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Amylase synthesis in *Aspergillus flavus* and *Aspergillus niger* grown on cassava peel

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SUMMARY

Aspergillus flavus and *Aspergillus niger* produce extracellular amylase into the culture medium when grown on basal medium containing 2% (w/v) soluble starch or cassava peel as the sole carbon source. On soluble starch the highest amylase activities were 1.6 and 5.2 mg of starch hydrolyzed/min per mg protein for *A. flavus* and *A. niger*, respectively. When grown on cassava peel, the highest amylase activity in the culture filtrate of *A. flavus* was 170-times higher than that on soluble starch, while that of *A. niger* was 16-times higher. The mycelial dry weight for both organisms was not significantly affected by the carbon sources. Maximum enzyme activity was obtained at the growth temperature of 29.0 ± 1 °C and pH 7 for both organisms. It is concluded that cassava peel might be a better substrate for the production of amylase by *A. flavus* and *A. niger* than commercial soluble starch.

INTRODUCTION

The need for improved efficiency of industrial processes catalyzed by enzymes has necessitated the use of isolated enzymes as opposed to whole cells. One such enzyme is amylase, used in brewing and bakery industries and in the industrial manufacture of glucose, invert sugars and high fructose syrup [7]. Amylase primarily converts starch into sugars. The production of amylase has been demonstrated in bacteria [3,5,16,19] and fungi [8,10,13–16]. In most cases, commercial soluble starch is used as the carbon source for the growth of the organisms, although sugars such as maltose, lactose and galactose may be used [2]. These substrates are expensive for large-scale culture of amylase-producing organisms, hence the need for alternative cheaper carbon substrates for amylase production. Cassava peel is rich in starch and in Nigeria it is available in abundance as a waste produced from cassava during its processing into various fermented foods such as 'gari', 'lafun' and 'fufu'. In this study, therefore, the possibility of high amylase production in *A. flavus* and *A. niger* using cassava peels in comparison with soluble starch was investigated, with a view to obtaining a cheaper carbon substrate for the production of amylase for industrial uses.

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MATERIALS AND METHODS

Source and maintenance of organisms

Aspergillus flavus and *Aspergillus niger* were from the culture collection of the Department of Biological Sciences, University of Ilorin, Nigeria. They were maintained on Potatoe Dextrose Agar (PDA).

Sample treatment

Freshly harvested cassava tubers (*Manihot utilissima*) were washed thoroughly with tap water and the outer corky bark was removed by scraping. The peel was carefully removed without cutting into the pulp. After washing with distilled water, the peel was dried in an oven at 80 °C and then ground into very fine powder. The powder was dried to constant weight in an oven at 60 °C. The dried cassava peel powder (containing 60% carbohydrates, 5.6% protein) was stored in a desicator until required.

Culture method and culture filtrate preparation

Spores of 48-h-old cultures of *A. flavus* and *A. niger* were harvested by washing slants with 10 ml of sterile distilled water [1]. An aliquot of 0.5 ml of spores suspension was used to inoculate 50 ml of growth medium containing per liter: $NaNO_3$ (3.0 g); K_2HPO_4 (1.0 g); $MgSO_4$ (0.5 g); KCl (0.5 g); $FeSO_4$ (0.01 g) and 20 g of either soluble starch or cassava peel powder. The cultures were incubated at 29.0 ± 1 °C on a rotary shaker at 80 rpm. At intervals of 24 h, cultures were suction filtered through

dried No. 1 Whatman filter paper to obtain the culture filtrate; the mycelia were dried to constant weight and used for growth measurements. Soluble protein in the culture filtrate was determined by the method of Lowry et al. [11]. The method of Dubois et al. [6] was used to determine the total sugar; residual starch was measured by the colorimetric method of Smith and Roe [18]. The pH of the culture filtrates were measured with a Pye Unicam pH meter (292 MK2).

Assay of amylase activity in the culture filtrates

A method which involved the reduction of starch-iodine color with increasing amylase activity as detailed by Coleman and Eliot [4]. Manning and Campbell [12] and Smith and Roe [18] was used. Amylase activity was expressed as the quantity of starch hydrolyzed (mg) $min^{-1} \cdot mg\ protein^{-1}$.

Effect of temperature on growth and amylase production

Cultures of *A. flavus* and *A. niger* were incubated at 10, 30, 40 and 50 °C for 6 days. At the end of 6 days, culture filtrates were prepared and analyzed for protein, sugar, pH and amylase activity. Growth was also determined from the mycelial dry weight.

Effect of pH on growth and amylase production

Buffered basal medium containing 2% (w/v) of either cassava peel powder or soluble starch were used to determine the effect of pH on growth and amylase production by *A. flavus* and *A. niger*. Buffering was done with citric acid-disodium hydrogen phosphate solutions of pH 3, 4, 5, 6, 7 and 8. All flasks were incubated at 29.0 ± 1 °C for 6 days after which they were analyzed for growth and amylase activity.

RESULTS AND DISCUSSION

Effect of commercial soluble starch and cassava peel on growth and amylase production by A. flavus and A. niger

Both species of *Aspergillus* produced extracellular amylase in basal medium containing either 2% (w/v) soluble starch or cassava peel as the sole carbon source. In both cultures the highest amylase activity was obtained during the late stationary phase of growth (Fig. 1a and b). The production of high amounts of the enzyme in both organisms during stationary phase may be in response to depletion of carbon source; induction of enzyme in response to limiting substrates in growth medium have been demonstrated in some organisms [9,17]. On soluble starch, the highest amylase activity for *A. flavus* was 1.6 mg of starch hydrolyzed/min per mg protein, while it was 120.0 mg starch hydrolyzed/min per mg protein when grown on cassava peel as the sole carbon source (Fig. 1a). Similarly, the highest amylase activities in the culture filtrates of *A. niger* grown on soluble starch and on cassava peel were 5.2 and 82.0 mg of starch hydrolyzed/min per mg protein, respectively (Fig. 1b). Under these conditions the growth pattern and growth rates of the organisms were not significantly affected by the carbon sources (Fig. 1a and b).

Effect of the composition of growth medium on growth and amylase production by A. flavus and A. niger

To determine whether the high level of amylase expressed in both organisms when grown on cassava peel as against soluble starch was substrate-specific, media of different compositions were used to grow the organisms for enzyme production (Table 1). On basal medium without added carbon source, growth of the organisms was poor, such that mycelial dry weights of *A. flavus* and *A. niger*

TABLE 1

Effect of composition of growth medium on biomass and amylase production by *A. flavus* and *A. niger*

Medium	*A. flavus*		*A. niger*	
	Maximum amylase activity (mg of starch hydrolyzed/ min per protein)	Maximum mycelia dry wt (g/50 ml culture)	Maximum amylase activity (mg starch hydrolyzed/ min per protein)	Maximum mycelia dry wt (g/50 ml culture)
Basal medium	0.00	0.031 ± 0.002	0.00	0.065 ± 0.004
Basal medium + soluble starch (2% w/v)	1.6 ± 0.1	0.359 ± 0.02	5.9 ± 0.1	0.430 ± 0.02
Basal medium + 2% (w/v) cassava peel	120.1 ± 0.3	0.497 ± 0.01	81.8 ± 0.2	0.496 ± 0.02
Cassava peel alone (2% w/v)	0.22 ± 0.01	0.368 ± 0.1	0.25 ± 0.01	0.407 ± 0.01
Soluble starch alone (2% w/v)	0.04 ± 0.001	0.214 ± 0.02	0.08 ± 0.002	0.284 ± 0.03
Cassava peel (2% w/v) + basal medium-organism	0.00	no growth	0.00	no growth

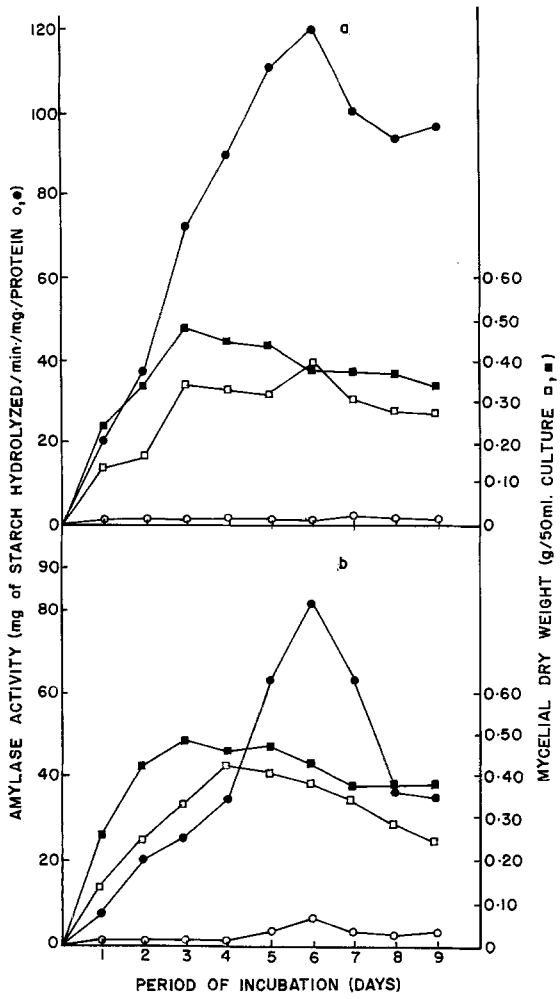

Fig. 1. Effect of soluble starch and cassava peel on the growth and amylase production by (a) *A. flavus* and (b) *A. niger*. Dry weight of mycelia on soluble starch (□), dry weight of mycelia on cassava peel (■), amylase activity on soluble starch (○) and on cassava peel (●).

were 0.031 and 0.065 g/50 ml culture, respectively. Under this growth condition, there was no amylase synthesized by either organism (Table 1). A suspension of cassava peel without minimal salts supported the growth of both organisms such that mycelial dry weights of 0.368 and 0.407 g/50 ml culture were obtained for *A. flavus* and *A. niger*, respectively. However, under this growth condition, amylase activities in the culture filtrates were 0.22 and 0.25 mg starch hydrolyzed/min per mg protein for *A. flavus* and *A. niger*, respectively. These activities were low compared with 120.0 and 81.8 mg starch hydrolyzed/min per mg protein obtained, respectively, for *A. flavus* and *A. niger* grown on cassava peel in basal medium. When grown on soluble starch alone, growth was similar to that on cassava peel, although very low levels of amylase were produced by both organisms. This ability of cassava peel to cause increased accumulation of extracellular amylase in the medium may be due to the presence of other compounds, such as non-starch carbohydrates, proteins, amino acids and other compounds. This is in contrast to commercial soluble starch that contains only starch.

Effect of temperature of growth and amylase production by A. flavus and A. niger grown on soluble starch and cassava peel

Cultures of *A. flavus* and *A. niger* grown on 2% (w/v) cassava peel produced low levels of amylase at 10 °C but increased to a maximum of 120.2 ± 0.3 and 90.1 ± 0.2 mg starch hydrolyzed/min per mg protein for *A. flavus* and *A. niger* respectively, at an incubation temperature of 30.02 ± 0.1 °C. Amylase levels were much lower at 50 °C; 12.0 and 6.0 mg starch hydrolyzed/min per mg protein for *A. flavus* and *A. niger*, respectively (Fig. 2a and b).

Initial experiments had indicated the sixth day as the ideal period to harvest for temperature-dependent growth and amylase activity assay, as the pattern was not different from shortened or prolonged incubation periods. Mycelial dry weights of both organisms were highest at 30 °C and lowest at 50 °C (Figs. 2a and b). The low levels of enzyme at 10 and 50 °C may be due to poor growth of both organisms at these temperatures as evident from the low mycelial dry weights of both organisms at the two temperatures (Fig. 2a and b). At all temperatures studied, enzyme activities in the culture filtrates of organisms grown on soluble starch were consistently very low, ranging from 0.01 to 1.6 and 0.1 to 5.2 mg starch hydrolyzed/min per mg protein for *A. flavus* and *A. niger*, respectively (Fig. 2a and b).

Effect of pH on amylase production by A. flavus and A. niger grown on soluble starch and cassava peel

Buffered basal media containing 2% (w/v) cassava peel or soluble starch as carbon source were used to determine the effect of pH on amylase production by *A. flavus* and *A. niger*. Amylase activity was found to progressively increase in the culture filtrates of both organisms as pH was increased from 3.0 to 7.0, reaching a maximum of 121.9 and 85.2 mg starch hydrolyzed/min per mg protein at pH 7 for *A. flavus* and *A. niger*, respectively, when grown on cassava peel (Fig. 3a and b). At pH 8.0 enzyme levels were 83.0 mg starch hydrolyzed/min per mg protein for both organisms. However, the final pH of the cultures were found to differ from the initial pH; the initial pH 3 of the *A. flavus* and *A. niger* cultures increased to 6.8 and 5.0, respectively, while pH 8 decreased to 7.3 for *A. flavus* and 6.8 for *A. niger* cultures. Other initial pHs increased to a range of 7.1 to 7.3 for both organisms. Amylase activities

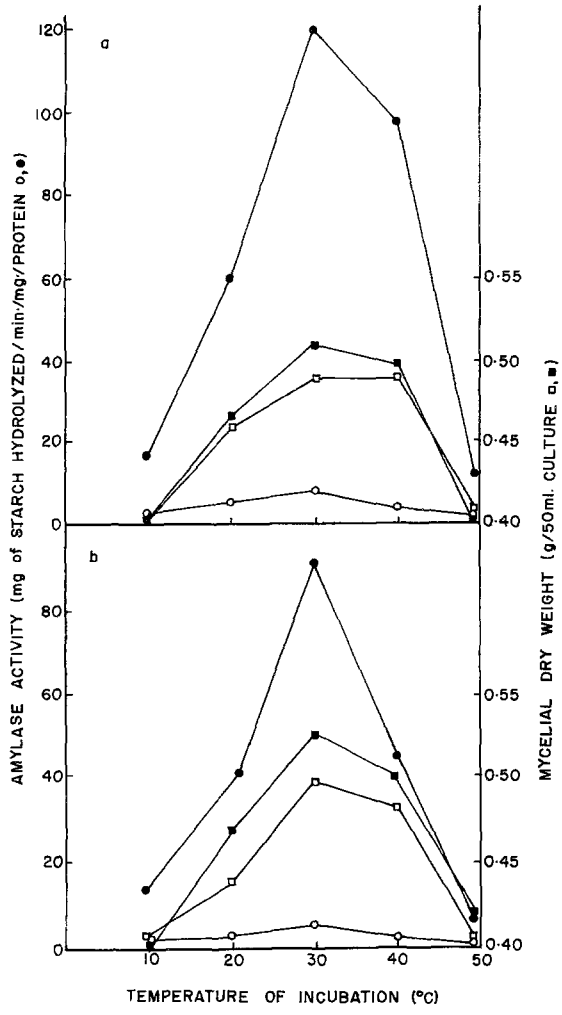

Fig. 2. Effect of temperature of incubation on the growth and amylase production by (a) *A. flavus* and (b) *A. niger* grown on soluble starch and cassava peel. Dry weight of mycelia on soluble starch (□) and on cassava peel (■). Amylase activity on soluble starch (○) and on cassava peel (●).

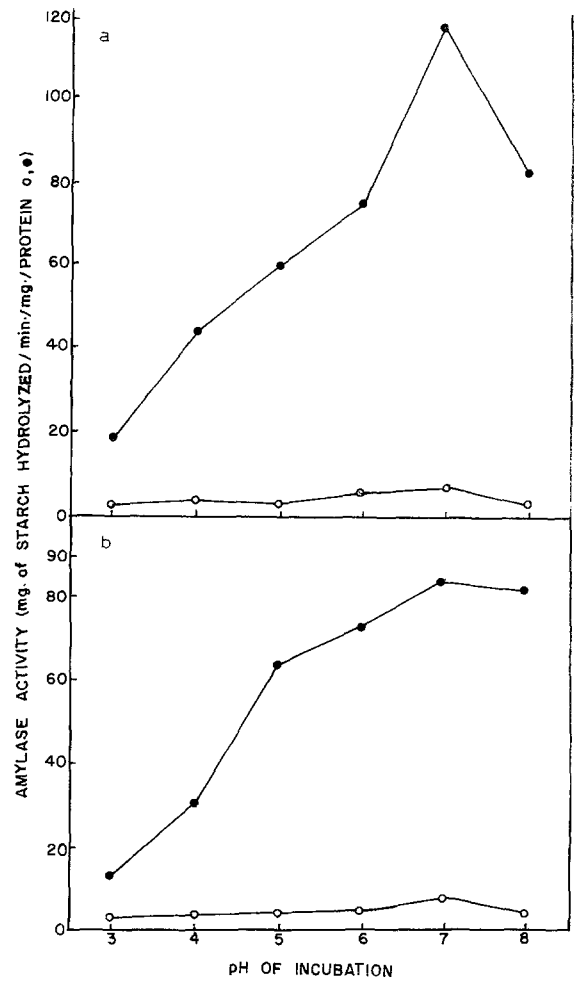

Fig. 3. Effect of pH of growth medium on amylase production by (a) *A. flavus* and (b) *A. niger* grown on soluble starch and cassava peel. Amylase activity on soluble starch (○) and on cassava peel (●).

were consistently low at all pHs studied when soluble starch was used as the carbon source (Fig. 3a and b).

In conclusion, it has been shown in this study that cassava peel may be a better carbon substrate than commercial soluble starch for amylase production by the two organisms investigated. However, cassava peel is known to contain high levels of cyanogenic substances [20]. Investigation is, therefore, in progress to determine cyanide levels and any mycotoxin produced by these organisms in the culture filtrates during their growth on cassava peel. Other materials and wastes are also being tried with some other amylase-producing microorganisms with a view to obtaining the most economical carbon substrate for the production of amylase for industrial use.

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