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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF MICROORGANISMS INVOLVED IN DEGRADATION OF SAWDUST WASTE IN RIVERS STATE, NIGERIA.

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The isolation and characterization of microorganisms involved in the degradation of sawdust waste in Rivers State was carried out. The sawdust waste samples were collected from various locations in Rivers State. The media used were nutrient agar for total aerobic plate count, Sabouraud dextrose agar for fungal count, MacConkey agar for coliform count and cellulolytic medium for cellulolytic count. The pour plate technique was employed. Colonial morphology, Gram staining and tests biochemical were used for the identification and characterization of the microorganisms. The analysis of variance (ANOVA) was used to know the significant difference. The mean aerobic plate count ranged from $5.53 \pm 0.87 Log_{10}$cfu/g to $5.83 \pm 0.10 Log_{10}$cfu/g while the mean fungal count ranged from $5.29 \pm 0.96 Log_{10}$cfu/g to $5.92 \pm 0.10 Log_{10}$cfu/mL. The mean coliform count ranged from $5.53 \pm 0.94 Log_{10}$cfu/g to $5.76 \pm 0.19 Log_{10}$cfu/g while the cellulolytic count ranged from $4.51 \pm 0.3 Log_{10}$cfu/g to $4.85 \pm 0.16 Log_{10}$cfu/g. The bacterial genera isolated were *Enterobacter* species, *Pseudomonas* species, *Micrococcus* species, *Staphylococcus aureus*, *Klebsiella* species, *Esherichia coli* and *Cytophaga* species, *Cellulomonas* species and *Bacillus* species. The fungi isolated were *Mucor, Aspergillus* species*, Penicillum* species, *Geotrichum* species and *Keratinomyces* species. It was observed that sawdust waste can be degraded by indigenous microbial population.

Key words: Isolation, characterization, microorganisms, degradation, sawdust, waste.

INTRODUCTION

Sawdust is composed of fine particles of wood. This material is produced from cutting with a saw hence its name. It is the main by-product of wood processing in sawmills and is generally regarded as a waste, unless reprocessed into particle board, burnt in sawdust burner or used to make heat for other milling operations. Sawdust may collect in piles and add harmful leachates into water systems and block the water ways thereby creating an environmental hazard.

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Water-borne bacteria digest organic material in leachate, but use up much of the available oxygen. This high "biological oxygen demand" can suffocate fish and other organisms. There is an equally detrimental effect on beneficial bacteria, so it is not at all advisable to use sawdust within home aquariums, as was once done by hobbyists seeking to save some expense on activated charcoal (Liu *et al*., 1998). Sawdust if burnt, produce very thick smoke with high environmental consequences. Wastes and their disposal is a subject of environmental concern worldwide especially when they are non biodegradable to useful goods and

services (Banjo and Kubuoye, 2000). Wood is made up of cellulose and lignin. Lignin makes up of about a third of the mass of typical wood and also adds great strength to the wood. It is a complex set of aromatic molecules. Cellulose is a polymer of sugars (Anderson *et al*., 2007). Cellulose fibrils have high tensile strength which is used in the textile industry, paper and miscellaneous materials like vulcanized fibre, plastic filters, filtering media and surgical cotton. Other uses include adhesives, explosives, thickening agents, coated paper, cellophane, artificial leather, films and foils (Hitchner and Leatherwood, 1982).

Biodegradation is the natural process of breaking down organic pollutants by microorganisms to harmless compound or recycling wastes to nutrients, which can be used by other organisms. Degradation is carried out by huge assortment of bacteria, fungi, insects, worms and other organisms that eat materials and recycle them into new forms (Singleton and Sambury, 1998). The end products of effective biodegradation are non-toxic such carbon dioxide and water and can be accommodated without harm to the environment and living organisms. The microorganisms also multiply in numbers in the process (Okpokwasili, 1994). The economic uses of sawdust include usage in ice houses to keep ice frozen during the summer, until the advent of refrigerator. It is used as platforms in poultry houses, cow pens and horse stalls and it is mixed with dirt and chicken manure for compositing. Sawdust is also used for energy production in the United States of America (Clasen and Gaddy, 2002, Rose, 2002). The aim of this work is to isolate and characterization of microorganisms involved in the degradation of sawdust waste in Rvers State, Nigeria.

MATERIALS AND METHODS

Collection of Samples

The sawdust waste samples were collected from sawmills located at Ikwerre, Eleme, Port Harcourt and Obio/Akpor Local Government Areas of Rivers State, Nigeria. A total of fifty samples were collected using sterile black cellophane bags. They were transported to the laboratory in an ice parked cooler and immediately analyzed on reaching the laboratory.

Chemical Reagents

The chemical reagents employed in the study were of analytical grade and were products of BDH chemicals, Pooles England and Sigma Chemical Company St. Louis Missouri, USA. The microbiological media used were products of Oxoid and DIFCO Laboratories, England. They included nutrient agar used for the estimation of total heterotrophic aerobic bacteria, purification of isolates and for stock culture; Sabouraud dextrose agar used for the isolation of fungi and MacConkey agar for the isolation of coliforms. The celluloytic medium was compounded and used for the isolation of cellulolytic microorganisms.

Enumeration of Total Heterotrophic Bacteria and Fungi

Samples of the sawdust wastes were serially diluted in ten folds. Total viable heterotrophic aerobic counts were determined using pour plate technique. Then the molten nutrient agar, Sabouraud dextrose agar and MacConkey agar at 45^0C were poured into the Petri dishes containing 1mL of the appropriate dilution for the isolation of the total heterotrophic bacteria and fungi and coliforms respectively. They were swirled to mix and colony counts were taken after incubating the plates at room temperature for 48h and preserved by sub culturing into nutrient agar slants which were used for biochemical tests.

Enumeration of Cellulolytic Bacteria

The cellulolytic medium according to Cruickshank *et al.*(1975) was used for the enumeration of the cellulolytic organisms It comprised $CaC0_3$,2g; $MgS0_4.7H_20$, 1g; K_2HP0_4,1g; $(NH4)_2S0_4$,1g; cellulose powder, 5g and agar, 15g in IL of distilled water. The celluloytic organisms were then enumerated after plating in duplicate using pour plate technique, 1mL of the appropriate dilution of the samples in Petri dishes. The molten medium was poured accordingly in the respective

Petri dishes for the isolation of these organisms. They were swirled to mix and allowed to solidify. Enumeration of these organisms was performed after incubation at room temperature for 2 days. Colonies of cellulolytic bacteria growing on agar plates were counted, isolated, purified by streaking on the fresh cellulolytic medium and kept on the medium slants as stock cultures for characterization and identification.

Characterization and Identification of Isolates

Bacteria isolates were characterized and identified after studying the Gram reaction as well as cell micro morphology. Other tests performed were spore formation, motility, oxidase and catalase production; citrate utilization, oxidative / fermentation (O/F) utilization of glucose; indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges Proskaur reaction and urease production. The tests were performed according to the methods of (Cheesbrough, 2005; Adeoye, 2007; Agwung-Fobellah and Kemajou, 2007; Ochei and Kolhatkar, 2007). Microbial identification was performed using the keys provided in the *Bergeys Manual of Determinative Bacteriology* (1994).Fungal isolates were examined microscopically and macroscopically using the needle mouth technique. Their identification was performed according to the scheme of Cheesbrough (2005). .

RESULTS

The results of the mean counts of microorganisms isolated from the sawdust waste are shown in Table 1. The total aerobic plate count ranged from 5.76 $\pm$ 0.87Log_{10}cfu/g to 5.83 $\pm$ Log_{10}cfu/g while the coliform count ranged from 5.53 $\pm$ 0.94Log_{10}cfu/g to 5.76 $\pm$ 0.19Log_{10}cfu/g. The fungal count ranged from 5.29 $\pm$ 0.96 Log_{10}cfu/g to 5.92 $\pm$ Log_{10}cfu/g. The cellulolytic count ranged from 4.51 $\pm$ 0.28 Log_{10}cfu/g to 4.85 $\pm$ 0.61 Log_{10}cfu/g. The ANOVA, $P > 0.05$ showed that there was no significant difference in the mean count among the organisms in the locations. Table 2 shows the microorganisms isolated and their percentage occurrence. The bacterial genera isolated showed that *Pseudomonas* species had the highest occurrence of 26.9% while *Klebsiella* species had the least occurrence of 8.6%.The fungal genera isolated showed that *Mucor* species had the highest occurrence of 46.8% while the *Cephalosporium* species had the least occurrence of 2.1%.

Table 1. Mean Counts of Microorganisms isolated from the Sawdust Waste Log_{10}cfu/g

Location	TAPC	CC	FC	CLC
ELGA	5.63	5.53	5.29	4.85
	$\pm$ 0.91	$\pm$ 0.94	$\pm$ 0.96	$\pm$ 0.061
KELGA	5.53	5.64	5.63	4.75
	$\pm$ 0.87	$\pm$ 0.81	$\pm$ 0.90	$\pm$ 0.28
PHALGA	5.83	5.76	5..92	4.61
	$\pm$ 0.26	$\pm$ 0.19	$\pm$ 0.10	$\pm$ 0.43
OBALGA	5.81	5.69	5.88	4.51
	$\pm$ 0.28	$\pm$ 0.24	$\pm$ 0.28	$\pm$ 0.3

Legend: ELGA = Eleme Local Government Area, KELGA = Ikwerre Local Government Area, PHALGA = Port Harcourt Local Government Area, OBALGA = Obio/Akpor Local Government Area, TAPC = total aerobic plate count, CC = Coliform count, FC = Fungal count, CLC = Cellulolytic Count

Table 2. Microorganisms Isolated from the sawdust waste and their percentage occurrence

Bacteria	No. of isolates	% occurrence
Enterobacter species	20	13.8
Escherichia coli	18	12.4
Pseudomonas species	25	17.2
Micrococcus species	12	8.3
Staphylococcus aureus	10	6.9
Klebsiella species	8	5.5
Cytophaga species	10	6.9
Cellulomonas species	22	15.2
Bacillus species	20	13.8
Fungi		
Mucor species	22	46.8
Aspergillus species	14	29.8
Geotrichum species	6	12.8
Penicillum species	2	4.3
Keratinomyces species	2	4.3
Cephalosporium species	7	2.1

DISCUSSION

The mean bacterial and fungal counts were high for all the samples from the sawdust waste sites. The high count recorded may be attributed to the level of contamination of sawdust waste in the area as a result of human and other activities. The high count could facilitate the degradation of the waste from that environment. Environmental stresses brought about by the contamination could be adduced for the reduction in microbial diversity but increasing

the population of few surviving species (Eze and Ikeri, 2010). The presence of *Enterobacter* species, *Escherichia coli* and *Klebsiella* indicated the possibility of faecal contamination of the sawdust waste. This could have been enhanced by unhygienic practices and well as poor sanitary conditions (Deeble and Lee, 2007).

The presence of *Staphylococcus* aureus must have also been due to poor sanitary condition of the site of collection of the sawdust waste. *Pseudomonas* species, *Micrococcus* species and the fungi isolated are well known wastes degraders (Eze and Okpokwasili, 2010, Eze and Ikeri, 2010). The presence of these organisms should be of concern to avoid the outbreak of gastroenteritis and aspergillosis. Hitchner and Leaderwood (1982) reported the ability of cellulase enzyme in the degradation of cellulose. Cellulose is mainly produced by the enzyme cellulase. This enzyme is produced by several microorganisms mainly bacteria and fungi. It has been reported that fungal genera for example *Trichoderma* and *Aspergillus* are taught to be cellulase producers and crude enzyme produced by these organisms are commercially available for agricultural use. The bacterial cellulase is constitutively produced whereas fungal cellulase is produced in the presence cellulose (Jones and Lee, 2008). The efficacy of fungi in cellulose degradation has also been reported. They are capable of utilizing sawdust waste as their source of carbon and energy for growth (Deeble and Lee, 1981). This is line with the findings of Kelsey and Shafizadeh (1980) and Lennox *et al*. (2010), when they reported the reduction in carbon content of sawdust when subjected to microbial degradation. Goodliving and Yoshitoshi (2002) reported that bacteria and fungi are involved in the degradation of wood sawdust. It has been reported that when natural environments are contaminated with pollutants the indigenous microbial communities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading the contaminating waste. Degradation of macromolecules in waste to smaller molecules is enhanced by soil microorganisms which produce a tremendous range of potentially useful enzymes that help in breaking down or decomposition of these macromolecules (Bartha and Atlas, 1977; Adesemoye *et al*., 2006; Eze and Ikeri, 2010). The bacteria and fungi isolated in this study were in line with the works of Hawker and Linton, 2000; Jones and Lee, 2008).

Some features of wood sawdust are known to aid their degradation. These are crystalinity, lignifications and the capillary structure of cellulose to cellulolytic enzyme (Bonnarme and Jeffries, 2001; Goodliving and Yoshitoshi, 2002). When sawdust is dumped in sawmill sites, unless reprocessed to particle boards, burnt or used to make heat for other milling operations, we must greatly on the activities of microorganisms to break it down to humus. Therefore, man can depend on microorganisms in making the world a better place to live as a result of their degradative potential of recycling wastes thereby maintaining environmental quality.

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