β -amylase Associated with the Green Mould Rot of *Dioscorea rotunda*

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Abstract: *Aspergillus flavus* L. is associated with the green mould rot of yam (*Dioscorea rotunda*). It grew in a synthetic growth medium with starch as the sole carbon source. β -amylase activity was detected in the growth medium. Optimum activity was observed on the 10th day of incubation at 27°C. The optimum activity of the enzyme was at 35°C and pH 5.0. The enzyme was heat labile, losing 90% of its activity after 25 min of heating at 80°C. Activity of the enzyme was stimulated by the monovalent cations, Na⁺ and K⁺ and divalent cations, Mg²⁺ and Ca²⁺.

Key words: *Aspergillus flavus* L., green mould rot, β -amylase, *Dioscorea rotunda*

INTRODUCTION

Aspergillus flavus Link. belongs to the Genus *Aspergillus*, Subdivision Deuteromycotina, Super Kingdom Eukaryota, Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Eurotiomycetes, Order Eurotiales and Family Trichocomaceae (Alexopoulos *et al.*, 1996). It is the leading cause of aflatoxicosis (Nester *et al.*, 2004). Aflatoxin produced by this fungus is both hepatotoxic and carcinogenic (Nester *et al.*, 2004; Payne *et al.*, 2006). *Aspergillus flavus* genomics may lead to improvement in human health, food safety and sustenance of agricultural economy (Yu *et al.*, 2005).

A variety of enzymes are produced extracellularly by this fungus (Olutiola and Cole, 1981, 1980; Olutiola and Okonkwo, 1982; Layokun *et al.*, 1990; Solomon *et al.*, 1990; Ajayi *et al.*, 2007).

This study describes the production of β -amylase by *Aspergillus flavus* L., isolated from yam (*Dioscorea rotunda*) in a synthetic growth medium with starch as sole carbon source. Attempts were made to determine some of the properties of the enzyme.

MATERIALS AND METHODS

Organism and culture conditions: The isolate of *Aspergillus flavus* L. (NSPR.108) used in this research was from yam (*Dioscorea rotunda*). It was obtained from the culture collection of Professor P.O. Olutiola of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The stock collection was routinely grown and maintained on 1% malt yeast extract agar slants.

Preparation of inoculum and synthetic growth medium:

A subculture of *Aspergillus flavus* Link. was prepared on 1% malt yeast extract agar slants and incubated at 25°C for 72 h. After the incubation period, spore suspension was prepared from the inoculum by adding 10 mL of sterile distilled water to each test tube slant containing the culture organism. The spores on the surface agar medium were dislodged by scraping with sterile inoculating loop. This was done carefully to avoid scrapping the agar medium. The spore suspension was diluted to have a final concentration of approximately 10⁵ spores per mL. The 100 mL of growth medium containing 1% starch, K₂HPO₄, MgSO₄·7H₂O, L-Cysteine, KNO₃, KH₂PO₄, FeSO₄·7H₂O, thiamine, biotin was inoculated with 1 mL of the spore suspension (Adejuwon and Olutiola, 2007). The inoculated growth medium was incubated at 27°C and observed daily for growth. Samples of the extract were taken on a daily basis and analysed for β -amylase activity.

β -amylase assay: β -amylase activity was determined by measuring the amount of reducing sugar using the modified DNSA method (Murao *et al.*, 1979). The 0.5 mL of the enzyme preparation was added to 2 mL of 0.1% starch in 0.02 M citrate phosphate buffer (pH 4.8). Controls contained only 2 mL of 0.1% starch. Incubation was at 35°C for 20 min. The reaction was terminated with 3 mL DNSA reagent. 0.5 mL of enzyme was then added to the controls. The reaction mixture was heated at 100°C (boiling) for 15 min and cooled. The solution was diluted with distilled water. The reaction mixture was allowed to stand for 15 min at room temperature. Optical density was measured at 540 nm.

One unit of enzyme activity was arbitrarily defined as the amount of enzyme which liberated 1 μ mole of maltose

in 1 mL of reaction mixture per minute under assay conditions.

β -amylase properties: Some properties of the β -amylase produced by the fungus were determined. These were the effects of temperature, pH, heat, substrate concentration and the effects of certain cations. The cations were Na^+ , K^+ , Mg^{2+} and Ca^{2+} .

RESULTS AND DISCUSSION

Aspergillus flavus Link. grew in a synthetic growth medium containing starch as sole carbon source. The structure of starch polymer is shown in Fig. 1. Culture filtrate of the isolate exhibited β -amylase activity from the 3rd day of incubation with optimum enzyme activity occurring on the 10th day (Fig. 2). Similar observations have been reported by Sani *et al.* (1992) on amylase production by *Aspergillus niger* and *Aspergillus flavus*.

The effect of concentrations of starch on β -amylase activity was directly proportional to enzyme activity indicating an increase in enzyme activity with increase in starch concentration (Fig. 3). According to Dixon and Webb (1971), if more substrate molecules are present, an enzyme binds substrate more often and the reaction velocity (usually expressed in terms of the rate of product formation) is greater than at a lower substrate concentration. Thus, the rate of enzyme-catalyzed reaction increases with substrate concentration. Eventually, further increase in substrate concentration does not result in greater reaction velocity because the available enzyme molecules are binding substrate and converting it to products as rapidly as possible. That is, the enzyme is saturated with substrate and operating at maximal velocity (V_{max}).

β -amylase activity increased gradually with increase in temperature. Optimum activity was observed at 35°C

after which activity declined (Fig. 4). Some researchers have reported similarly for amylases from some fungal isolates (Abou-Zeid, 1997; Cherry *et al.*, 2004). The decline may be attributed to the formation of incorrect conformation due to processes such as hydrolysis of the peptide chain, destruction of amino acid and aggregation (Rawn, 1989). Dixon and Webb (1971) reported that both functional and structural aspects of enzymes are affected when subjected to a temperature above the optimum.

β -amylase activity was gradually lost with increase in time of heating when the enzyme was heated at 80°C for a period of 30 min. The enzyme lost 90% of its activity after 25 min (Fig. 5).

Enzymes are mostly proteins with a labile nature. Inactivating agents such as heat impair their native conformation so affecting their catalytic activity (Dixon and Webb, 1971).

In this study, β -amylase activity increased slightly from pH 3.0 to 3.5 after which a rapid increase was observed from pH 3.5 to 5.0. Optimum activity was observed at pH 5.0 (Fig. 6). A noticeable decline was observed from pH 5.0 to 7.0.

Mamo and Gessesse (1999) reported optimum amylase activity at pH 5.0-5.5 for *Aspergillus* sp.

pH is an important factor that determines growth and morphology of microorganisms since they are sensitive to the concentration of hydrogen ion present in their medium of growth. Earlier studies have revealed that fungi require slightly acidic pH for optimum growth. pH is known to affect the synthesis and stability of amylase. Each enzyme functions most rapidly at a specific pH (Abou-Zeid, 1997). When the pH deviates too greatly from that of the enzyme's for optimum activity, activity slows and the enzyme may be damaged (Abou-Zeid, 1997).

With increase in concentration of each of the cations: Ca^{2+} , Mg^{2+} , Na^+ and K^+ from 0-25 mM, there was a gradual increase in the activity of the β -amylase in this study.

Fig. 1: Structure of starch polymer with possible sites of hydrolysis

Fig. 2: Effect of growth period on the activity of amylase produced by *Aspergillus flavus*

Fig. 5: Effect of time of heating (80°C) on the activity of amylase produced by *Aspergillus flavus*

Fig. 3: Effect of concentration of starch on the activity of amylase produced by *Aspergillus flavus*

Fig. 6: Effect of pH on the activity of amylase produced by *Aspergillus flavus*

Fig. 4: Effect of temperature on the activity of amylase produced by *Aspergillus flavus*

Fig. 7: Effect of concentration of cations on the activity of amylase produced by *Aspergillus flavus*

Optimum activity was observed at 25 mM concentration with each of the four cations (Fig. 7). According to Ajayi *et al.* (2005), these cations stimulate α -amylase activity. Ajayi and Fagade (2003) reported that Ca^{2+} was stimulatory to β -amylase produced by *Bacillus subtilis* with Na^+ being less effective for enzyme activity.

CONCLUSION

The results of this investigation show that *Aspergillus flavus* L. produce β -amylase which must have contributed to its role in the degradation of starch components of yam (*Dioscorea rotunda*) from which this strain of *Aspergillus flavus* L. was isolated. However, earlier research carried out (Ajayi *et al.*, 2005) has shown that yam contains endogenous α -amylase. The results of this earlier investigation by Ajayi *et al.* (2005), with the results of this investigation suggest that the β -amylase produced by *Aspergillus flavus* L. coupled with endogenous α -amylase of yam play a role in the degradation of the starch components of yam. Degradation of starch components of yam is therefore not solely by this phytopathogen.

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