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Production of Glucoamylase by *Aspergillus niger* in Solid State Fermentation

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Abstract: Glucoamylase is a starch-degrading enzyme widely used in the food industry to produce high glucose syrup, and also in fermentation processes for production of beer and ethanol. In this study, *Aspergillus niger* was used to produce glucoamylase in a solid state fermentation using wheat bran, cassava starch and soybean flour as substrate. The effect of incubation period, temperature, pH and metals on the activity of the enzyme was evaluated. The enzyme showed high activity of 80 U/ml at 48 hours of fermentation and the lowest activity (53U/ml) was recorded at 24 hours of fermentation. Glucoamylase presented highest activity (140U/ml) at temperature 60°C while the lowest enzyme activity (32 U/ml) was recorded at 90°C. The enzyme produced has the highest activity (152 U/ml) at pH 6.0 and the lowest activity (78 U/ml) at pH 8.0 while $MgCl_2$ produced the highest glucoamylase activity of 153 U/ml. This study showed that wheat bran, cassava starch and soybean flour can be used as substrate for the production of glucoamylase.

Key words: Glucoamylase · *Aspergillus niger* · Fermentation · Enzyme · Wheat Bran

INTRODUCTION

The amylase family of enzymes has been well characterized through the study of various microorganisms [1]. Two major groups, endo- and exo-amylases, have been identified and are among the enzymes most studied [2]. These enzymes represent about 25-33% of the world enzyme market, second after proteases [2]. Amylase enzymes are important enzymes employed in starch processing industries for hydrolysis of polysaccharides into simple sugars [3].

Microorganisms including a number of fungal species have been used to produce amylases more economically than from other sources. Glucoamylase is useful in food industries for saccharification of starch and other related oligosaccharides. Glucoamylase (GA) consecutively hydrolyzes α-1,4glycosidic bonds from the non-reducing ends of starch, resulting in the production of glucose. To a lesser extent, it also has the ability to hydrolyze α-1,6 linkages, also resulting in glucose as the end-product [4]. A number of organisms are used to produce glucoamylase, *Aspergillus niger*, *Aspergillus awamori* and *Rhizopus oryzae* have been considered the most important for industrial application [5].

The principal industrial use of Glucoamylase is in the production of glucose, which in turn serves as a feedstock for biological fermentations in the production of ethanol or in the production of high fructose syrups [6] it also improves barley mash for beer production [7]. Glucoamylase is a key enzyme too in the production of sake and soy sauce. Specially, in sake brewing the enzyme is considered to be most important because the rate of fermentation is dependent on the activity of the GA [8].

In industrialized countries, glucoamylase is produced in economic quantities for industries by using fungal organisms grown on agricultural wastes such as wheat or rice bran. In a number of developing countries including Nigeria, using microorganisms such as fungi to produce glucoamylase for industrial activities have received very little attention. The outcome is the importation of glucoamylase for commercial production of products such as alcohol. A number of small scale alcoholic beverage industries in Nigeria for example, have stopped production because of the high cost of imported glucoamylase. A number of surviving alcoholic beverage producing industries in Nigeria for example, depend on starch-rich staple foods such as maize, rice, millet and sorghum as sources of amylases for starch hydrolysis [6].

The use of staple food for enzyme production on large scale has the potential of increasing prices of staple foods and can potentially affect food security. It is therefore, very necessary that efforts are made to produce

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amylases using microorganisms grown on cheaper substrates. This study was aimed at isolation and characterization of glucoamylase from *Aspergillus niger* in a solid state fermentation system using a combination of cassava starch, wheat bran and soya bean flour as carbon source.

MATERIALS AND METHODS

Sample Collection and Preparation: Wheat bran, cassava starch and soybean flour were all obtained from a local market at Osiele, Abeokuta, Nigeria. The samples were dried, ground into powder form and stored in an air-tight jar.

Isolation of *Aspergillus niger*: Soil samples were obtained from a cake factory at Osiele, Abeokuta, Nigeria. The samples were serially diluted and inoculated on sterile Potato Dextrose Agar and incubated at 28°C for 48-72 hours. The colonies were characterized and named with reference to Barnett and Huntter [9].

Screening for Glucoamylase Production: Plate screening was done by supplementing potato dextrose agar with one percent of soluble starch. The plates were inoculated and incubated at 28°C for 48 hours. After incubation, the plates were flooded with Lugol's iodine solution. The fungal colonies with clear zones indicated a positive glucoamylase production [10].

Formulation of Fermentation Medium: The fermentation medium used was formulated as described by Akpan *et al.* [10] with some modifications. Ten grams of wheat bran, 1g of cassava starch and 3g of soybean flour served as the substrate while mineral water, made up of KH_2PO_4 (14g/l), NH_4NO_3 (10g/l), KCl (0.5g/l), $MgSO_4.7H_2O$(0.1g/l) and $FeSO_4.7H_2O$(0.01g/l) was added to the fermentation medium to serve as source of other nutrients apart from carbon.

Testing for Glucoamylase Activity: The glucoamylase activity was determined according to the method of Akpan *et al.* [10]. Crude amylase was mixed with 4% (w/v) gelatinized cassava starch in 0.1 M acetate buffer (pH 4.5) in ratio (1:4 v/v) and incubated at 60°C for 1hour. The reaction was stopped by heating the tube at 100°C for 2 minutes to inactivate the enzyme. Glucoamylase activity was determined using dinitrosalicylic acid method, and glucose was used as the standard [10]. One unit of enzyme activity was defined as the amount of enzyme which liberate 1 micromole of glucose from starch in a 1 mL reaction mixture at 60°C for 1hour. Specific activity was expressed as units of enzyme activity per mg of protein.

Effect of Assay pH and Temperature on Enzyme Activity: The effect of temperature on glucoamylase activity was determined between 40°C and 90°C using Cassava starch(4% w/v) in 0.1 M acetate buffer (pH 4.5) as the substrate.The effect of pH on enzyme activity was evaluated by carrying out the reactions in 0.1 M acetate buffer between pH3.0 and 5.5 at 60°C for 1h.

Effect of Incubation Period: The effect of incubation period on enzyme activity was determined by making assay on enzymes extracted from the fermentation medium after 24, 48, 72, 96 and 120 hours of inoculation of *Aspergillus niger*

Effect of Metal Ions: The effect of metal ion was examined by putting 50mM of metals (NaCl, $CaCl_2$, $MgCl_2$, KCl) into the fermentation medium and cultured for 48 hours, the enzymes were harvested from the different fermentors and assay was made on them individually.

RESULTS

Results obtained from this study showed that *Aspergillus niger* survived well on a substrate formed by combining wheat bran, soy bean flour and cassava starch. Fig. 1 shows the effect of assay temperature on the activity of glucoamylase, the enzyme produced had the highest activity at 60°C with the activity as 140U/mL while the lowest activity (32U/ml) was recorded at 90°C). There was a gradual increase in activity from 40-60°C then the activity started going down at 70°C, then there was a very sharp decrease in the temperature from 80-90°C showing that the glucoamylase produced do not work well at high temperature.

Fig. 2 shows the effect of assay pH at 60°C and the activity was found to be highest at pH 6.0 with the activity152U/ml.

Fig. 3 reveals the effect of incubation period on glucoamylase assayed at pH 7.4 and 50°C the result clearly shows that the glucoamylase produced in this study had the highest activity after 48 hours. Fig. 4 shows the effect of metals on glucoamylase activity, Magnesium chloride produced the highest activity of 127 U/ml.

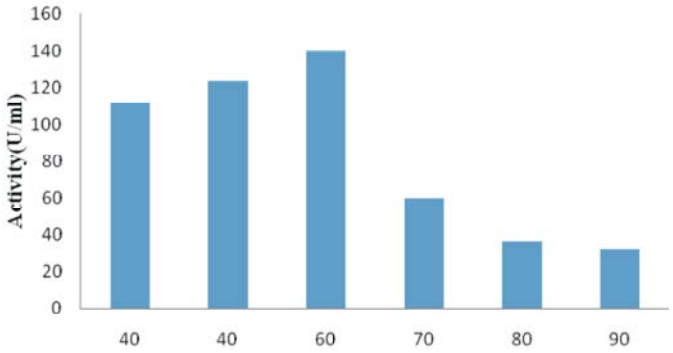

Fig. 1: Effect of assay temperature on glucoamylase activity

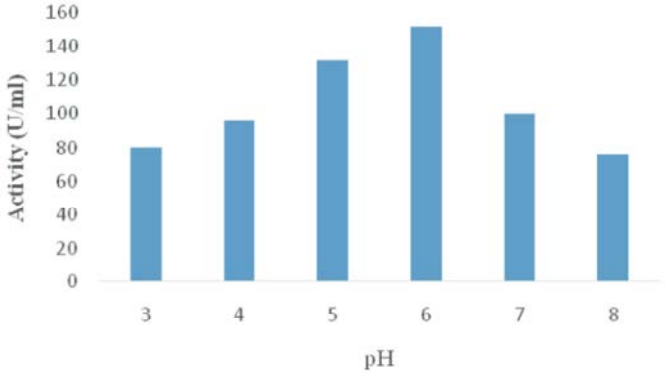

Fig. 2: Effect of assay pH on glucoamylase activity

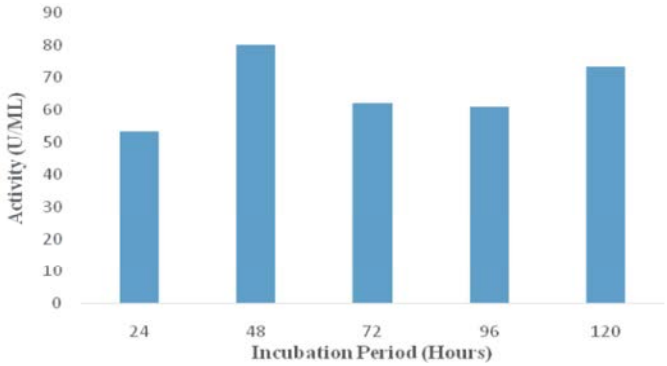

Fig. 3: Effect of incubation period on glucoamylase activity

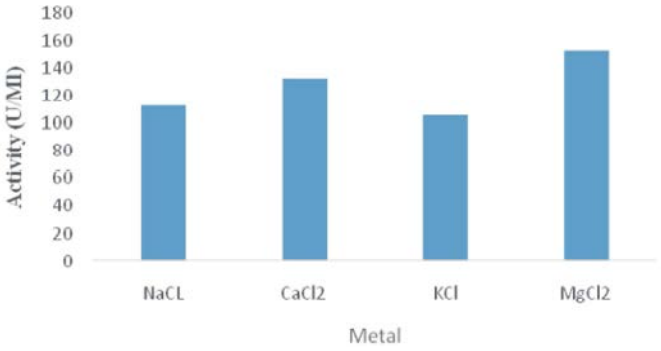

Fig. 4: Effect of metals on glucoamylase activity

DISCUSSION

Aspergillus niger investigated in this study was able to produce glucoamylase on a substrate formulated by adding wheat bran, soy bean flour and cassava starch. The activity of glucoamylase produced by this fungal species varied under different conditions. *Aspergillus niger* produced a glucoamylase of the highest activity at 60°C, this result agrees with the result of Deshmukh *et al.* [11] who reported maximum activity at 55°C. The small observable difference in the temperature of maximum activity may be attributed to the difference in the substrate used. In a similar study, Kareem *et al.* [12] used *Rhizopus oligosporus* to produce glucoamylase and reported that the maximum activity was found to be at a higher temperature of 80°C.In this study, assay temperature, assay pH and incubation period of culture media influenced the production and activity of glucoamylase. There was a gradual increase in activity from 40 to 60°C and a steep decline in activity from 70 to 90°C. [11], also reported that temperature influenced the activity of glucoamylase. In this study, highest glucoamylase activities were recorded at pH 6.0.This is in agreement with Ellaiah *et al.* [1] who reported that pH influenced glucoamylase activity with the highest glucoamylase activity achieved at pH 5 for *Aspergillus niger*.

In this study, highest glucoamylase activities were achieved with *Aspergillus niger* at incubation period of 48 hours. Deshmukh *et al.* [11] demonstrated that the incubation period influenced glucoamylase activity. In their work, highest glucoamylase activity was achieved at 72 hours of incubation period. By comparing the best glucoamylase activities achieved in this study with what have been reported by other workers, activities in this study were low. Magnesium chloride was found to have the highest positive effect on the activity of glucoamylase in this study in which the activity was found to be 153 U/ml.

CONCLUSIONS

This study showed that glucoamylase can be produced by *Aspergillus niger*. It showed that the substrate used which is the combination of wheat bran, soy bean flour and cassava starch could potentially be used for the production of glucoamylase by Solid-State Fermentation process. The study pointed out that the nature of substrate, incubation period, incubation temperature and pH of the culture medium used all affected production of glucoamylase in Solid State Fermentation.

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