International Journal of Advance Research in Science and Engineering
Volume No.06, Special Issue No.(01), December 2017
www.ijarse.com
IJARSE
ISSN: 2319-8354

ISOLATION, CHARACTERIZATION AND PURIFICATION OF AMYLASE ENZYME

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ABSTRACT

Nowadays, the use of α-amylase in some industries especially in food, beverage, textiles, leather and paper industries is increasing; there is a need for other source of the enzyme to be discovered as Nigeria is a tropical country which is rich of natural resources, particularly the microbes as enzyme producers. Amylase activity of pure culture was assayed by starch hydrolysis test. The enzyme production was done by using amylase production media at 37°C in shake flask culture. The enzyme was extracted by centrifugation, and then it was purified by ammonium sulfate precipitation 10 to 65%. The precipitated protein sample has been subjected to dialysis by using phosphate buffer. The enzyme activity of crude protein extract was determined by DNS method and maximum activity was 0.32 AU/ml. Crude protein and purified samples were subjected to 10% SDS-PAGE.

I INTRODUCTION

Amylase is an enzyme which hydrolyses starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units [1]. This enzyme is of great significance in present day biotechnology with applications ranging from food, fermentation, textile to paper industries. Although amylase can be derived from several sources; including plants, animals and microorganisms, microbial enzymes generally meet industrial demands [2]. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industries.They are also used as a partial replacement for the expensive malt in the brewing industry, to improve flour in the baking industry, and to produce modified starches for the paper industry. In addition to this, they are used to remove starch in the manufacture of textiles (desizing) and as additives to detergents for both washing machines and automated dishwashers (DhanyaGangadharan, 2006).Present study is focused on extraction, purification,optimization and characterization of amylase from *Bacillusamyoliquefaciens*.

II MATERIALS AND METHODS

Media Preparation The nutrient agar, Sabouraud Dextrose agar and Starch agar medium used for this work were prepared (Oyeleke S. B. *et al*, 2011).

Maintenance of culture

*Bacillus amyloliquefaciens*ATCC23842 was grown on nutrient agar (Hi-media, Mumbai,India) slants at 37 °C for 24 h. The fully grown slants were stored at 4 °C and were sub cultured every two weeks. A volume of 50mL of nutrient broth taken in a 250-mL Erlenmeyer flask was inoculated with a loopfull of cells from a 24-hour-old slant and kept at 37 °C in a rotary shaker. After 18 h of incubation, 1mL of this nutrient broth culture was used as the inoculums (DhanyaGangadharan, 2006).

Enzyme assay

The amylolitic activity of the test isolates was determined by using the starch agar plate method, by inoculating the identified *Bacillus* species into Nutrient Agar medium which was supplemented with 1g of starch. The agar plates were then incubated at 37℃for 24hrs. After the incubation period, freshly prepared iodine solution was added to the culture plate to identify the zones around the cultures. The diameter formed after the addition of iodine solution was measured to represent the amylolytic activity (ElifDEMĐRKAN, 2010), (Oyeleke S. B. *et al*, 2011), Khan et al(2011).

Production and Extraction of enzyme at lab scale

The amylase production was carried out in 250 mL conical flasks containing 50 mL medium with the following composition: 5g/L soluble starch, 5g/L yeast extract, 2.5g/L (NH4)2SO4, 0.2g/L MgSO4·7H2O, 3g/L KH2PO4, and 0.25 g/L CaCl2·2H2O incubated at 50°C under shaking conditions (200rpm) and inoculated with 2.5% of 24th old culture (Yasser R. Abdel Fattah, 2013), (Ajayi A., 2008).After incubation, the production medium was centrifuged at 6000rpm for 30 min to separate the cells. The supernatantwas collected as it contained the crude enzyme and stored at 4oC till further use, (Varalakshmi et al, 2008), (Oyeleke S. B.*et al*, 2011).

Amylase assay

Amylase activity was estimated by the activity of reducing sugar released during hydrolysis of 1%(w/v) starch in 0.1M phosphate, pH 6.5,at 250c for 20 min by dinitrosalicylicmethod(DNS) method(Miller,1959).One unit of amylaseactivity was defined as the amount of enzyme that release 1mmol of reducing sugar as glucose per min assay condition (Varalakshmi et al,2008).

Enzyme optimization

Optimization for different parameter such as time, temperature, pH on enzyme activity was carried out. Effect of temperature on enzyme activity was determined at different temperature from 10-600c and effect of pH on enzyme activity was determined at different pH within range 4-9 and also the effect of time on enzyme activity was determined at different time intervals from 10- 60min(K. JAYA PRIYA,2011),(Shembaker et al,2013),(Tippsmwat et al,2006).

Enzyme purification and characterization

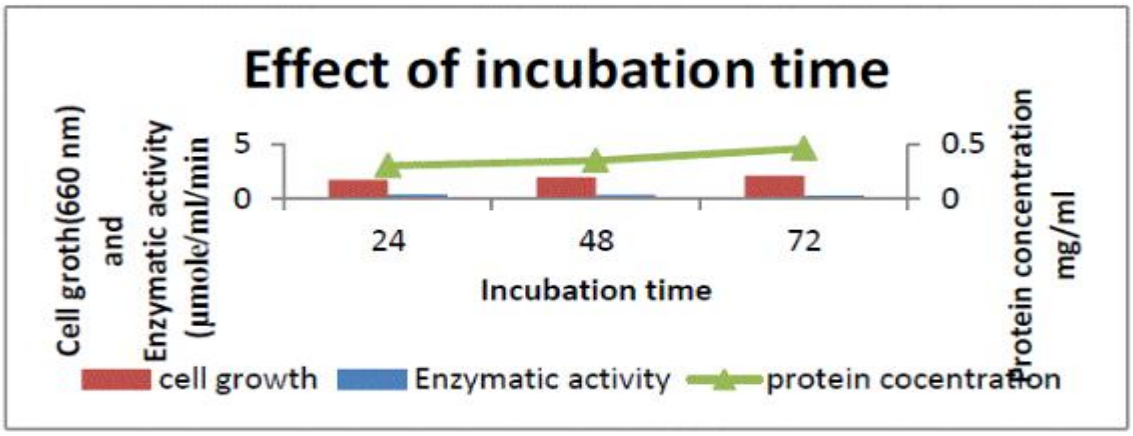
Enzyme was purified by ammonium sulphate (40%) method.The precipitate obtained by centrifugation was dissolved in 0.1M phosphate buffer(pH7) and dialysed overnight against 0.01M phosphate buffer. The experiment was carried out under low temperature 40c to prevent denaturation. The sample obtained thus subjected to polyacrylamide gel electrophoresis using specific markers such as albumin, oval albumin(Varalakshmi et al, 2008).

Protein Estimation

Concentration of protein in crude and purified extracts was determined by Lowry's method of protein estimation wherein 0.5 ml of enzyme was made upto 1ml with distilled water and reacted with Lowry's reagent C and D as per the protocol given by (Lowry *et al*., 1951). The absorbance (550nm) obtained after the reaction was compared with the standard graph plotted by reacting a standard protein (BSA; 0.02-0.2mg/ ml) with Lowry's reagents and the concentration of protein in crude and purified extracts was obtained.

III RESULTS

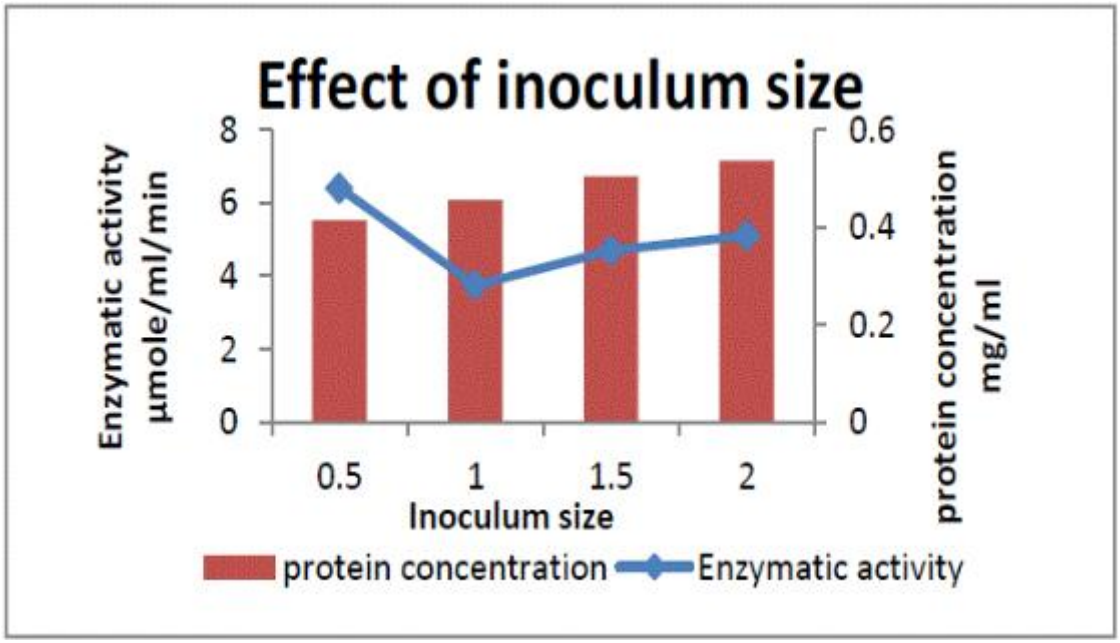
Optimize the incubation time for maximum amylase production sample was harvested at 24 h interval for 72 h from the production medium and centrifuged at 10,000 rpm for 10 minute. Supernatant was used for the amylase assay as described in methods and protein estimation (Lowery et al., 2005). The growth of culture was also checked in terms of O.D. The results obtained are shown in the following graph (Figure.). From graph it was concluded that maximum amylase production (0.41µmole) was observed at the end of 24 h and it decreased with increase in time.



Effect of different incubation time on amylase production

To study the effect of different inoculum size on amylase production

Effect of inoculum size for maximum amylase production was checked. Different inoculum size such as 0.5 O.D.660/100 ml, 1 O.D. 660/100 ml, 1.5 O.D. 660/100 ml, 2 O.D. 660/100 ml was inoculated in production medium and sample was harvested at the interval of 24, 48, 72 h. The results obtained are shown in the following graph. Since maximum enzyme production was obtained at 24 h, graphical representation of enzyme and protein production at the end of 24 h only is depicted.

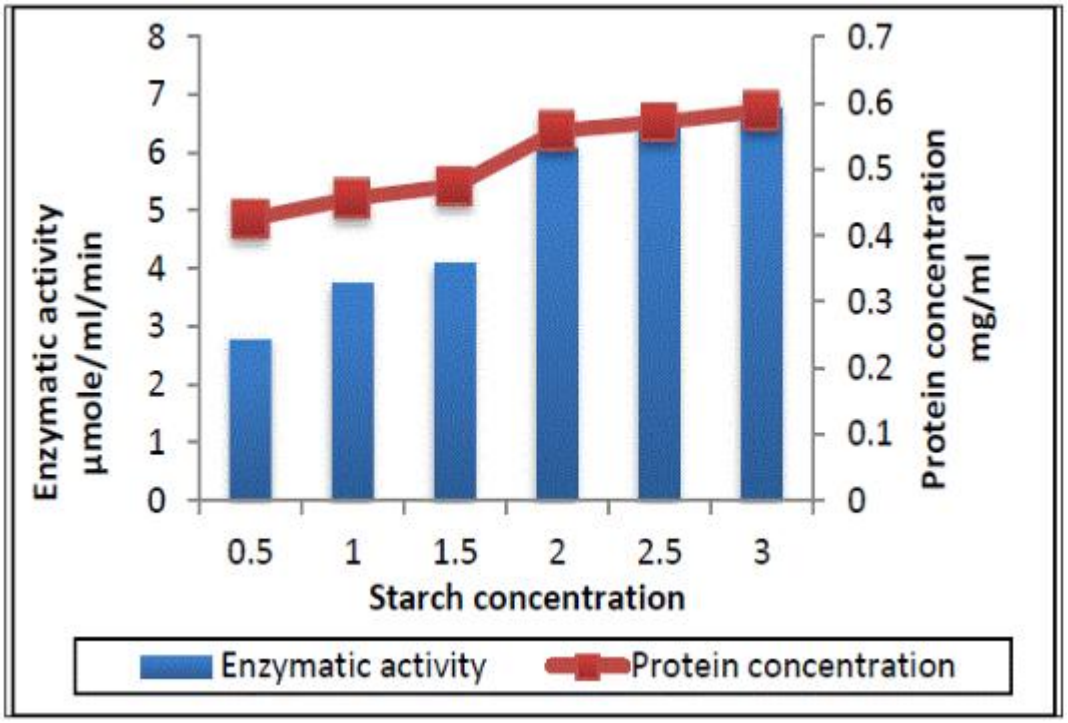


Effect of different inoculum size on amylase production

From graph it was concluded that inoculum size of 0.5 O.D. /100 ml is optimum for maximum enzyme production (6.40 µmole/ml/min). With increase in inoculum, increase in the extracellular protein content was observed but similar pattern was not observed for the amylase production.

To study the effect of different concentration of starch on amylase production

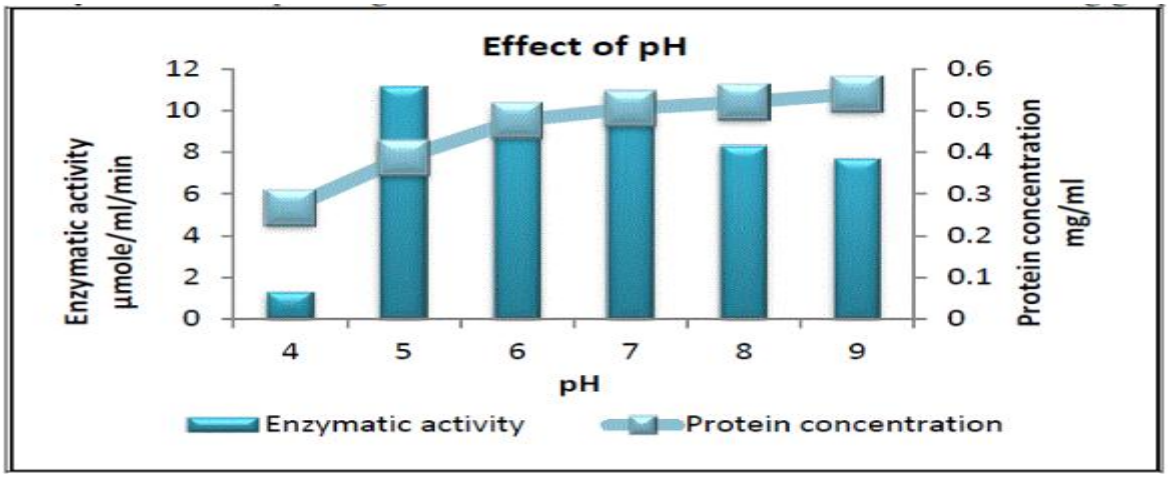
Bajpai et al.,(1989) reported that carbon source greatly influence amylase production and most commonly used substrate is starch. In this paper, the effect of different concentration of soluble starch on amylase production was studied, for this study production medium having different concentration of starch were prepared. Our strain shows a stable increase in the enzyme production with increase in starch concentration. Highest amount of amylase (6.77 U) was produced with 3% starch in production medium



Effect of different concentration of starch on amylase production

To study the effect of different pH on amylase production

Production media was set at different initial pH in order to check the effect of pH on amylase production by Pseudomonas sp. B5. Different initial pH ranging from 4 to 9 was tested. Our strain gives highest enzymatic activity at pH 5 and yield good enzyme at initial pH range 5 to 7. The results are as shown in the following graph.



Effect of different pH on amylase production

IV CONCLUSION

The usefulness of an enzyme from any organism for starch hydrolysis depends upon its potential to degrade native starch to oligosaccharides, glucose and other products at high temperatures and over a wide range of pH. The ability of Bacillus sp. to degrade native starch, at a wide range of pH and the thermal stability of amylase are the attractive attributes which make this bacterial strain to be a potential source of this enzyme for starch hydrolysis, especially for many applications ranging from bread and baking industry, starch liquefaction and scarification, textile desizing, paper industry, detergent application, analysis in medicinal and clinical chemistry.

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