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Isolation and Characterization of Cellulolytic and Proteolytic Microorganisms Associated with Municipal Solid Wastes in Jos Metropolis, Nigeria

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Abstract

The research was conducted to isolate, identify and screen for effective cellulolytic and proteolytic bacteria and fungi associated with segregates of biodegradable municipal solid wastes from three locations in Jos Metropolis, Nigeria. Thirty samples of the segregated wastes were collected and cultured in Nutrient agar fortified with meat extract for proteolytic bacteria, Carboxymethyl cellulose agar (CMCA) for cellulolytic organisms and Sabouraud dextrose agar (SDA) for fungi growths. Microbial loads were determined for each sample respectively. The growths were purified by repeated sub-culturing and identified using standard microbiological techniques. The various isolates of bacteria and fungi were tested for their proteolytic and cellulolytic potentials by measuring the zones of clearing of the individual organisms cultured on Cesien agar and CMCA respectively. The results of the study showed that cumulative mean counts for cellulolytic and proteolytic bacteria were 2.9 x 10^9 Cfu/g and 4.4 x 10^9 Cfu/g respectively. The percentage frequencies (%) of occurrence and zones of clearing (in millimeters) for cellulolysis and proteolysis (all in brackets) of the bacteria and fungi involved were: *Bacillus sp.* (6.67:39:21), Coagulase negative *Staphylococcus* (33.33:20:19), *Bacillus subtilis* (6.67:17:0), *Aeromonas schubertii* (13.33:16:0) and *Klebsiella aerogenes* (16.67:8:22), *Staphylococcus aureus* (6.67:0.00:20.00), *Rhizopus* sp. (13.33:43:36), *Mucor* sp. (30.00:29:52), *Fusarium* sp. (6.67:29:16), *Aspergillus niger* (13.33:28:29), *Aspergillus fumigatus* (3.33:26:32) and *Cylindrocarpon* sp. (3.33:20:0). While 5 (55.6%) of the isolated bacteria and 6 (100%) fungi had cellulolytic activity, 4 (44.4%) bacteria and 5 (86.3%) fungi demonstrated proteolytic activity when grown on their respective selective media. The results of this study imply that a high percentage of cellulolytic and proteolytic microorganisms which hold promise for use as industrial and bioremediation agents can be sourced locally from municipal solid wastes.

Keywords: Municipal Solid Wastes, Cellulolytic, Proteolytic Microorganisms.

INTRODUCTION

The United States Environmental Protection Agency (2005) defined municipal solid waste as trash or garbage consisting of everyday used and thrown away items, such as product packaging, grass clippings, furniture, bottles, food scraps, newspapers, and appliances from homes, schools, hospitals and businesses.

In Nigeria, municipal solid wastes abound as house and commercial refuse, construction and demolition debris, garbage and electronic wastes. These items are dumped and heaped indiscriminately on roadsides, any available open pits or landfills irrespective of the health implication on the masses. An approximated twenty-five million (25,000,000) tonnes of municipal solid wastes are said to be generated annually in Nigeria and waste generation rates range from 0.66kg/cap/day in urban areas to 0.44kg/cap/day in rural areas as opposed to 0.7-1.8kg/cap/day in developed countries (Abila *et al.,* 2013). 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 (Egbere *et al*., 2014).

With the increasing human population and urbanization in Nigeria, municipal solid waste has een expectedly being on

a geometric increase and due to lack of dumping sites and inefficient management systems in our climes, these waste products are indiscriminately piled up in large quantities in the environment (major streets and open spaces) and they cause a great deal of pollution and contamination. As a result of untreated and improperly managed municipal solid waste, it has served as a reservoir and carrier of many pathogenic microorganisms such as bacteria, fungi, viruses, protozoa and parasites which threatens life and enhances the spread of human diseases such as cholera, typhoid and dysentery (Egbere, *et al*, 2002). Municipal solid wastes could lead to concomitant increased mortality rates in such given population (Zaved *et al*., 2008).

Apart from these organisms being pathogenic, some play vital roles in the biodegradation of the organic components of municipal solid waste into simple monomers which in turn are used as biofertilizers, biofuels and other products of industrial microbiology such as enzymes (Santra *et al*., 2014). All these are achievable by specific species of bacteria, fungi and other microbes which actually grow within the solid waste. Presently, there is little or no commercial production of cellulases, proteases and other enzymes 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 (Egbere, *et al*., 2014)

Considering the tremendous need for waste management and utilization (conversion of wastes to wealth) and bioremediation of our environments, this study was carried out with intent to isolate and screen for both bacteria and fungi that hold potentials for intentional use for production of industrial enzymes and bioremediation agents.

MATERIALS AND METHODS

Study Location and Collection of samples

Ten samples each of municipal solid wastes were collected from three different locations namely, 'Rock Haven', 'Laranto' and University of Jos quarters respectively in Jos Metropolis, Nigeria, (making a total of 30 samples). Each sample was collected aseptically in labeled separate sterile polythene bags using the ingeniously constructed waste collection bin by Egbere, (2014). The samples were placed in ice -packed plastic containers, transported to the laboratory for microbial analysis within three hours.

Delivery and Sorting

The waste collected were immediately transported to the laboratory and segregated into biodegradable and non-biodegradable wastes. The biodegradable portions were used for further microbiological analyses.

Media Preparation

Five solid media were prepared according to manufacturers' instruction and used. They are Carboxymethyl cellulose agar, Casein agar, Sabaroud Dextrose Agar, Nutrient agar supplemented with meat extract, MacConkey agar and Blood agar.

Isolation and Enumeration of Microorganisms

The microorganisms were isolated using methods described by Santra (2014). Ten-fold serial dilution was carried out in all. This was to reduce the microbial load contained in the samples. For each of the sample, 1g was aseptically transferred into 9mls of sterile normal saline in contained in Universal bottles to form the stock. This was homogenized thoroughly and allowed to stand for 10 minutes. Six test tubes containing 9ml of sterile normal saline were set and labeled 10^{-1}-10^{-6} for each sample. Using a micropipette, 1ml of each stock sample was aseptically transferred into the test tube labeled 10^{-1}dilution. Another 1ml was taken from 10^{-1}dilution and transferred aseptically to the second test tube (10^{-2}) also containing 9ml of sterile normal saline and was mixed thoroughly. This procedure was repeated serially until a dilution of 10^{-6} was reached.

Culturing of Samples

Dilutions of 10^{-4}, 10^{-5} 10^{-6} were plated out on Nutrient agar fortified with meat extract and Carboxymethyl cellulose agar respectively. This was done by aseptically pipetting 0.02 ml of each dilution unto the agar plates, spread uniformly using

an improvised spreader and incubated at 37°C for 24 hours for proteolytic bacteria and 48 hours for cellulolytic bacteria respectively.
In the case of fungi, 0.5g of each of the samples was inoculated onto Sabouroud Dextrose agar which was incubated at room temperature for 3 days in the case of fast growing fungi and 5 days for slow growing fungi.

Purification of Bacteria

At the end of the incubation period, the plates were observed for bacterial growth and the numbers of colonies present on each plate were counted to obtain the microbial counts of each sample and recorded as Colony forming units. The colonies were purified by sub-culturing on nutrient agar until pure colonies were obtained.

Purification of Fungi

At the end of the incubation period, the plates were observed for fungal growth. The colonies were purified by sub-culturing on Sabouroud Dextrose agar plates aseptically until pure colonies were obtained.

Identification of Bacteria

Morphological and microscopic examinations were done as described by Cheesbrough, (2006). Biochemical tests such as catalase, coagulase, oxidase, Indole, Citrate, Lactose fermentation,Sucrose fermentation,Glucose fermentation and gas production and Triple Sugar Iron Agar (TSI) were carried out for identification of bacteria using procedures described by Ochei et al. (2000) and Cheesbrough (2006).

Identification of Fungal Isolates

Filamentous fungi isolated were identified on the basis of the following: growth rate (i.e the time required for the fungus to produce visible growth on culture medium), colonial morphology (i.e physical appearance such as colour. texture, colony topography and diffusible pigment) and microscopic morphology as described by Ochei *et al*. (2000).

Screening for the Cellulolytic Activity

Screening of the microbial isolates for cellulolytic activity was carried out using methods described by Choudhary and Jain (2012). The pure bacterial and fungal isolates were inoculated onto Carboxymethyl cellulose agar. Inoculated bacterial test plates were incubated at 37°C for 48 hours while the fungal plates were incubated at 37°C for 3-5 days. At the end of the incubation period, the diameter of the zones of clearing around colonies was measured using a transparent meter rule and recorded in millimeters.

Screening for the Proteolytic Activity

Screening of the microbial isolates for proteolytic activity was carried out using methods described by Choudhary and Jain, 2012. The pure bacterial and fungal isolates were inoculated onto Casein agar. Inoculated bacterial test plates were incubated at 37°C for 48 hours while the fungal plates were incubated at 25± 2°C for 3-5 days. At the end of the incubation period, the diameter of the zones of clearing around colonies was measured using a transparent meter rule and recorded in millimeters.

RESULTS

The results showed that the locations where Municipal Solid wastes were collected had cellulolytic and preoteolytic microorganisms. The cellulolytic bacterial counts ranged from 2.5×10^4 - 6.9×10^9 CFU/g with samples collected from Rock Haven (3.4×10^9 CFU/g) having the highest mean count while Laranto (2.3×10^9 CFU/g) had the lowest mean

count. The proteolytic bacterial counts ranged from 1.1 x 10^7-1.9 x 10^{10} CFU/g with samples collected from the University Senior Staff quarters (5.8 x 10^9 CFU/g) having the highest mean count while samples from Laranto (3.1 x 10^9 CFU/g) had the lowest mean count. (Table 1)

A total of 9 bacteria were isolated from all samples collected as shown on Table 2. The organisms isolated include *Staphylococcus aureus, Bacillus* species*,* Coagulase negative *Staphylococcus, Klebsiella aerogenes, Aeromonas schubertii, Aeromonas hydrophila, Streptococcus* species*, Pseudomonas maltophilia and Bacillus subtilis.* Coagulase negative *Staphylococcus* had the highest frequency of occurrence (33.3%), this was followed by *Bacillus species* (26.6%) while *Streptococcus* species and *Pseudomonas maltophilia* had the lowest frequency of occurrence (2.6%). Also, a total of 6 fungi were isolated from all the samples as shown on Table 3. The fungi isolated include *Rhizopus* Species*, Mucor* species*, Aspergillus niger, Aspergillus fumigatus, Fusarium* species and *Cylindrocarpon* species. *Mucor* species had the highest frequency of occurrence (30.0%), this was followed by *Rhizopus* Species and *Rhizopus* Species (13.3%) and *Aspergillus fumigatus* and *Cylindrocarpon* species had the lowest frequency of occurrence (3.3%).

Among the 9 bacteria isolated, 5 (55.6%) demonstrated cellulolytic activity while all the fungi demonstrated celluolytic activity. The bacteria, *Bacillus* species had the highest zone of clearing (39mm), followed by Coagulase negative *Staphylococcus* (20mm) while *Klebsiella aerogenes* had the lowest zone of clearing (8mm) (Table 4). The fungus, *Rhizopus* species had the highest zone of clearing (43mm), followed by *Mucor* species (29mm) and *Fusarium* species (29mm) while *Cylindrocarpon* species had the lowest zone of clearing (20mm) (Table 5).

Among the 9 bacteria isolated, 4 (44.4%) showed proteolytic hydrolysis and of the 6 fungi isolated, 5 (83.3%) showed proteolytic activity. The bacteria, *Klebsiella aerogenes* had the highest zones of clearing (22mm), followed by *Bacillus species* (21mm) while Coagulase negative *Staphylococcus* had the lowest zone of clearing (19mm) (Table 4). The fungi, *Mucor* species had the highest zone of clearing (52mm), followed by *Rhizopus* species (36mm) while *Fusarium* species had the lowest zone of clearing (16mm) (Table 5).

Table 1. Bacterial Loads in Samples of Municipal Solid Wastes Collected from Jos Metropolis, Nigeria

S/NO.	Staff Quarters		Laranto		Rock Haven	
	Counts of cellulolytic bacteria (CFU/g)	Counts of proteolytic bacteria (CFU/g)	Counts of cellulolytic bacteria (CFU/g)	Counts of proteolytic bacteria (CFU/g)	Counts of cellulolytic bacteria (CFU/g)	Counts of proteolytic bacteria (CFU/g)
1	4.0 x 10^9	5.6 x 10^7	3.0 x 10^9	4.3x 10^9	1.9 x 10^9	4.5x 10^9
2	2.8 x 10^8	1.1 x 10^7	4.5 x 10^9	6.3 x 10^6	6.9 x 10^9	3.2 x 10^9
3	1.1 x 10^7	3.4 x 10^7	4.3 x 10^7	6.9 x 10^7	3.9 x 10^9	6.4 x 10^9
4	6.6 x 10^9	1.4 x 10^9	4.2 x 10^9	1.3 x 10^8	6.4 x 10^8	1.7 x 10^9
5	2.1 x 10^8	3.4 x 10^7	3.8 x 10^9	8.8 x 10^9	3.8 x 10^8	8.9 x 10^7
6	5.9 x 10^9	1.6 x 10^{10}	2.4 x 10^9	5.1 x 10^9	8.1 x 10^9	1.4 x 10^8
7	5.2 x 10^9	1.5 x 10^{10}	3.7 x 10^8	5.5 x 10^9	3.8x 10^9	5.4 x 10^8
8	2.5 x 10^4	5.6 x 10^5	3.2 x 10^8	3.7 x 10^9	3.5 x 10^8	9.3 x 10^9
9	1.5 x 10^9	7.7 x 10^9	3.5 x 10^8	1.8 x 10^8	3.0 x 10^9	8.8 x 10^9
10	5.6 x 10^9	1.6 x 10^{10}	4.2 x 10^9	3.5 x 10^9	4.5 x 10^9	8.0 x 10^9
Mean	2.9 x 10^9	5.8 x 10^9	2.3 x 10^9	3.1 x 10^9	3.4 x 10^9	4.3 x 10^9
STD	2.6 x 10^9	7.3 x 10^9	7.1 x 10^9	3.0 x 10^9	2.7 x 10^9	3.6 x 10^9

Cumulative mean for cellulolytic bacteria (n=30)= 2.9 x 10^9
Cumulative mean for proteolytic bacteria (n=30)= 4.4 x 10^9

Table 2: Frequency of Occurrence of Bacteria Associated with Samples of Municipal Solid Wastes Collected from Jos Metropolis, Nigeria

	Staphylococcus aureus	***Bacillus* sp.**	**CoN. *staphylococcus***	***Klebsiella aerogenes***	***Aeromonas hydrophila***	***Aeromonas schubertii***	***Pseudomonas maltophilia***	***Bacillus subtilis***	***Streptococcus* sp.**
Staff Quarters	2(20.00)	5(50.00)	4(40.00)	2(20.00)	0(0.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Laranto	0(0.00)	3(30.00)	5(50.00)	3(30.00)	0(0.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Rock Haven	0(0.00)	0(0.0)	1(10.00)	0(0.00)	5(50.00)	4(40.00)	1(10.00)	2(20.00)	1(10.00)
Mean	6.67	26.67	33.33	16.67	16.67	13.33	3.33	6.67	3.33

CoN- Coagulase Negative
*Values in parenthesis are percentages

Table 3. Frequency of Occurrence of Fungi associated with Samples of Municipal Solid Wastes Collected from Jos Metropolis, Nigeria

	Aspergillus niger	***Aspergillus fumigates***	***Fusarium* sp.**	***Rhizopus* sp.**	***Cylindrocarpon* sp.**	***Mucor* sp.**
Staff Quarters	0(0.00)	1(10.00)	0(0.00)	0(0.00)	0(0.00)	3(30.00)
Laranto	3(30.00)	0(0.00)	2(20.00)	2(20.00)	0(0.00)	3(30.00)
Rock Haven	1(10.00)	0(0.00)	0(0.00)	2(20.00)	1(10.00)	3(30.00)
Mean	13.33	3.33	6.67	13.33	3.33	30.00

*Values in parenthesis are percentages

Table 4. Cellulolytic and Proteolytic Activity of Bacterial Isolates from Municipal Solid Wastes Collected from Jos Metropolis, Nigeria

Bacteria	**zone of cellulolysis (mm)**	**zone of proteolysis (mm)**
Bacillus sp.	39	21
CoN. *staphylococcus*	20	19
Bacillus subtilis	17	-
Aeromonas schubertii	16	-
Klebsiella aerogenes	8	22
Staphylococcus aureus	-	20
Aeromonas hydrophila	-	-
Streptococcus sp.	-	-
Pseudomonas maltophilia	-	-

Table 5. Cellulolytic and Proteolytic Activityof Fungal Isolates from Municipal Solid Wastes Collected from Jos Metropolis, Nigeria

Fungi	**zone of cellulolysis (mm)**	**zone of proteolysis (mm)**
Rhizopus sp.	43	36
Mucor sp.	29	52
Fusarium sp.	29	16
Aspergillus niger	28	29
Aspergillus fumigates	26	32
Cylindrocarpon sp.	20	-

DISCUSSION

The bacterial load in this study ranged between 2.50 x 10^4-1.60 x 10^{10} CFU/g. This was higher than that reported by Obire *et al*. (2002) in Port Harcourt, Nigeria. The difference in the count could be as a result of differences in locations as well as increase in population and changing trends since the past fourteen years in the two locations. Generally, the mean count of proteolytic organisms were higher than those of cellullolytic organisms. This could be indicative of the fact that substrates for proteolytic activity was higher in abundance or that proteolytic organisms were more prolific than than the cellulolytic ones. This suggests high protein content in the solid wastes samples collected. Almost all foods and biodegradables contain a certain degree of protein notwithstanding the class of food. This can be seen in a research conducted by Beltrán-García *et al*. (2001) who reported the composition of cornstalk: hemicelluloses (43%), lignin (29%), protein (7%), ash (5%) and other constituents (16%).

The relative abundance of both cellulolytic and proteolytic bacteria per location also varied. While Laranto had the lowest bacterial loads, the counts of bacterial loads were higher in the wastes collected from the University of Jos Staff Quarters. This may be attributed to the standard of living. A more comfortable residence may demand for a variety of organic foods which may results in the generation of complex and more nutritive food waste segregates for microorganisms compared to residence that are not well to do economically. Therefore, the type and composition of waste generated will not be the same.

The variation in the relative abundance (occurrence) of each isolate is an indication that the nutrient composition is more contributory and favourable for the growth of the bacteria, Coagulase Negative *Staphylococcus* and the fungi, *Mucor* species while the bacteria, *Streptococcus* species and *Pseudomonas maltophila* and the fungi, *Aspergillus fumigatus*

and *Cylindrocarpon* species are less favoured.

Some of the bacteria and fungi isolated showed no positive radial zone of hydrolysis. They are said to be non-cellulolytic and non-proteolytic. The non-cellulolytic bacteria observed in this study were *Bacillus subtilis*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Streptococcus species* and *Pseudomonas maltophilia* while the non-proteolytic bacteria were *Aeromonas schubertii*, *Aeromonas hydrophila*, *Streptococcus* species, *Pseudomonas maltophilia* and fingi, *Cylindrocarpon* species.

The results of this study showed that microbial enzymes of industrial and bioremediation significance can be obtained typically from biodegradable solid wastes. The nature of these enzymes is dependent on the available biomass or substrate concentration acting as carbon source. Also, the difference in the activities of the microorganisms may be a function of the different enzyme complexes possessed by each microorganism (Deswal *et al*., 2011 and Reczey *et al*., 1996). This explains the differences in the zones of hydrolysis implying that each organism is able to hydrolyze substrate based on different enzyme complexes.

Bacillus sp. and *Rhizopus* sp. were found to be the highest cellulase-producing bacteria and fungi respectively. This is similar to a research by Kim *et al. (*2012) who isolated *Bacillus* sp. as cellulolytic bacteria but Chandel *et al.* (2013) reported *Rhizopus* sp. as the least cellulose-producing fungi, a contrast to what was obtained in this study.

Bacillus subtilis was found to be cellulolytic in this research work which is in contrast to some other research works that reports the bacterium as a leading proteolytic bacterium (Watanabe and Hayano, 1993, Oyeleke *et* al., 2011, Ravishankar *et* al., 2012 and Vanitha *et al*., 2014). This may have resulted due to variation in strains of the bacterium.

Klebsiella sp. and *Mucor* sp. were the highest protease-producing bacteria and fungi respectively. *Klebsiella* sp. was isolated by Rodarte *et al.* 2011 and reported the organisms as non-proteolytic which is in contrast with this study while *Mucor* sp. are among the leading fungal organisms used for production of proteases by some researchers(Choudhary and Jain, 2012; Sethi and Gupta 2015). Most of the commercially used proteases are produced by *Bacillus, Pseudomonas, Clostridium, Proteus* species and also fungi. Among these, Bacilli is the major protease producer with application in pharmaceuticals, food, leather processing, medicine, 10 molecular biology, tannery, detergent, metal recovery and peptide (Vijayalakshmi, *et al.* 2013). During the recent years, efforts have been directed to explore the means to reduce the protease production cost through improving the yield, and the use of either cost free or low cost feed stocks (Kuberan, *et al*. 2010). Variations in the genetic profiles of organisms of same species with differing genetic codes for synthesis of different enzymes explains the variation of the reports of the different researchers above. Induced or natural mutation of the organisms due to environmental hazards or exposure could also be a factor in stratifying organisms of same species in producing or not producing a particular enzyme.

The activities of the proteolytic and cellulolytic microorganisms in this study can be properly harnessed through proper waste collection and management agencies and used in future treatment plants in Nigeria. The organisms could be used to accelerate the bioconversion of waste as a source of enzyme producing microorganisms and as source of organic fertilizers thereby boosting the agricultural sector.

Some of the microorganisms isolated in this study have been implicated for causing opportunistic infections. This infers that both pathogenic and non pathogenic microorganisms survive in wastes. These microorganisms can pose a threat to the health of people living around the environment where the wastes are been disposed.

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Conclusion

It can be concluded from this research that substantial number of cellulolytic and proteolytic bacteria and fungi could be obtained from residential and native localities in Jos, Nigeria, with *Bacillus* sp. and *Rhizopus* sp. being the leading cellulolytic bacterium and fungus and *Klebsiella* sp. and *Mucors* sp. being the leading proteolytic bacterium and fungus in this study. Furthermore, the cellulose and protease enzyme obtained could be useful for a wide range of industrial purposes as well as for bioremediation in the environment. Therefore, sourcing these organisms locally and utilizing them for the local production of cellulase and protease 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.

Recommendations

Since microorganisms have high and safer enzyme producing capacity and can be obtained locally, the organisms with novel traits should be identified to their species and strain levels.

These organisms should be mutated and genetically modified to enhance higher yield and efficacy of the enzymes as it is being done presently in some of our other studies.

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