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Characterization of extracellular amylase produced by *Aspergillus fumigatus* isolated from rice husk waste dumpsite

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ABSTRACT

Amylases are a class of enzymes (hydrolases) that are capable of digesting the glycosidic linkages found in starch or glycogen. In this study, *Aspergillus fumigatus*, isolated from a local rice husk waste dumpsite was used for the production of extracellular amylase enzyme. The optimum pH for the activity of the amylase produced was pH 7 with activity of (0.51mg/ml). Optimum temperature for amylase activity was 70°C with an activity of (0.55mg/ml). Maximum amylase activity was attained after 144hours incubation period with activity of 1.42mg/ml. this study has shown that amylase produced by the isolated *Aspergillus fumigatus* may have practical applications in the starch industry. Further studies to purify and characterize the amylase complexes produced by this strain should be investigated.

KEYWORDS

Amylase;
Aspergillus fumigatus;
Enzymes;
Optimum;
Activity.

INTRODUCTION

Amylases are widely distributed and are one of the most studied enzymes. Amylases are a class of enzymes (hydrolases) that are capable of digesting the glycosidic linkages found in starch or glycogen. Under aqueous conditions, amylases act on glycosidic bonds present in starch to liberate glucose, maltose, and maltotriose[1]. They are produced by a variety of living organisms ranging from bacteria, fungi to plants and humans[2]. Amylases are among the most important enzymes in present day biotechnology and they have almost completely replaced chemical hydrolysis of starch in starch producing industry and have a great significant demand as they hold approximately 25% of the enzyme market[3]. Although amylases can be derived from several sources, microbial enzymes generally meet industrial demand. These enzymes have found wide applications in a number of industrial processes such as food, fermentation, textile and paper industries[4].

Bacteria and fungi secrete amylases to the outside of their cell to carry out extracellular digestion. When they have broken down the insoluble starch, the soluble end products such as (glucose or maltose) are absorbed into their cells. Soil fungi are a very good source of amylase production[5].

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Many chemical transformation processes used in various industries have inherent draw-backs from a commercial and environmental point of view. In particular, a greater awareness of conservation issues has forced industries to consider alternative, cleaner methods. With this regards, the use of enzymes as industrial catalysts is becoming the best option, and microbial enzymes are gradually replacing chemical catalysts in many areas of industry[5]. Microbial enzymes are becoming increasingly important for their technical and economic advantages[6]. Recent discoveries of starch degrading enzymes have led to increase in the application of amylase in various industrial processes. Wastes can also be used as a good source for amylase activity[7].

The ability to use starch as a carbon and energy source is widely distributed among different organisms. Since this polymer is water insoluble and too large to pass across the cell membrane, biodegradation occurs extracellularly. The properties of starch degrading enzymes however, vary with the source organism, and different organisms produce one kind or a mixture of these amylolytic enzymes[5].

Currently, the screening and identification of filamentous fungi capable of secreting extracellular enzymes with biotechnological potential are activities of great importance. The confirmation of the potential for enzyme secretion by a species and the analysis of the conditions of production lead to a possible improvement of the environmental conditions favoring the maximal exploration of this capacity. The present study was undertaken to produce amylase by *Aspergillus fumigatus* and to determine the effect of pH, temperature and incubation time on amylase production.

MATERIALS AND METHODS

Sample collection

Ten grams of soil were collected in sterile polythene bags from a local rice husk dumpsite along Minna-Bida road in Minna, Niger State and where taken to the Microbiology laboratory at Federal University of Technology, Minna for analysis.

Isolation and identification of fungi

The fungal isolates were isolated from soil by the serial dilution method of Gillman[8]. Ten gram of the soil sample was suspended in test tubes containing 9ml of sterile distilled water. After the serial dilution, 0.1ml from the 10^6 dilution was spread on potato dextrose agar plates. The plates were incubated at room temperature for 5 days. The colonies that developed were subcultured unto Sabouraud Dextrose Agar (SDA) and were identified on the basis of color of arial and substrate hyphae.

Qualitative test for amylase

Screening for the amylolytic activity of the fungal isolate

The amylolytic activity of the fungi isolate was determined using the starch agar plate method as described by Bertrand[9]. The isolate was inoculated unto Sabouraud Dextrose agar medium which was supplemented with 1g of starch. The plates were then incubated at room temperature for 72 hours. After the incubation period, 1% of Lugol's iodine solution was layered on the agar plates. Zones formed were measured using graduated ruler and these represented the amylolitic activity of the isolate[10].

Quantitative test for amylase

Growth conditions

The liquid minimal media for amylase production contained the following composition in grams per liter; soluble starch, (20.0), $MgSO_4.7H_20$ (0.1), $KH_2\ PO_4$ (1.4), KCl (0.5), $FeSO_4.7H_20$ (0.01) and NH_4NO_3 (10.0); pH 6.5, in distilled water[11]. This was autoclaved and allowed to cool.

Enzyme extraction

Twenty two (22ml) of 0.1M phosphate buffer saline (pH 7) was added to each of the inoculated substrate beds and was vigorously shaken in rotary shaker for 15 minutes at 120rpm. The mixture was filtered through cheese cloth and centrifuged at 8000rpm for 15min. The filtrate was used as the crude enzyme preparation.

Enzyme assay

Activity of the amylase was determined by dinitrosalicyclic acid (DNS) method as described by Okolo[12], by pipetting 0.5ml of culture extract enzyme into test tubes and 1ml of 1% soluble

starch in citrate phosphate buffer having a pH of 6.4. The reaction mixture was incubated in a water bath at 40°C for 30 minutes. A blank, consisting of 1ml of soluble starch in citrate phosphate buffer (pH 6.4) was also incubated in a water bath at the same temperature and time with the test tubes. The reaction was terminated by adding 1ml DNSA reagent in each test tube and then immersing the tubes in a boiling water bath for 5 minutes. They were allowed to cool and 5ml of distilled water was added. The absorbance was then measured at 540nm with spectrophotometer (JENWAY, 6305). The amount of glucose produced was calculated by referring to the standard plot using glucose as the reducing sugar. Enzyme activity was defined as the amount of enzyme required to liberate a unit of glucose per minute (mg/glucose/ml/min).

Estimation of soluble protein

Soluble protein concentration was determined in aqueous extract fermented substrate using Bovine serum as standard[13].

Optimization of culture conditions for production of amylase

Different culture conditions like pH, temperature and incubation time were optimized for production of amylase from *Aspergillus fumigatus.* Amylolytic activity was measured under standard assay conditions. The crude enzyme extract was taken and used for the characterization. The effect of pH (4,5,6,7,8,9,10) and temperature (30,40,50,60,70,80,90,100) on the activity of crude amylase enzyme was studied. The crude enzyme was incubated for 20 minutes and enzyme activity was measured before and after the treatment under standard assay conditions. Adjustments of the pH were done by addition of hydrochloric acid (0.1N) and sodium hydroxide (0.1N) to achieve acidity and alkalinity respectively. Thermo-stability of the enzyme was determined by maintaining the enzyme solution in a water bath at the different temperatures.

Effect of incubation time

The effect of incubation period on enzyme production was investigated by checking the enzyme activity at 72, 96, 120 and 144 hours of incubation at pH 7 and at room temperature.

RESULTS AND DISCUSSION

There are several factors which affect enzyme production process. Among these, (physical, chemical and biochemical) are crucial factors[14]. In the present work the selection of *Aspergillus fumigatus* was based on the fact that fungal amylases are produced mainly by *Aspergillus* sp. It is also known that the amylase of *Aspergilli* species is known to produce more sugar than bacterial amylase[15].

Qualitative and quantitative analysis

Aspergillus fumigatus was found to produce amylase on the basis of clear zones around the fungal colonies on SDA agar medium supplemented with 1g of starch at 37°C after 5 days. The clear zones were due to the hydrolysis of the substrate. Maximum production of amylase by *Aspergillus fumigatus* was 1.42mg/ml after 144 hours, at 37°C. The production was 0.60mg/ml, 0.71mg/ml and 1.23mg/ml after 72, 96 and 120 hours, respectively.

Optimization of culture conditions for amylase production

Temperature and pH are the most important factors, which markedly influence enzyme activity.

Effect of pH

Production of amylase was observed at various pH values from pH 4 to 10. The maximum production of amylase was 0.51mg/ml at pH 7 (Figure1). *Aspergillus fumigatus* produced amylase that was active at pH range 5-8 with two sharp peaks, one acidic (pH5) and one basic (pH8). This suggests that the enzyme will be useful in processes that require wide range of pH changes from slightly acidic to slightly alkaline range. It also suggests the presence of at least two amylolytic activity in the crude enzyme preparation, an alpha and a glucoamylase[16,17]. Some of these enzymes act synergistically in starch degradation[17]. As the strain was seen growing in wide range of differences in pH, this represents their adaptability and tolerance in both acidic and basic conditions thus producing large amount of enzymes unaffected. Similarly, multiple pH optima were observed for amylolytic activities in the crude amylase preparation in various literatures[5].

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The maximum activity of amylase at pH 7 agrees with the result of Chakraborty[18] who also recorded maximum amylase activity at pH 7.

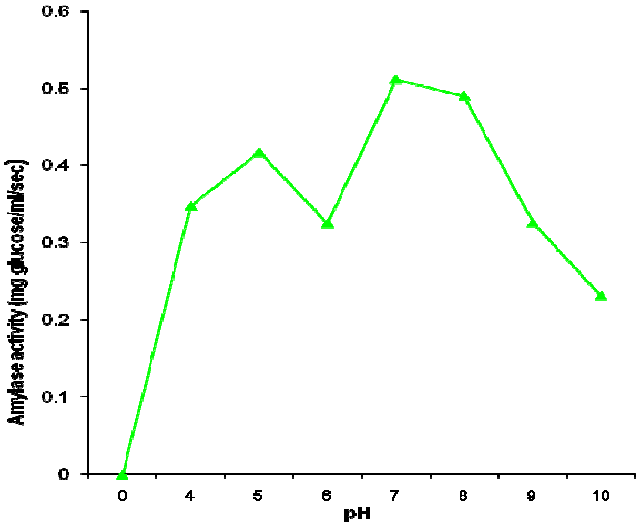

Figure 1 : Effect of pH on the activity of amylase produced by *Asepergillus fumigatus*.

Effect of temperature

Only few microbial strains are able to grow at elevated temperatures[19]. The effect of various temperatures (30-100°C) on amylase activity was studied. It was observed that amylase activity was maximum (0.55mg/ml) at 70°C (Figure 2). Further increase in temperature resulted in decrease in the activity of amylase. Omemu[20] as well as Olajuyigbe and Ajele[21] obtained similar results. The result is also similar to the works of Oyeleke[21] and Daniel[22], who stated that during isomerisation, temperature is preferably maintained within the range 20°C-90°C and the best activity is obtained within 50-70°C. The decrease in enzyme activity at higher temperatures might be due to the destruction of an enzyme at certain temperatures.

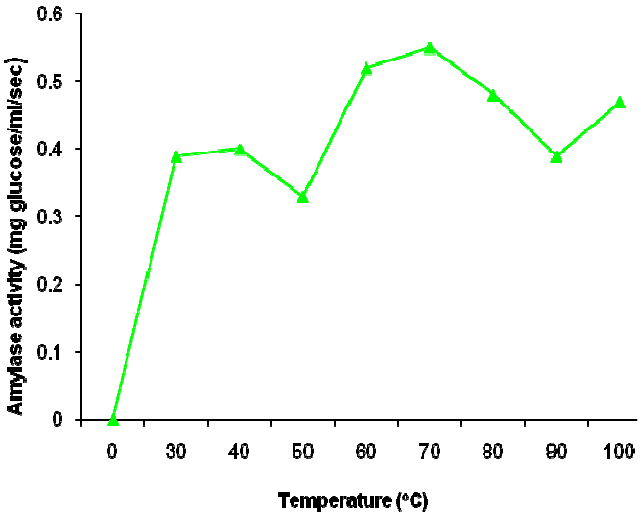

Figure 2 : Effect of incubation time on activity of amylase produced by *Aspergillus fumigatus*.

Effect of incubation time

Amylase production has been found to be growth associated. The incubation time for achieving maximum enzyme yield is governed by the characteristics of the culture and based on growth rate and enzyme production[23]. Amylase production by *Aspergillus fumigatus* reached its maximum level (1.42mg/ml) after 144 hours of incubation (Figure 3). In the optimization of incubation period the actual production of amylase during growth phase of the fungal strain was known.

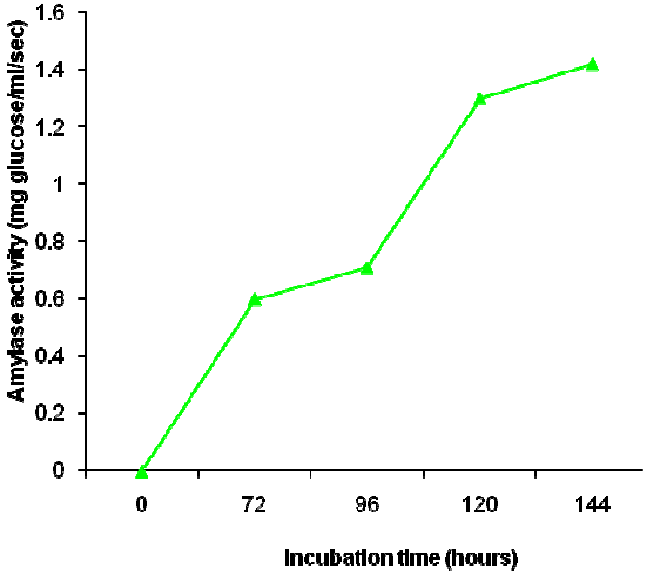

Figure 3 : Effect of temperatureon the activity of amylase produced by *Aspergillus fumigatus*.

CONCLUSION

In conclusion, this study has shown that the amylolytic enzymes produced by the isolated *Aspergillus fumigatus* may have practical applications in the starch industry on account of the stability at acidic and alkaline pH, and also high temperature. Further studies to purify and characterize the amylase complexes produced by this strain will be investigated.

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