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Optimization of Process Parameters for Amylolytic *Bacillus Subtilis* LC060260 Isolated from Almora Region of Central Himalayas

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Abstract

Amylase producing bacteria were isolated from soil of Almora district of Uttarakhand, India. Twenty bacterial isolates were checked for their amylolytic activity on starch agar medium, strain that showed utmost zone of clearance was identified biochemically. Genomic analysis confirmed the strain as Bacillus subtilis. It was submitted to gene bank with accession LC060260. Different culture parameters were optimized for maximum alpha amylase activity and enhanced alpha amylase production 121.22 U/ml/min was observed under incubation period of 72 hr at 40^{0}C and medium pH 7.0.

Keywords: Alpha-amylase, Bacillus subtilis, Almora, Starch agar

Introduction

Alpha-amylase is an extracellular hydrolytic enzyme that break α 1-4 glucosidic linkages of starch and other related substrates in an endo-fashion resulting saccharides of lesser molecular weight such as alpha limit dextrin, matose and glucose[1]. Now a day, field of alpha amylase utility has extended to areas of analytic chemistry, medical and clinical diagnostics viz., acute pancreases inflammation, mumps, ulcers of pelvic, macro-amylasemia etc.[2] Because of these applications, research is being done to search more proficient techniques to enhance alpha amylase production. Microbial genra of fungi and bacteria are broadly exploited for alpha amylase production yet Bacillus sp. is more reliable for amylase production. Industrial economy of enzyme production is dependent on number of factors that include strain type, culture medium composition, and conditions of fermentation, availability of carbon, nitrogen and mineral salts. Because 30-40% industrial enzyme cost is controlled by that of growth medium used[3], it is very important to optimize culture conditions so as to get maximum enzyme and microbial growth[4]. Physiological parameters like incubation period, optimum temperature and pH range are very important for production of enzyme[5].

Materials and Methods

Sample collection: The soil samples are collected randomly during October 2011 from the sites of Almora District, Uttarakhand, India by using standard procedure. Samples were dried under shade, mixed and representative samples were collected in a clean zip lock bag.

Strain isolation: Bacterial isolation was done by serial dilution and spread plate technique. Concentration range from 101 to 106 was made by serially diluting one gram of soil in sterilized distilled water. By following spread plate technique, 0.1 ml of each dilution was transferred aseptically to different starch agar plates. Plates were incubated for 24 hr at 37^{0}C. Isolates were sub cultured and slants of pure culture were maintained at 4^{0}C.

Starch hydrolysis test for amylolytic bacteria: The bacterial strain was centrally streaked on starch agar plates and kept for incubation at 37^{0}C for 72 hr. Later, the plate was flooded with iodine solution for 30 sec. Appearance of clear zone around the streaked culture shows a positive result that can be selected for alpha amylase production.

Identification of amylolytic bacteria: Bacterial strain showing maximum zone of hydrolysis was selected for identification of bacteria by performing and different biochemical tests as per Bergey's manual. The genomic analysis via 16sRNA was done.

Production medium: A loopfull of bacterial culture was suspended in nutrient broth medium and kept in incubator shaker for 24 hr.

Enzyme recovery: For extraction of enzyme three ml of production medium was taken to centrifuge for 20 min at 5000rpm. The supernatant was used as crude enzyme.

Optimisation parameters: Incubation time: To find out optimum time of incubation for amylase production, 50 ml of broth media was inoculated with the 100 µl fresh grown broth culture of selected bacterial strain and incubated at 37ºC and 150 rpm. 1 ml samples were withdrawn at 24, 48, 72, 96 and 120 to measure enzyme activity.

Temperature: To find out optimum temperature for amylase production 50 ml of broth media, inoculated with the 100 µl fresh grown broth culture of selected bacterial strain and incubated at different temperatures (30, 40, 50, 60^0C) in shaker incubator (150 rpm). A 1 ml sample was withdrawn and the enzyme activity was measured.

pH: pH of medium was varied to 4.0, 5.0, 6.0, 7.0 and 8.0 by addition of 0.1N HCl / NaOH. The 50 ml broth media having different pH values (*i.e.* 4.0, 5.0, 6.0, 7.0, and 8.0) was separately inoculated and allowed to incubate in rotary shaker incubator 150 rpm.

Assay of Amylase activity: Amylase activity was determined by DNS method. Soluble starch was used as substrate at a concentration of 1% in 0.05M phosphate buffer of Ph 6.9. The tube containing reaction mixture 0.5ml of crude enzyme and 0.5ml of substrate was incubated for 10min at 37^0C after which the reaction was arrested by adding 1ml DNS. The tube then kept in boiling water bath for 5 min and cooled. The optical density was read at 540 nm against blank (without enzyme). Maltose was used to plot standard curve. Single unit of amylase activity was defined as the amount of enzyme giving 1mg of sugar in 1min[6].

IU/ml/min= Amylase activity x1000/ Molecular weight of maltose x Incubation time

Results and Discussion

The selected screened amylolytic bacterial strain showing the maximum zone of hydrolysis isolated from soil of Almora, Uttrakhand India was taken for its morphological and biochemical identification. The strain was short rods with single arrangement having a discrete, off white non pigmented colony and was positive for starch hydrolysis, Gram reaction, Voges-Proskauer, catalase and glucose fermentation, while gave negative results for Nitrate reduction and Methyl red. It was gnomically confirmed and submitted to gene bank with accession as *Bacillus subtilis* LC060260.

Optimization parameters: Incubation time: Figure-1 revealed the effect of incubation period (24 - 120 hrs) on alpha amylase production via *Bacillus subtilis* LC060260. Enzyme production was enhanced as fermentation period was augmented and reached maximum after 72 h. However, slightly declined but nearly similar amylase activity (106.66 U/ml/min) was examined when the organism was allowed to be fermented in production medium for 96h, thereafter decreased dramatically. Later on, depletion in nutrients occurs that lead bacteria to stationary growth phase, producing secondary metabolites[7] causing lesser enzyme production. Bacterial growth at this stage may also be decreased because of other side products or toxic discharge of substances in the fermentation medium**)**. Optimum fermentation period of 72 hrs was found best for *A. oryzae* in submerged state fermentation when incubated at 45ºC, pH 7 [8].

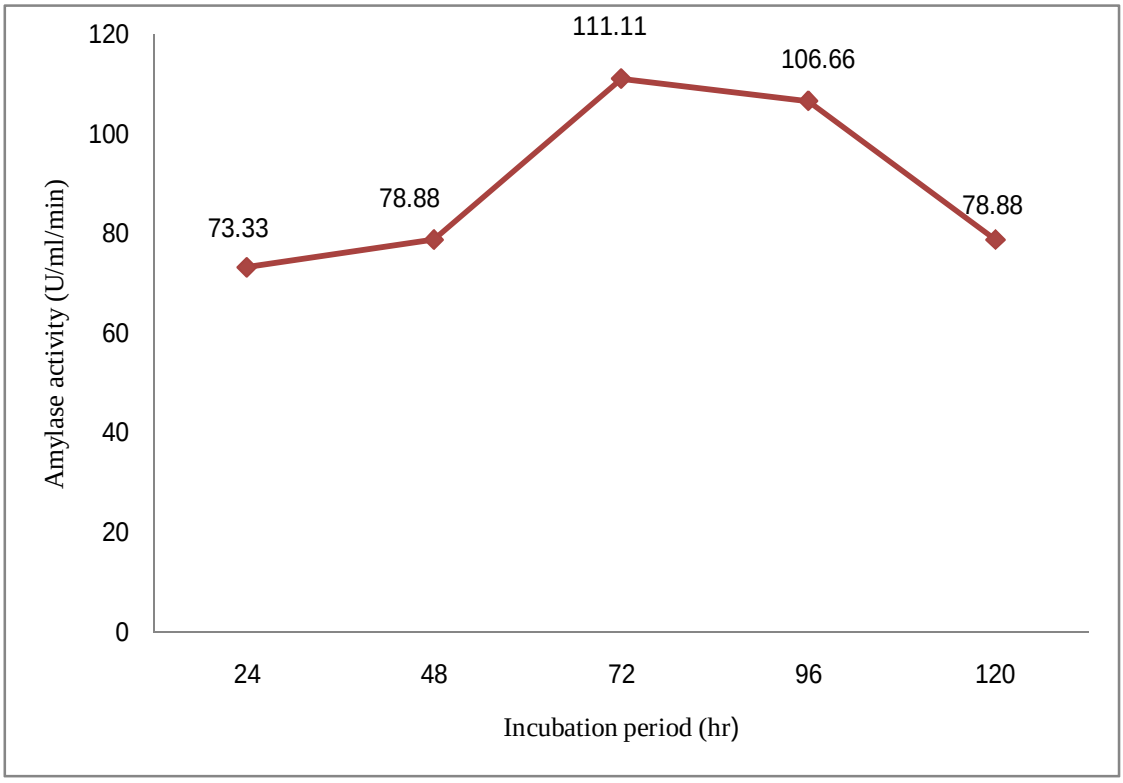

Figure-1
Effect of incubation period (hr) on amylase activity from *Bacillus subtilis* LC060260

Temperature: Temperature influences both the growth of microorganisms and biological activities. Range of temperature for maximum amylase production usually varies from one organism to another but generally optimum temperature and stability was observed between 30-50^{0}C. Figure-2 depicts the influence of temperature on alpha amylase activity at incubation time of 72 hrs.

Bacterial growth, enzyme production and optimum temperature are found to be correlated. Highest yield of amylase was achieved at 40^{0}C (120.33U/ml/min). Bacterial growth and enzyme biosynthesis by changing incubation temperature either sides of optima was found decreased gradually. Similar pattern in repression of bacterial growth as well as enzyme formation due to change in temperature below or above optimum have been reported [9].

However, the higher temperature i.e. 50ºC favors less reduction in enzyme formation (117.77 U/ml/min) than the lower temperature 30ºC (93.33 U/ml/min). The temperature of fermentation medium beyond 50ºC inhibited the production. It might be because of increased temperature that inhibited bacterial growth hence, enzyme formation was also prohibited[10]. Our results are in consonance with the previous reports[11].

pH: pH is one of the important physical parameter largely influencing both growth and morphologic characteristics of microorganism affecting enzyme yields. It is reported earlier that optimum pH range of 6.0-7.0 resulting in maximum culture growth and enzyme production.

Figure-3 shows different pH and their effect on alpha amylase production at optimum incubation temperature of 40ºC and incubation time 72 hrs. Amylase production was found to increase till pH 7.0 (121.22 U/ml/min) than decreased. Though, optimum value of pH varies with microorganism.

It is reported that variation in pH on either side of optimum value lead to decline in microbial growth ultimately lowering production. Most of bacterial cultures require neutral pH for optimum growth. Similar results were reported previously researchers[12].

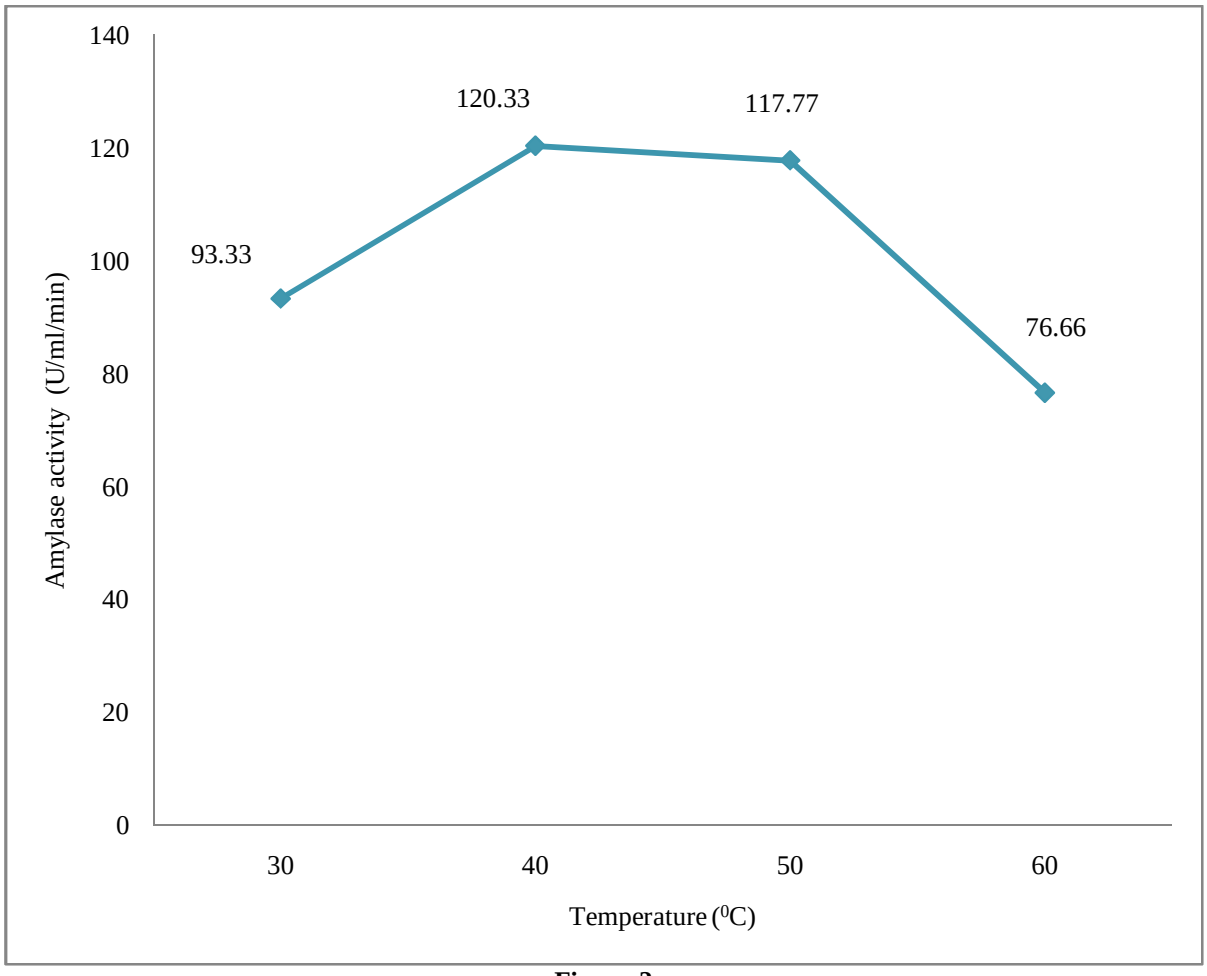

Figure-2
Effect of temperature (^{0}C) on amylase activity from *Bacillus subtilis* LC060260

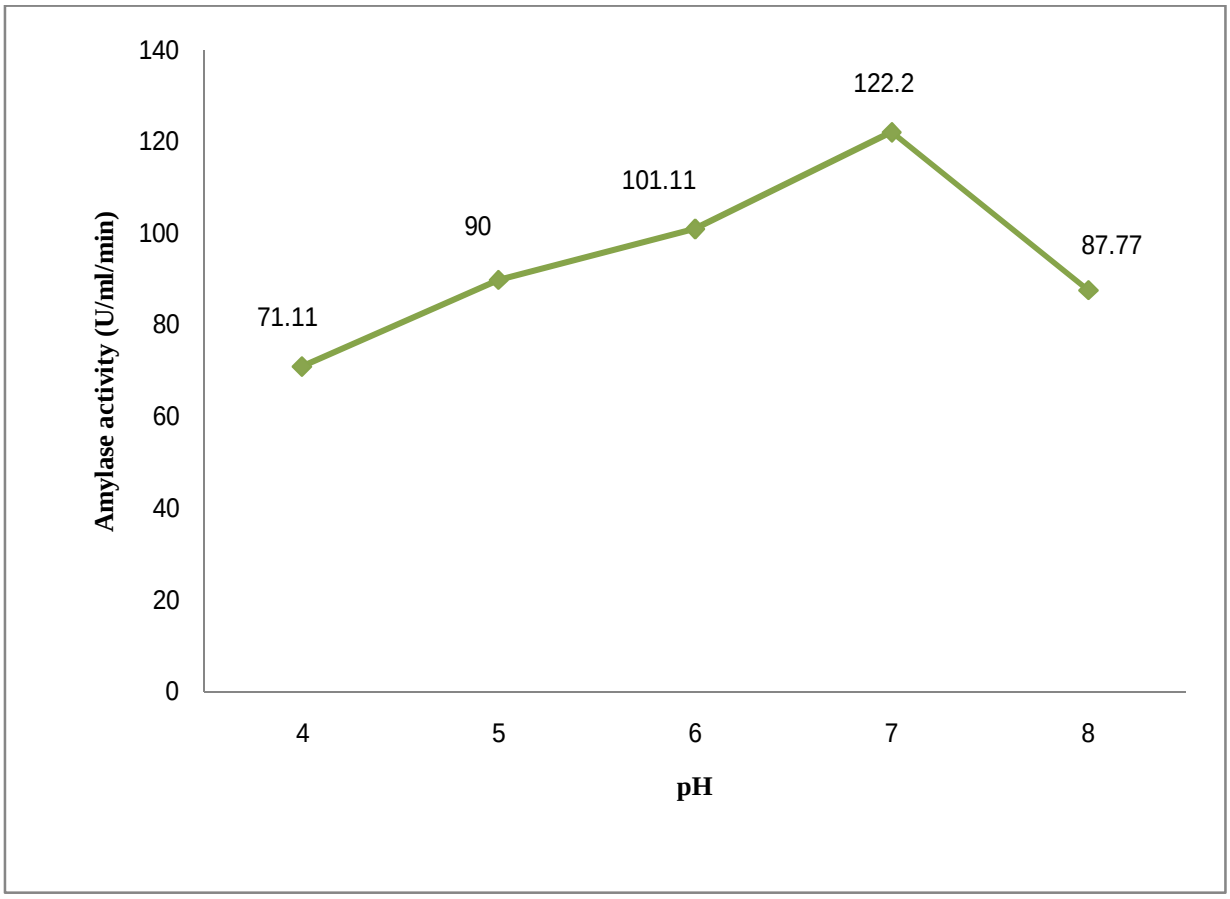

Figure-3
Effect of pH on amylase activity from Bacillus subtilis LC060260

Conclusion

The present study concluded that soil served as a rich source of numerous hydrolytic enzymes, can be a source to isolate many potent native microorganisms. The genus *Bacillus* produces a wide range of economically important enzymes including alpha amylase. *Bacillus subtilis* LC060260 was used to determine the enzyme production and found able to secrete high levels of a-amylase of industrial importance in economic culture medium by SmF.

References

1. Acharya D.K., Shah I.J., Gami1 P.N. and Shukla R.M. (2014). Optimization for α-amylase production by Aspergillus oryzae using submerged fermentation technology Basic. *Research Journal of Microbiol.,* 1(4), 1-10.

2. Ajayi A.O. and Fagade O.E. (2006). Growth pattern and structural nature of amylases produced by some *Bacillus* species in starchy substrates. *Afr. J. Biotechnol.,* 5(5) 440-444.

3. Bansod S., Thigale D., Dure P. and Sohoni H. (2014). Production, purification and enzyme kinetics of amylase from bacteria isolated from a soil of solapur, maharashtra state, India. *Int. Journal of Bioassays,* 3(2), 1785-1788.

4. Calik P. and Ozdamar T.H. (2001). Carbon sources affect metabolic capacities of *Bacillus* species for the production of industrial enzymes. *Biochem. Eng. J.,* 8(1), 61–81.

5. Chengyi W.H., Ming M. and Jiang R. (1999). Studies on the properties of alpha–amylase produced by *Bacillus pumilus* 289 (PBX96). *Acta. Microbial. Sin,* 32, 400–404.

6. Chimata M.K., Sasidhar P. and Challa S. (2010). Production ofextracellular amylase from agricultural residuesby a newly isolated *Aspergillus*species in solidstate fermentation. *Afri. J. Biotechnol.,* 9(32), 5162-5169.

7. Gupta R., Gigras P., H. Mohapatra, VK Goswami and Chauhan B. (2003). Microbiol α-amylase: a biotechnological perspective. *Process Biochem.,* 38(11), 1599–1616.

8. Haq I., Riaz N., Ashraf H., Qadeer M.A. and Iqbal J. (2002). Effect of inorganic salts on the biosynthesis of

alpha amylase by Bacillus subtilis. *Indus. J. Plant Sci.*, 1, 115–21.

9. Joo H.S., Ganesh C.K., Park G.C., Kim K.T., Paik S.R. and Chang C.S. (2002). Optimization of the production of an extracellular alkaline protease from Bacillus horikoshii. *Process Biochem.*, 38(2), 155-159.

10. Lonsane B.K. and Ramesh M.V. (1990). Production of bacterial thermo stable amylase by solid state fermentation: a potential tool for achieving economy in enzyme production and starch hydrolysis. Advances in Appl Microbiol., Academic press, Inc., California, 35, 1-56.

11. Ramachandran S., Patel A.K., Nampoothiri K.M., Chandran S., Szakacs G., Soccol C.R. and Pandey A. (2004). Alpha amylase from a fungal culture grown on oil cakes and its properties. *Brazilian Archives of Biol. and Technol.*, 47(2), 309-317

12. Singh P., Gupta P., Singh R. and Sharma R. (2012). Factors affecting alfa Amylase Production on Submerged Fermentation by Bacillus sp. *Int. J. of Pharm. and Life Sci.*, (3)12, 2243-2246.