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Mycelial growth and cellulase activities of some thermophilic fungi isolated from municipal solid wastes and palm-kernel stacks in Nigeria

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Abstract
A total of 16 species of fungi were isolated from 3 locations viz:- two refuse-dump sites and palm kernel stack in Ibadan, Nigeria. Out of the 16 isolates obtained at 45^{o}C incubation temperature, 9 were found to be thermotolerant while the other 7 were found to be thermophilic. The thermophilic and thermotolerant fungi were separated from the others by incubating at a low temperature of 12^{o}C at which the thermotolerant fungi grew, while the thermophilic did not grow at this temperature. All the fungi (thermophilic and thermotolerant) were screened for possible antimicrobial activities using different media sources. However, none of the screened organisms showed sign of antimicrobial activity by having no clear zone of inhibition against the test organisms. The test organisms used were *Bacillus subtilis* and *Staphylococcus aureus*. The conditions necessary for the production of extracellular enzymes of thermophilic fungi isolated were determined using a stationary liquid medium of Oat-meal-chaff. Cellulases were produced by all the thermophilic fungi. The cellulase activities of all the fungi used were determined at pH 5.5. The peak activities for the enzyme (cellulase) were shown to be at 45^{o}C. The possible use of extracellular enzymes from these fungi for various purposes is discussed.

1. Introduction

Thermophilic fungi are a small assemblage in mycota that have a minimum temperature of growth at or above 20 ^{o}C and a maximum temperature of growth extending up to 60 to 62 ^{o}C (Maheshwari *et al.*, 2000). As the only representatives of eucaryotic organisms that can grow at temperatures above 45 ^{o}C, the thermophilic fungi are valuable experimental systems for investigations of mechanisms that allow growth at moderately high temperature yet limit their growth beyond 60 to 62 ^{o}C.
Thermophilic fungi are potential sources of enzymes with scientific and commercial interests. Enzymes of thermophilic fungi include cellulase, amylase, xylanase, polygalacturonase, glucoamylase, protease, lipase, α –amylase, Cellobiose dehydrogenase, phytase, D - Glucosyl transferase. Examples of media for the growth of thermophilic fungi are Potato dextrose agar, Starch-yeast- extract agar, Malt extract agar, Saboraud dextrose agar.

Genes of thermophilic fungi encoding lipase, protease, xylanase and cellulase have

been obtained for elucidation of the mechanisms of their intrinsic thermostability and catalysis (Maheshwari *et al.*, 2000).

Thermophilic fungi are the chief components of the microflora that develops in heaped masses of plant materials, piles of agricultural and forestry products and other accumulations of organic matter where-in the warm, humid, and aerobic environment provides the basic conditions for their development (Allen and Emerson, 1949). They constitute a heterogeneous physiological group of various genera in the phycomycetes, ascomycetes, fungi imperfecti, and mycelia sterilia (Mouchacca, 1997]. Thermophilic fungi reproduce by producing male and female spores that come in contact with each other to produce a new organism.

Municipal Solid Waste (MSW), commonly called "trash" or "garbage", includes wastes such as durable goods, e.g., tires, furniture; non-durable goods, e.g., newspapers, plastic plates/cups; containers and packaging, e.g., milk cartons, plastic wrap; and other wastes, e.g. yard waste, food. This category of waste generally refers to common household waste, as well as office and retail wastes, but excludes industrial, hazardous, and construction wastes. (Center for Sustainable Systems, 2009).

Sha'Ato *et al*.(2007) describe composition of municipal solid waste in Nigeria as follows:

Composition of municipal solid waste in Nigeria.(Sha'Ato et al., 2007)

Waste	**Waste category (%)**							
Source	**Putrescibles**	**Plastics**	**Paper**	**Metals**	**Glass**	**Textiles**	**Fines**	**Others**
LD	57.5	6.10	4.30	2.50	2.30	2.90	21.0	3.40
MD	53.7	7.10	4.10	2.01	1.70	2.40	27.1	1.70
HD	36.4	8.04	2.59	1.75	0.86	3.67	41.0	5.73
COMM	27.9	10.20	10.90	3.40	6.90	1.20	36.4	3.10
INS	44.8	5.90	8.90	0.90	1.20	0.30	36.4	3.10

LD = low density; MD = medium density; HD = high density; COMM = commercial; INS = institutional; Putrescibles include food remnants, and decomposable matters.

The disposal of refuse in cities has always presented a serious problem and the odours that characterize the outskirts of many large cities confirm the fact that the problem has frequently been solved in a manner that is not only crude but also a health hazard. (Sharma, 1989).

Presently, the importance of environmental sustainability cannot be over-emphasized. The status of municipal solid waste disposal is in a way indicative of a good primary health care and how sustainable the quality of life and living in any municipality. This research work was therefore designed with the aims and objectives to:

(1) Isolate and identify thermophilic fungi from municipal solid wastes (refuse dumps) and palm kernel stacks (agricultural waste);

(2) Determine the growth rate of the isolates;

(3) Screen for the antimicrobial activities of the thermophilic fungi if any;

(4) Assay for the production of cellulase enzyme by some selected thermophilic fungi isolated from refuse dumps.

2. Materials and Methods

2.1. Location and Collection of Samples

Samples for this research work were collected from Apete and Bodija refuse dump sites at Ibadan, Nigeria. Samples were also collected from the teaching and research farm, University of Ibadan. Decomposing refuse samples were collected aseptically from the three locations chosen for this work. Samples were collected by using clean shovel to dig about 70cm depth of the dump sites and samples collected into sterile polyethylene bags.

Temperature was noted with a thermometer. The samples were collected at varying different locations on each dump site to obtain a very good representative sample for the work. Each sample collected contained about 1000g of refuse.

2.2. Preparation of Glasswares/Materials

Glasswares:- All glass wares used in the experiment were washed, dried and sterilized in the hot air oven at 160^{o}C for 2 hours.

Open- flame:- Other materials like wire loop, inoculating needles, neck and mouth of beakers and conical flasks, were sterilized by flaming using spirit lamp.

70% ethanol:- This was always used with the aid of absorbent cotton wool to wipe the working table before each experiment to ensure an aseptic work condition.

2.3. Preparation of Culture Media

All culture media used in the experiments were aseptically prepared according to the manufacturers specifications. They were sterilized by autoclaving at 121 ^{o}C for 15 minutes before dispensing into sterile plates or universal bottles to make slants.

2.4. Isolation and Total Fungal Count

One gram of the refuse sample from each of the three

locations was suspended in 9ml of sterile distilled water. It was vigorously shaken for 15 minutes to dislodge all the mycelia and spores and keep them in suspension in water. Pasteur pipette was used to pipette 1ml of the sample (suspension/supernatant) to 9ml of sterile distilled water, i.e. 10 – fold serial dilution; this was then shaken vigorously.

Selected dilutions were plated out on Potato Dextrose Agar (PDA) medium using the pour plate method and done in triplicates. Uninoculated plate of the medium served as the control .The plates were then incubated at 45^{o}C for 3 days after which the fungal colonies were observed and counted.

2.5. Maintenance of Fungal Isolates / Cultures

Pure cultures of fungal species isolated from the refuse dumps and agricultural waste were grown on slants of PDA contained in sterile McCartney bottles and were kept in the refrigerator at 4^{o}C as stock cultures.

2.6. Identification of Fungal Isolates

Fungal isolates were subjected to both macroscopic and microscopic observations. Cotton blue -in-lactophenol was used for fungal microscopic examination on slides which were viewed under the x10 and x40 objectives. Identification was made with reference to the compendium of soil fungi Volume 1 by K.H. Domsch, W. Gams and Traute – Heidi Anderson.

2.7. Distinguishing the Thermophilic from Thermotolerant Isolates

All the fungal isolates obtained at 45^{o}C were incubated at a low temperature of 12^{o}C for 5 days to distinguish the truly thermophilic ones from the thermotolerant organisms. The organisms that grew at this temperature were then taken to be the thermotolerant organisms, while those that did not show any sign of growth at this temperature after 5 days of incubation were the thermophilic organisms.

2.8. Source of Test Organisms

The test organisms used for the antimicrobial investigation, *Bacillus subtilis* and *Staphylococcus aureus*, were collected from Dr Adenike Ogunshe of the Department of Microbiology, University of Ibadan, Nigeria.

2.9. Screening for Antimicrobial Activity of Isolates

All the fungal isolates were screened for their antimicrobial activity. This was done by first culturing all the isolates for 5 days in Petri- dishes at their optimal temperature of 45 ^{o}C to obtain a good growth .

Perpendicular lines were drawn at the base of all the Petri-dishes needed for this experiment to ease measurement of zone of inhibition in millimeters (mm).

The test organisms were inoculated into nutrient broth in McCartney bottles and incubated at 37 ^{o}C for 24 hrs and 10 – fold serial dilution was performed on the stock culture in broth. Selected dilutions were subsequently transferred onto sterile Nutrient Agar (NA), Potato Dextrose Agar and Mueller Hinton Agar (MHA) medium using the pour plate method which was then allowed to solidify. The different culture media sources were used so as to obtain the best medium for the antimicrobial activity of the fungal isolates. Uninoculated plates for each of the media sources served as the control.

One centimeter cork borer was used to remove a disk from the fungal plates each and inoculated at the centre of the plates already seeded with the test organisms.

Incubation was initially done at 5^{o}C for 24 hrs to allow the fungus diffuse its metabolites on to the medium. Thereafter, the plates were incubated at 37^{o}C for 24 hrs.

The plates were then observed for any zone of inhibition around the fungal mycelium at the centre of the plate.

2.10. Determination of Cellulase Activity

An Oatmeal-chaff medium was used to study the production of the cellulase enzyme. Oatmeal – chaff was obtained by running distilled water over Quaker oats on a muslin cloth. This washed off most of the starch, leaving the chaff which was then dried in an oven at 85^{o}C for 48 hours. The oatmeal – chaff medium was prepared by suspending 20g of the dry chaff in a liter of distilled water together with 5g yeast extract (pH 6.8). This was dispensed into 150ml conical flasks in 30ml amounts per flask, care being taken to shake the medium well before dispensing into each flask. The flasks were then autoclaved at 121 ^{o}C for 15 minutes.

To obtain the inoculum, the fungus was grown for 3 days at 45^{o}C in Petri dishes on starch –yeast – extract agar (SYEA). Each flask of the chaff medium was inoculated with one disk (1-cm diameter) of agar and mycelium obtained by using a sterile cork borer.

Uninoculated flasks of the chaff medium served as the control.

After growth had been allowed to proceed for 5 days at 45^{o}C, the cultures were filtered and the cellulolytic activity of the filtrates was determined using the method of Reese and Mandels (1963).

The assay medium was 0.55% carboxymethyl cellulose (CMC) in 0.55M acetate buffer (pH 5.6) and 9ml of this were incubated with 1ml of the fungus filtrate for 1 hour at 45^{o}C. Filtrates of the uninoculated control were also obtained and similarly assayed.

To estimate the amount of reducing sugars released, 1ml of dinitrosalicylic acid (DNSA) reagent was added to 1ml of the filtrate – CMC reaction mixture and the transmittance was determined at 540nm using a spectrophotometer. Transmittance was set at 100% with the filtrate –CMC reaction mixture of the uninoculated control.

Dinitrosalicylic acid reagent was prepared by combining 1.0g DNSA with 20ml 2N NaOH and 20g potassium sodium tartrate in 100ml distilled water. The transmittance

of standard aqueous solutions of D- glucose of various concentrations (0-10mg per ml) was determined and used to construct a graph of % transmittance as related to mg of glucose per ml. The amount of reducing sugar produced by 1ml of fungus filtrate from the CMC assay medium was calculated from this graph.

Cellulolytic activity of the filtrates was then expressed in terms of the amount of total reducing sugars (RS) in mg per ml. Three replicate determinations were carried out in each case and the mean of the three values was taken.

2.11. Mycelial Growth Determination with Time at 45 °C

This was carried out by employing the method of Oso (1979). At 2-day intervals for a 10-day period, mycelial weight of the culture filtrates were recorded. The mycelium produced in each flask(3 for each fungus) were filtered in pre-weighed filter paper, dried in an oven at 85°C for 2-4 hours, cooled in a desiccator and weighed.

3. Results

3.1. Cellulase Activity of the Isolates

Five isolates were used for the determination of cellulase activity. These were: *A. corymbifera*, *T. helicus*, *Chaetomium elatum*, *Humicola* sp. and *R. pusillus*. The result of cellulase activities of the culture filtrates at 45°C shows that all the fungi produce extracellular cellulases (Tables 4a-e).

Table 1. *Location and Frequency of Fungi Isolated at 45 °C.*

Probable Names of Isolates.	Refuse Dump Sites.		
	Bodija	Apete	U.I. Farm
Aspergillus fumigatus	+	-	+
Aspergillus sp.	+	+	+
Aspergillus clavatus	+	-	-
Absidia corymbifera	+	-	-
Acremonium sp.	+	-	-
Gilmaniella humicola	+	+	-
Humicola sp.	+	-	-
Rhizomucor pusillus	+	+	-
Chaetomium elatum	+	-	-
Chaetomium sp.	+	-	-
Talaromyces helicus	+	-	-
Aspergillus candidus	-	+	-
Scopulariopsis brevicaulis	-	+	-
Scopulariopsis sp.	-	-	+
Chalara elegans	-	+	-
Trichosporiella cerebriformis	-	+	-

Key: + = Present at location. - = Absent at location.

Table 2. *Thermotolerant and Thermophilic Fungi.*

Probable Names of Isolates.	Refuse Dump Sites.		
Thermotolerants.	Bodija	Apete	U.I. Farm
Aspergillus fumigatus	+	-	+
Aspergillus clavatus	+	-	-
Aspergillus sp.	+	+	+
Acremonium sp.	+	-	-
Scopulariopsis brevicaulis	-	+	-
Scopulariopsis sp.	-	-	+
Chalara elegans	-	+	-
Trichosporiella cerebriformis	-	+	-
Aspergillus candidus	-	+	-
THERMOPHILICS.			
Absidia corymbifera	+	-	-
Gilmaniella humicola	+	+	-
Humicola sp.	+	-	-
Rhizomucor pusillus	+	+	-
Chaetomium elatum	+	-	-
Chaetomium sp.	+	-	-
Talaromyces helicus	+	-	-

Key: + = Present at location. - = Absent at location

Table 3. *Comparison of Mycelial Growth with Time at 45 °C.*

Days of Incubation	*A. corymbifera* (mg/30ml)	*T. helicus* (mg/30ml)	*Chaetomium elatum* (mg/30ml)	*Humicola* sp. (mg/30ml)	*R. pusillus* (mg/30ml)
2	10.0	30.0	60.0	70.0	60.0
4	30.0	40.0	70.0	250.0	100.0
6	40.0	40.0	80.0	870.0	830.0
8	70.0	60.0	90.0	600.0	500.0
10	20.0	30.0	30.0	590.0	500.0

Table 4a. *Cellulase Production by Absidia corymbifera; and pH of Medium when Grown on Oat-Meal-Chaff Medium at 45 °C.*

Days of incubation	Cellulase Units	^{pH} culture[a] filtrate
2	2.0	7.54
4	3.0	5.47
6	3.2	7.21
8	4.0	5.68
10	3.5	5.87

[a] Initial pH=6.8

Table 4b. *Cellulase Production by Talaromyces helicus; and pH of Medium when Grown on Oat-Meal-Chaff Medium at 45 °C.*

Days of incubation	Cellulase Units	^{pH} culture[a] filtrate
2	9.0	4.77
4	9.5	4.76
6	10.0	5.30
8	9.0	5.08
10	8.0	5.45

[a] Initial pH=6.8

Table 4c. *Cellulase Production by Chaetomium elatum; and pH of Medium when Grown on Oat-Meal-Chaff Medium at 45 °C.*

Days of incubation	Cellulase Units	^{pH} culture[a] filtrate
2	5.0	5.42
4	5.5	5.44
6	7.1	6.07
8	5.0	5.49
10	4.9	5.41

[a] initial pH=6.8

Table 4d. *Cellulase Production by Humicola sp.; and pH of Medium when Grown on Oat-Meal-Chaff Medium at 45°C.*

Days of incubation	Cellulase Units	^{pH} culture[a] filtrate
2	8.5	6.22
4	9.0	6.01
6	10.0	6.77
8	9.5	6.06
10	9.0	4.55

[a] initial pH=6.8

Table 4e. *Cellulase Production by Rhizomucor pusillus; and pH of Medium when Grown on Oat-Meal-Chaff Medium at 45 °C.*

Days of incubation	Cellulase Units	pH culture[a] filtrate
2	3.0	4.24
4	3.5	5.55
6	5.0	6.65
8	3.0	5.93
10	1.0	6.86

[a] initial pH=6.8

Table 5a. *Anova Table for the Cellulase Yield .*

S.V	d.f	S.S	M.S	F-cal	F-tab
Cellulase yield /day	4	9.56	2.39	4.938	3.01 *
Individual isolate's yield.	4	183.45	45.86	94.75	3.01 **
Error	16	7.75	0.484		
Total	24	200.76			

** - Highly significant at 0.05 level of significance.
* – significant.

Table 5b. *Anova Table for the Mycelial Growth .*

S.V	d.f	S.S	M.S	F-cal	F- tabulated (α-0.05)
General weight / day of incubation	4	344,584	86,146	2.931	3.01 n.s
Isolate's individual dry weight .	4	932,264	233,066	7.930	3.01*
Error	16	470,256	29,391		
Total	24	1,747,104			

*- Significant at 0.05 level of significance.
n.s- Not significant

Table 6a. *Duncan Multiple Range Test I.*

Treatment	Cellulase Yield	Dry Weight Mycelium. (mg/30ml)
A. corymbifera	3.14^{a}	34.0^{a}
T.helicus	9.10^{b}	40.0^{a}
C.elatum	5.50^{c}	66.0^{a}
Humicola sp.	9.20^{b}	476.0^{b}
R.pusillus	3.10^{a}	398.0^{b}

-Means followed by the same alphabet vertically are NOT significantly different from each other.

Table 6b. *Duncan Multiple Range Test II.*

Treatment	Cellulase yield
Day 2	5.50^{a}
Day 4	6.10^{ab}
Day 6	7.06^{b}
Day 8	6.10^{ab}
Day 10	5.28^{ab}

- Mean followed by the same alphabet vertically are not significantly different from each other.

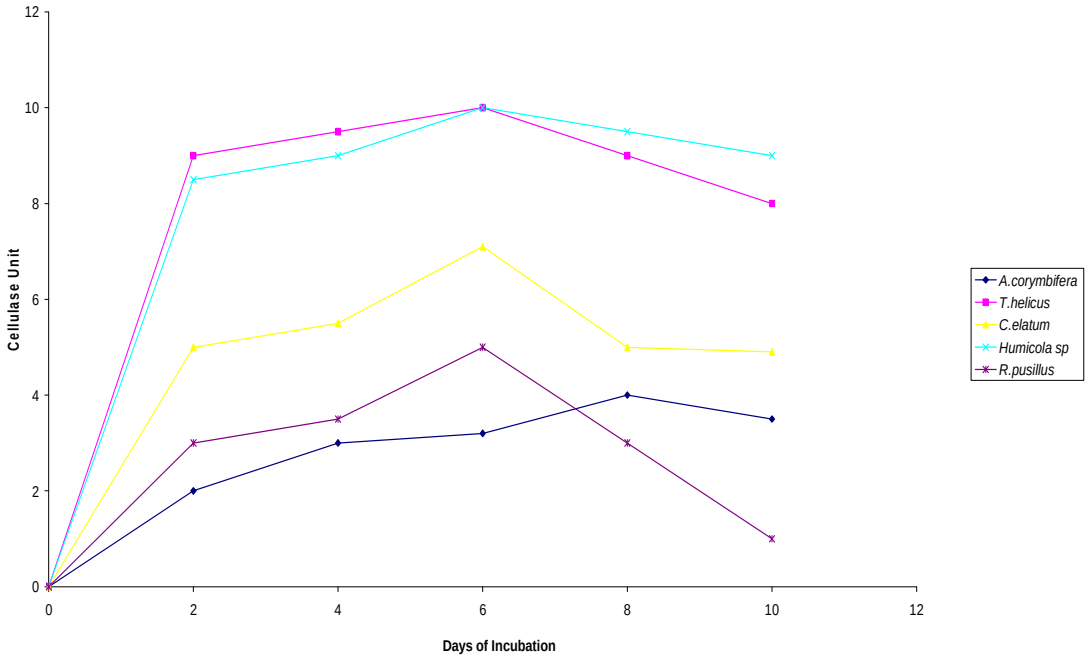

Fig. 1. *Cellulase Activities of Culture Filtrates at 45 °C.*

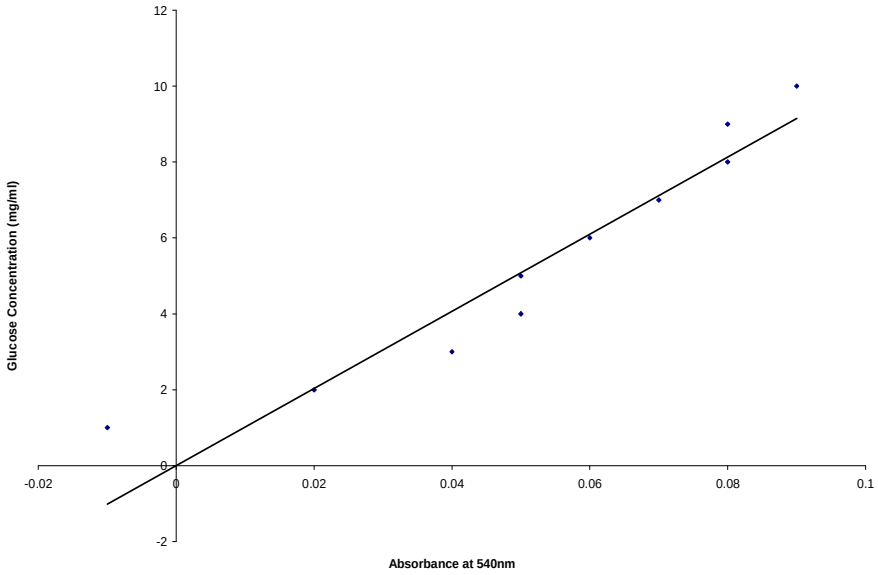

Fig. 2. *Standard Curve of Percent Absorbance as Related to mg of Glucose per ml.*

4. Discussion

The isolation of the fungi; all totaling 16 at 45^{o}C conforms to earlier reports by Kane and Mullins(1973), and Chang and Hudson (1967). In view of the fact that 3 locations Apete, Bodija and University of Ibadan Farm were used for the sources of the samples used in this research study; the frequency of occurrence of the fungi isolated at 45^{o}C from the various locations can be seen in Table 1. Eleven (11) fungi were isolated from Bodija which were: *Aspergillus fumigatus, Aspergillus sp, Aspergillus clavatus, Absidia corymbifera, Acremonium sp., Gilmaniella humicola, Humicola sp, Rhizomucor pusillus, Chaetomium elatum, Chaetomium sp, Talaromyces helicus*; Seven (7) fungi were isolated from Apete which include *Aspergillus sp, Gilmaniella humicola, Rhizomucor pusillus, Aspergillus candidus, Scopulariopsis brevicaulis, Chalara elegans, Trichosporiella cerebriformis* whereas three (3)fungi were isolated from the University of Ibadan Farm which include *Aspergillus fumigatus, Aspergillus sp, Scopulariopsis sp.*

Moreover, it can also be noticed in Table 1 that *Aspergillus sp, Gilmaniella humicola* and *Rhizomucor pusillus* were common to both Bodija and Apete refuse dump sites respectively.

Aspergillus fumigatus was isolated from both Bodija and University of Ibadan Farm; while *Aspergillus sp* was isolated from all the three locations (that is, Apete, Bodija and the University of Ibadan Farm).

The thermophilic fungi were subsequently separated from the thermotolerant fungi by incubating at 12^{o}C for 5 days and the result shown in Table 2, represent clearly that those fungi which grew at 12^{o}C as well as their location and frequency of occurrence were the following thermotolerant fungi; *Aspergillus fumigatus, Aspergillus sp, Aspergillus clavatus Aspergillus candidus, Acremonium sp, Scopulariopsis brevicaulis, Scopulariopsis sp., Chalara elegans, Trichosporiella cerebriformis* whereas those that did not grow were the following thermophilic fungi *Gilmaniella humicola, Humicola sp, Rhizomucor pusillus, Absidia corymbifera,Chaetomium elatum, Talaromyces helicus and Chaetomium sp*. Which compared favourably and was in line with reports of growth of thermotolerant fungi in a temperature range of 12.5 to 45^{0}C (Finstein and Morris, 1975).

All the fungi isolated from the dump sites were screened for their antimicrobial activities of which 3 different media used were Nutrient Agar (N.A), Sabouraud Dextrose Agar (S.D.A) and Mueller Hinton Agar (M.H.A) and *Bacillus subtilis* and *Staphylococcus aureus* served as the test/indicator organisms. After the screening, no zone of inhibition were seen around the fungal mycelium seeded at the center of all the Petri dishes used for this study which indicated that none of all the fungi isolated was an antibiotic producing fungi. This is with particular reference to the fact that attention have been drawn to the potential health hazards these fungi pose to man and his animals as well as understanding that most of the major groups of antibiotics that are used to ameliorate these health problems posed by these fungi have been discovered by exhaustive screening of thousands of cultures from natural sources (Frobisher and Fuerst, 1983). Table 3 shows the comparison of mycelial growth with time at 45^{o}C. The mycelial growth of the fungi with time (days of incubation) was determined to find out whether there was a correlation between mycelial growth and enzyme production.

For *Humicola sp* and *Rhizomucor pusillus*, there was a rise in mycelial weight with time reaching the peak on the 6th day, after which there was a decline. Whereas *Chaetomiun elatum, T. helicus and Absidia corymbifera* reached their peak on the 8th day after which their was a decline. The decline in mycelial weight may be as a result of decrease in the availability and supply of nutrients in the medium for use by the fungi. It may also be as a result of

autolysis. This result compare favourably with previous work by Oso(1979) and suggest that the fast metabolic rates of these fungi with its mycelial production is directly proportional to its extracellular enzyme production which can be related to the biodegradative roles of these fungi in nature and especially in refuse dumps. These properties can be of tremendous use in industrial fermentation processes. Tables 4a-e shows the cellulase productions of the thermophilic fungi and the pH of the medium when grown at 45^{o}C. The determination of the cellulase activities of the culture filtrates of the fungi shown in figure 1 indicates that all the fungi produced cellulases. *Humicola sp* and *Talaromyces helicus* produced the highest amounts of cellulases on the 6th day. *Chaetomium elatum* and *Rhizomucor pusillus* also produced their peak amounts on the 6th day during the 10-day incubation period. It is also worthy to note that *Absidia corymbifera* reached its peak production at the 8th day during the 10-day incubation period. A standard curve of percent (%) absorbance as related to milligram (mg) of glucose per ml as seen in figure 2 was used to calculate the amount of reducing sugar released by 1ml of fungus filtrate from the carboxymethyl cellulose (CMC) assay medium.

Moreover, the Analysis of Variance (ANOVA) table for the cellulase yields (Table 5a) shows that there is significant difference in the cellulase yield of all the fungi used for the study. From the table, the variance ratio (F_{tab}) at degree of freedom 4 and 16 at 0.05 level of significance = 3.01. F_{cal} for yield per day of incubation was 4.938. Therefore, test is significant. The value of the variance ratio calculated (F_{cal}) for isolate's yield(94.75) was greater than the value of the F_{tab}. Therefore, the test is significant for each isolate. That is, there is significant cellulase yield for each isolate throughout the 10-day incubation period. Table 5b shows the ANOVA table for the mycelial growth. From the table, the variance ratio (F_{tab}) at degree of freedom 4 and 16 at 0.05 level of significance = 3.01. F_{cal} for general weight of mycelia produced per day of incubation was 2.931. Therefore the test is not significant. The value of the variance ratio calculated (F_{cal}) for isolate's individual dry weight(7.930) was greater than the value of the F_{tab} (3.01). Therefore, the test is significant for each isolate. That is, there is significant mycelia yield for each isolate throughout the 10-day incubation period.

Tables 6a and 6b shows the result of the Duncan multiple range tests. The parameters tested were: cellulase yield and mycelial dry-weight. Mean yields followed vertically by the same alphabets on the tables are not significantly different from each other.

5. Conclusion & Recommendation

From the above observations, it can be concluded that lignocellulose's degradation in refuse dumps and composts involves the activity of a range of hydrolytic and oxidative enzymes.

Further studies will be needed by the enzymologists to explore the suitability of the enzymes of thermophilic fungi in environmental bioprocesses; and to also determine the possible use of cellulolytic thermophilic fungi from refuse dumps in the large scale enzymic production of glucose and maltose from starch.

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