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Synthetic Production of Amylase from *Aspergillus niger* Isolated from Citrus Fruit

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Abstract: During the growth of a citrus fruit isolate, *Aspergillus niger*, in a synthetic medium containing starch as sole carbon source, proteins which exhibited amylase activity were produced. Production of enzyme increased with increase in days of incubation, with optimum occurring on the tenth day. After partial purification by ammonium sulphate precipitation and dialysis, activity of amylase increased with increase in concentration of starch, optimum at 35°C and pH 6.5. Activity was stimulated by Na^+, K^+, Mg^{2+} and Ca^{2+}, maximally at 25mM concentrations. Activity was inhibited, when the enzyme was subjected to periodic heating, at 80°C. Approximately 80% of activity was lost within two minutes of heating at this temperature.

Key word: *Aspergillus niger* · Growth medium · Amylase · Citrus fruit

INTRODUCTION

Amylases are important enzymes employed in processing industries for the hydrolysis of starch into simple sugars [1]. Evidences of amylase production by moulds, yeasts and bacteria have been reported and their properties documented [2,3]. Fungi among many microbes are good sources of amylolytic enzymes [4,5]. Amylases hydrolyse the alpha 1-4-glucosidc bonds of amylopectin, glycogen are related compounds [6].

This article describes the ability of *Aspergillus niger* isolated from citrus fruit to produce amylase in a synthetic growth medium. Attempts were made to characterize the enzyme after partial purification.

MATERIALS AND METHODS

Organism and Culture Conditions: The isolate of *Aspergillus niger* used in this investigation was part of a culture collection of the department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. It was isolated from decaying citrus fruit. The stock was routinely grown and maintain on 1% malt yeast extract agar slants.

Preparation of Incoculum: A subculture of *Aspergillus niger* was prepared on 1% malt yeast extract agar slants and incubated at 25°C for 72hours. After the incubation period, spore suspension was prepared from the inoculum by adding 10ml of sterile distilled water to each test tube slant containing the culture of the organism. The spores on the surface of the agar medium were dislodged by carefully scraping them with sterile inoculating loop. The scraping was done carefully to avoid scraping the agar medium. The spore suspension was filtered into steriled flasks through four layers of sterile muslin cloth and employed as inoculum in all experiments. The spore suspension was diluted to have a final concentration of approximately 6×10^7 spores per ml [7].

Inoculation: One hundred milliliter of growth medium, prepared as described by Adejuwon and Olutiola [7], but with starch as sole carbon source, was dispensed into 250ml conical flasks. The final concentration of starch was 1% (w/v). The contents of each flask was inoculated with the spore suspension, prepared as described above.

Enzyme Preparation: On the tenth day of incubation, contents of each flask was filtered using glass fibre filter paper (Whatman G/A). This served as the enzyme prepration. Amylase activity [8] and protein content [9] of the preparation were determined.

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Ammonium Sulphate Fractionation: The crude enzyme was subjected to ammonium sulphate fractionation at 90% saturation. Precipitation during salt saturation was at 4°C for 24hr. It was subjected to cold centrifugation at 10,000g for 30minutes at 4°C using a high speed cold centrifuge (Optima LE-80K Ultracentrifuge, Beckman, USA). The supernatant was discarded and the precipitate reconstituted in 0.02m citrate phosphate buffer pH 6.0. The enzyme was dialysed using acetylated dialysis tubing (Visking dialysis tubing). Acetylation was as described by Whitaker *et al.* [10]. Dialysis was carried in a multiple Dialyser (Pope Scientific Inc. Model 220, USA) at 4°C for 24hr against several changes of buffer. Amylase activity and protein content of fractions were thereafter determined.

Amylase Assay: Amylase activity was assayed using the modified method of Pfueller and Elliott [8]. The reaction mixture was 2ml of buffered (0.02m citrate phosphate buffer pH 6.0) soluble starch (Sigma) and 0.5ml enzyme. Incubation was at 35°C for 20 minutes. The reaction was terminated with 3ml of IN HCl. 2ml of the terminated reaction mixture was added to 3ml of 0.1N HCl. Colour was developed by adding 0.1ml iodine solution. One unit of amylase activity was defined as the amount of enzyme which produced 0.01% reduction in the intensity of the blue colour of the starch-iodine complex under assay conditions.

RESULTS

Aspergillus niger grew in a synthetic medium containing starch as sole carbon source. During this period of growth, proteins which exhibited amylase activity were produced. Optimum activity was observed on the tenth day.

Aspergillus niger was able to degrade starch as substrate, within a concentration range of 0.1% - 0.6% (W/V). Optimum activity was observed at 0.6% (W/V).

Aspergillus niger produced amylase activity within a pH range of 4.0-8.0. There was a gradual increase in activity from pH 4.0 to pH 6.5. Optimum activity was observed at pH 6.5 after which there was a decline.

Aspergillus niger exhibited amylase activity within a temperature range of 20°C to 45°C. There was a gradual increase in activity as temperature increased from 20°C. Optimum activity was observed at 35°C, after which there was a decline.

Table 1: Partial purification of amylase from *Aspergillus niger* isolated from citrus fruit

Fraction	Amylase activity (Units)	Yield	Purification (fold)
Crude	88	100	1
Ammonium sulphate Precipitation	84	94.5	2.6

When the amylase produced by *Aspergillus niger* was heated at 80°C within 0 and 30 minutes, activity was lost. There was a gradual decline within two and twenty-five minutes. There was complete loss of activity within thirty minutes.

Na^+, K^+, Mg^{2+} and Ca^{2+} stimulated amylase activity produced by *Aspergillus niger* in a synthetic medium containing starch as sole carbon source. For each of the cations, activity increased gradually from OmM to 25mM concentration. Optimum activity was observed at 25mM concentration for each of the cations. The amylase activity, yield and purification (fold) of fractions are expressed in Table 1.

DISCUSSION

The results of this investigation showed that *Aspergillus niger* grew on a medium containing starch as sole carbon source producing amylase, capable of degrading glucosidic bonds of starch, hydrolytically.

Cultural conditions have an influence on enzyme production [11].

In this study, amylase activity increased with increase in hydrogen ion concentration indicating that the amount of starch hydrolysed increased with increase in hydrogen ion concentration. Optimum activity was at pH 6.5 after which there was a decline.

Amylases are stable over a wide range of pH from 4 to 11, however, some amylases have a narrow stability range [12]. A change in pH will affect the ionic character of the amino and carboxylic acid of the enzyme which will in turn affect both the catalytic site and conformation of the enzyme [13]. Low and high pH values can also cause a considerable denaturation and hence inactivation of the enzyme [6].

Temperature changes had an effect on amylase activity produced by *Aspergillus niger*. Optimum activity was at 35°C after which there was a decline in activity.

The rate of enzyme catalysed reactions increase with temperature. This occurs only within the temperature range at which an enzyme is stable and retains full activity [14]. Effect of temperature on the activity of an enzyme

may be dependent on: the stability of the enzyme-substrate affinity. It might however have an effect on increase in reaction rate and increase in thermal denaturation. [6].

In this study amylase activity was gradually lost with increase in the time of heating at 80°C. Within thirty minutes, there was complete loss of activity.

Thermostabilities are affected by factors such as substrate concentrations and cations. [11]. Enzymes are heat labile and denaturation of the enzyme protein by heat results in gradual loss of their catalytic properties [15].

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