

“ Production and Partial Purification of α -Amylase from Bacterial Strains ”

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Abstract

The Aim of Study is to produce α - Amylase by using bacterial strains namely *Streptomyces cyaneus* and *Streptomyces diastaticus*. These Enzymes have many Industrial applications and have a very wide scope. Both the bacterial species are well known for the production of amylases. Crude enzyme was obtained from these strains. Various enzyme purification techniques used were ammonium sulfate precipitation and dialysis. Pure enzyme was obtained after dialysis and filtration. Enzyme activity and Specific activity of enzyme was observed to be more in pure enzyme i.e. after dialysis.

Keywords: α - Amylase , ammonium sulfate precipitation and dialysis.

1. Introduction

Amylases are one of the main enzymes used in industry. Such enzymes hydrolyze the starch molecules into polymers composed of glucose units. They can be obtained from several sources, such as plants, animals and microorganisms. Amylases have potential application in a wide number of industrial processes such as food, fermentation and pharmaceutical industries. The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics. Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market .

2. Materials and Methodology

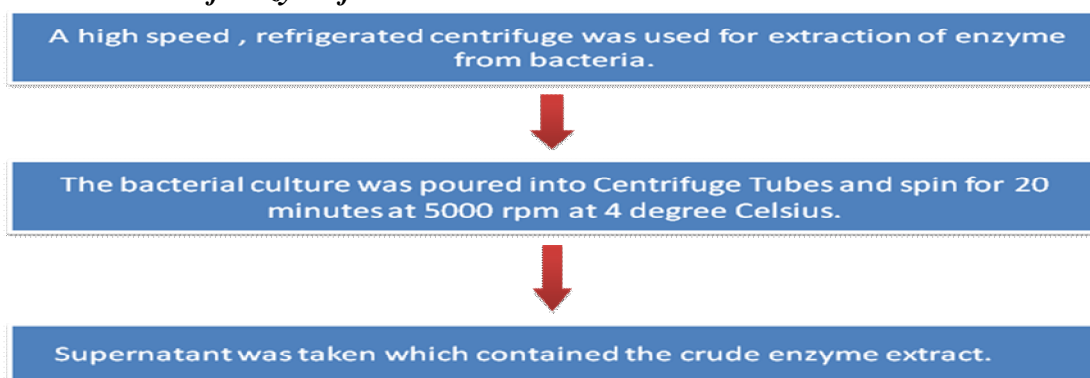
Materials Required :- SCA (STARCH,CASEIN,AGAR) Media.

Bacterial strains :- *Streptomyces cyaneus* ,*Streptomyces diastaticus*.

Chemicals Required :- Di-Nitro Salicylic Acid (DNS) , Sodium Potassium Tartarate , Bovine Serum Albumin (BSA) , Lowry's Reagent and Folin-Ciocalteu (FC) Reagent.

Inoculation :- Bacterial Strains T1 (*Streptomyces cyaneus*) and T2 (*Streptomyces diastaticus*) were inoculated in SCA Media in B.O.D incubator at 40 degree Celsius for 48 hours. These Bacterial strains were procured from Modern Biotech, Samta Colony, Raipur. After Observing Growth , a loopful of Bacterial Culture was added to the BAP (Bacterial Amylase Production) media. 40 ml of media along with culture was kept in Shaking incubator for 2 days.

2.1 Extraction of Enzyme from Bacteria



2.2 Standard plot for maltose

100 ml DNS solution was prepared by adding 1 gram DNS, 2M NaOH . Different concentration of maltose was taken for the preparation of a working standard solution (0.5ml,1ml,1.5ml, 2ml). Thus, a standard graph was plotted by taking OD at 540nm. Increased amylase activity produces more maltose.

Test tube samples :-

1. Blank,Control.
2. 4 Standards (S1, S2, S3, S4).

Test Samples :-

(T1) *Streptomyces cyaneus* (TS 22), (T2) *Streptomyces diastaticus* (TS 35).

Test tubes Containing samples were incubated in boiling water bath for 15 mins.

After incubating the samples were cooled and O.D was taken at 540 nm.



Figure.1 Samples for concentration of maltose

2.3 Protein Estimation by Folin – Lowry Method

Test tube samples :-

1. Blank.
2. 5 Standards (S1,S2,S3,S4,S5) with different concentration of working BSA (wBSA).



Figure.2 Standard samples for protein estimation.

Test Samples :-

- (T1) *Streptomyces cyaneus* (TS 22) ,(T2) *Streptomyces diastaticus* (TS 35)
1ml of Enzyme was added to each of the sample. O.D was taken at 660 nm.



Figure.3 Test Samples {T1 (TS35), T2 (TS22)}

3. Partial Purification Of Enzyme

3.1 Ammonium Sulphate Precipitation

About 18 ml of Crude enzyme was brought to 80% saturation with solid Ammonium Sulphate . The Mixture was left overnight at 4 degree Celsius in a Magnetic Stirrer. Centrifuge the Mixture at 10,000 rpm for 10min. Pellet was dissolved in 10ml of 50mM sodium acetate buffer (pH – 5.5) for further purification. Amount of ammonium sulphate for 1 litre cell free extract was calculated from the table. Reference – Analytical biochemistry & separation techniques by Dr. P. Palamivelu , School of Biotechnology, Madhurai University.

1. For 80% saturation
1 litre = 561 gm
9ml =5gm.
2. For 40% saturation
1 litre =242 gm
9ml =2.17 gm.

80% & 40% saturation of proteins was done by carrying out maltose test & Folin-Lowry test for estimation of proteins.

3.2. Dialysis

It is necessary to remove the ammonium sulphate before you can proceed to subsequent steps in the purification process. The simplest way to achieve this is to dialyse the solution.

The enzyme solution is placed in a bag of selectively permeable membrane. It is immersed in a large volume of buffer that is stirred and maintained at about 4⁰C . The membrane has pores that will permit small molecules such as ammonium and sulphate ions to cross, and hence equilibrate in the larger volume of buffer outside, while not permitting large protein molecules to cross. If the buffer is changed several times, allowing several hours each time for the ammonium sulphate to equilibrate, more or less all of it will be removed from the protein solution. Dialysis will increase the volume of the enzyme solution.

4. Formula and Results

4.1. Enzyme Activity ,

$$\text{Unit/ml of enzyme extract} = \frac{\text{mg of maltose released}}{\text{volume of enzyme used}} \times 30$$

4.2. Specific Activity

$$= \frac{\text{Unit/ml of enzyme extract}}{\text{mg protein/ml of enzyme extract}}$$

Enzyme Unit = One Unit of Enzyme will liberate 1mg of maltose from 1% starch in 30 min under defined conditions.

Table 1. Enzyme Purification Table

Samples	Enzyme Activity (unit/ml of protein)	Specific Activity (U)
Crude		
TS 35 (T1)	18.6	100
TS 22 (T2)	29.4	150
After 80% Saturation		
TS 35 (T1) (sup.)	31.2	288.9
TS 35 (T1) (pellet)	21.6	291.9
TS 22 (T2) (sup.)	40.2	242.16
TS 22 (T2) (pellet)	27.6	255.56
After Dialysis		
TS 35 (T1) (sup.)	78	722.25
TS 22 (T2) (sup.)	100.5	605.4

5. Conclusion

α -Amylase was produced from the obtained bacterial strains. It was observed that amylase enzyme produced from *Streptomyces diastaticus*(T2) had high enzyme activity (unit/ml) & specific activity as compared to amylase produced from *Streptomyces cyaneus* (T1). We also observed that the unit/ml of protein increased after 80% saturation of enzyme by ammonium sulphate precipitation. Almost pure enzyme was obtained after dialysis with the increase in the specific activity(U) upto 3 times. Thus we conclude that this bacterial species *Streptomyces cyaneus* (T1) & *Streptomyces diastaticus* (T2) can produce sufficient amount of amylase having a great economic importance.

References

- [1] Analytical biochemistry & separation techniques by Dr. P. Palamivelu, School of Biotechnology, Madurai University.
- [2] Althoff, K., Schütt, C., Steffen, R., Batel, R., Müller, W.E.G.: Evidence for a symbiosis between bacteria of the genus *Rhodobacter* and the marine sponge *Halichondria panicea*: harbour also for putatively toxic bacteria? *Mar. Biol.* 130 (1998), 529–536.
- [3] Bailey, J.E., Ollis, D.F.: *Biochemical Engineering Fundamentals*. Tokyo:

- McGraw-Hill Kogakusha Ltd., 1977, 155–220.
- [4] Burja, A.M., Webster, N.S., Murphy, P.T., Hill, R.T.: Microbial symbionts of Great Barrier Reef sponges. *Mem. Queensl. Mus.* 44 (1999), 63–76.
 - [5] Chandrasekaran, M.: Industrial enzymes from marine microorganisms: The Indian Scenario. *J. Mar. Biotechnol.* 5 (1997).
 - [6] Müller, W.E.G., Zahn, R.K., Kurelec, B., Lucu, C., Müller, I., Uhlenbruck, G.: Lectin, a possible basis for symbiosis between bacteria and sponges. *J. Bacteriol.* 145 (1981).
 - [7] Outtrup, H., Boyce, C.O.L.: Microbial proteinases and biotechnology. In: *Microbial Enzymes and Biotechnology.* (Fogarty, W., Kelly, C. T., eds.). London and New York: Elsevier Applied Science, 1990, 227–254.
 - [8] Pomponi, S.A.: The bioprocess-technological potential of the sea. *J. Biotechnol.* 70 (1999).
 - [9] Vengadaramana, A. Balakumar, and S. Vasanthi Arasaratnam, Dept. of Botany, Faculty of Science, Anna university.
 - [10] Wiseman, A.: *Handbook of Enzyme Biotechnology.* New York: Ellis Horwood Ltd., 1985, 274–379.]