Vol 4, Issue 4, 2017 ISSN 2349-7041

Research Article

BACTERIAL α-AMYLASE ACTIVITY ON THE STARCH PRESENT IN MAIZE KERNEL.

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ABSTRACT

Introduction: The purpose of the present research work was to investigate the feasibility of production of alpha amylase enzyme from three bacterial species *Bacillus subtilis*, *Bacillus licheniformis* and *Lactobacillus acidophilus* and to check their applicability on maize starch hydrolysis. The present study was focused on the alpha amylase production from three bacterial species and comparative study of them to hydrolyse the maize starch. **Method**: For the growth of bacteria and amylase enzyme production from them, they were grown in a nutrient medium. These bacteria can produce alpha amylase enzyme in a nutrient medium. This amylase is responsible for starch hydrolysis, through this study we can find the alternative mean for the starch digestion. As with the age Alpha, amylase production becomes reduced in the human body. The enzyme alpha amylase is required to digest the starch. So, scarcity of this enzyme in the body may cause many diseases associated with carbohydrate, like diabetes and colon cancer. So, when the metabolic rate becomes down, and alpha amylase production is not properly achieved than to digest the food starch in our body we can give this extracted alpha amylase from bacteria in some form. Amylase activity was checked by the Iodine assay and DNSA assay method. **Result**: Bacteria *Bacillus subtilis* has shown the highest **α**-amylase activity on the starch present in Maize kernel.

Key words: *Bacillus subtilis*, Bacillus licheniformis, *Lactobacillus acidophilus*, and the alpha amylase.

INTRODUCTION

The enzyme alpha amylase is required to digest the starch. So, scarcity of this enzyme in the body may cause many diseases associated with carbohydrate, like diabetes and colon cancer. So, when the metabolic rate becomes down, and alpha amylase production is not properly achieved than to digest the food starch in our body, we can give this extracted alpha amylase from bacteria in some form. The present study was focused on the alpha amylase production from three bacterial species and comparative study of them to hydrolyse the maize starch.

In the present study, ***Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus*** were studied. The alpha amylase production and their activity on maize starch, from all selected bacteria, were also observed.

Bacillus species is fast and easy growing bacteria even in simple and cheaper media, and so they secreted enzymes directly into the growth agar medium. This reduces the cost of production. So in the present work, we have selected these bacteria for alpha amylase enzyme production study by using DNSA assay technique and checked their applicability on maize starch hydrolysis. By checking bacterial amylase activity, we can find out which bacterial amylase has highest potential to digest the starch in maize.

MATERIALS AND METHODS

Maintenance of Bacteria

Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus bacteria were and taken for study; grown separately on nutrient agar plates and slants at 37°C and sub cultured at 24h, 48h, 72h and 96hours.

The composition of nutrient agar media was (g/l) Peptic digest of animal tissue (Peptone) 10.00**,**Beef Extract 10,Sodium Chloride 5.00,Agar 12.00 and pH was set at 7.0±0.1. Agar is not added in nutrient broth, it was prepared for production of enzyme.

For each bacterial species the following scaling up technique was done separately. The inoculum was prepared by the adding bacteria into sterile distilled water. From this 0.5ml of suspension was inoculated in to 3ml of sterilized nutrient broth and incubated for 24hours. From this 1 ml culture was inoculated into 9ml of nutrient Broth to make 10 ml cultures and incubated for 24hours. From this 3 ml culture was inoculated into 27ml of nutrient broth to make 30 ml cultures and incubated for 24hours. From this 10 ml culture was inoculated into 90ml of nutrient broth to make 100 ml cultures and incubated at for 24hours.

Collection of maize kernel

Maize kernels were purchased from various markets in Mandsaur (M.P.)

Growth Profile

Growth absorbance was measured by taking the absorbance of cultures at 600 nm using a Spectrophotometer Model DIGISPEC-200Gl. The un-inoculated broth was used as a control, against broth with growth.

Assay for enzyme study

The activity of the enzyme produced from each species of bacteria in the media was checked separately using enzymatic assay method at regular time intervals of 24h, 48h, 72h, and 96h, to find the time period and the medium with the substrate that showed the highest enzyme production.

The incubated culture broth was centrifuged separately for each bacterial species at 8000 rpm for 20 minute in centrifuge. The collectedsupernatantwas used as enzyme extract for assaying enzyme activity estimation by using DNSA method.

The method was proposed by Bernfield in 1955.The DNSA method is to detect the saccharifying action of α-amylase enzyme. In this method, the dinitrosalicylic was used. The aliquots of the substrate stock solution are mixed with the enzyme. It is followed by different time intervals and incubation at room temperature.DNSA reagent is added to the test tube to stop the reaction; the mixture is incubated in boiling water bath for 5 minutes. After cooling in cold water to room temperature, the absorbance of the supernatant at 540 nm is measured.

The absorbance value for the substrate and enzyme mixture are subtracted to the enzyme blanks are analyzed. It gives a measure of

extent of saccharification of starch by α-amylase. This method is also known as dinitrosalicylic acid assay.

Determination of growth activity of Maize Cereal

Determination of germination capacity

We have checked the germination capacity of taken seeds of cereals, by viability test. Then malting procedure was carried out for taken seeds.

Selection of grains

Seeds were collected, selected randomly, cleaned and finally unfilled seeds were discarded. 10 grains of maize counted and weighed.

Steeping

All the kernels of maize were washed with detergents, and they were rinsed 4-5 times with double distilled water. Maize was steeped for 24 hours in water, in a beaker. From the beaker, water was drained and the grains separately distributed in a compact single layer on a petri dish. Compact single layer on petri dish over lay with moistened filter paper.

Germination

Petri plate was covered separately and placed in the dark at room temperature for germination. Moisture content in each grain was maintained by adding few drops of water to the filter paper, occasionally. After 72 hours, seeds having shoot longer than 1 cm. were considered as viable. Under the controlled condition, wet grains grow, and its internal structure changes its sugar, starch amount. Viable seeds were counted and taken for α-amylase production in taken cereals**.**

Kilning

During this procedure, color and flavor of grains are changed so, warm air is passed to the grain, due to which growth of grain was halted and dry grain becomes stable. Dry grains stability is required to observe the saccharification activity of starch hydrolysis by α-amylase enzyme.

Fig.1: Determination of Germination Capacity of Maize Kernels.

Application of α-amylase from *Bacillus species* for breakdown of grain starches to maltose

Maize cereal kernels were taken, having starch as proved by various scientists. Maize starch contains larger content of amylopectin.

Using amylase enzyme for breakdown of maize starch, applicability for food uses can be demonstrated. However, since we were not confirmed of the extent of liquefaction/saccharification that can take place after enzyme binding on maize kernels, an exploratory experiment was set up. The extent of breakdown may be indicated by the higher saccharifying activity, as checked by assaying the reducing sugars released (as reducing end groups).

We took sieve to get different size of grain kernels. Then crush and sieved some maize kernels.Maize kernels were separated into two categories that are uncrushed kernel and crushed kernel.

Uncrushed kernels were weighed and crushed kernels were sieved and weighed.Equal amounts of uncrushed and crushed kernels in glass tubes were taken. 10ml. of supernatant enzyme extract from 96h culture growth of each bacterial species were taken from a 100ml scale growth, and added to the crushed/semi crushed/crushed maize kernels in separate tubes. These were allowed to heat in boiling water bath for five mins. One test tube of each was kept blank in which no enzyme was added. It was incubated overnight at room temperature.Then, centrifuge the overnight incubated sample at 8000rpm for 20 minutes in cooling centrifuge. The suspension was then assayed for reducing sugars released during maize starch hydrolysis activity. Bernfield's method with DNSA reagent for enzyme assay was done.

RESULTS AND DISCUSSION

Bacterial species

All the bacteria isolated were further processed for enzyme activity and their applicability on maize starch hydrolysis.

Growth profile

Separately when all bacterial species ***Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus*** were inoculated on nutrient agar, it remained in lag phase for few hours then it starts depleting. It may be due to depletion of nutrients in the medium and due to the scarcity of the water.

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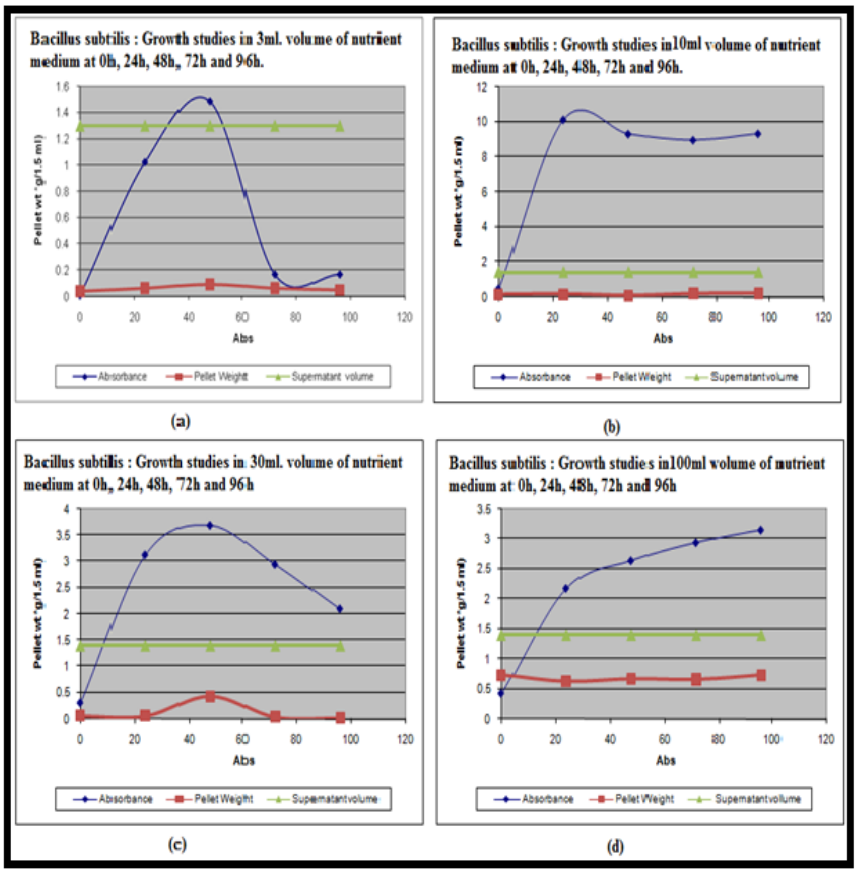

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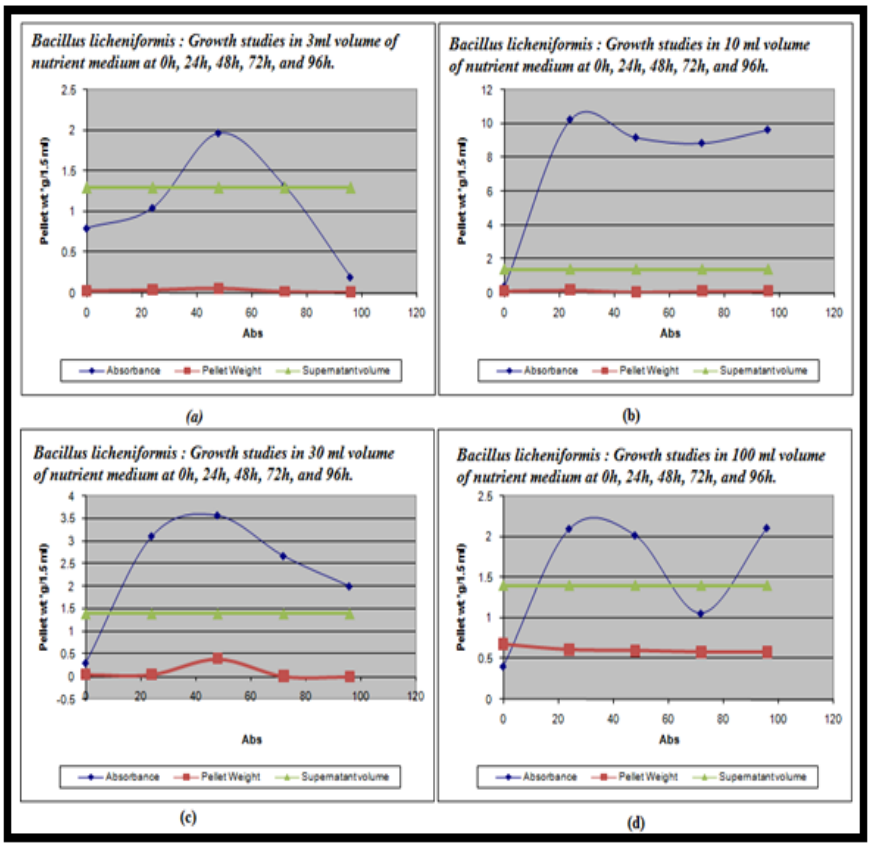

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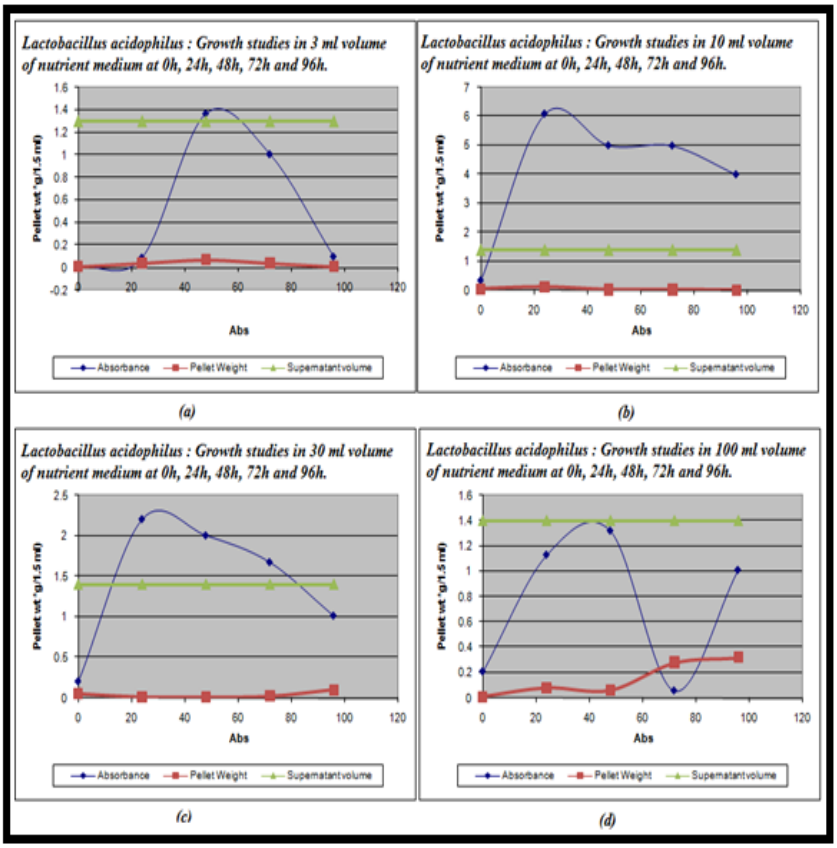


(c)

Fig.2: Growth Absorbance (at 600 nm), Pellet Weight and Supernatant Volume as observed during different growth volume of *(a) Bacillus subtilis, (b) Bacillus licheniformis and (c) Lactobacillus acidophilus* (a) 3ml, (b) 10ml, (c) 30ml and (d) 100ml.

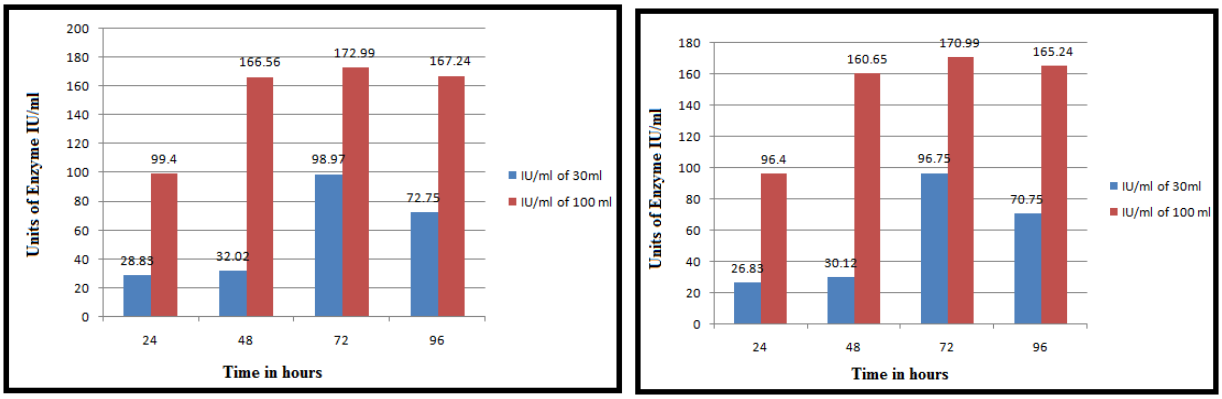
Production of Enzyme

Saccharifying action of α- amylase enzyme in nutrient broth showed that the enzyme excreted in higher volume (100 ml), reduced during the period between 24 h to 96 h of growth saccharifying action of α-amylase enzyme from

Bacillus subtilis in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 28.83 U/ml, 32.02 U/ml, 98.97 U/ml and 72.75 U/ml; Liquefying action of α-amylase enzyme from *Bacillus subtilis*in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 99.4 U/ml, 166.56 U/ml, 172.99 U/ml, and 167.24 U/ml respectively.

Bacillus licheniformis in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 26.83 U/ml, 30.12 U/ml, 96.75 U/ml and 70.75 U/ml; Liquefying action of α-amylase enzyme from *Bacillus licheniformis* in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 96.4 U/ml, 160.65 U/ml, 170.99 U/ml, and 165.24 U/ml respectively. *Lactobacillus acidophilus* in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 10.83 U/ml, 12.11 U/ml, 18.17 U/ml and 19.75 U/ml; Liquefying action of α-amylase enzyme from *Lactobacillus acidophilus* in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 15.4 U/ml, 95.56 U/ml, 96.99 U/ml, and 90.24U/ml respectively.

The enzyme excreted in lower volume (30 ml), did not reduce to the extent as in higher volume (100 ml) during the period between 24 h to 72 h of growth and the reason that may attribute to this behavior by the organism is the excretion of the other metabolites (like proteases in higher levels in higher volume of broth) and that heterologous proteins (amylases) are often rapidly degraded in the presence of such extracellular proteases. However, confirmation to this reasoning needs to be performed and proven in this study yet.



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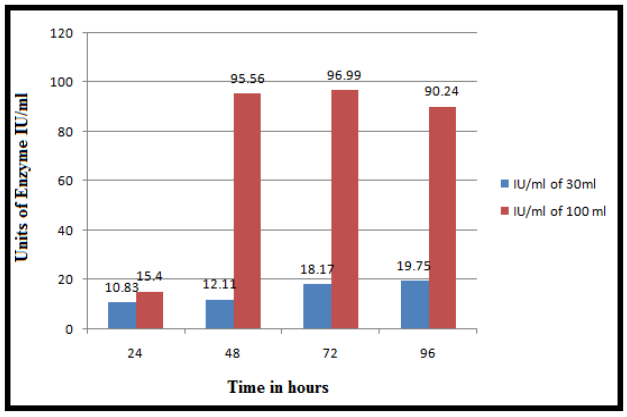


Fig.3: Enzyme α-amylase (assayed as Saccharifying activity) secreted during growth of (a)*Bacillus subtilis (b)Bacillus licheniformis (c)Lactobacillus acidophilus* in 30 ml and 100ml volumes of the Nutrient broth.

3.4 Application of α-amylase enzyme activity to check its starch hydrolysis working in maize cereals.

Germination capacity of Maize was studied and observed good for maize. Previous research workers have confirmed that cereal germination capacity of up to 80% was recommended as viable.

Alpha amylase from *Bacillus subtilis* has shown highest saccharifying action on powder form of maize granule that is 60.197 and least in pure maize granules that are 40.32. It has suggested that in powder form starch gets easily be broken by alpha amylase, so it shows the highest hydrolysis.

Alpha amylase from *Bacillus licheniformis* has shown highest saccharifying action on powder form of maize granule that is 50.18 and least in pure maize granules that are 34.32. It has suggested that in powder form starch gets easily be broken by alpha amylase, so it shows the highest hydrolysis.

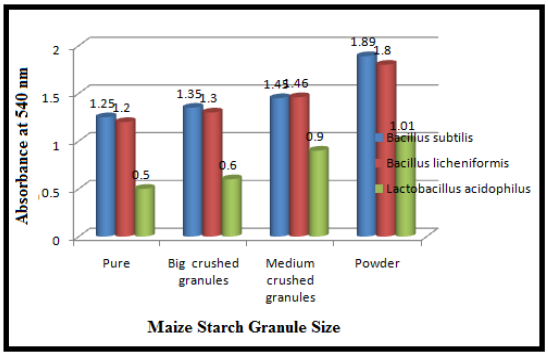
Alpha amylase from *Lactobacillus acidophilus* has shown highest saccharifying action on powder form of maize granule that is 29.88 and least in pure maize granules that are 21.06. It has suggested that in powder form starch gets easily be broken by alpha amylase, so it shows the highest hydrolysis.

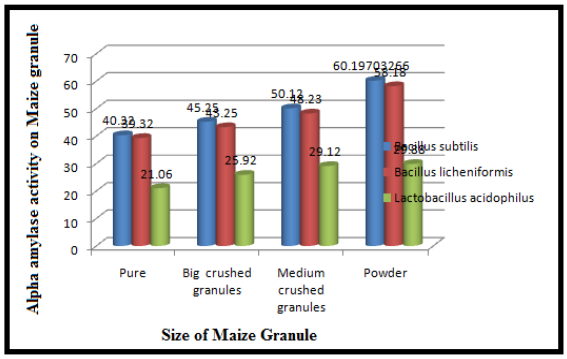
α-amylase production in sprouting cereals was more from ***Bacillus subtilis*** in compare to ***Bacillus licheniformis*** and ***Lactobacillus acidophilus*** Sprouting maize showed peak amylase activity at three days.

The present studies showed that Maize must be sprouted 72 hours, and for 72-96 hours for maximum amylase production. At the peak amylase production, the amylase activities were observed to produce maltose/ glucose per minute for sprouting Maize.

The present result showed that maize has the best yield of alpha amylase at peak amylase production.

This observation shows that maize starch is highly hydrolyzed into from alpha amylase yield at peak amylase production from ***Bacillus subtilis***.





(a) **(b)**

Fig. 3.3 Maize Starch hydrolysis (a) Absorbance and (b) Enzyme Activity

Considering the amylase production observed in the present work, it is possible to conclude that the characteristics of α-amylase from different cereals differ and so may have different levels of usefulness in the food industries. Maize may be a better source for amylase production and can be a substitute for other α-amylase in industrial processing.

ACKNOWLEDGEMENTS

The author is grateful to Pacific Academy of Higher Education and Research (PAHER), Udaipur (Rajasthan) for providing the lab facilities to carry out this work & also thankful to Dr.MrunalShirsat who guided her for this work. She is grateful to God, her parents, brother, and staff who have given her support throughout Ph.D. research work and financial support.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. AjitaSundarram, ThirupathihalliPandurangappa Krishna Murthy .(2014). α-Amylase Production and Applications: A Review.*Journal of Applied & Environmental Microbiology*, 2 (4), pp 166-175
2. Brock &Medigan (2003).Enzyme production by the microorganism. Biology of microorganism pp 377
3. Bernfeld (1955). Amylases α- and β-methods. Enzymology 1:pp.149-158
4. Crabb, W.D., Mitchinson, C., 1997. Enzymes involved in the processing of starch to sugars. TIBTECH., 15, 49-352.

5. Collins &Lyne (1996). Laboratory technique series microbial method. Butterworth Publication pp 100
6. GoyalNidhi, J.K. Gupta, & S.K. Soni. (2005).A novel raw starch digesting thermostable α-amylase from *Bacillus sp. I-3* and its use in the direct hydrolysis of raw potato starch. Enzyme & Microbial Technology. 37:723-734
7. Harwood CR (1989) Introduction to the Biotechnology of *Bacillus*. In: Harwood CR (eds) Biotechnology handbooks, *Bacillus*, vol 2. Plenum, New York, pp 1–4.
8. Hamilton Lynn M., Catherine T. Kelly, and William M. Fogarty. (1999). Production and properties of the raw starch-digesting α-amylase of *Bacillus sp.IMD 435*. Process Biochemistry 35:27-31
9. Huang Hanjing, Darin Ridgway, TingyueGu,n& Murray Moo-Young. (2003). A segregated model for heterologous amylase production by *Bacillus subtilis*. Enzyme & Microbial Technology. 32, 407-413
10. Kobayashi Y, Kawamura F (1992) Molecular Cloning. In: Doi RH, Mcgloughlin M (eds) Biology Bacilli Applications To Industry. Butterworth-Heinemann. USA. pp 123–188.
11. Konsula Z. and M.Liakopoulou-Kyriakides. (2004). Hydrolysis of starches by the action of α-amylase from *Bacillus subtilis*. Process biochemistry. 39:1745-1749
12. Madigan MT (1997) Genetic engineering and biotechnology. In: Madigan MT, Martinko JM, Parker J (eds) Brock Biology of microorganisms, 8th edn. Prentice Hall, New Jersey.
13. Shrivastava R.A.K. and J.N. Baruah (July,1986). Culture conditions for production of Thermostable Amylase by *Bacillus stearothermophilus.* Applied and Environmental Microbiology. 52:1, 179-184
14. Schallmey, M., A. Singh, and O. P. Ward. 2004. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. 50:1-17.
15. Stanier RY, Adelberg EA & Ingraham JL (1985) Microbial growth. In: General Microbiology. MacMillan Pub. Ltd. Houndmill, Basingstoke, Hampshire. pp 275-279.
16. V.Ishwaran- IARI Delhi. A treatise on media & method used in Bacteriological Technique. Today & Tomorrow's printers & publishers, New Delhi; pp 165
17. Wrint Rick and Ahns Peter Stegbauer.(1947). Measurement of reducing groups.In Methods of Enzymatic analysis. 2:885
18. Windish, W. W., &Mhatre, N. S. (1965). Microbial amylases. In W. U. Wayne (Ed.). Advances in applied microbiology (Vol. 7, pp. 273–304). New York: Academic Press.