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Statistical Analysis of Growth and Amylase Production by *Aspergillus flavus* grown on Different Carbon Sources

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Abstract

The mycelial dry weight and dinitrosalicylic acid (D.N.S.A.) method was used to determine growth and amylase production by *Aspergillus flavus* grown on different carbon sources. Growth of the fungus was determined at 24 h intervals over a period of six days by the dry mycelial weight methods, while the amylase activity in the culture filtrates of *A. flavus* was determined by the D.N.S.A method. A total of 45 samples were prepared to determine growth and amylase activity of *Aspergillus flavus* grown on different carbon sources. The concentration of the various carbon sources ranges between 0.4 to 2% W/V. Duncan's multiple range test was used to determine the level of significance of the different carbon sources for effective growth and amylase production by *Aspergillus flavus*. *Aspergillus flavus* demonstrated the capability to produce significant growth and amylase activities in the medium containing soluble starch, sorghum and cassava peel as sole carbon source. The amount of mycelial dry weight produced from soluble starch, sorghum and cassava peel is significantly higher than those produced from other carbon sources. The data revealed that there is a correlation between growth and amylase production by *Aspergillus flavus*. The available data from this study showed that soluble starch is the best carbon source for optimum growth and amylase production by *A flavus* while sorghum and cassava peel are close substitute for optimum growth and amylase production by *Aspergillus flavus*.

Keywords: Growth, amylase activity and *Aspergillus flavus*

Introduction

Amylase is one among such enzymes that are very important in the field of biotechnology. It constitutes a class of industrial enzymes having approximately 25% of enzyme market. Amylase enzymes are distributed in all the animal, plant and microbial kingdoms. α – Amylase enzymes are important enzymes employed in starch processing industries for hydrolysis of polysaccharides such as starch in to simple constituent Akpan *et al* (1999).

Extracellular enzymes, such as cellulases, amylase, proteases as well as lipases have been detected in the culture filtrates of thermophilic fungi, and their activities examined (Barnet and Fergus, 1971; Ogundero and Osunlaja 1986; Ogundero 1987; Ueki *et al*., 1991). Enzymes of microbial origin, have been exploited in medical, food, pharmaceutical, textile and biotechnology based industries (Lim *et al.,* 1984; Ali *et al.,* 1989; Oyewale 2006; 2009; 2010; 2012; 2013.

Starch degrading enzymes like amylase have received great deal of attention because of there perceived technological significance and economic benefits. Moulds are capable of producing high amount of amylase. *Aspergillus niger* is use for commercial production of α – Amylase. Studies on fungal amylase especially in developing country have concentrated mainly on *Aspergillus niger*

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probably because of their ubiquitous nature and non fastidious nutritional requirement of these fungi. (Abu et al 2005)

This present investigation aims at optimization of a medium for comparative analysis of growth and amylase production by *Aspergillus flavus* grown on different carbon sources.

Materials and methods

Preparation of inoculum

With aid of sterile corkborer 5mm disc of an advancing edge of a 4-day old fungal culture was inoculated on to an agar slant prepared with Czapekdox agar containing 2% (W/V) soluble starch. The cultures were then incubated at 30°C for 48 h before use.

Culture methods

Spores of 48 h old cultures of *Aspergillus flavus* were harvested using the methods described by Akinyosoye and Akinyanju (1989). The 48 h old cultures of *Aspergillus flavus* was washed with 10 mL of sterile distilled water, by shaking to obtain a suspension of spores. An aliquot (0.5 mL) of spore suspensions was aseptically used to inoculate 50 mL of liquid medium, containing any of the carbon sources, and then incubated at room temperatures (29 ± 1°C) on a rotary shaker at 80 rpm. Cultures were then suction filtered through a pre-weighed Whatmann No. 1 filter paper and the filtrate obtained was used as crude extract. At intervals of 24 h the protein content as well as the amylase activity in the culture filtrate were determined. The mycelium retained on the pre-weighed Whatmann No. 1 filter paper was used for growth measurements.

Determination of fungal growth

Growth of fungus was determined at intervals of 24 h over a period of seven days by the dry mycelial weight method. The filter pads used to harvest the mycelial were first oven dried at 80°C to a constant weight. After filtration the mycelial on the filter paper were oven dried at 80°C. The weight was expressed in mg/50 mL of culture.

Determination of amylase activity

The amylase activity in the culture filtrate of *Aspergillus flavus* was determined by the D.N.S.A. methods (Ogundero, 1979, 1982, a & b); 1 mL of culture filtrates was added to 3 mL of standard soluble starch in 0.002 M Na_2HPO_4, and 0.006M NaCl (pH 6.9) and incubated at 45°C for 1 h (Ogundero, 1979). The reducing sugars produced were determined by addition of 3 mL of dinitrosalicylic acid (D.N.S.A) reagent, which contained D.N.S.A (1.0 g); 2 M NaOH, (20 mL); potassium sodium tartarate (30g); in 100 mL of distilled water and boiled for five minutes to complete the reactions. The absorbance of the cooled solution was then measured at 540 nm with a W.P.A. S106 spectrophotometer. The reaction mixtures of the uninoculated control were used to set absorbance readings at zero. The amylase activity of the culture filtrates was expressed as total reducing sugars released/min/mg protein.

Determination of protein contents

The method of Lowry *et al* (1951) was used for the determination of soluble proteins in the culture filtrates of *Aspergillus flavus*.

Statistical analysis of growth of *Aspergillus flavus* on different carbon sources

The various data obtained during the growth of *Aspergillus flavus* on various carbon sources experiment expressed as mycelial dry weight (mg/50 mL culture) was subjected to the new Duncan's Multiple Ranges test to determine the level of significance (Table 1).

The amylase activity expressed as mg of total reducing sugars released/min/mg protein) were subjected to New Duncan's Multiple range tests, to determine the level of significance (Table 2).

Table 1: Growth production by *Aspergillus flavus* grown on different carbon sources at concentration ranging from 0.4 - 2% w/v.

Concentration	Solubles starch	Sorghum	Maize	Cassava	Sucrose	Galactose	Xylose	Raffinose	Lactose
0.4	21	28	15	24	11	10	15	18	14
0.8	42	39	28	36	23	14	20	24	23
1.2	53	47	37	42	34	20	22	32	28
1.6	55	50	43	46	38	25	25	36	30
2.0	90	60	45	50	35	30	30	40	35

Table 2: Amylase production by *Aspergillus flavus* grown on different carbon sources at concentration ranging from 0.4-2% w/v.

Concentration	Solubles starch	Sorghum	Maize	Cassava	Sucrose	Galactose	Xylose	Raffinose	Lactose
0.4	0.44	0.31	0.22	0.19	0.15	0.28	0.15	0.16	0.11
0.8	0.64	0.41	0.26	0.13	0.32	0.43	0.18	0.18	0.14
1.2	1.06	0.54	0.39	0.38	0.31	0.41	0.19	0.25	0.19
1.6	1.10	0.64	0.53	0.48	0.35	0.45	0.19	0.23	0.33
2.0	1.10	0.69	0.56	0.51	0.47	0.48	0.25	0.23	0.34

Results

Statistical analysis of growth of *Aspergillus flavus* on different carbon sources

The various data obtained during the growth of *Aspergillus flavus* on different carbon sources were subjected to the new Duncan's multiple rage test to determine the level of significance. The result in Table 3 showed the statistical analysis of growth of *Aspergillus flavus* on different carbon sources.

The best substrate for the growth of *A. flavus* is soluble starch with a value of 52.60 mg/50 mL culture. Starch, sorghum and cassava peel produced growth, which are not significantly different from each other. Sorghum and cassava peel are considered to be close substitute for commercial soluble starch. Maize, raffinose and sucrose produced growth, which are not significantly different from each other but are inferior raw materials for growth of *Aspergillus flavus*.

Finally lactose, xylose and galactose are the least in their ability to produce growth hence they are least recommended for the growth of *Aspergillus flavus* (Table 3).

Table 3: Statistical analysis of growth of *Aspergillus flavus* on different carbon sources.

Carbon Sources	Mean Mycelial dry weight (mg/50 mL culture)
Soluble Starch	52.60^{a}
Sorghum	44.80^{ab}
Cassava Peel	39.60^{abc}
Maize	33.60^{bd}
Raffinose	30.00^{bd}
Sucrose	29.20^{bd}
Lactose	26.00^{cd}
Xylose	22.40^{cd}
Galactose	20.80^{cd}
Control	02.00

Means followed by similar letters are not significantly different at P = 0.05 according to the Duncan's Multiple range tests.

Statistical analysis of amylase production by *Aspergillus flavus* on different carbon source

The best substrate for the production amylase is soluble starch with value of 0.87 mg T.R.S released/min/mg protein while sorghum and cassava peel are close substitute to soluble starch for amylase production by *Aspergillus flavus.*

Moreover other raw materials like maize, galactose and sucrose with inferior substrates for amylase production by *Aspergillus flavus.* Lactose as well as raffinose and xylose are the poorest raw material for production of amylase by *Aspergillus flavus.*

Soluble starch, sorghum and cassava peel produced mycelial dry weight that was significantly different from that produced by maize raffinose and Sucrose. While growth on lactose xylose and galactose were not significantly different from one another (Table 3).

Mean amylase activity produced by *Aspergillus flavus* grown on soluble starch, was significantly different from that produced on other carbon sources, while amylase activity produced on sorghum and cassava peel was significantly different from that produced on maize, galactose and sucrose. Growth on lactose, raffinose and xylose had mean amylase activities that were not significantly different from one another (Table 4).

Table 4: Statistical analysis of amylase production by *Aspergillus flavus* grown on different carbon sources

Carbon Sources	Mean amylase activity (mg reducing sugar release/min/mg protein)
Soluble Starch	0.87[a]
Sorghum	0.52[b]
Cassava Peel	0.41[bc]
Maize	0.39[bcd]
Galactose	0.37[bcd]
Sucrose	0.32[bcd]
Lactose	0.22[cd]
Raffinose	0.21[d]
Xylose	0.19[d]
Control	0.00

Discussion

The results obtained in this investigation showed that *Aspergillus flavus* is capable of producing extracellular amylase into the culture medium when grown on basal medium containing various carbon sources as substrates.

Aspergillus flavus demonstrated the ability to degrade a wide range of substrates, i.e. soluble starch, sorghum and cassava peel, and convert them into simple sugars. Amount of reducing sugars produced from soluble starch, sorghum and cassava peel is significantly higher than those produced from maize, raffinose, sucrose, lactose, xylose and galactose respectively.

The ability of *Aspergillus flavus* to induce growth and amylase production from various carbon sources has been demonstrated. Maximal growth and amylase activities was establish at 2% weight/volume

Mean Amylase activity produced by *Aspergillus flavus* grown on soluble starch, was significantly different from that produced on other carbon sources, while amylase activity produced on sorghum and cassava peel was significantly different from that produced on maize, galactose and sucrose. Growth on lactose, raffinose and xylose had mean amylase activities that were not significantly different from one another (Table 4).

The fungal strain also demonstrated the ability to produce significant growth in the medium containing; soluble starch, sorghum and cassava peel as sole carbon source. The amount of mycelial dry weight produced from soluble starch, sorghum and cassava peel is significantly higher than those produced from other carbon sources.

The reason for increased mean amylase activity may be due to secretions of more enzymes into the medium to hydrolyze more of the starch into forms that can be utilized for the synthesis of cellular materials. These findings are similar to those of Pestana and Castillo (1985); Oyewale (2010; 2012).

Soluble starch, Sorghum and Cassava peel produced mycelial dry weight that was significantly different from that produced by maize raffinose and Sucrose. While growth on Lactose xylose and galactose were not significantly different from one another (Table 3).

The ability of the carbon sources to support the growth of *A. flavus* decreased in the following order: soluble starch, sorghum, cassava peel, maize, raffinose sucrose, lactose xylose and galactose. Similarly the ability of the carbon soruces to cause high levels of amylase production by *A. flavus* decreased in the following order soluble starch, cassava peel, maize, galactose, sucrose, lactose, raffinose, and xylose.

Higher levels of amylase production during growth on complex sugars, as compared with simple sugars may be a response to a need to convert the complex sugars into simple sugars before utilization by the organism. These findings are similar to those of Pestana and Castillo (1985); Akinyosoye and Akinyanju (1989); Ali et al (1990); Oyewale (2006, 2009, 2010).

The maximal growth and amylase production was obtained on sixth of incubation at pH 7, temperature of 29 ± 1°C and 80 r.p.m.

Aspergillus flavus exhibited high growth and amylase production on some polysaccharides including soluble starch sorghum and cassava peel. The data revealed that there is a correlation between growth and amylase production by *Aspergillus flavus* the ranking of the mean amylase activities decreases in the following order soluble starch, sorglum, cassava peel, maize galatose, and sucrose while the ranking of the mean mycelia dry weight decreases in the following order soluble starch, sorghum, cassava peel etc.

This result implies that soluble starch is the best carbon sources for optimum production of amylase by *Aspergillos flavus* while sorghum and cassava peels are close substitutes for optimum production of amylase enzymes.

The readily availability of sorghum and cassava peel, at affordable prices may make the use of these agricultural raw material a cheaper alternative to soluble starch for large scale production of Amylase enzymes.

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