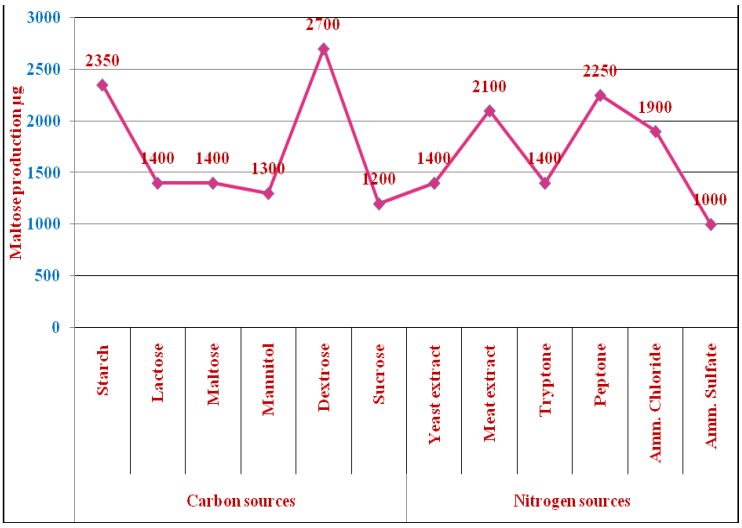

Fig. 2: Effect of C: N sources on production of alkaline α- amylase from *B. licheniformis*

Similar findings of Mukesh Kumar in (2012) effect of different carbon and nitrogen sources were studied in order to determine the optimum conditions for amylase production by *Bacillus* sp. Effects of various carbon and nitrogen on α-amylase production were examined. Alpha amylase enzyme was produced 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. 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.

CONCLUSION

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. licheniformis* is a potential producer of extracellular α-amylase which could find applications in industry and biotechnology. The optimum alkaline amylase production was observed in the 1% starch as a carbon source and 0.5% peptone and meat extracts as a nitrogen source**.** The culture conditions and media components were optimized for better production of both the enzymes. The enzyme thus is produced presently under optimization. Hence amylase would have a potential application in the food and pharmaceutical industry for the production of maltohexaose. The amylase activity from the bacteria is

comparable with the activity of maltohexaose producing amylases from other organisms.

REFERENCES

1. Aygan Ashabil, Burhan Arikan, Hatice Korkmaz, Sadik Dinçer, Omer Çolak (2008). Highly thermostable and alkaline α-amylase from a halotolerant-alkaliphilic *Bacillus* sp. AB68. *Braz. J. Microbiol*. 39 (3): 547-553.

2. Bhutto M. Aqeel and Dahot M. Umar (2010). 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)*: 85-90.

3. Boyer, E. W. and M. B. Ingle (1972). Extracellular alkaline amylase from a *Bacillus* species. J. Bacteriol. 110:992–1000.

4. Grant, W. D., and K. Horikoshi (1989). Alkaliphiles, p. 346–366. *In* M. S. Dacosta, J. C. Duarte, and R. A. D. Williams (ed.), Microbiology of extreme environments and its potential for biotechnology. *Elsevier Science Publishers Ltd*., Essex, England.

5. Hayashi, T., T. Akiba and K. Horikoshi (1988). Production and purification of new maltohexose-forming amylases from alkalophilic *Bacillus* sp. H-167. *Agric Biol Chem*, 52:443–8.

6. Holt. G.J., Noel Krieg R., Peter Sneath H.A., James Stanley and Williams T. (1994). *Bergy's manual of determinative bacteriology*, ninth edition, 559-561.

7. Horikhishi, Koki (1971) Production of Alkaline Enzymes by Alkalophilic Microorganisms Part II. Alkaline Amylase Produced by Bacillus No. A-40-2. *Agr. Biol. Chem*., 35(11): pp. 1783-1791.

8. Horikoshi, K. (1999). Alkaliphile: Some application of their products for biotechnology. *Microbiol. Mol. Biol. Rev.*, 6: 735-750.

9. Ito, S. (1997). Alkaline cellulases from alkaliphilic *Bacillus*: enzymatic properties, genetics, and application to detergents. *Extremophiles*, 1:61–66.

10. Kim, T.U., Gu, B.G., Jeong, J.Y., Byun, S.M. and Shin, Y.C. (1995). Purification and characterization of a maltotetraose forming alkaline a-amylase from an alkalophilic *Bacillus* strain GM8901. *Appl. Environ. Microbiol*. 61(8): 3105–3112. PMID: 16535108.

11. Manning, G. B., and L. L. Campbell (1961). Thermostable α -amylase of *Bacillus stearothermophilus*. *J. Biol. Chem.* 236:2952–2957.

12. McTigue, M.A., C.T. Kelly, E.M. Doyle, and Fogarty, W.M. (1995). The alkaline amylase of the alkalophilic *Bacillus* sp. IMD 370. *Enzyme Microb. Technol.*, 17: 570-573.

13. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem*., 31: 426–428.

14. Mrudula, S. and R. Kokila (2010). 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*, 8(1): 1-16.

15. Mukesh Kumar, D.J., Jayanthisiddhuraj, B. Amutha, D. Monica Devi, M.D. Bala Kumaran and P.T. Kalaichelvan (2012). Purification and Characterization of α-Amylase and β-Galactosidase from *Bacillus* Sp. MNJ23 Produced in a Concomitant Medium. *Am-Euras. J. Agric. & Environ. Sci.,* 12 (5): 566-573,

16. Nakai, R., T. Sato and K. Okamoto (1986). Manufacture of alkaline amylase with *Streptomyces*. *Japanese Kokai Koho patent*. 86: 209, 588.

17. Ogbonnaya N. and A. Odiase (2012). Influence of media composition on the production of alkaline α-amylase from *Bacillus subtilis* CB-18. *Acta Sci. Pol., Technol. Aliment*. 11(3): 231-238.

18. Olajuyigbe, Folasade M and Joshuao Ajele (2005). Production dynamics of extracellular protease from Bacillus species. *African. J. Biotechnol*., 4(8): 776-779.

19. Ozaki, A., and A. Tanaka (1990). Heat-stable alkaline amylase from *Bacillus*. *Japanese Kokai Koho patent* 9: 049, 584.

20. Saito, N. (1973). A thermophilic extracellular α -amylase from *Bacillus licheniformis*. *Arch. Biochem. Biophys*., 155:290–298.

21. Upadek, H., and B. Kottwitz (1997). Application of amylases in detergents, p. 203–212. *In* J. H. van Ee, O. Misset, and E. J. Baas (ed.), Enzymes in detergency. Marcel Dekker, Inc., New York, N.Y.

22. Yamamoto, T. (1988). Bacterial α -amylase (liquefying- and saccharifying types) of *Bacillus subtilis* and related bacteria, p. 40–45. *In* The Amylase Research Society of Japan (ed.), Handbook of amylases and related enzymes. *Pergamon Press, Oxford*, England.

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