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Partial Purification and Immobilization of novel α-amylase from streptomyces enissocaesilis

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ABSTRACT
Streptomyces enissocaesil is were isolated from marine samples collected from Visakhapatnam (Bay of Bengal), Andhra pradesh, India. They were used for the production of α-amylase. α-amylase production maximum on 5th day of incubation. (44.21 U/gds) by Streptomyces enissocaesilis was partially purified with ammonium sulfate precipitation by salting out technique about 70% (partially purified) 1 fold purification of the enzyme yield was 9.05 U/mg. The purified α-amylase enzyme produced was immobilized in calcium alginate beads. This immobilized enzyme is proceeded to assay procedure the maximumα-amylase activity from the immobilized enzymeis 41.32 U/gds.

I. INTRODUCTION

Actinomycetes are known to produce several enzymes, degrading complex organic materials in marine sediments Gluve and Desmukh, 2011, reported various enzymes such as protease, gelatinase, amylase, pectinases, cellulases and ureases from the coastal segments.

Partial purification of a particular enzyme involves removal of other substances (proteins as well as non-proteins) present in the preparation. Purification of an enzyme protein is generally a multi-step process exploiting range of biophysical and biochemical characteristics such as its relative concentration in the source, solubility, charge, size (molecular weight), hydrophobicity/ hydrophobicity of the target protein.

Designing the purification procedure, initially emphasis is given on concentrating the protein concentration in the sample rather than purification. After concentration, emphasis is given to purification (removal of unwanted proteins) and lesser loss of enzyme activity of the targeted enzyme. Commonly, the first step in enzyme purification is based on fractionation of proteins on the basis of solubility of proteins in aqueous solutions of salts or organic solvents. Immobilization is defined as the imprisonment of the cell or enzyme in distinct support or matrix. The matrix on which the enzymes are immobilized allows the change of medium containing substrate or inhibitor molecule for the production.

An immobilized enzyme is an enzyme attached to an inert, insoluble material—such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride This can provide increased resistance to changes in conditions such as pH or temperature It also lets enzymes be held in place throughoutthereaction,followingwhichtheyareeasily separatedfromtheproductsandmaybeused again - a far more efficient process and so is widely used in industry for enzyme catalyzed reactions. An alternative to enzyme immobilization is whole cell immobilization.

II. MATERIALS AND METHODS

Purification of α-amylase enzyme

A fraction (20ml) of supernatant produced under SSF was separated for partial purification by employing Ammonium sulphate precipitation and followed by dialysis. Ammonium sulphate at a 70% final concentration was used to precipitate the protease enzyme from the crude extract (Ravi kumar G. et al., 2012). The precipitated proteins were separated by centrifugation at 10,000 rpm at 4 0C for 15min. These were dissolved in phosphate buffer (pH 7.0) and stored at 4 0C. The precipitated sample was dialyzed against phosphate buffer (pH 7.0) at 4 0C for 24 h with buffer changes at regular intervals (Alagarasamy Sumantha et al., 2005).

Ammonium sulphate precipitation Requirements:
Ammonium sulphate
Ice tray
Magnetic bead and stirrer
Centrifuge

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Procedure:

A 20ml of clarified supernatant (crude extract) was taken and transferred in to anice cold beaker with a magnetic bead. Exact amount of finely powdered ammonium sulphate (436gm/liter of solution at 0 0C) was taken to bring the supernatant to 70%saturation. The beaker was placed in an ice tray to maintain low temperature and beaker was transferred on to magnetic stirrer along with the ice tray. Now exact amount of salt weighed for 20 ml of solution is slowly added with stirring. Add a small amount at a time and then allow it to dissolve before further addition. Keep it on the stirrer for 1hr precipitation to occur in ice. After that, centrifuge the resultant solution at 10,000 rpm for15min at 4 0C. The pellet contains the precipitated protein which could be dissolved in suitable buffer phosphate buffer (pH7) and stored in a refrigerator for further analysis and purification. For a second round of precipitation of a different protein, the supernatant is again used and the above same steps are followed.

Immobilization of Pure Enzyme

Entrapment of protease enzyme in calcium alginate beads

A 5ml of enzyme solution was transferred into 20ml of sodium alginate solution and kept on a rotary shaker for 10min. The mixture of alginate and enzyme solution was taken into a sterile syringe containing 23 grid needle and extruded gently drop wise into a0.2M $CaCl_2$ solution from 5cm height. The beads thus formed were stored in the same solution for an hour in refrigerator, after curing the beads washed with sterile water, drained and reused for further studies.

Results and Discussion

Fig:1 Crude α- amylase enzyme

Purification of α-amylase enzyme

An extracellular amylase was purified from the 20ml of culture filtrate of Streptomyces enissocaesilis. The enzyme was partially purified by ammonium sulphate precipitation (70%). From the results it was observed that by ammonium sulphate precipitation (Fig 1) about 70% of the enzyme yield (partially purified) was obtained with 2.3 purification fold and maximum amylase activity observed was shown in Table 1(9.05 mg).

Table: 1 Various ranges of ammonium sulfate precipitation and the respective activity of amylase

Ammonium sulfate concentration (%)	Activity of amylase (U/mg)
50	2.3
60	5.62
70	9.05
80	3.1

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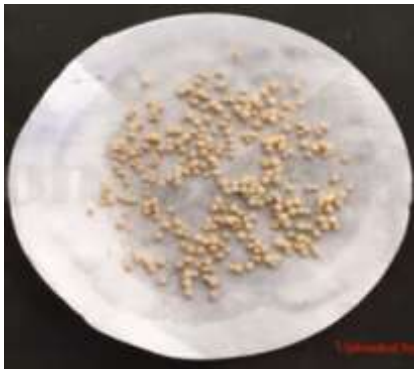

Fig: 2 Crude α-amylase enzyme entrapped in to calcium alginate beads

Immobilization of the α-amylase enzyme

The purified protease enzyme produced was immobilized in calcium alginate beads .this immobilized enzyme is proceed to assay procedure The maximum protease activity from the immobileenzymeis (Fig 2) 41.01 U/gds.

III. CONCLUSION

Partial purification of amylase, partial concentration of enzyme was achieved through 50-80% $(NH_4)_2SO_4$. Developing amylase purification techniques, which enable applications in pharmaceuticals and clinical sectors which require high purity amylases.

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