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Full Length Research Paper

A survey of cellulolytic mesophilic fungi in the soil environment of Keffi Metropolis, Nasarawa State, Nigeria

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An investigation was carried out to determine the species of cellulolytic fungi present in the soil environment of Keffi metropolis. Keffi town is the Headquaters of Keffi Local Government Area of Nasarawa State, Nigeria. Soil samples of 200 g each were collected at random from 10 different locations at dump sites of Keffi town. Malt extract agar and potato dextrose agar were used for the isolation of mesophilic filamentous fungi at incubation temperature of 25°C. All the isolates were again inoculated on chemically defined cellulose agar medium to determine their cellulases producing abilities. The results revealed that the cellulolytic mesophilic fungi were present in the soil environment of Keffi metropolis and they included *Aspergillus niger, Aspergillus flavus, Tricoderma viride, Penicillium* sp. and *Fusarium solani.* The fungal isolates could be harnessed as inocula for use in solid waste management involved in the biodegradation of cellulose-containing materials. The results further demonstrate that the isolates could also be harnessed for the industrial production of cellulases.

Key words: Cellulases, mesophilic fungi, soil environment, Keffi, Nigeria.

INTRODUCTION

Cellulases are a group of hydrolytic enzymes capable of hydrolyzing cellulose to smaller sugar components of -glucose units. Celulases refer to a class of enzymes that catalyze the cellulosis (or hydrolysis) of cellulose. Cellulose is an organic compound with the molecular formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand linked glucose units (Crawford, 1981; Young, 1986; Updegraff, 1969). Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the Oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on earth. Although, a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolyzing crystalline cellulose *in vitro*.

Cellulases play an important role in natural biode-gradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and even protozoa. In the industries, these enzymes have found novel applications in areas such as the production of fermentable sugars and ethanol (Olsson and Haling-Hagerdalu, 1997; Levy et al., 2002; Van Wyk and Mohulatsi, 2003; Luo et al., 1997), detergents and other chemicals (Oksanen et al., 2000), pulp and paper processing involving de-inking of fiber surfaces and improvement of pulp drainage (Oksanen et al., 2000; Suurnakki et al., 2004), textile production (Cavaco-Paul and Cuibitz, 2003; Nierstrasz and Warmveskerken, 2003; Miehinen_Oinonen et al., 2004), animal feed production (Ishikuro, 1993), food processing (Penttila et al., 2001, Urlaub, 2002), cellophane processing, as well as biotransformation of cellulose- containing waste to fermentable sugar (Van Wyk and Mohulasti, 2003). Cellulases are also important in protoplast production and fermentation of biomass into bio-fuels (Davis, 1985; Mandels et al., 1974 and Bhat,

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2000). The demand for more thermostable, highly active and specific cellulases is on the increase. Fungi are well-known agents of decomposition of organic matter in general and cellulose substrates in particular (Lynd et al., 2002). Fungal cellulases are inducible enzymes that are usually excreted into the environment (Bhat and Bhat, 1997) and this depends on the type of cellulose (amorphous or crystalline) being acted upon (Ortega et al., 2001). The role of the fungi such as *Acremonium* sp., *Chaetomium* sp*., Trichoderma reesei, Trichoderma viride, Penicillium pinophilum, Phanerochaete chrysosporium (Sporotrichum pulvenlentum), Fusarium solani, Talaromyces emersonii, Trichoderma koningi, Fusarium oxysporium, Aspregillus niger and Rhizopus oryzae* in the cellulose degradation process in various environment has been well documented (Kuzmanova et al., 1991; Bhat and Bhat, 1997; Schulein, 1997; Murashima et al., 2002; Mach and Zielinger, 2003).

Klyosov (1990) described three major types of cellulases: endoglucanase (endo-cellulase), which breaks down internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains; cellobiohydrolase (exo-cellulase) which cleaves 2 - 4 units from the ends of the exposed chains produced by endocellulase and cellobiase (Beta-extracellulase) which either hydrolyses cellobiose into individual monosaccharides such as glucose or depolymerizes cellulose by radical reactions. Enari (1983) reported that these enzymes are extracellular and inducible in nature. The ability to produce cellulases is widespread among fungi, which has become the subject of extensive investigation.

Fungi of the genera *Trichoderma* and *Aspergillus* have been reported to be the best cellulase producers and crude enzymes produced by these microorganisms are made commercially available for agricultural use. Fungi of the genus *Trichoderma* produce relatively large quantities of endo- -glucanase and exo- -glucanase, but only low levels of -glucosidase, while those of the genus *Aspergillus* produce relatively large quantities of endo- -glucunase and -glucosidase with low levels of exo- -glucanase production. Enzyme producing companies such as Dyadic International, Inc. (http://www.dyadic-group.com/) have been using fungi to develop and manufacture cellulases in large industrial fermenters.

Mesophilic fungi are organisms that grow best in moderate temperatures, typically, between 25°C and 40°C. They are involved in decomposition of organic matter in natural environment. This group of fungi is widely distributed within diverse habitats which include soil, dead organic matter, plant and animal residues.

Mesophilic fungi degrade organic material aerobically. Virtually, all the fungi that have been reported for the production of cellulases are mesophilic fungi, and the best known cellulase producers include *Trichoderma,* sp., *Aspergillus* sp*., Acremonium* sp*., Penicillium* sp., *Rhizopus* sp*. F. solani* and *Chaetomium* sp., among other mesophiles (Kuzmanova et al., 1991; Tecrei and Koivala, 1995; Bhat and Bhat, 1997; Schulein, 1997). This investigation was aimed at screening for cellulase producing mesophilic fungi in the soil environment of Keffi metropolis, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study area

This investigation was carried out in the soil environment of Keffi metropolis, the headquarters of Keffi local area, Nasarawa State, Nigeria. Keffi is 68 km from Abuja, the Federal Capital of Nigeria; and it is 128 km from Lafia, the Capital town of Nasarawa State. Keffi is located on latitude 8°5'N and longitude 7°50'E and situated on altitude 850 m above sea level (Akwa et al., 2007). It is located North-West of Lafia.

Samples collections

Soil samples of 200 g were collected from 10 different dump sites in different locations in Keffi metropolis. The samples were collected with small sterile shovels into sterile plastic containers. The soil samples were conveyed to the laboratory within 30 min for analyses.

Physicochemical properties of the soil

Soil types

The soil types were determined by the method of sieve analysis (Akwa et al., 2007), in the Laboratory of the department of Geology and Mining, Nasarawa State University, Keffi. The sieve used had pores sizes which range from 0.20 to 2.00 mm (Pettijohn, 2009).

pH

The pH of soil samples were determined using digital pH meter (model 127) as recommended by association of analytical chemists (AOAC, 1990). The pH meter was inserted into the soil solution mixture and was allowed to stay for 2 min, after which the pH reading was obtained. The average of two consecutive readings was recorded for each site.

Temperature

The temperature of the soil was determined by the use of field thermometer. The thermometer was inserted into the soil to a depth of 5 cm and allowed to stay for 5 min, after which the temperature reading was obtained. The average of two consecutive readings was recorded for each location.

Isolation and identification of soil fungal isolates

Potato dextrose agar (PDA) and malt extract agar (MEA) were employed for the isolation of mesophilic fungi by both pour plate and spread plate methods using serial dilution technique. 30 mg each of penicillin and streptomycin were incorporated into all the media just before pouring into Petri dishes which inhibited the growth of bacteria and thus promoted the growth of fungi. All the plates were incubated at 30°C for 7 days.

Table 1. Physicochemical properties of soil samples.

Location	Soil types	pH	Temperature
AL	Loamy	6.0 ± 1.4	26.0 ± 0.8
AM	Sandy-loamy	5.3 ± 2.1	25.0 ± 0.2
AR	Sandy	8.6 ± 1.2	25.0 ± 0.2
BCG	Sandy	8.2 ± 0.8	24.0 ± 1.2
HC	Sandy-loamy	8.6 ± 1.2	26.0 ± 0.8
KH	Loamy	5.4 ± 2.0	25.0 ± 0.2
LC	Sandy	9.6 ± 2.2	26.0 ± 0.8
MC	Sandy	7.1±0.3	26.0 ± 0.8
TA	Loamy	7.9 ± 0.5	24.0 ± 1.2
TK	Sandy	7.3 ± 0.1	25.0 + 0.2

AL, Angwan Lambu; AM, Angwan Mada; AR, Angwan Rimi; BCG, BCG; HC, High court; KH, New Keffi Hotel; LC, Low Cost; MC, Main campus; TA, Tudun Amama; TK, Tsho Kasuwa.

Table 2. Total fungal counts in the soil environment of Keffi using two different media.

Location	Fungal count (TFC/ml)
AL	4.4×10^3
AM	6.3×10^3
AR	3.7×10^3
BCG	3.8×10^3
HC	4.9×10^3
KH	6.3×10^3
LC	4.0×10^3
MC	5.0×10^3
TA	4.3×10^3
TK	2.8×10^3

AL, Angwan Lambu; AM, Angwan Mada; AR, Angwan Rimi; BCG, BCG; HC, High court; KH, New Keffi Hotel; LC, Low Cost; MC, Main campus; TA, Tudun Amama; TK, Tsho Kasuwa.

Fungal isolates were identified and characterized according to the current keys of identification using both cultural and microscopic features (Raper and Funell, 1997; Domsch et al., 1980; Samson et al., 2000; Pitt, 1979; Carmicheal et al., 1980).

Screening of cellulolytic fungi

Chemically defined medium that had cellulose as the sole source of carbon was prepared containing 1.0 g potassium sulphate, 0.5 g aluminium chloride, 0.5 g DL-asparagine, 0.5 g yeast extract, 0.2 g magnessium sulphate, 0.1 g calcium chloride, 20 g agar-agar powder and 2% cellulose dissolved in 1 L of distilled water. This was used for the selection of cellulases-producing fungi.

Cellulolytic fungi were screened on the basis of their ability to hydrolyze cellulose by forming diameter zone of clearance around the fungal colony on the chemically defined cellulose agar (Mandel's and Reese, 1967; Bradner et al., 1999).

RESULTS AND DISCUSSION

Table 1 shows the results of the physicochemical properties of the soil of the 10 different sites, while Table 2 shows the total fungal counts of the sites. Table 3 shows the percentage occurrence frequencies of the fungal isolates from the 10 different sites studied, while Table 4 shows the results of cellulase-producing potential by fungal isolates.

The results of physico-chemical properties of the soil types revealed that the soil of Keffi environment is sandy in Angwan Rimi, BCG, Low Cost, Main Campus and Tsho Kasuwa; loamy in Angwan Lambu, New Keffi hotel and Tudun Amama and sandy-loam in Angwan Mada and High Court. The soil pH of the different locations of Keffi ranged from 5.3 - 9.6. The mean temperature of Keffi soil during the time (July, 2008) of this investigation (rainy season) was in the range of 24 - 26°C.

The results of total fungal count shows that Angwan Mada and New Keffi hotel had the highest count of 6.3×10^3 TFC/ml, followed by Main Campus, High Court, Angwan Lambu, Tudun Amama, Low Cost, BCG and Angwan Rimi, which had 5.0×10^3, 4.9×10^3, 4×10^3, 4.0×10^3, 3.8×10^3 and 3.7×10^3 TFC/ml, respectively, while Tsho Kasuwa had the lowest fungal count of 2.8×10^3 TFC/ml.

The percentage occurrence frequencies of fungal isolates from the different locations indicate that *A. niger* and *Penicillium* species had the highest percentage of 60%, followed by *A. flavus* and *Rhizopus stolonifer* which had 50%, respectively. *A. fumigatus* and *Absidia corymbifera* respectively had 40%, *F. solani* had 30%, *Mucor* sp had 20%, while *R. oryzae* had the lowest percentage occurrence frequencies of 10% respectively.

The results of screening of fungal isolates for cellulase production which was demonstrated by growth on chemically defined medium that contained cellulose as its only source of carbon shows that *Acremonium* sp., *A. niger, T. viride, R. oryzae, F. solani* and *Penicillium* sp. produced cellulases, while *Aspergillus flavus, A. fumigatus, R. stolonifer, A. corymbifera* and *Alternaria alternata* did not.

Conclusion

Acremonium sp, *A. niger, T. viride, R. oryzae, F. soloni* and *Penicillium* sp. produced cellulases. This investigation may lead to the development of strains of soil fungi that would be used locally for the biodegradation of cellulose materials. These organisms are so recommended as source of cellulases which may be harnessed for industrial production of the enzyme, as well as management of solid wastes containing cellulose. The results of this investigation are thus useful to industries that use cellulases (textile, laundry, detergents, pulp and paper industries). The results would also be

Table 3. Percentage occurrence frequencies of fungi isolates at different locations of the soil environment of Keffi.

Fungal isolates	Occurrence at the different locations										Occurrence
	AL	AM	AR	BCG	HC	KH	LC	MC	TA	TK	frequency (%)
Aspergillus niger	+	-	+	+	-	+	+	-	-	+	60
Aspergillus fumigatus	-	+	-	+	-	+	-	+	-	-	40
Aspergillus flavus	-	+	-	+	+	-	+	-	-	+	50
Absidia corymbifera	+	-	+	-	-	-	+	-	-	+	40
Alternaria alternata	-	-	-	-	-	+	-	-	-	-	10
Acremonium sp.	-	+	-	-	-	+	-	-	-	+	30
Penicillin sp	+	-	+	+	+	-	+	+	-	-	60
Trichoderma viride	+	-	-	-	+	-	-	+	-	-	30
Rhizopus stolonifer	+	+	-	+	-	-	-	+	-	+	50
Fusarium solani	-	+	-	-	+	-	-	-	+	-	30
Mucor sp.	-	-	-	-	+	-	-	-	-	+	20

AL, Angwan Lambu; AM, Angwan Mada; AR, Angwan Rimi; BCG, BCG; HC, High court; KH, New Keffi Hotel; LC, Low Cost; MC, Main campus; TA, Tudun Amama; TK, Tsho Kasuwa.

Table 4. Production of cellulase by fungal isolates.

Fungal isolates	Cellulase production
Aspergillus niger	+
Aspergillus fumigatus	-
Aspergillus flavus	+
Absidia corymbifera	-
Alternaria alternata	-
Acremonium sp.	+
Penicilliun sp.	+
Trichoderma viride	+
Rhizopus stolonifer	-
Fusarium solani	+
Mucor sp	-

useful to environmental agencies that are concerned with the management of solid wastes.

However, further studies need to be carried out to determine quantitatively the catalytic activity of the cellulases produced by each of the cellulases producing fungal isolates. Environmental agencies can take advantage of the result of this investigation and then invest in further studies which could lead to the isolation and characterization of specific high-yielding fungal strains found in the local soil environments. This will go a long way in reducing the cost of production in such industries as well as the cost of management of solid waste by environmental agencies.

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