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ISOLATION, OPTIMIZATION, PRODUCTION AND PURIFICATION OF ALPHA AMYLASE FROM SOIL BACTERIA

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Abstract - *Amylase is a hydrolytic enzyme that is used widely in industries. It is easier to produce enzymes from bacteria than any other organism. The present work focused on the production of amylase enzyme from bacteria isolated from soil samples. The amylase producing bacterial strain screening was performed using starch agar plates. Various biochemical tests were carried out to confirm the strain and was identified as B.subtilis. Fermentation conditions such as temperature and P^H were optimized for maximum enzyme production from B.subtilis. The optimum P^H was found to be 7.0 and the temperature was found to be 37° C. The enzyme was partially purified by ammonium sulfate precipitation method and it was purified by dialysis. The SDS-PAGE was also performed to determine the molecular weight of amylase enzyme produced.*

Key Words: **Amylase, *Bacillus subtilis*, ammonium sulfate, dialysis, SDS-PAGE**

1. INTRODUCTION

α-Amylase is a protein enzyme E(.3.2.1.1) that hydrolyses alpha bonds of large, polysaccharides, such as starch and glycogen , yielding glucose and maltose[1]. They can specifically cleave the O-glycosidic bonds in starch. Starch depolymerization by amylases is the basic mechanism for used in the preparation of glucose syrups, Bread making and brewing. Thus it is a key enzyme in the production of starch derivatives and is also used in desizing fabrics, in pharmaceuticals and detergents. Submerged fermentation (SmF) has been traditionally used for the production of industrially important enzymes because of the ease of handling and greater control of environmental factors such as temperature and p^H[2].

Solid-state fermentation dominates over submerged fermentation in aspects such as better yield, simple technique, low capital investment, lower levels of catabolite repression, high stability and better product recovery[3]. Agro-residues are generally considered as the best substrate for the solid-state fermentation processes[4].

Many agro-industrial by-products such as wheat bran, rice bran, molasses, barley bran, maize meal, soybean meal, potato peel and coconut oil cake have been screened as low cost solid substrates for microbial production of α-amylase in solid-state fermentation[5]. The major factors that affect microbial synthesis of enzymes in a solid-state fermentation system include the selection of a suitable substrate, microorganism, inoculum concentration, particle size and moisture level of the substrate.

There are different sources from which the enzymes can be produced. Plants, animals and microbial sources can produce amylases[6]. Microorganisms produce different kinds of industrial enzymes. Because of their biochemical diversity and the case with environment and genetic manipulation, they have replaced enzymes, which traditionally have been isolated from complex eukaryotes[7]. Microorganisms utilize various substrates as a nutrient source for their growth and metabolic activities and subsequently produce metabolism related products.

However, fine tuning of nutrient concentrations regulate the microbial metabolism and associated metabolic product formation. Balancing of nutrient concentration with minimum experimentation and other cultural parameters is an art in microbial metabolism to optimize enzyme production[8]. Enzyme production is dependent on a number of factors that include strain type, culture medium composition and condition of fermentation, availability of carbon, nitrogen, mineral salts etc.,

The growth and enzyme production of the organisms are strongly influenced by medium composition. Thus, optimization of media components and cultural parameters is the primary task in a biological process [9]. The main strategy used is media engineering for which the optimal operating condition of a parameter is standardized by changing one parameter at a time and keeping the others at a constant level[10]. The optimization studies do not consider the interaction effects among the variables as any process is influenced by several variables .The present study focused on the isolation, optimization, production and purification of α-amylase from soil bacteria.

2. MATERIALS AND METHODS

2.1. Isolation of microorganisms

The soil samples were collected from Jai Nagar Park, Arumbakkam, Chennai, Tamil Nadu. The soil samples were subjected to serial dilutions and 10^{-2} and 10^{-3} dilutions were swabbed on agar plates. They were incubated at 37° C for 48 hours.

2.2. Screening

The grown culture was again streaked on 1% starch agar plates to screen the amylase producing bacteria. They were incubated overnight. The hydrolyzed zone was identified by adding grams iodine.

2.3. Identification of bacterial strain:

The isolated strain was subjected to various biochemical tests such as Urease test, Citrate test, Triple Sugar Ion test, Indole test, Motility test, Oxidase test, Catalase test and gram staining to identify the species.

2.4. Optimization of fermentation parameters:

2.4.1. Effect of P^H:

The media was prepared and the p^H was adjusted as 5, 6, 7, 8, and 9 in different tubes by adding 1N HCl and 1 N NaOH. 0.1 ml of 24 hours grown culture was inoculated and incubated at 37° C for 48 hours.

2.4.2. Effect of carbon sources:

Five different carbon sources were taken such as Dextrose, Maltose, Sucrose, Lactose and Mannitol and were analyzed at 1% concentration. The media was prepared with respective carbon sources and 0.1 ml of 24 hours grown *B.subtilis* culture was inoculated to the medium and incubated at 37° C for 48 hours.

2.4.3. Effect of nitrogen sources:

Five different nitrogen sources such as Ammonium chloride, Sodium Nitrate, peptone, Protease peptone and Calcium Nitrate were analyzed at 1% concentration. The media was prepared with respective nitrogen sources and 0.1 ml of 24 hours grown *B.subtilis* culture was inoculated to the medium and incubated at 37° C for 48 hours.

2.5 Production medium:

The production media was prepared based on the optimized conditions and 24 hours grown *B.subtilis* culture was added and incubated at 37° C for 48 hours.

2.6. Estimation of amylase enzyme assay:

Amylase activity was determined by DNS method. Enzyme activity was assayed by reducing sugar formed by the enzymatic hydrolysis of soluble starch. Starch was used as the substrate at a concentration of 1% in 0.05 M phosphate buffer (p^H 6.9). Crude enzyme sample was mixed in a test tube and substrate solution incubated at 37° C for 10 minutes. The reaction was controlled by adding 1ml of Di Nitro Salicylic acid solution. The test tube kept in boiling water bath for 5 min and cooled. The absorbance was read at 540 nm against blank (without enzyme).

2.7. Partial purification:

2.7.1. Ammonium sulfate precipitation method:

The 48 hours grown bacterial culture was centrifuged at 10000 rpm for 15 minutes. The supernatant was collected separately and the enzyme were precipitated by ammonium sulphate salt. To the crude extract 70% of the NH_4SO_4 was added. Then it was incubated for 24 hours and centrifuged at 10000 rpm for 15minutes and the supernatant was decanted.

2.7.2. Dialysis:

The partially purified enzyme was further purified by dialysis. The dialysis tube was boiled in distilled water for few minutes. Then the pellet was mixed with Tris-HCl buffer and the solution was transferred to the dialysis tube. Then it was placed in a beaker containing 500 ml of buffer for 24 hours. Due to osmosis, the impurities were removed and the same process was repeated for 48 hours.

2.7.3. SDS-PAGE

SDS-PAGE method was used to determine the molecular weight of purified enzyme. The samples were mixed with loading dye. The sample and marker were loaded on the respective wells and was run for 1 hour. The gel was observed for determination of Molecular weight.

3. RESULTS AND DISCUSSION:

3.1. Isolation and Identification:

The bacterium producing Amylase enzyme was isolated and identified from the soil sample as *B.subtilis*. They were screened by the starch hydrolysis and they showed a clear zone of hydrolysis of starch shown in Fig.1.This was in accordance with the results[11].

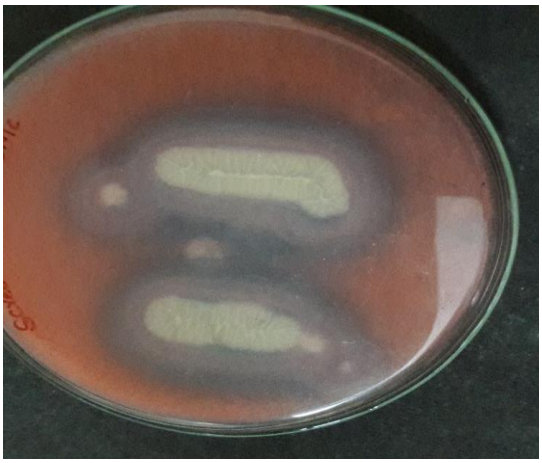

Fig.1. Screening

They were further confirmed as *B.subtilis* using Gram staining and biochemical assays.

Table 1:

Biochemical Test	Results
Urease test	Negative
Citrate test	Positive
Triple Sugar Ion(TSI)	Negative
Indole test	Negative
Motility test	Positive
Oxidase test	Negative
Catalase test	Positive
Gram staining	Positive

3.2. Optimization:

P^H is one of the important parameters largely influencing growth of the microorganism and affecting enzyme yields. The variation in p^H on either side of the optimum value lead to the decline in microbial growth[12] . The optimum p^H for the production of α-amylase from *B.subtilis* was found 7.0. This was in accordance with Mantsala (1989), Hamitton *et al*.., (1999), Saito (1973) and khoo *et al*.., (1994)[13] . In the present study, the maltose was the best carbon source for the production of amylase enzyme from *B.subtilis* is 0.351 units/ml. The production of amylase enzyme from *Bacillus megaterium* using maltose as carbon source shown 0.280 units/ml[2]. The protease peptone was the best nitrogen source for the production of amylase enzyme is 0.7585 units/ml. Qader *et al*.., (2006) reported that maximum enzyme production was found with 1 % peptone[14]. Earlier studies reported[15] (Bajpai *et al*.., 1989).

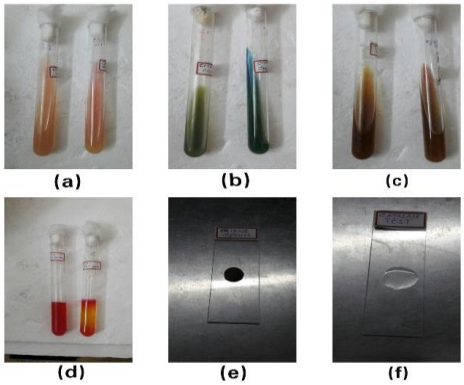

Fig.2.Biochemical tests

(a)Urease test (b) Citrate test (c) Triple Sugar Ion test

(d) Motility test (e) Oxidase test (f) Catalase test

The optimally grown 150 ml bacterial culture was centrifuged at 10000 rpm for 10 min (4° C). The supernatant was collected separately and 70 % ammonium sulphate and incubate overnight at 4° C. The crude enzyme was obtained from the pellets by centrifugation. The total protein was estimated using Lowry's method and it was found to be 112µg/ml. The crude enzyme was purified by dialysis. Then the molecular weight of purified enzyme was found to be 53 kda as estimated by SDS-PAGE.

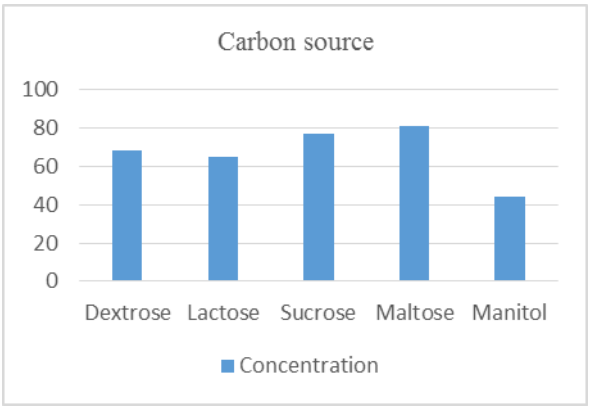

Fig.3.various concentrations of carbon source

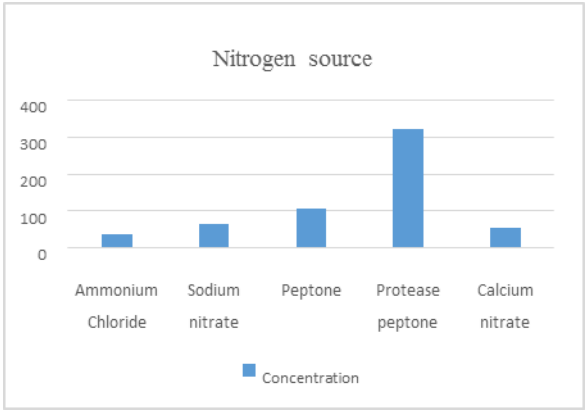

Fig.4.Various concentrations of nitrogen source

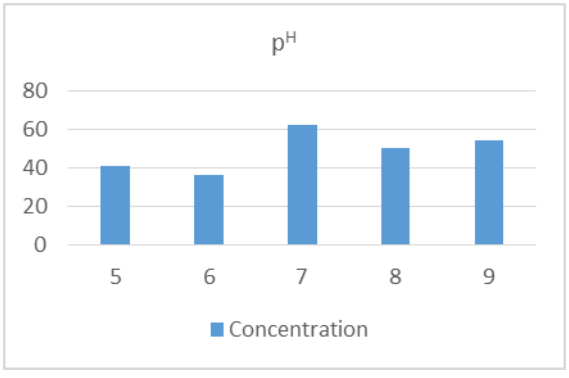

Fig.5.Various concentrations of P^H

α-Amylase activity was also determined[16] using Bernfeld method (1955). One unit of amylase activity is defined as the number of micro moles of maltose liberated by 1 ml of enzyme solution per minute. Amylase production was expressed as units per gram of dry substrate[17]. (Kaliappan

kalaiarasi and Ramasamy Parvatham, 2013).The α-amylase activity was found to be 84 µg/ml.

4. CONCLUSION

Amylases are extensively used in industrial applications like starch modification and food processing. Amylase producing bacteria was isolated from soil sample and identified as *B.subtilis* by various biochemical tests. The strain was found to be a potent producer of amylase enzyme with high activity after 24 hours at 37° C. The enzyme was purified by ammonium sulphate(70%) precipitation. The present study concluded that soil served as a rich source of numerous hydrolytic enzymes and can be a source of many potent microorganisms.

5. REFERENCE

[1] Maureen Barlow Pugh., ed. (2000). Stedmans Medical Dictionary (27 ed). Baltimore, Maryland,USA: Lippincott Williams and Wikins . p .65. ISBN 978-0-683-40007-6.

[2] M.Sankareswaran, K.Kalaiselvi, S.Anbalagan, M.Suganya, K.Abirami (2015). Production, cahracterisation and immobilized dye decolorization of amylase enzyme produced by Bacillus megabacterium isolated from soil sample. International Journal of advanced Research(2015),Volume 3,Issue 8,295-305.

[3]Babu KR and Satyanarayana T. Folia Microbiol 1993;38:77-80

[4] P Elliah ,B Srinivasalu and K Adinarayana (2002).A Review on Microbial Alkaline Proteases. JSIR Vol, 61,sep 2002, pp 690-704

[5]Jyoti Shukla and Rita Kar. Potato peel as a solid substrate for thermostable α-amylase production by thermophilic *Bacillus* isolates. World Journal of Microbiology and Biotechnology. May 2006, Volume 22, Issue 5, pp 417-422.

[6] Sivaramakrishnan S, Gangadharan D, Nampoothiri KM, Soccol CR, Pandey A: alpha -amylases from microbial sources. An overview on recent developments. Food technol biotechnol, 44, 173-184, (2006).

[7] Pandey,A., P.Nigam,C.R. Soccol, V. T. Soccol, D. Singh and R. Mohan., (2000). Advances in Microbial amylases Biotechnol. Appl, Biochem.31 (2):85-89.

[8] Prakasham R S, Subba Rao C H,Seenivas Rao R, SSarma PN (2007). Enhancement of acid amylase production by an isolated *Aspergillus awamori.* J.Appl. Microbiol. 102:204-211.

[9]Djekrif-Dakhmouche S, Gheribi-Aoulmi Z, Meraihi Z, Bennamoun L(2006). Application of a statistical design to the optimization of culture medium for α-amylase production by Aspergillus niger ATCC 16404 grown on orange waste powder. J. Food Eng. 73: 190-197.

[10] Bing-Lan Liu and Yew-Min tzeng. Optimization of growth medium for the production of spores from Bacillus thuringiensis using response surface methodology. Springer June 1998. Volume 18, Issue 6, pp 413-418.

[11] Nanik Rahmani ,Yopi, Ade Andriani,Alex prima(2011). Production and Characterisation of amylase enzyme from marine bacteria. Proceedings of the second International seminar on chemistry (255-259).

[12] Singh P., Gupta P.,Singh R. and sharma R.(2012).Factors affecting α-amylase production on submerged fermentation by Bacillus sp. International journal of pharmaceutical and life science. (3)12, 2243-2246.

[13] Mantsala (1989), Hamitton et al.(1999), Saito (1973) and khoo et al.(1994), purification and properties of raw starch degrading α-amylase of Bacillus sp. IMD 434, Biotechnol. Lett.21(1999) 111-115.

[14] Qader SAU, bano S, Aman A, Syed N, Azhar A (2006).Enhanced production and extracellular activity of commercially important amylolytic enzyme by a newly isolated strain of Bacillus sp. AS-1.Turk. J.Biochem.31:135-140.

[15] Bajpai,p. and Bajpai, p.(1989). High-temperature alkaline α-amylase from Bacillus licheniformis TCRDC-B13.Biotech.Bioengc.33:72-78.

[16] Bernfeld P(1955).Amylases,α and β. In: Methods in Enzymology. Academic Press, New York, USA, pp. 149-158.

[17] Kaliappan kalaiarasi and Ramasamy parvatham(2013). Optimization of process parameters for α-amylase production under solid-state fermentation by Bacillus cereus MTCC 10202. African journal of Microbiology Research.Vol.7(45),pp.5166-5177