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Full Length Research

Screening of Cellulolytic and Amyloytic Fungi Associated With Corncobs in Refuse Dumps Within Jos, Nigeria

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Screening of cellulolytic and amylolytic fungi that colonize corncobs collected from fifteen refuse dumps in Jos Metropolis, Nigeria was undertaken. The samples were cultured on Potato Dextrose Agar and the fungi were isolated and identified. The isolates were then sub-cultured on Modified Starch Agar and Modified Cellulose Agar to determine their amylase- and cellulase producing abilities respectively. The starch-iodine reaction test was used to determine amylase activity while cellulase activity of the organisms was measured using the radial diameter of their colonies. The organisms were further cultured on modified agar prepared using various cellulosic wastes such as paper, cornstalk and wood sawdust. A mean fungal count of 1.17×10^6 cfu/g was obtained for the corncobs, showing no significant difference between the fungal loads in the locations (P≥0.05). A total of 15 different fungi and an actinomycetes were isolated. They were : *Streptomyces sp. (*actinomycetes*)* (15.93%), *Aspergillus sp.* (14.16%), *Rhizopus sp.* (11.50%), *Mucor sp.* (10.62%), *Geotrichum sp.* (9.74%)*, Trichophyton sp.* (8.85%), *Penicillium sp.* (7.08%), *Trichoderma sp.* (6.2%), *Microsporum sp.* (5.31%), *Fusarium sp.* (4.43%), *Chaetomium sp.* (1.77%), *Monilia sp.* (1.77%), *Nocardia sp.* (0.88%), *Aureobasidium sp.* (0.88%), and *Madurella sp.* (0.88%) respectively with the percentage frequency of occurrence in parenthesis. While 8 (53.33%) of the isolated microorganisms had high amylolytic activity, 10 (66.67%) demonstrated cellulolytic activity when grown on modified starch agar and modified cellulose agar respectively. The results of this study implied that a high percentage of cellulolytic and amylolytic fungi could be obtained locally from corncobs and most of the microorganisms isolated hold varied industrial and bioremediation potentials.

Key words: Cellulolytic**,** Amylolytic, Fungi, Corncob.

INTRODUCTION

Celluloytic and amylolytic enzymes are used in industries for the production and processing of chemicals, foods and manufactured goods such as paper, rayon and cellophane, among others. For instance, cellulases have been extensively utilized for extraction of valuable components from plant cells, improvement of nutritional values of animal feed and the preparation of plant protoplasts in genetic research (Kader *et al*., 1999). Amylases are enzymes that hydrolyze starch, releasing several products including dextrins and small polymers of glucose units (Onofre *et al.,* 2011). Fungi have been the main source of cellulolytic enzymes. *Trichoderma sp.* Have been shown to be the most efficient cellulase

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producers and *Penicillicum funiculosum* and *Fusarium solani* have also been shown to possess equally potent cellulases (Okafor, 2007). Amylases are found in virtually every living cell and the property and substrate pattern of these amylases vary according to their source (Okafor, 2007). Although, amylases can be derived from several sources such as plants, animals and microorganisms, the enzymes from fungal sources are preferred in the industrial sector and a large number of them are available commercially (Sidkey *et al*., 2011).

The wastes generated during agricultural and industrial processes contribute to environmental pollution and therefore, the local production of such enzymes using locally available agricultural wastes as substrates may reduce the cost of importation and encourage self-reliance (Milala *et al*., 2005). These wastes which include cereals, straw, leaves, and corncobs among others, are highly underutilized in Africa, including Nigeria. In most parts of the country, these materials are used mostly as animal feeds. A large quantity is left on farmlands to be decomposed by microorganisms. Proper biotechnological utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products and therefore ameliorate the problems they cause (Milala *et al*., 2005). Amylases are amongst the most important industrial enzymes and account for over 25% of industrial enzymes (Nwagu and Okolo, 2011). These enzymes are required as feedstock in the Nigerian industries and are all imported at great foreign exchange cost (Federal Ministry of Science and Technology, 2004).

Presently, there is little or no commercial production of cellulases and amylases for large-scale industrial use within the country, Nigeria. Industrialists have thus resorted to importation of microbial enzymes for their industrial processes and therefore, huge amount of foreign exchange is spent annually in importing enzymes needed (Egwim and Oloyede, 2006). The availability of huge amount of cellulosic materials in Nigeria underlines the need to explore the potentials of the natural decomposers of the plant cell-wall polymers for the transformation of these wastes into useful products. Strains of the viable organisms could be used for the production of cellulases and other cell-wall hydrolyzing enzymes needed for industrial saccharification of cellulosic materials (Nwodo-Chinedu, 2005). The capacity for the local production of bioreactors for use in enzyme production in Nigeria has been demonstrated. As culture substrates are readily available and the operating conditions for production are also attainable (Federal Ministry of Science and Technology, 2004), this study seeks to explore the relative potentials of cellulolytic and amyloytic organisms that could be sourced from a very readily available substrate, corncobs in Jos, Nigeria. This could be a means to solving the issue of importation of these enzymes. The study was designed to isolate and screen fungi from corn cobs in refuse dumps of Jos metropolis for their potentials to produce cellulase and or amylase enzymes.

MATERIALS AND METHODS

Sample collection

Fifteen samples each were collected from four different areas in Jos North LGA of Plateau State, Nigeria, (a total of 60 in all) using precise aseptic techniques .The four areas include, University of Jos, Farin Gada, Gada Biu, and Terminus market. Each sample was collected aseptically from a rubbish dump using a clean polythene bag. Hand gloves were used to avoid contamination and infections. The samples were placed in ice -packed plastic containers, transported to the laboratory and analyzed within three hours.

Preparation of Media and Samples

Potato Dextrose Agar was prepared according to the manufacturer's instructions. Chloramphenicol antibiotic was incorporated at a 1% concentration weight /volume. Modified cellulose agar and Modified Starch Agar were prepared according to the methods adopted by Pointing (1999). Fourty grams per litre of Cellulose and Starch substrates were incorporated into solid agar media respectively. Chloramphenicol, 1% v/v was thereafter, added aseptically before pouring into Petri dishes. Each sample of corncob was aseptically blended using a sterile blender to ensure homogenization of the sample.

Isolation of Fungi

The pour plate method as described by Cheesbrough (1999) was employed. One gram of each sample was aseptically weighed and placed in a test tube containing sterile water. It was then allowed to stand for 30 minutes. One ml of the stock solution was serially diluted and 10^{-5} dilution of each sample was plated out. The plates were incubated at 25°C for a period of three, five, and seven days during which they were carefully monitored and examined for growth.

Identification and Characterization of isolates

Each growth was observed macroscopically for features including colour of the growth, colour of the reverse side, texture, and consistency. Each fungus was examined microscopically using the Lactophenol Cotton Blue (LCB) staining technique as described by Aneja (2003). Micro-scopic features that were observed include presence of septates, presence of arthroconidia, conidia and conidia bearing hyphae, macroconidia and spores, branching, shape and size of structures. The fungi were identified by comparison with pictures of fungi in an Atlas for fungi (Larone, 1995).

Table 1. Fungal Load of Corncob Samples Collected from Various Locations in Jos North, Nigeria.

S/N	UJ	FG	GB	T
	$\times 10^5$cfu/g	$\times 10^5$cfu/g	$\times 10^5$cfu/g	$\times 10^5$cfu/g
1	7.4	4.9	5.4	7.1
2	22.6	8.7	6.1	6.2
3	39.7	17.7	3.4	2.31
4	5.0	6.5	22.0	4.2
5	13.4	5.1	8.7	26.9
6	6.4	5.8	12.0	6.8
7	3.9	6.0	4.8	5.9
8	4.6	5.6	5.3	7.0
9	15.4	4.5	6.8	11.6
10	9.0	24.3	5.2	13.2
11	4.2	3.9	47.5	41.1
12	5.4	5.9	8.5	31.5
13	6.2	4.5	8.9	5.4
14	2.8	6.0	29.1	9.4
15	27.3	4.8	25.8	14.1
Means ± S.D	**11.55±10.62**	**7.61±5.69**	**13.30±12.46**	**12.85±11.33**

UJ = University of Jos; FG = Farin Gada; GB = Gada Biu; T = Terminus market; S.D = Standard Deviation

Screening for Cellulase and Amylase producing fungi

Growths observed on the cellulose agar were a positive indication of the presence of cellulolytic fungi. The radial diameter of the growths on the agar were measured in millimeters. All isolates from the cellulose agar were further tested for their cellulolytic activity on three different media incorporated with paper, maize stalk and sawdust respectively. Growth of fungi on the media containing these three substrates were a confirmation that cellulolytic fungi were present. The fungi genera were then determined by macroscopic and microscopic features.

Gram's iodine was added to each growth to screen for amylase production. A positive result was indicated by a yellow colour or a clearing, while a blue-black colour was observed in all areas where starch has not been degraded (Sohail *et al.*, 2005).

Statistical Analysis

The results on the fungal loads of corncobs in the various refuse dumps were subjected to simple statistical analysis including mean, standard deviation and significant difference among the means according to methods described by Keller and Onyeka (1992)

RESULTS AND DISCUSSION

Mean fungal loads of corncobs

A mean fungal count of 1.17×10^6 cfu/g was obtained for the corncobs collected from various areas in Jos North, Showing no significant difference between the fungal loads in the locations ($P\geq0.05$). The results are shown in Table 1. Although, the mean values of the fungal loads on corncobs collected from different locations in Jos North (Table 1) show that the samples from Gada Biu were more contaminated (1.33×10^6 cfu/g), followed by those from Terminus (1.29×10^6 cfu/g), University of Jos (1.16×10^6 cfu/g), and Farin Gada (7.61×10^6 cfu/g), respectively, the difference in the means were not significant ($P\geq0.05$). The results imply that there is no difference in the relative abundance level of fungi on corncobs in the city. The fungal loads observed in this study (with a gross mean load of 1.7×10^6 cfu/g), is much higher than those obtained by Nwagu and Okolo (2011) (1.6×10^4cfu/g) for soil samples in Eastern Nigeria. The high fungal load in the samples implies that corncobs are a ready source for various fungi that could be of industrial benefits. It could be inferred that the relatively lower temperature of Jos town lying between 26 and 30°C could account for the high fungal loads of corncobs in the waste dumps there. This is because optimal growth temperature for fungi lies within this temperature regime (Adams and Moss, 1999).

Frequencies of occurrence of organisms in corncobs

A total of 15 fungi were isolated from all the samples as shown in Table 2, while Table 3 shows the percentage frequency of occurrence of each isolate in the various locations. The organisms isolated include *Streptomyces*

Table 2. Radial Diameter of Fungi on Modified Cellulose Agar Medium.

S/n	Fungi	3 days (mm)	5 days (mm)	7 days (mm)
1	*Aspergillus flavus*	8	9	13
2	*Aspergillus fumigatus*	3	4	6
3	*Aspergillus niger*	5	8	9
4	*Aureobasidium sp*	6	8	13
5	*Chaetomium sp*	2	6	9
6	*Fusarium sp*	3	5	6
7	*Geotrichum sp*	12	15	16
8	*Monillia sp*	2	3	5
9	*Mucor sp*	2	8	15
10	*Penicillium sp*	5	7	8
11	*Rhizopus sp*	7	8	19
12	*Trichoderma sp*	14	17	20

Table 3. Radial Diameter of Fungi on Modified Paper, Cornstalk and Wood sawdust Agar Media.

Fungi	Modified Paper Agar (mm)			Modified Cornstalk Agar (mm)			Modified Sawdust Agar (mm)		
	5	**7**	**10**	**5**	**7**	**10**	**5**	**7**	**10**
Chaetomium sp	9	11	12	5	8	13	-	2	6
Aspergillus sp	-	-	-	-	-	-	4	7	8
Aspergillus niger	5	8	14	4	9	13	-	-	-
Aspergillus fumigates	-	-	-	5	7	14	-	-	-
Aspergillus flavus	4	5	8	4	8	11	-	-	-
Geotrichum sp	4	5	7	7	11	17	-	5	8
Fusarium sp	3	7	10	14	17	20	3	4	7
Aureobasidium sp	-	-	-	-	7	9	-	-	-
Mucor sp	8	10	17	6	7	10	-	-	-
Penicillium sp	2	5	10	2	3	7	-	-	-
Monillia sp	-	-	-	7	12	15	-	-	-
Rhizopus sp	3	7	13	8	12	14	-	-	-
Trichoderma sp	-	-	-	12	15	17	4	6	12

- = Nil

sp. (15.93%), *Aspergillus sp.* (14.16%), *Rhizopus sp.* (11.50%), *Mucor sp.* (10.62%), *Geotrichum sp.* (9.74%), *Trichophyton sp.* (8.85%), *Penicillium sp.* (7.08%), *Trichoderma sp* (6.2%), *Microsporum sp.* (5.31%), *Fusarium sp.* (4.43%), *Chaetomium sp.* (1.77%), *Monilia sp.* (1.77%), *Aureobasidium sp.* (0.88%), *Madurella sp.* (0.88%) and *Nocardia sp.* (0.88%). Out of the 15 fungi that were isolated from the 60 corncob samples, the most abundant was *Streptomyces sp.* (15.93%), followed by *Aspergillus sp* (14.16%), while the least abundant were *Aureobasidium sp., Madurella sp.,* and *Nocardia sp.* (0.88% each). The variations in the relative abundance of each isolate are an indication that the nutrient composition of the rubbish dumps is more favorable to the growth of *Streptomyces* and *Aspergillus*, while growth of *Aureobasidium*, *Madurella*, and *Nocardia* are less favoured.

Cellulolytic and Amylolytic Activities of the Organisms Isolated

The radial diameters of the fungi cultured on Modified Cellulose Agar Medium are depicted in Table 3. Out of the 15 fungi isolated, 10 (66.67%) demonstrated cellulolytic activity. These fungi include *Aspergillus sp.*, *Mucor sp.*, *Chaetomium sp., Penicillium sp., Geotrichum sp., Monilia sp., Fusarium sp., Aureobasidium sp.,* and *Rhizopus sp.,* with *Trichoderma sp.* showing the highest activity (20mm) and *Monilia sp* with the least activity (5mm) after seven days of incubation at room temperature. The radial diameters of these fungi grown

Table 4. Amylolytic Activity Based on Starch-Iodine Reaction Test for Fungi Cultured on Modified Starch Agar Medium.

Fungi	Zone of clearance (mm)	Starch-Iodine reaction test
Streptomyces sp	48	+ve
Aspergillus fumigatus	46	+ve
Rhizopus sp	45	+ve
Mucor sp	44	+ve
Aspergillus flavus	42	+ve
Fusarium sp	40	+ve
Geotrichum sp	38	+ve
Penicillium sp	37	+ve
Aspergillus niger	35	+ve
Monillia sp	30	+ve
Trichophyton sp	-	-ve
Microsporum sp	-	-ve
Chaetomium sp	-	-ve
Trichoderma sp	-	-ve
Nocardia sp	-	-ve
Aureobasidium sp	-	-ve
Madurella sp	-	-ve

+ve - Positive for starch iodine reaction test
-ve - Negative for starch iodine reaction test

on Modified Paper, Cornstalk and Wood Sawdust Agar Media are shown in Table 4, depicting the growth of the fungi within a period of 5-10 days. The results of fungal growth on modified cellulosic waste products showed that cornstalk supported the highest growth followed by paper, while wood sawdust supported least growth, ($P \geqq 0.05$).

The amylolytic activity based on starch-iodine reaction test of fungi grown on Modified Starch Agar Medium is shown in Table 5. Out of the 15 fungi isolated, 8 (53.33%) showed amylolytic activity on Modified Starch Agar Medium. These organisms include *Streptomyces*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucor, Rhizopus, Penicillium, Fusarium sp.*, *Geotrichum sp*, and *Monilia sp*. *Streptomyces sp.* demonstrated highest amylase activity, as shown by the zone of clearance (48 mm), followed by *Aspergillus fumigatus* (46mm), while *Monilia sp.* showed the lowest amylase activity (30mm). However, 7(46.67%) of the fungi grown on the Modified Starch Agar and tested for their Amylase activity based on the Starch-iodine reaction test were negative and did not show any zone of clearance. These fungi include *Microsporum sp., Chaetomium sp., Trichoderma sp., Nocardia sp., Aureobasidium sp., Trichophyton sp.* and *Madurella sp*.

The results of this study implied that a high percentage of cellulolytic and amylolytic can be obtained locally from corncobs and most of these isolated hold varied industrial and bioremediation potentials. The growth of fungi on various cellulosic wastes showed that although, paper, cornstalk and wood sawdust supported fungal growth, cornstalk supported the highest growth followed by paper, while sawdust supported the least growth. This might be due to the fact that although, cornstalk is composed of a high percentage of cellulose and hemicellulose (43%) and lignin (29%) it also contains a small proportion of proteins (7%), ash (5%) and other constituents (16%) (Beltrán-García *et al*., 2001), which is higher than that in paper or wood sawdust. Therefore, these proteins, ash and other constituents allow for higher and more growth of the fungi, at a higher rate in the corncob than on paper and wood sawdust. This is in agreement with studies by Beltrán-García *et al*. (2001), where cornstalk extract supported faster growth of fungi than other substrates used by the authors. Paper on the other hand supported more growth than wood sawdust because paper is made of cellulosic materials which have undergone extensive treatment, leading to exposure of the lignocellulosics to attack (Alava and Niskanen, 2006). This makes it is easier to degrade than wood sawdust which is composed mainly of cellulose (50%), and hemicellulose and lignin (50%) that have not been broken down (Subramaniyan and Prema, 2002).

Streptomyces was found to be highest in number than the other amylase-producing fungi because they could be more numerous in the environment than the fungi. Also, *Aspergillus* was the second in abundance and is one of the leading fungi used for industrial production of amylases (Adams and Moss, 1999). This is in accordance with previous studies that demonstrated

Streptomyces and *Aspergillus* as leading fungi in the production of amylases for industrial use (Okafor, 2007). *Trichoderma* sp. had highest cellulolytic activity on Modified Cellulose Agar Medium and this is in agreement with previous studies which have demonstrated *Trichoderma resei* and *T. harzanium* to be the leading producers of the cellulase enzyme. The fungi that were negative for the starch-iodine reaction test is obviously as a result of the fact that the strains of the fungi that were isolated were non-amylase producing strains.

It can be concluded from this research that a considerable number of cellulolytic and amylolytic fungi may be obtained from corncobs found in Jos North, Nigeria, with *Trichoderma sp.* being the leading cellulolytic fungus, and *Streptomyces sp.* being the leading amylolytic actinomycetes in this study. The growth of cellulase-producing fungi was more favored on Modified Cornstalk Agar Medium than on Modified Paper or Wood sawdust Agar Media. This implies that corncob can be further developed into a selective medium for the screening of cellulolytic fungi and could indeed be processed for use as a substrate in the industrial production of these enzymes. Furthermore, the amylase and cellulase enzymes obtained could be useful for a wide range of industrial purposes as well as for bioremediation in the environment. Sourcing these fungi locally and utilizing them for the local production of cellulase and amylase enzymes which are in high demand will go a long way to boosting the local industries as well as the economy, as importation will be reduced. Since fungi that have high cellulase- and amylase-producing capacity can be obtained locally, these fungi should be identified to their species and strain levels and genetically modified to be able to produce these enzymes more efficiently.

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