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Hydrolysis of Tuber Peels and Sorghum Chaff by Cellulolytic Culture Filtrates of *Aspergillus Niger* AC4 Isolated from Agricultural Waste Dumpsites

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Abstract
Six of the twenty strains of *Aspergillus niger* isolated from tuber peels and sorghum chaff collected from twenty two agricultural waste dumpsites show strong capacity for producing cellulase enzyme using PDA plate supplemented with 2% (w/v) carboxylmethylcellulase incubated at room temperature. Cellulase production was detected by staining the plates with 2% congo red solution and then measuring the zone of clearance around the fungal colonies on the plates. *Aspergillus niger* AC4 produced the largest zone of clearance (3.63cm) on the PDA congo red stained plate and also gave the highest cellulase activity (240U/ml) on hydrolysis hence it was selected for further studies. Culture filtrates of the *Aspergillus niger*AC4 had optima temperature, concentration and pH at 35^{o}C, 4% and 5.0 respectively. Culture filtrates of *Aspergillus niger*AC4 was also used to hydrolyze yam peel, cocoyam peel, sweet potato peel, sorghum chaff and carboxylmethylcellulase (CMC) in comparison. Hydrolysis was significantly dependent on cellulose source and length of incubation ($P<0.05$). cellulose activities differ significantly in the cellulose sources ranging from 240U/ml in cassava peel to 54U/ml in sorghum chaff. It is concluded that cassava peel might be a better substrate for the production of cellulase by *Aspergillus niger* than commercial carboxymethlcellulase (CMC).
Key words: Tuber Peels, Sorghum Chaff, cellulolytic filtrate and hydrolysis.

1.0 Introduction
The recent thrust in bioconversion of agricultural and industrial wastes to chemical feedstock has led to extensive studies on cellulolytic enzymes produced by fungi and bacteria. Cellulose is a potentially valuable resource for fibre, fuel and feed. Investigations into ability of microbes to degrade native and modified cellulose so far have revealed that only a few fungi possess ability to degrade native cellulose (Moo- young et.al.,1987). A majority of microbes can however degrade modified cellulose. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes (Sani et.al.,1992; Doppelbauer et al.,1987). Cassava, yam, sweet potatoes, cocoyam and sorghum are important staple foods and they are source of food for about 200 – 300 million people in tropical areas (FAO, 1986).The first step in the processing of these tubers is the removal of the peels which are the two coverings of the tubers. These peels have been found to be very rich in cellulose.The possibility of high cellulase production from selected tubers and sorghum chaff by Aspergillus nigeris investigated in this study with a view to obtain a cheaper carbon substrate for the production of cellulase for industrial uses.

2.0 Materials and Methods
2.1 Sample treatment
Cassava, yam, cocoyam and sweet potato peels as well as sorghum chaff were collected from twenty two agricultural waste dumpsites in Ilorin metropolis, Kwara state. They were then dried in an oven at 80^{o}C and then ground into very fine powder separately. Each of the ground powder was dried into constant weight in an oven at 60^{o}C and their proximate analyses determined by standard methods. There was no significant difference in the dry matter of all the samples with cocoyam having the highest dry matter content and cassava with the least. Highest crude protein was from sorghum chaff and it is significantly different from the others with sweet potato having the lowest value of

crude protein. Cassava peel has the highest carbohydrate content with the least from sorghum chaff. Cyanide was absent from all the samples except cassava peels with 1.74mg/ 100ml of the cassava peel. They were stored in different desiccators until required.

2.2 Source and Maintenance of Organisms

Five grams each of the freshly obtained peels and chaff was taken from the sealed cellophane bags (with which they were collected from source) into sterile universal bottles containing 100ml of distilled water and shaken thoroughly. The settled supernatants were decanted off into separate test tubes serving as stock solutions. Serial dilutions of the stock were made with 0.1ml of the aliquot spread on Potato Dextrose Agar (PDA) plates containing 1% streptomycin.(to inhibit bacterial growth).The plates were incubated at room temperature (27 – 31^{0}C) for 48 hours (Berghem, 1976; Chahal, 1992; Lawal *et al*.,2005). After incubation, representative fungal colonies were picked from each plate and purified on fresh PDA plates. The purified isolates were transferred to PDA slants incubated at room temperature for 48 hours and stored at 4^{0}C. The isolates were identified according to the scheme of McGinnis (1980).

2.3 Screening for Cellulase Production

The method of Bisaria and Ghose,(1987) was used. Point inoculations of the spores of the isolates were grown on PDA supplemented with Carboxylmethylcellulase (CMC,2% W/V) medium. The plates were incubated at room temperature (27^{0}C- 32) for 72 hours after which it was stained with 2% Congo red solution for 15 minutes. Excess dye was removed by washing with 1M NaCl and the plates were fixed with 1M HCl. The production of extracellular cellulase by the organism was indicated by a zone of clearance around the fungal colonies on the plate. The zone of clearance was measured on each plate and the average determined. *Aspergillus niger* AC4 showing the biggest zone of clearance was selected for further studies.

2.4 Production of Crude Cellulase

Mineral salts media (MSM) for cultivation of fungal isolates was prepared with compositions as shown below {g/l}. KH_2PO_4 ,10g;$(NH4)_2SO4$,10.5 g ; $MgSO_4.7H_2O$, 0.3g; $CaCl_2$, 0.5 g; $FeSo_4$, 0.013g ; $MnSO_4.H_2O$ 0.04; $ZnSO_4.7H_2O$ 0.04; Yeast extract 0.5g; Carbon source (40g). The carbon sources were the delignified peels, chaff and carboxylmethylcellulase. One hundred and fifty milliliter of each medium was dispersed into conical flask and sterilized in the autoclave at 121^{0}C for 15 minutes. The final pHs of the medium was adjusted to 5.0with 0.1 M NaOH and 0.1M HCl using a pH meter (pye unicam pH). Spores of 72 hour old cultures of *Aspergillus niger*AC4 were harvested by washing slants with 10ml of sterile distilled water. An aliquot of 5ml of the spore suspension was used to inoculate 150ml of each of the prepared medium.

The culture media were incubated at room temperature (27-32^{0}C) in an orbital shaker (Gallenhamp, England) at 100rpm for seven days.

Hydrolysis of tuber peels and sorghum chaff. The ability of the crude enzyme to hydrolyze the tuber peels and sorghum chaff was studied using the grounded cassava,yam,sweet potato and cocoyam peels as well as sorghum chaff. Commercial carboxymethylcellulase (CMC) was used as the standard. Cellulase activity of the suction culture filtrate was determined colorimetrically by measuring the increase in reducing groups by the hydrolysis of Carboxylmethylcellulase (CMC) substrate and the other carbon sources (Panda 1989; Ali *et al*., 1991). Cultured samples were filtered through whatmann filter paper to remove the mycelia and other particle.The filtrates were used to assay enzymatic activity. The reaction mixture containing 0.5ml of the enzyme solutions and 1ml of 2% (w/v) of the crabon sources were incubated at a temperature of 35^{o}C for 20 minutes and the reactions stopped by adding 2.0ml of DNS Reagent materials (1.0g of 3,5-dinitrosalicylic acid, 20ml of NaoH, 30 g of sodium potassium tartarate in 100ml). The total mixture was then heated for 5 minutes, cooled and 20ml of distilled water added. The color intensity was determined at 560nm using a spectrophotometer (Jenwey 6405 UV/visible).

The effect of hydrolysis was also studied alongside time of incubation.One unit of cellulase activity was defined as the amount of enzyme which will release 1.0µmole or one unit of enzyme activity (U) of D- glucose or reducing sugar as glucose per millimeter of digest in 20 minutes under the specified conditions. It is also defined as the amount of glucose produced per ml in the reaction mixture per unit time

3.0 Results

From the result (Table 1), *Aspergillus niger* AC4 was considered to be the best cellulase producer with zone of clearance 3.36cm and cellualse activity of 240U/ml. It has the highest cellualse activity which is significantly different from the others ($P< 0.05$) and produced the largest zone of clearance hence it was selected for further studies.

3.1 Effect of temperature cellulase Activities

Cultures of *Aspergillus niger* AC4 were incubated at different temperature ranges of 25^0C to 70^0C using temperature regulated orbital shaker at 5^OC interval and cellulase production monitored in shake flask culture. Cellulase production increased progressively from 25^oC to the maximum at 35^oC above which there was decrease in cellulase production as shown in figure 1.

3.2 Effect of pH on Cellulase production

The effect of pH (2.0 -8.0) on cellulase production of *Aspergillus niger* AC4 was studied by adjusting pH of the hydrolyzing medium to various values ranging from 2 to 10 with 0.1 M NaOH and 0.1M HCl using a pH meter at 1.0 pH unit increments and cellulose activity monitored. Highest cellulase activity was obtained at pH 4.0 for sweet potato peel and 5.0 for cassava, yam, cocoyam peels, CMC and sorghum chaffand above these pHs, there were decrease in cellulase activities as shown in the figure 2

3.3 Effect of concentration cellulase production

Different concentrations of the substrates ranging from 1% to 9 % (w/v) were used in the preparation of the hydrolyzing medium with concentration unit increments of one. cellulase activities was found to be increasing up to 4% where it is maximum above which cellulase activities values started decreasing as shown in the figure 3.

The various optimal conditions for cellulase production were combined and the effect of time of incubation on the hydrolysis of the different carbon sources were studied under the determined optimal conditions as shown by the figure 4.

4.0 Discussion

The ability of only six out of the twenty strains of *Aspergillus niger* isolated from agricultural waste dumpsites confirm the earlier reports of Doppelbauer *et.al*., 1987; Moo-young *et.al*., 1987; Raji *et.al*., 1988; Desvaux *et.al*., 2000; that only a few fungi possess ability to degrade cellulose. All the isolates selected from the PDA-congo red stained plates produced detectable quantities of cellulase during hydrolysis of the raw tuber peels and sorghum chaff hence the use of PDA-Congo red stained plates present a simple and easy way of screening for cellulolytic microbes.

The study also showed that the susceptibility of the raw tuber peels and sorghum chaff to the crude enzyme of *Aspergillus niger* AC4 was significantly dependent on the carbon source and time of incubation. Cultures of *Aspergillus niger* AC4 grown on 2% (w/v) PDA carboxylmethycellulase produced low level of cellulase at 25^oC but cellulase production increased to the optimum at 35^oC for cassava and other carbon sources and 40^oC yam peel. Beyond these optimal temperatures, cellulase activities started reducing and eventually became zero at 55^oC probably because the enzymes are being denatured as temperature increases. Initial experiments by Sani*et.al*., 1992 and Omojasola *et.al*.,2009 had indicated the sixth and seventh day as the ideal period to harvest for temperature dependent growth but this work is at variance with their reports indicating the fourth day for yam, cassava, sweet potato peels and sorghum chaff while cocoyam peel and CMC had the fifth day as the ideal period for the harvest of the growth. When the determined optima conditions for cellulase production were combined in the submerged culture,cellulase production increased significantly and this agrees with the earlier reports of Ali *et.al.,*1991; Davies, 1994 that the extracellular production of microbial cellulase depend on a number of factor such as inoculums size,carbon source, pH, temperature, presence of inducers and or inhibitors, medium additives,batch size, aeration and growth time and that if these conditions are combined optimally, cellulase production will be enhanced.

The ability of the crude cellulase of *Aspergillus niger* AC4 to hydrolyze the tuber peels especially cassava and tuber peels presents a remarkable property since these tuber peels are abundantly available in the tropics and over thirty million tonnes of these tubers are lost as wastes yearly after harvesting due to poor storage (FAO,1986). Conversion of these tuber peels by this enzyme means that these wastes can be used by industries employing hydrolysis processes for value added products.(Sani *et.al*.,1992; Doppelbauer *et al*.,1987).

In conclusion, it has been shown in this study that cassava peel may be a better carbon substrate than commercial carboxylmethycellulase for cellulase production by the *Aspergillus niger* AC4 investigated. Other materials and wastes are also being tried with some other cellulase producing microorganisms with a view to obtaining the most economical carbon substrate for the production of cellulase for industrial use.

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Table 1: Cellulase production capacities of *A.niger* strains

Isolate code	Means of clear zones	Cellulase Activity (U/ml)
AC4	3.36	240[a]
AC2	2.27	161[b]
AY14	2.00	138[c]
AY19	1.82	130[c]
AC 12	1.80	129[d]
AP20	1.53	109[e]

[a,b,c,d,e]Means on the same column with different superscripts differ significantly. ($P<0.05$)

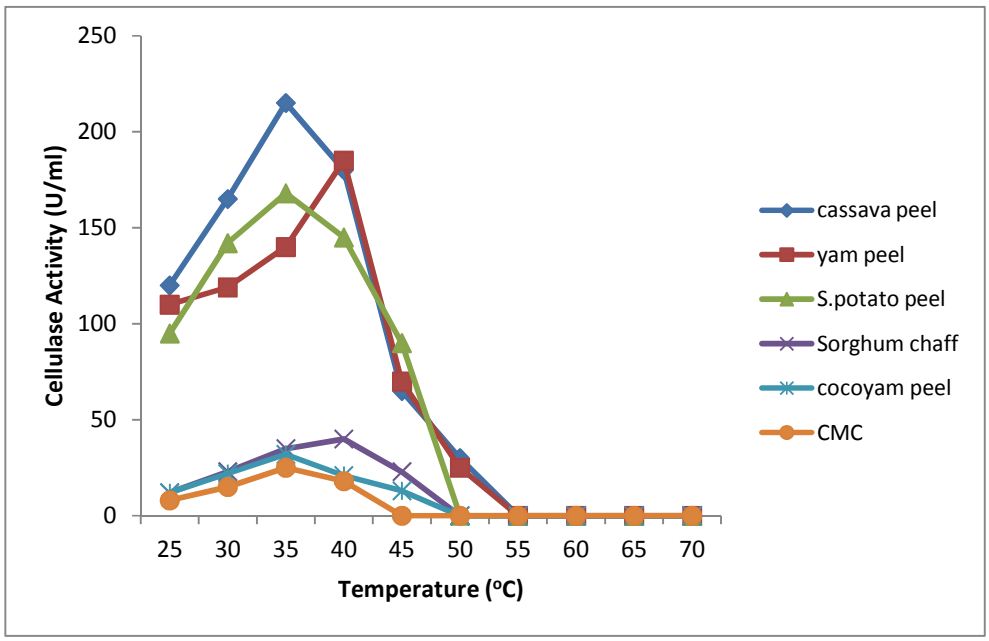

Figure 1: Effect of varying substrate concentration on cellulose activities of *A.niger* AC4

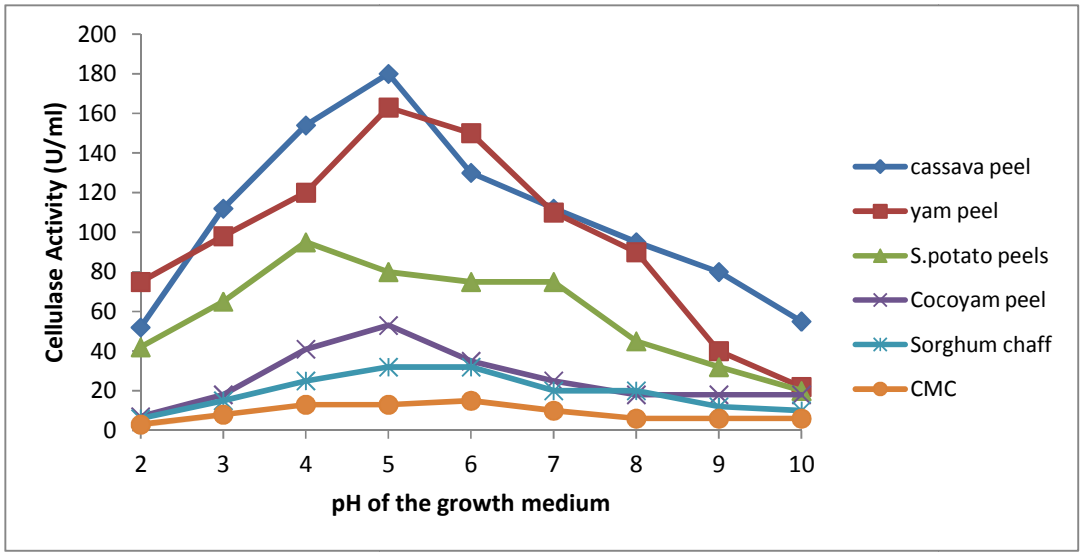

Figure 2: Effect of varying pH on cellulase activities of *A.niger* AC4 in shake flask culture

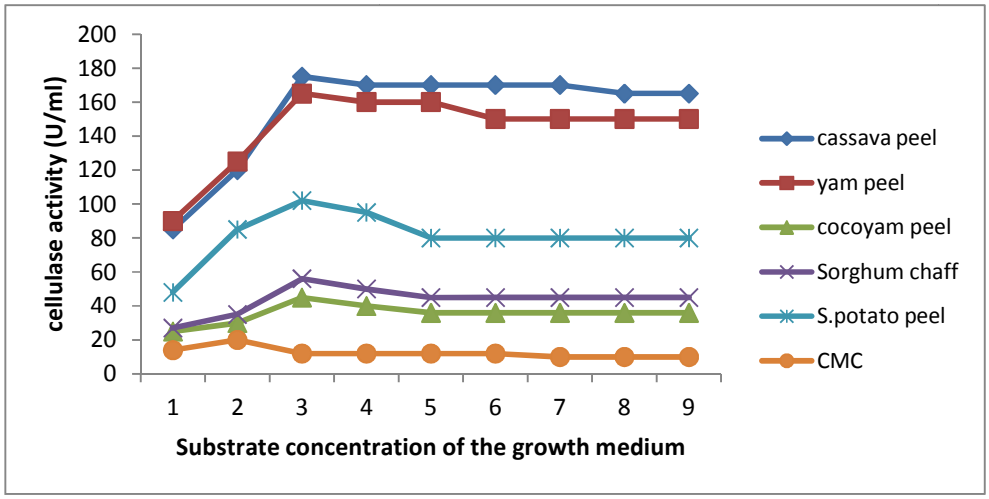

Figure 3: Effects of varying substrate concentrations on cellulase activities of *A.niger* AC4 in shake flask culture.

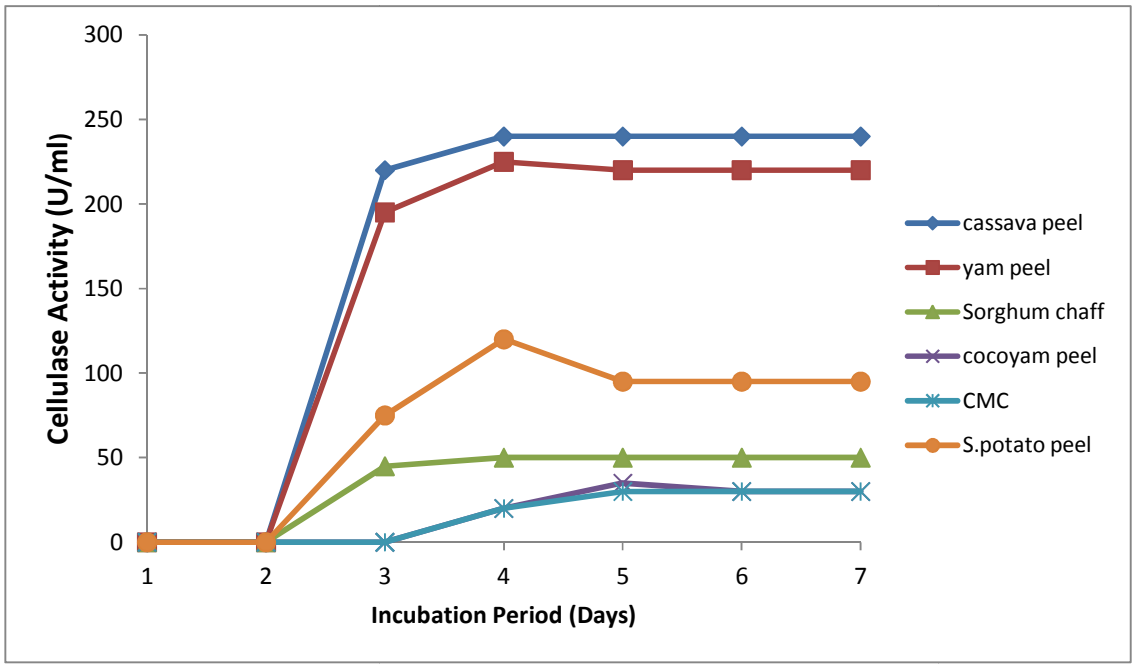

Figure 4: Enzyme activities of *Aspergillus niger* under optimal conditions of pH 4.0 for sweet potato peel and 5.0 for other carbon sources; substrate concentrations of 3% for CMC and 4% for other carbon sources and temperature 40^{o}C for cocoyam and 35^{o}C for other carbon sources.

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