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Optimization of α -amylase and glucoamylase production from three fungal strains isolated from Abakaliki, Ebonyi State

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ABSTRACT

*In the present study, optimization of medium composition and culture conditions for α -amylase and glucoamylase production by three fungal strains isolated from Abakaliki metropolis were investigated. The fungal isolates include *Aspergillus species* (AS-2a), *Mucor species* (MS-3) and *Rhizopus species* (RS-6b). The parameters such as optimal incubation period, pH, temperature, carbon sources and starch concentrations were considered in the study. Maximum enzyme activities of the isolates were attained at 120 hours incubation period. The isolates had their optimal amylase enzymes production at pH 4.0. The optimum temperature for α -amylase enzyme activities was 45°C while the maximum activities of glucoamylase were obtained at 30°C. Of the carbon sources tested, sweet potato starch gave the highest amylase activities. The optima starch concentrations for α -amylase and glucoamylase production by the isolates were 50 g/l and 80g/l, respectively. The enzymatic activities were measured quantitatively using potassium-iodide spectrophotometric method and glucose-oxidase/peroxidase method for α -amylase and glucoamylase respectively.*

Key words: Optimization, Amylase, Glucoamylase, Fungi, Culture, parameters.

INTRODUCTION

The potential of using microorganisms as sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activities in several microorganisms [Akpan *et al*, 1999; Pandey *et al*, 2000; Abu *et al*, 2005]. Amylase enzymes are of great importance in fermentation for hydrolysis of starch and other related oligosaccharides [Akpan *et al*, 1999; Pederson and Nielson, 2000]. Besides their use in starch saccharification, they have potential application in a number of industrial processes such as in food, baking, brewing, detergent, textile and paper industries. Microbial amylases have also successfully replaced chemical hydrolysis of starch in starch processing industries.

Amylases are enzymes produced by variety of organisms, such as bacteria, fungi, plants and animals. Bacteria and fungi secrete amylases to the outside of their cells to carry out extracellular digestion. Amylases degrade starch and other related polymers to yield products characteristic of individual amylolytic enzymes. Starch is a glucose polymer linked together by α -1,4 and α -1,6 glycosidic bonds. Amylases act by hydrolyzing the glycosidic bonds between adjacent glucose units of the polymer [Bernfeld, 1951].

Based on the points of attack on the glucose polymer chain, alpha-amylases can be classified into two categories, liquefying and saccharifying [Nigam and Singh, 1995]. The fungal α -amylase belongs to the saccharifying category and attacks the second linkage of the straight chain of starch molecule from the non-reducing terminal (i.e. C4 end), resulting in the splitting off of two glucose units at a time [Fadel, 2000]. The resulting product is a disaccharide called maltose. The starch chains are thus broken down into smaller units. Glucoamylase (amyloglucosidase) in turn hydrolyses α - 1, 4 - glycosidic bonds from non- reducing ends of starch molecules, resulting in the production of glucose. To a lesser extent, glucoamylase also has the ability to hydrolyze α - 1, 6 linkages, also resulting in glucose as the end product [Mertens and Skory, 2006]. Fungal α -amylase and glucoamylase may be used together to convert starch to simple sugars, which is used in the production of high fructose syrups, that in turn serves as sweetener for many food processing industries for production of beverages, bakeries, confectionaries, or may become feedstock for bioethanol production [Seviek *et al*, 2006].

Amylase enzymes are not yet commercially produced in Nigeria, making them too expensive for amylase utilizing industries. However, fungal organisms are known for their ability to produce extracellular enzymes, α -amylase and glucoamylase capable of hydrolyzing starchy substrates [Syu and Chen, 1997]. These fungal organisms are readily available in our environment. When these amylase producers are isolated, there is need to explore their production efficiency through studying of their best conditions for a higher productivity. These stimulated our interest in this research to optimize the cultural conditions of some isolated α -amylase and glucoamylase producing fungi to enhance their potentials for more effective industrial applications.

MATERIALS AND METHODS

Preparation of enzyme production media

The medium was constituted with the following nutrients in grams per litre: KH_2PO_4 , 14g; NH_4NO_3 , 10g; KCl, 0.5g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1g; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01g and soluble starch, 20g. This was adjusted to pH 6.9. The medium (100ml) was distributed into 250ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 minutes. They were allowed to cool to 25°C.

Cultivation of the Isolates for α -amylase and glucoamylase production

The isolates were inoculated into the enzyme production medium and incubated with shaking intermittently at 25°C for 3-5 days. The entire content of the culture were centrifuged and filtered to get the crude enzymes as culture filtrate. These were used to hydrolyze fresh starch solution for α -amylase and glucoamylase activities assay.

Measurement of α -amylase activity

The α -amylase activities of the isolates were assayed using the Caraway-Somogyi, iodine- potassium iodide (IKI) method [Cheesbrough, 2005].

Measurement of glucoamylase activity

Glucoamylase activity of each of the isolates was determined using Trinder's method [Trinder, 1959] known as the glucose oxidase/peroxidase (GOD/POD) method.

Time courses of α -amylase and glucoamylase production

The effect of cultivation period on α -amylase and glucoamylase production was determined by measuring the enzymes activities of the isolates after every 24 hours of incubation till the activities declined.

Effect of pH on α -amylase and glucoamylase production

Assay of the enzymes production was carried out at different initial pH of the medium. $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ (0.1M) buffer was used and the pH was adjusted using 0.1N HCl to variable range of pH 3.5 - 8.0 to determine the pH for maximum production. After 3-5 days cultivation, samples from each culture were tested for α -amylase and glucoamylase activities.

Effect of temperature on α -amylase and glucoamylase activities

The enzymes activities of the isolates crude enzymes were assayed at various temperature ranges of 20 - 60°C. The crude enzymes filtered from each culture were used to hydrolyze a fresh 1g/l starch solution. After 20 minutes of incubation, samples were collected from the cultures and tested for their enzymes activities.

Effect of carbon sources on α -amylase and glucoamylase production

The enzyme production media was prepared with starch from different sources and forms to assess their influences on α -amylase and glucoamylase production. These were gelatinized cassava flour, corn flour, potato flour, soluble starch and non-gelatinized soluble starch.

Effect of carbon source concentrations on α -amylase and glucoamylase production

The influence of carbon source was tested at different concentrations of starch with potato flour (10 g/l to 90 g/l) to determine the best concentration for maximum production of the extracellular α -amylase and glucoamylase by the isolates.

RESULTS**Time course of amylase production by the isolates**

To ascertain the effects of cultivation time on the amylase enzymes production by the selected fungal strains, the isolates were cultivated for different periods at 25°C and pH 4.0 in a medium containing 80 g/l starch concentration. The results of parallel triplicate experiments recorded maximum production of α -amylase and glucoamylase at 120 hours (5days) of cultivation [figures 1 and 2]. The activities of the amylase enzymes under study for the isolates were observed to have increased progressively from the first day until the fifth day when decline was recorded for them.

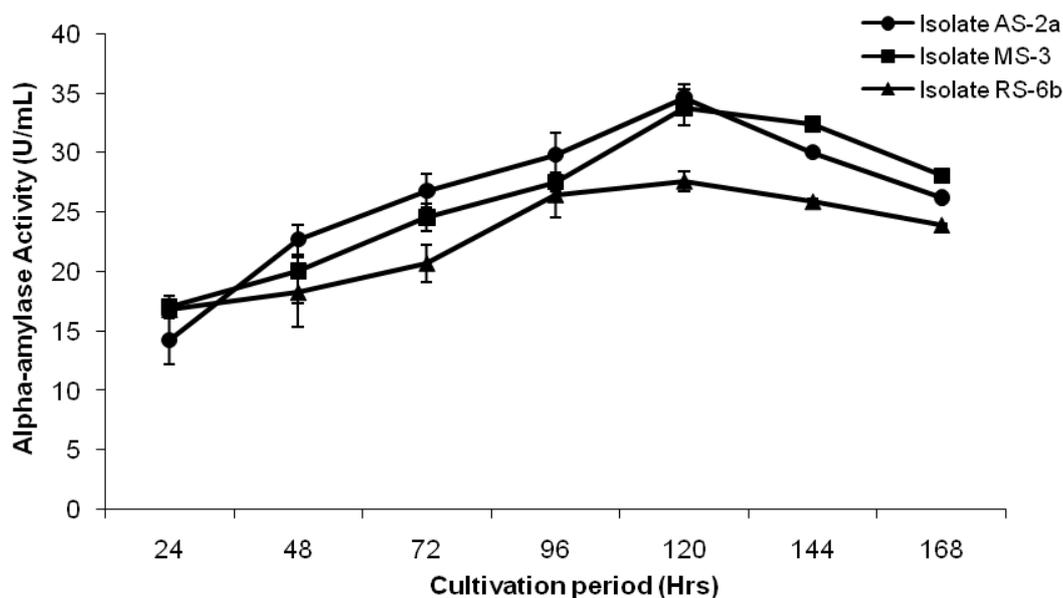


Figure 1. Time course of α -amylase production by the three isolates at 25°C and 80g/L starch concentration

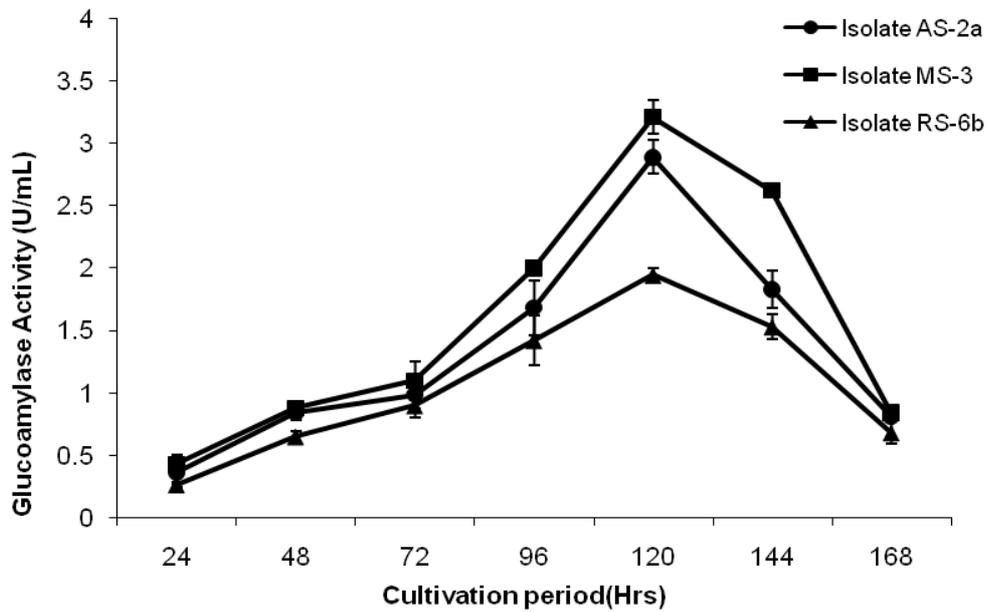


Figure 2. Time course of glucoamylase production by the three isolates at 25°C and 80g/l starch concentration

Effects of pH on amylase production by the isolates

Figures 3 and 4 present the effect of initial culture pH on production of the two amylases by the isolates. Each of the isolates produced the highest amylase activity at pH 4.0. The effect of initial medium pH was studied with pH values of 3.5, 4.0, 5.0, 6.0, 7.0 and 8.0, and the initial pH value of 4.0 was recorded best for the isolates for the production of both α -amylase and glucoamylase followed by pH 5.0 and 3.5.

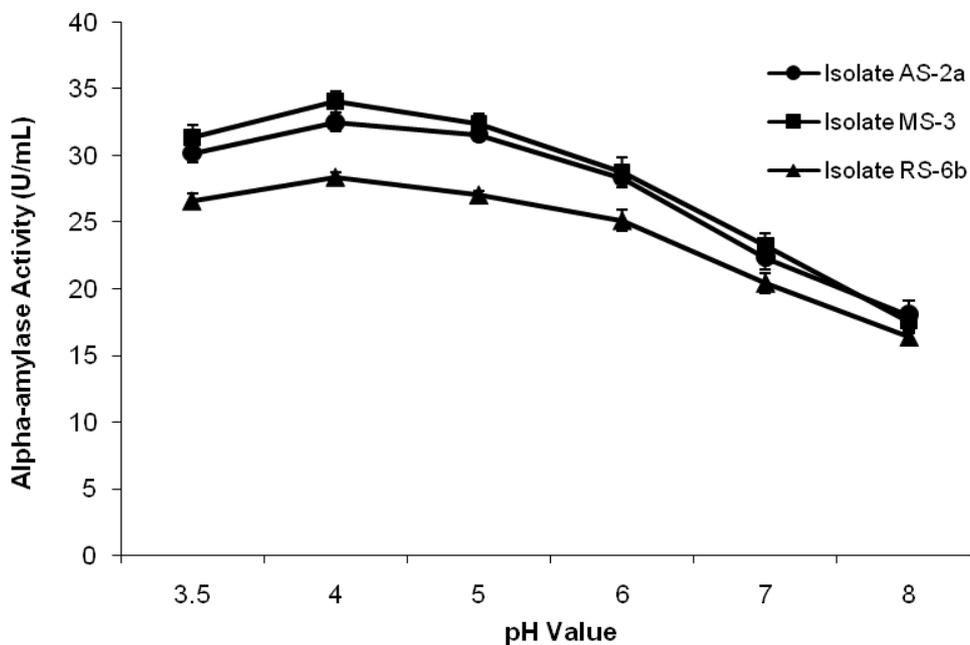


Figure 3. Evaluation of the effect of pH on the three isolates production of α -amylase at 25°C and 80g/L starch concentration

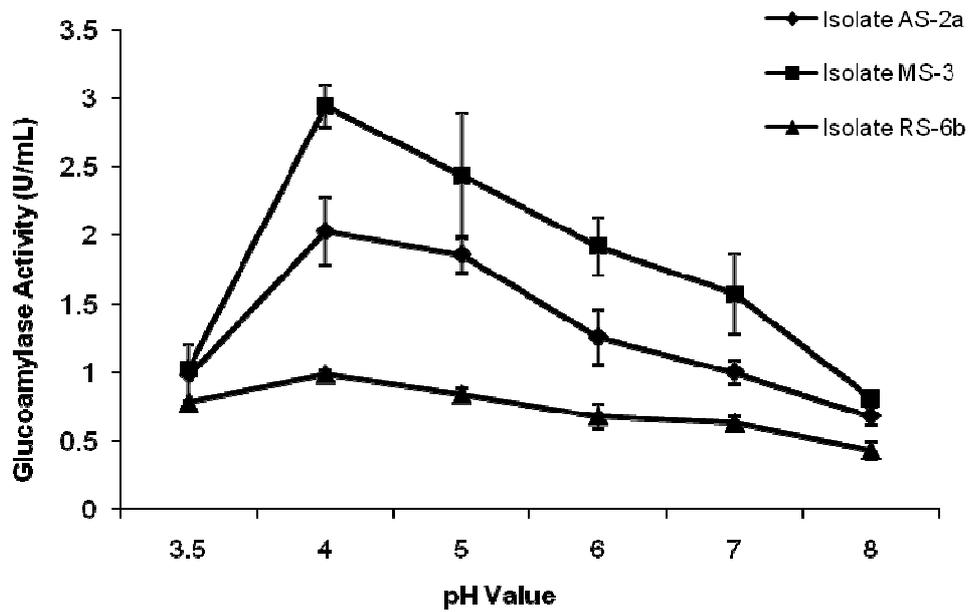


Figure 4. Evaluation of the effect of pH on the three isolates production of glucoamylase at 25°C and 80g/l starch concentration

Influence of temperature on amylase activities from the isolates

The activities of the amylase enzymes produced by the isolates were assayed at various temperatures as presented on figures 5 and 6. Maximum activity of α -amylase of the isolates was observed at 45 °C while the maximum activity of their glucoamylase was observed at 30 °C.

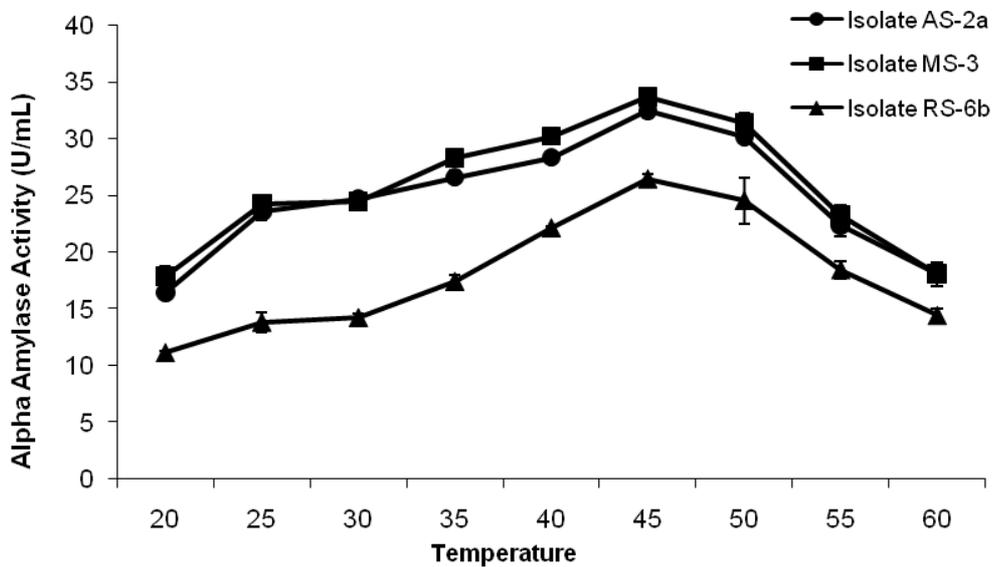


Figure 5. Effect of temperature on α -amylase activities of the three isolates cultivated for five days at 25°C and 80g/l starch concentration

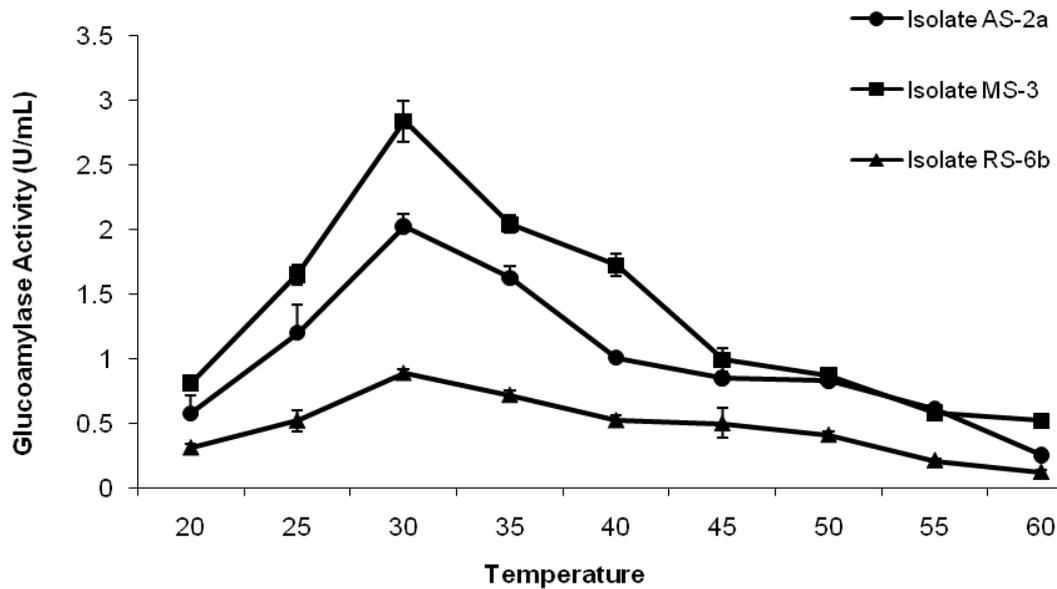


Figure 6. Effect of temperature on glucoamylase activities of the three isolates cultivated for five days at 25°C and 80g/l starch concentration

Influence of different carbon sources on amylase production

Figures 7 and 8 showed the influence of different substrates (carbon sources) on amylase production by the selected fungal isolates. The cultures with gelatinized potato flour produced the highest amylases. Other starch substrates such as corn flour, cassava flour and soluble starch were also utilized by the fungal isolates when gelatinized but they did not utilize non-gelatinized starch. The culture conditions were room temperature and 120 hours. Non-gelatinized starch was not soluble in water at the temperature and thus was not utilizable to the isolates.

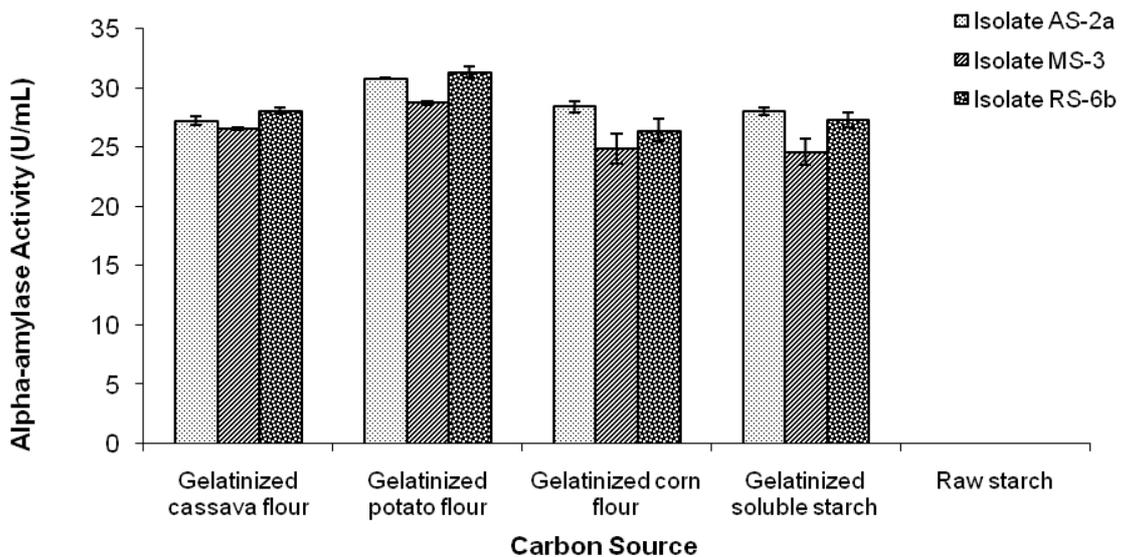


Figure 7. Effect of carbon source on the production of α-amylase by the three isolates cultivated at 25°C, pH4 and 80g/l starch concentration

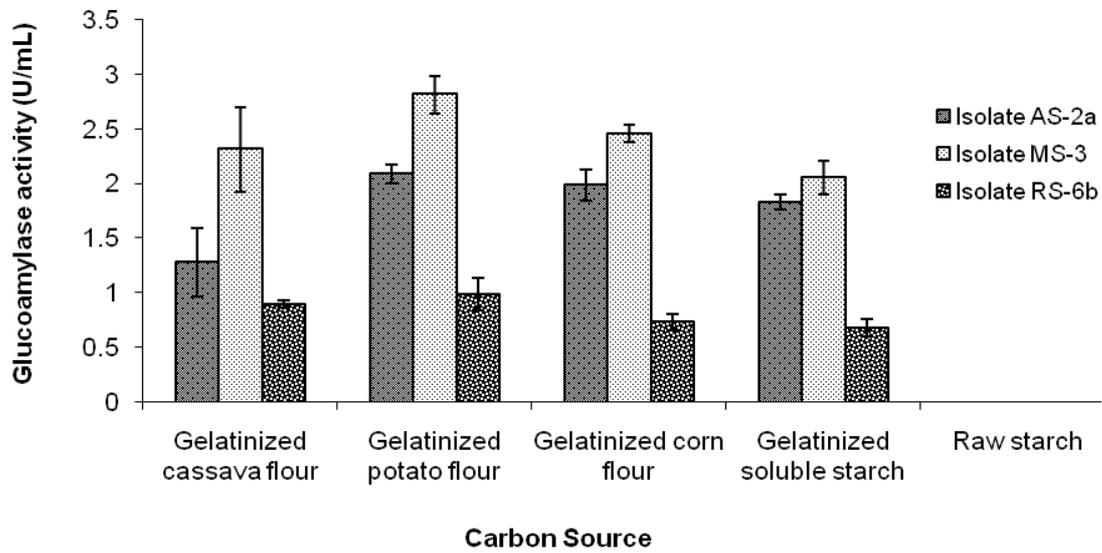


Figure 8. Effect of carbon source on the production of glucoamylase by the three isolates cultivated at 25°C, pH4 and 80g/L starch concentration

Effect of carbon source concentrations on the isolates amylase production

The effect of various starch concentrations on amylase production by the isolates revealed that the productivity of α -amylase peaked at 50 g/l starch concentration while the peak of glucoamylase production was observed at 80 g/l starch concentration [figures 9 and 10]. Further increase in starch concentration resulted to a decrease in the enzymes production. All experiments were run parallel in triplicates. The values differ significantly at $P < 0.05$.

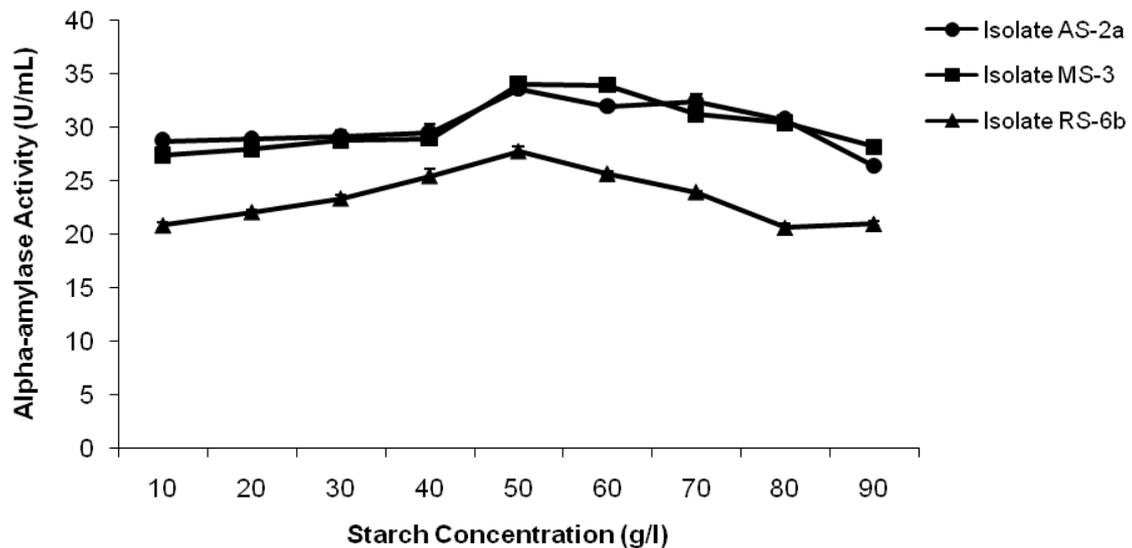


Figure 9. Assessment of carbon source concentration on α -amylase production by the three isolates cultivated for 5 days at 25°C

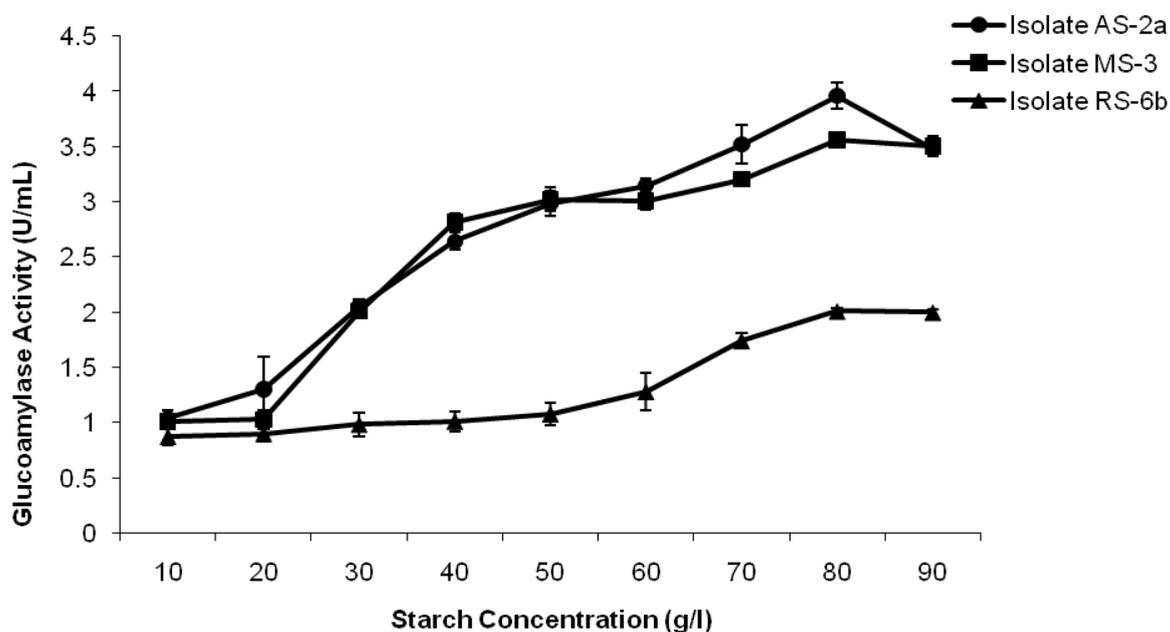


Figure 10. Assessment of carbon source concentration on glucoamylase production by the three isolates cultivated for 5 days at 25°C

DISCUSSION

A number of reports exist regarding the effects of various cultural and environmental conditions such as incubation period, pH, temperature, carbon sources and carbon source concentrations on the production of amylases by fungal strains. Environmental factors such as temperatures and pH has been reported to have some important influences on enzyme productivity by fungal organisms [Gupta *et al*, 2008]. In line with the above records, this investigation is made to enhance maximum productivity of α -amylase and glucoamylase by the isolated fungi strains. The optimization of their cultural conditions such as cultivation period, optimum pH, temperature, the best carbon sources and optimal starch concentrations, of the isolates were necessary.

Time courses of the amylase enzymes production were studied for the three fungal isolates at different periods in hours (24, 48, 72, 96, 120, 144, and 168). The productivity of the enzymes was peaked at 120 hours. Further Increase in incubation period beyond the optimum incubation time resulted in decrease in amylase activities [figures 1 and 2]. The decline in the productivity may be due to depletion of one or more essential nutrients in the medium or due to accumulation of toxic by-products.

In addition to the regulatory effect on gene expression, cultivation pH can also affect fungal morphology greatly [Whitaker and Long, 1973]. Thus, development of an optimal pH control strategy is helpful in obtaining higher enzyme productivity by the fungal strains. The effect of pH on the production of amylase enzymes in this study revealed that the amylase productivity by the isolates was at their maximum at pH 4.0 [figures 3 and 4]. This supports the earlier report that amylases are produced by fungal organisms maximally in acidic medium [Arti *et al*, 2010]. The activities of the enzymes decreased with further increase in pH values. This implies that the successful production of amylase enzymes depends on the maintenance of initial pH of the production medium. All experiments were run in parallel triplicates. The values differ significantly at $P < 0.05$. Temperature is a critical factor which markedly influences enzyme activities. The effect of temperature on amylase activities was shown in figures 5 and 6. In the present study, maximum α -amylase and glucoamylase activities of the fungal isolates were obtained at 45 °C and 30 °C respectively. This observation agrees with the earlier report that amylases differ in their temperature optimum, pH optimum and several other physiochemical properties depending on their origin. Hence, different enzymes have found specific application in different industries [Arti *et al*, 2010].

The study of the effect of carbon source showed that the best substrate for the production of α -amylase and glucoamylase by the three isolates is gelatinized sweet potato flour [figures 7 and 8]. This finding is in line with the report that the best carbon substrate for amylase production is sweet potato starch [Fabiana *et al.*, 2008]. Other starch substrates such as corn flour, cassava flour and soluble starch were also utilized by the fungal isolates when gelatinized but not non-gelatinized starch. The culture conditions were room temperature and 120 hours of incubation. Non-gelatinized starch was not soluble in water at the temperature and thus was not utilizable to the isolates.

The enzymatic activities of the isolates were tested from cultures of different starch concentrations. The results were shown in figures 9 and 10 for α -amylase and glucoamylase activities respectively. Though there was progressive increase in the productivity of α -amylase and glucoamylase by the isolates as the starch concentration increased, the maximum production of α -amylase was obtained at 50 g/l starch concentration and the productivity of glucoamylase was peaked at 80 g/l starch concentration. This supports the report that carbon sources like starch may have some inducing effects on amylase enzymes productions [Archer and Peberdy, 1997].

Finally, the present study established that for the amylases to be produced adequately by the fungal isolates, their culture should be regulated to their optimal conditions for maximal productivity. The factors such as incubation time, pH, temperature, carbon sources and carbon source concentrations need to be considered in cultivation of these fungal strains since it affects the enzyme production. The results of this study are informative in determining culture conditions for commercial scale production.

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