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CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH THE DEGRADATION OF SAWDUST AND WOODCHIPS

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ABSTRACT

Microorganisms play a vital role in the degradation of organic matters such as Sawdust and woodchips. In this study, the Serial dilution method and pour plate techniques were used according to microbiological standards. The media used were nutrient agar, sabouraud dextrose agar, and cellulolytic medium to identify microorganisms and inoculated them into the Sawdust and woodchips and kept for 30 days at 37°C and 25°C. The total viable bacterial count for Sawdust and woodchips ranged between 9.0×10^3 - $6.0\times10^3$and 1.96×10^3 - 1.48×10^3, respectively. A total of 12 organisms were identified according to the biochemical reactions, six bacteria, and six fungi. Bergy's manual of determinative bacteriology confirmed the organism as Escherichia coli, Pseudomonas aeruginosa, Klebsiella sp, Staphylococcus aureus, Bacillus cereus, and Cellulomona sp. The fungal species identified include Rhizopus sp, Mucor sp, Saccharomyces cerevisiae, Candida sp, Aspergillus niger, and Aspergillus flavus. The bacteria with the highest potential to degrade Sawdust and Woodchips are Cellulomonas sp. (20.9%), Klebsiella sp (17.8%), Escherichia coli (8.3%), Bacillus cereus (7.15%), and Staphylococcus sp (6.2%). Furthermore, the fungi capable of degrading the Sawdust and woodchips are Mucor sp (19.90%), Aspergillus flavus (17.8%), Aspergillus niger (17.3%), Rhizopus (16.9%), and Saccharomyces cerevisiae (15.55%). From these results, it can be concluded that some microorganisms could be used for the biodegradation of lignocellulose materials.

Keywords: Aspergillus sp, Biodegradation, Cellulomonas, Sawdust, Woodchips

INTRODUCTION

Biodegradation can be defined as process by which organic substances are broken down into smaller compounds by microorganisms or enzymes Nezha et al. (2013). However, sawdust is composed of fine particles of wood made up of three components namely: cellulose, hemicelluloses, and lignin Green (2006). Woodchips can be defined as small to medium-sized pieces of wood formed by cutting or chipping larger woods such as trees, branches, and wood waste Janssen et al. (2011). Sawdust and woodchip are used in poultry houses, cow pens, horse stalls, biofuels (bioethanol), wood pulp, paper production, and also mixed with chicken manure Eze et al. (2011).

Wastes and how it is disposed of is a subject of environmental issues worldwide especially when they are non-biodegradable to useful goods and services Banjo and Kubuoye, (2000). The natural way in which Sawdust and woodchips degrade is very slow and the degree of degradation of sawdust can be caused by environmental factors such as temperature, pH, oxygen supply, and moisture content Zheng et al. (2013a). Some microorganisms such as *Streptomycetaceae*, *Pseudomanas* sp, *Erwinia*, *Actinomycetaceae, Trichoderma,* and *Aspergillus* sp are capable of utilizing sawdust and woodchips as sole sources of carbon source; suggest that these organisms are responsible for its degradation Adeline and Ka, (2014); Jonnys, (2019). Sawdust and woodchips can generally be regarded as waste and therefore, causes a lot of health problems and environmental pollution Baran and Teul, (2007). The present study investigated Sawdust and woodchips biodegradation potential of indigenously characterized microorganisms.

MATERIALS AND METHODS

Sample Collection and Sample Preparation

Undecomposed sawdust and woodchips samples were collected from Shehu Wunbi Timbershed Market, Sokoto State, Nigeria in sterile polythene bags. The woodchips were reduced to a small size by grinding with mortar and pestle. One gram (1g) of Sawdust and woodchip was serially diluted in ten folds and 0.1ml of the prepared dilution was aseptically transferred onto the surface of nutrient agar (NA) and Sabouraud dextrose agar (SDA) plated using pour plate method. They were incubated at 37°C for 24hrs and 25°C ± 2 for five days, respectively. Discrete colonies were sub-cultured and stock cultures were prepared from the pure cultures and stored at 4°C until needed Lennox *et al.,* (2010).

Isolation and Characterization of Isolates

The method described by Oranusi *et al*., (2004) was used for the identification of bacterial isolates. The biochemical tests conducted include: catalase, methyl-red, citrate utilization, coagulase, indole, Voges-Prokauer, motility**,** triple sugar iron tests Cheesbrough (2003). The fungal isolates were characterized based on colonial morphological features and microscopic examination using lactophenol cotton blue-stained slide cultures. The result was compared with fungal atlas Oyeleke and Manga (2008).

Enumeration of Cellulolytic Microorganism

The method described by Christian *et al.* (2017) was used for the enumeration of cellulolytic organisms. The medium comprised of $CaCo_3$,2g; $MgSo_4.7H_20$, 1g; K_2HPo_4,1g; $(NH_4)_2So_4$,1g; cellulose powder, 5g, and agar, 15g in 1L of distilled water. The Cellulolytic microorganisms were enumerated in duplicate using the pour plate method. The molten medium was poured respectively in the Petri dishes for the isolation of these organisms. They were mixed aseptically and allowed to solidify. Enumeration of these organisms was performed after incubation at ambient temperature for two days. The Pure culture was made from the colonies of the cellulolytic microorganisms growing on agar plates by streaking on the fresh cellulolytic medium and kept on the medium slants as stock cultures for screening tests of the microbial isolates Christian *et al.,* (2017).

Screening test for degradation of Sawdust and woodchip using the microbial isolates

Two grams (2g) of Sawdust and woodchips were added to different test tubes and 18 ml of distilled water was also added to each of the test tubes Lennox et al., (2010). The content of the tubes was autoclaved at 121°C for 15 min. The tubes for the bacterial isolates were labeled and controlled, while those of fungal isolates were numbered and controlled. Each isolate was inoculated into each tube except the controls. The tubes that contained the bacterial isolates were incubated at 37°C for 30 days while those of fungal isolates were incubated at 25°C for 30 days. At the end of the 30 days, the liquid contents in the tubes were carefully poured out. The water content in each test tube was centrifuged and the absorbance of each test tube was obtained using a spectrophotometer as the final absorbance, the initial absorbance was also checked, the rate of degradation of cellulose was obtained using Beer lambert's law Lennox et al., (2010). The cellulose content of each tube was finally determined.

Rate of Degradation: Final ÷ Initial ×100 = Cellulose %

RESULTS AND DISCUSSIONS

Total Viable Bacterial Count

The result of this study revealed the total viable count of the bacterial isolates as shown in Table 1. A_2 has the highest bacterial count of 9.0×10^3Cfu/g in Sawdust and A_3 has the lowest bacterial count of 6.0×10^3 Cfu/g while woodchips have the highest bacterial count in B_1 1.96×10^3 Cfu/g and B_3 has the lowest bacterial count in woodchips 1.48×10^3 Cfu/g. It is not surprising the high counts of microorganisms indicate that Sawdust may contain a nutrient that is favorable for them to grow. The finding agrees with Eze *et al*., (2011) who reported enumerated microorganisms involved in degradation from Sawdust in River state.

Morphology Characterization of Bacteria from Sawdust and Woodchips

The result of biochemical characteristics and identification of the bacterial isolates from sawdust and woodchips samples are shown in Table 2 using the conventional biochemical techniques as described in Bergey's Manual of Determinative Bacteriology (Holt, 1994). The isolates were identified as *E. coli, Pseudomonas* sp, and *Klebsiella* in Sawdust sample, *Staphylococcus* sp, *Bacillus cereus,* and *Cellulomonas* sp in woodchips samples. Bacteria play an important role in the degradation of plant residues (lignin) and other organic matter. This finding is in line with the work of Lennox *et al.* (2010) who reported how Sawdust is degraded by microbes.

Table 1: Total Viable Count of the Bacterial Isolates from Sawdust and Woodchips

S/N	Sample	Colony Count Cfu/g	Mean & SD
1.	A1	8.0 X10^3	
2.	A2	9.0 X10^3	7.67 X 10^3 ±1.53
3.	A3	6.0 X10^3	
4.	B1	1.96 X10^3	
5.	B2	1.52 X10^3	1.65 X 10^3 ± 0.27
6.	B3	1.48 X10^3	

KEYS: A_1, A_2 and A_3 = Sawdust B_1, B_2, and B_3 =Woodchips SD = Standard deviation

Table 2: Biochemical Characterization of the Isolates from Sawdust and Woodchips Samples.

Isolate Code	GR	Shape	Cat	Coa	Ind	Mot	Cit	Ure	Mr	Glu	Suc	Lac	H_2s	Gas	SUSPECTED ORGANISMS
A_1	-	R	+	+	-	-	+	+	-	+	+	+	-	+	*E. coli*
A_2	-	R	+	-	-	+	-	+	-	+	+	+	+	-	*Pseudomonas* sp
A_3	-	R	+	-	-	-	+	+	-	+	+	-	-	-	*Klebsiella* sp
B_1	+	C	+	+	+	-	-	+	+	-	NA	NA	NA	-	*Staphylococcus* sp
B_2	+	R	+	-	-	+	-	+	+	-	NA	NA	NA	+	*Bacillus cereus*
B_3	+	R	+	-	-	+	+	+	-	+	NA	NA	NA	-	*Cellulomonas* sp

KEYS: GR: Gram reaction; R: Rod; C: Cocci; Cat: Catalase; Mot: Motility; Cit: Citrate; Coa: Coagulase; Mr: Methyl red; Vp: Voges-Prokeur; Glu: Glucose; Suc: Sucrose; Lac: Lactose; H_2S: Hydrogen sulfide; NA: Not Applicable; +: Positive; -: Negative;
Sample A_1, A_2 and A_3 = Sawdust
Sample B1, B_2, and B_3 =Woodchips

Percentage Occurrence of the isolated Bacteria

The percentage occurrence of the isolated bacteria is presented in Figure 1. The result shows that *Escherichia coli* and *Klebsiella* sp has the highest percentage occurrence of 20% in Sawdust followed by *Pseudomonas* sp which has the lowest percentage occurrence (10%) in Sawdust. In the same vein, *Bacillus cereus* has the highest percentage occurrence of 23.3% in woodchips followed by *Staphylococcus aureus* with a percentage occurrence of 16.7%, and *Cellulomonas* sp which has the lowest occurrence (10%) in woodchips. The presence and abundance of *Bacillus* sp may not be surprising because Bacillus is one of the most predominant bacteria residents in soils, some plants, and in breaking down of Sawdust. This result is in agreement with the work of Idu *et al.* (2019) who worked on isolation and identification of bacteria from waste generated from a sawmill.

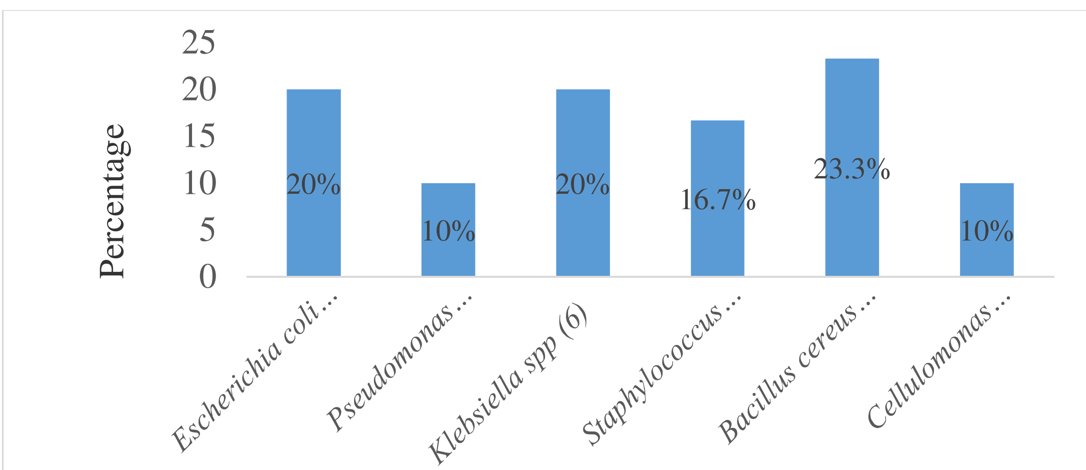

Figure 1: Shows the Percentage Occurrence of the isolated Bacteria

Characterization of Fungi Isolates

The identification and Characterization of isolated fungi which are shown in Table 3 from the result *Rhizopus*, *Mucor* sp, and *Saccharomyces cerevisiae* were the main fungi isolated from the sawdust sample. *Candida* sp, *Aspergillus niger,* and *Aspergillus flavus* were isolated from the woodchips sample. This shows that some of the isolates are capable of utilizing Sawdust as their source of carbon and energy for growth and these fungal isolates have different abilities in how they degrade the cellulose component of Sawdust. This conforms to Godliving and Yoshitoshi (2002) who reported that fungi are involved in the degradation of wood sawdust.

Table 4: Identification of fungi

S/N	MICROSCOPY	MACROSCOPY	ORGANISMS
1.	Sporangia contain spores, have rhizoid spotted with black color	Cotton like white growth	*Rhizopus*
2.	Sporangia contain spores, do not have rhizoid	Cotton like white growth spotted with black color	*Mucor* sp
3.	Unicellular cocci or ovoid shape, larger than bacterial cells	Flat, smooth, moist, glistering, or dull and cream to tannish cream in color	*Saccharomyces cerevisiae*
4.	Unicellular cocci or ovoid shape larger than bacterial cells.	Flat, smooth, large colonies	*Candida* sp
5.	Non-branched conidiophores with bulb end carries conidia like sun rays	Pin like black growth	*Aspergillus niger*
6.	Non-branched conidiophores with bud end carries conidia	Pin like green growth	*Aspergillus flavus*

Degradation of Cellulose in Sawdust and Woodchips by Bacteria

The rate of degradation of cellulose in Sawdust and woodchip by bacteria is presented in Table 4 revealed *Klebsiella* has the highest percentage cellulose degradation of 17.8% in Sawdust followed by *E.coli* with a percentage cellulose degradation of 8.3% and *Pseudomonas* sp which has the lowest percentage degradation of Sawdust 2.69%, *Cellulomonas* sp has the highest percentage of 20.9% in woodchips, *Bacillus cereus* with percentage cellulose degradation of 7.15% and *Staphylococcus aureus* has the lowest percentage cellulose degradation of 6.2%. The ability of these organisms to degrade Sawdust and woodchip can be harnessed to produce useful products, *Cellulomonas* sp had the highest capacity to generate cellulase enzyme and subsequently break down cellulose. This result is in line with the work of Lennox *et al*., (2010) who reported the degradation of Sawdust by microorganisms and also detect an increase in the percentage degradation of cellulose by bacteria.

Table 4: The Rate of Degradation of Cellulose in Sawdust and Woodchips by Bacteria

Microbial Isolate	Organism	Final cellulose	Initial cellulose	Cellulose (%)
A1	*E. coli*	0.011	0.133	8.3
A2	*Pseudomonas* spp	0.026	0.966	2.69
A3	*Klebsiella* spp	0.034	0.191	17.8
B1	*Staphylococcus aureus*	0.064	1.025	6.2
B2	*Bacillus cereus*	0.061	0.853	7.15
B3	*Cellulomonas* sp	0.038	0.181	20.9

KEYS: A_1, A_2 and A_3 = Sawdust B_1, B_2, and B_3 =Woodchips

Degradation of Cellulose in Sawdust and Woodchips by Fungi

The rate of degradation of cellulose in Sawdust by fungi is shown in Table 5 revealed *Mucor* sp has the highest percentage (19.90%) in cellulose degradation of Sawdust followed by *Rhizopus* sp with the percentage degradation of 16.9% and the least being *Saccharomyces cerevisiae* 15.55%, *Aspergillus flavus* has the highest percentage in cellulose degradation of woodchip 17.8% followed by *Aspergillus niger* with percentage degradation of 17.3% and *Candida* sp has the lowest percentage in cellulose degradation of woodchip (16.16%). Some of these fungi are cellulase producers which make it easy to degrade Sawdust and woodchips, fungi have a strong capacity for cellulose degradation thereby making way to produce useful and good products. This is contrary to the work of Idu *et al.* (2019) and Lennox *et al.* (2010) who detect the percentage degradation of cellulose in fungi.

Table 5: The Rate of Degradation of Cellulose in Sawdust and Woodchips by Fungi

Microbial Isolate	Organism	Final cellulose	Initial cellulose	Cellulose (%)
A_1	*Rhizopus*	0.124	0.731	16.9
A_2	*Mucor* sp	0.060	0.301	19.90
A_3	*S. cerevisiae*	0.070	0.450	15.55
B_1	*Candida* sp	0.141	0.872	16.16
B_2	*Aspergillus niger*	0.085	0.491	17.3
B_3	*Aspergillus flavus*	0.094	0.527	17.8

KEYS: A_1, A_2, and A_3 = Sawdust B_1, B_2, and B_3 =Woodchips

CONCLUSION

It can be concluded that in some cellulose rich materials such as Sawdust and woodchip, indigenous utilizing microbes exhibited various biodegradation ability towards Sawdust and woodchip. It can further be concluded that the present study has validated the promising potentials of microbes such as *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Klebsiella, Mucor* sp, *Rhizopus* sp, *Saccharomyces cerevisiae, Aspergillus flavus, Aspergillus niger* and *Candida* sp as cellulose utilizing microbes as their sole source of carbon and energy. Equally, the study revealed that there is a high load of microorganisms in Sawdust and woodchips which could be identified using molecular method of identification for further biodegradation study owing to improving environmental sanitation and sustainability.

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