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Immobilization of α-Amylase by Pseudomonas sp isolated from Indian Chicken

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ABSTRACT

Control of α-Amylase formed by a Pseudomonas sp isolated from Indian Chicken feather was deliberate. Partly cleansed enzyme with 486.77 IU and specific activity of 811.28 units mg (protein) was used for immobilization study. An easily obtainable and cheap 3% Sodium alginate matrix with easy control gel entrapment procedure was used for tricking the enzyme showed 555.5 IU activity. The optimization was carried out to study the catalytic properties which showed the optimum pH, temperature and substrate concentration at pH 7, 450C and 16mg, respectively. The reusability of the arrested enzyme preparation presented its use in incessant starch hydrolysis for up to 10 cycles. This immobilized enzyme can be used as a standby of profitable enzyme since it has shown same greater operational flexibility and enzymatic activity of the pure enzyme.

Keywords: Immobilization, Calcium alginate, Immobilization, Pseudomonas sp.

INTRODUCTION

Enzymes are biocatalyst, whose operation has appeared as chief plans towards the ecofriendly and energy saving chemical processes (Om Prakash, 2011). Amylase is ubiquitous enzymes, which are found in animals, plants, fungi and bacteria and have been used in food, paper, textile, baking and detergent industry (Devendra Kumar, 2012; Sachin Talekar, 2012). In spite of the wide circulation of amylases, microbial sources, namely fungal and bacterial amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and ease of process modification and optimization (Dev, 2003). Control is a process of attachment of enzyme on solid supports is a very effective way to increase enzyme stability. Among the various matrices available, frequently used is gel entrapment within porous matrices, such as alginate in the form of beads (Dey, 2003 and Elif demirkan, 2011).

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This is a method for protecting and stabilizing the enzymes, thereby enhancing their properties and reasonably simple, cheap, safe, and offer high porosity, good mechanical strength, for substrate and product diffusion (Bucke, 1987; Vandana Singh, 2014). It is expected that starch hydrolysis reaction could occur more successfully if an enzyme bound to surface (Lily, 2007). The use of enzymes in a soluble form must be considered as less economic, wasteful since the enzyme usually cannot be improved at the end of the reaction. Feathers are produced in major bulk as a biological waste by poultry processing industries in India and cause serious environmental issue (Khardenvis, 2009). In order to solve the problem biodegradation of feathers are finished by microorganisms and are recycled as rich meal for animals and nitrogenous fertilizers for plants (Venkatesh, 2014). It has been also reported use of feathers as Low cost building materials (Menandro, 2011) and for construction biodiesel (Janhavvi, 2014). But feathers as a source of microorganisms and enzyme have not been studied. Hence, in the present study, new source of amylase producing microorganism was isolated from Indian chicken and its activity were considered and equated with profitable purified enzyme.

MATERIALS AND METHODS

Enzyme

Amylase producing microorganism was isolated from Indian chicken feather collected from the local market and was incompletely cleansed by ammonium sulphate and dialysis.

Screening and Identification of amylase creating bacteria.

Several methods have been proposed for showing of amylase. These methods either straight use the microorganism under study or quantity the enzyme activity in the crude or cleansed culture preparations. In the present study, the screening of bacterial strain from Indian chicken feather obained from Warangal districts were achieved as per standard serial dilution technique and plated on starch agar media. Plate recognition method was used for the observation of hydrolysis of starch through the presence of clear zones around bacterial strain. Biochemical characterizations of bacterial strains were carried out as per Bergys Manual to identify the bacteria 14. Extracellular extraction and purification of amylase Extraction of enzyme process was followed as per Devi 15 the 24 hr old culture bacterial strain was inoculated into the broth containing soluble starch, yeast extract, peptone, $MgSO_4.7\ H_2O$, NaCl and $CaCl_2$ and was incubated at 37^0C for 48 hrs. After 48hrs of incubation the broth is centrifuged at 10000 x g rpm for 10 min at 0^0C. Supernatant is used as crude extract of amylase.

The enzyme was partially purified by ammonium sulphate precipitation followed by dialysis using phosphate buffer (0.1M, pH 7.0). The Enzyme activity and specific activity of selected bacterial strain was determined by Dinitrosalicilyis acid (DNSA) method and Lowry's method respectively 16. The partially purified enzyme was used further for immobilization studies.

Entrapment of crude amylase extract on calcium alginate beads

The Incompletely purified enzyme was immobilized in Calcium alginate beads in 1:10 ratio of enzyme and 3% of Sodium alginate solution. The beads are formed by adding the sodium alginate solution into 0.2M CaCl2 at height of approximately 4-5cm through the burette. Beads are left in the solution for 3 hrs time. The calcium alginate beads containing the enzyme were thoroughly washed with distilled water in order remove excess calcium chloride solution on the surface of the beads and later it is preserved in the phosphate buffer (0.1M, pH 7.0)2. Same protocol was carried out for commercially available amylase enzyme procured from Himedia for the comparison.

Immobilized Enzyme Assay

Assay of immobilized α-Amylase was set up according to protocol described by Kumar (Kumar, 2006). For enzyme assay, four beads of calcium alginate of crude enzyme (2.4 µg protein/bead) and commercial enzyme adjourned in reaction mixture consist of 0.4 ml assay buffer (phosphate buffer, pH 7.0) was incubated with 1.6 ml of 1% starch solution at room temperature for 3 min and the reaction was stopped with 3 ml of 3, 5- dinitrosalicylic acid followed by heating the reactants in a boiling water bath for 10 min and then cooling down to room temperature. Solution was thinned by adding 9 ml of distilled and the amount of reducing sugar (maltose) produced was determined spectrophotometrically at 540 nm. Amylase activity was expressed in terms of IU. The amount of reducing sugar released during the reaction was estimated with the help of the maltose standard curve.

Optimization of temperature, pH and Substrate concentration

The optimum temperature for crude α-Amylase was resolute by hatching the reaction mixture of 14.5 ml comprising 0.1 ml of soluble enzyme and 4 beads of calcium alginate at numerous temperature 0, 30, 40, 45, 50, 60 and 70^0C for 10 min. The enzyme assay reaction was immobile by removing the beads and incubating the reaction mixture in boiling water bath for 10 min. The activity of amylase was recite at 540 nm spectrophotometrically. The Enzyme assay for determining the optimum pH was carried out by varying pH (4-10) at optimum temperature and for optimization of substrate (starch) concentration, varying concentrations (4-30 mg) of substrate was used.

RESULTS AND DISCUSSION

α-Amylase, an enzyme which fits to hydrolase family is formed by several bacteria and fungi. In our study amylase were removed from the source Indian chicken feather using starch media as a substrate. The isolate which exhibited the maximum clear zone of hydrolysis around the bacterial colony when flooded with iodine solution on the starch plate at 37^0C incubated for 48 hr indicates the presences of amylase activity and was selected for further studies. The bacterial isolates were characterized on the basis of cultural characteristics, microscopic characteristics and biochemical tests as per Bergys manual and the isolate were identified has *Pseudomonas* sp.

The crude α-Amylase enzyme was extracted, hastened upto 70% using Ammonium sulphate solution and dialyzed in phosphate buffer (pH 7.0) showed the enzyme activity of 486.77 IU and exact activity of 810.28 units mg protein. This partially purified enzyme was immobilized in calcium alignate and it showed the enzyme activity of 555.5 IU. The commercial amylase presented 610.11 IU and 1018.5 units mg protein-1 for soluble enzyme analysis and for immobilized 780.22 IU. The experimental results of optimization of crude *Pseudomonas* amylase and commercial amylase has shown in fig 1, 2, 3. The soluble enzyme of pure and crude amylase showed maximum activity at 45 0C, 7 pH and 16mg protein concentration. The immobilized enzyme showed variation in optimum pH, there was a slight shift towards both acidic and alkaline direction. The kinetics of the enzyme varies based on the

microenvironment has been cited in various reports (Sachin Talekar, 2012). One of the important characteristics of an immobilized enzyme is its stability and reusability over an extended period of time. The repeated use of the halted amylase for hydrolysis of starch was studied. It has been reported that the loss of activity of entrapped enzyme was due to leakage of enzyme from the beads during washing of beads at end of each cycle.

Figure-1. Variation in Enzyme activity due to Temperature

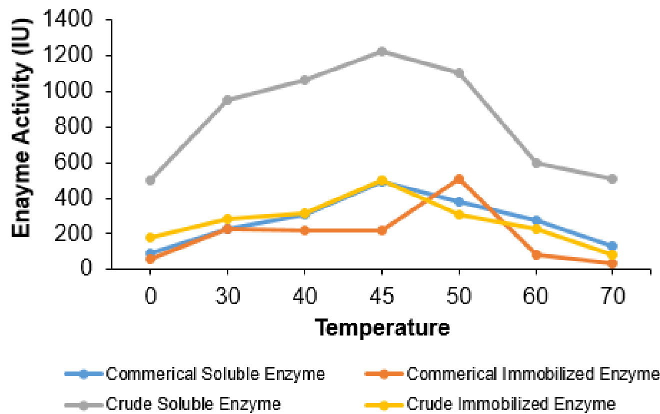


Figure-2. Variation in Enzyme activity due to pH

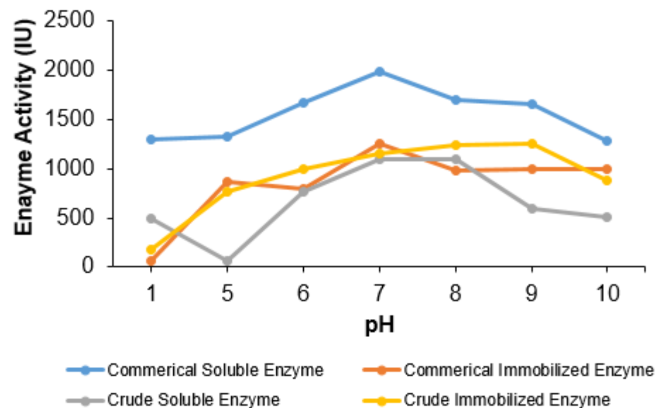
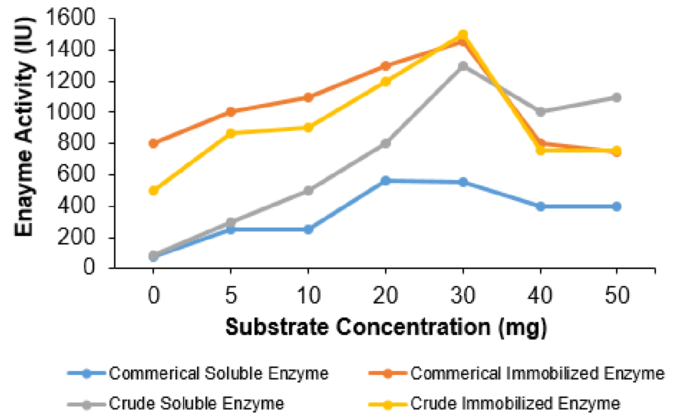


Figure-3. Effect of substrate concentration on α-amylase activity



CONCLUSION

The halted crude α-Amylase from *Pseudomonas* sp can be used as a replacement to commercial enzyme, since it showed activity very near to the commercial enzyme. The working parameter were same as marketable enzyme, it showed the greater satiability in high temperature and pH. The use of crude enzyme and reusability of enzyme is the one of the cost cutting strategies which can be employed for industrial use.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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