Isolation, Characterization and Identification of Bacteria emanating from Sawdust generated in Ahiake Saw mill, Umuahia,Abia State, Nigeria

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ABSTRACT

The isolation, identification and characterization of bacteria from sawdust was carried out. Sawdust waste samples were collected from different spots in the Ahiake saw mill, Umuahia. The media used were nutrient agar for total aerobic plate count, McConkey agar for coliform count and cellulolytic media for cellulolytic count. The pour plate method was employed. Colonial morphology, gram staining and biochemical tests were used for the identification of the bacteria. The total aerobic plate for the sawdust waste ranged from 2.33×10^7 to 8.2×10^6, coliform count ranged from $7.6 \times 10^6$to 1.7×10^6Cfu/g and cellulolytic count ranged from 4.7×10^6 to 2.6×10^6Cfu/g. The bacteria isolated from the sawdust waste sample were *Pseudomonas* spp., *Bacillus* spp., *Escherichia coli*, *Staphylococcus* spp., *klebsiella* spp., *Micrococcus* spp., and *Cellulomonas* spp. The study concludes that microorganisms occur in a large number and variety in sawdust wastes.

Key words: Bacteria, pour plate technique, zoonoses, sawdust, Gram staining, Umuahia,cellulose

I. Introduction

Saw dust or wood dust could be referred to as the by-product of cutting, grinding, drilling, sanding or otherwise pulverizing wood with saw or other tool. It is composed of fine particles of wood. Saw dust is made up of three major components; Cellulose, Hemicellulose and Lignin (Alexander, 1997; Erikson *et al*., 1990). Lignin is the most recalcitrant and protects the cellulose and hemicellulose from enzymatic attack by some microorganisms (Bornnarne and Jeffries, 1998). Cellulose constitute one-third to one-half of the approximately 150 billion tones of organic matters synthesized annually (Shewale and Sardana, 1978; Bayer and Moray, 1994). Hemicellulose is an ill-defined group of carbohydrates and is of the major plants constituents, second in quantity to cellulose. Cellulose is totally insoluble in water and has about 2000-10,000 glucose subunits with molecular weight determination value ranges from 200,000 to about 2.4 million (Gilkes *et al.,*1988; Wu *et al.,* 1998). Cellulose fibrils have high tensile strength which is used in the textile industry, paper and miscellaneous materials like vulcanized fiber, plastic filters, filtering media and surgical cotton. Other uses include adhesives, explosives, thickening agents, coated agents, cellophane, artificial leather, films and foils (Hitchner and Leatherwood, 1982). Saw dust can be sourced from the saw mills (Eze *et al*., 2011).

Saw dust is commonly used for the production of particle board; course saw dust may be used for pulp. Saw dust have

a variety of other practical uses, including serving as much as an alternative to clay cat litter, or as a fuel. Prior to the invention of refrigeration, it was often used in ice house to keep ice frozen during the summer. It has been used in artistic displays, and as scatter. It is also sometimes used to soak up liquid spills, allowing the spill to be easily collected or swept aside. As such, it was formally common on bar room floors.

Cellulose, fiber starch which is known to be indigestible to humans and filler in some low calorie food, can be and is produced from saw dust, derived cellulose has also be used as a filler in bread.

The inhaling and accumulations of sawdust poses a great treat of health and safety hazards. Wood dust tends to become a potential health problem when for example the wood particles from processes such as sanding become airborne and are inhaled is known as a human carcinogen, produce severe adverse reactions.

Saw dust when left unprocessed into varying products such as particles board,fuel for combustion or utilized in the production of heat piles,tend to release harmful leachates into local water system consequently leading the proliferation of pathogenic bacteria species hence, posing great environmental hazard and leading to the spread of diseases capable of leading to the loss of human capital. This has placed small sawyer, and environmental agencies in a dead lock.

The need in making vital relevant statistics in tackling health menace and challenges posed by the release of unprocessed sawdust into water ways and other physical environments triggered this research work.

II. MATERIALS AND METHODS

Umuahia is the capital city of Abia State located in the southeastern region of Nigeria. It is known to lie on the geographical coordinates of 5°32′N 7°29′E. It is located along the rail road that lies between Port Harcourt to its south and Enugu city to its north. The city has a population of 359,230 according to the 2006 Nigerian census conducted by the National Population Commission. Umuahia is predominantly occupied by the ibo speaking race. The town is known to be an agrarian town little wonder the siting of Nigeria's National Root Crops Research Institute, at Umudike.

Sample Collection

The saw dust waste used for this study was collected from the dumping yard located in timber market at Ahiaeke Ndume, Ibeku, Umuahia North local government area, Abia state, Southeast, Nigeria. The samples were collected into sterile black cellophane bags at 10 different spots randomly and transported aseptically to the laboratory for

microbiological analysis. The saw dust were blended into smaller particles/bits for further analysis.

Chemical Reagents

Chemical reagents utilized in the study were of analytical grade and were products of BDH

chemicals, Pooles England and Sigma Chemical Company St. Loius Missouri, USA. The

microbiological media utilized included nutrient agar used for the estimation of total

heterotrophic aerobic bacteria, purification of isolates and for stock culture;thiosulphate

bilesalt agar (TCBS)for the isolation of Vibrio cholerae and McConkey agar for the

isolation of coliforms all products of Oxoid and Difco Laboratories England.The cellulolytic medium was compounded and utilized for the isolation of cellulolytic bacteria.

Enumeration of Total Heterotrophic Bacteria from Saw Dust

One gram of the saw dust was mixed with 9 ml of sterile distilled water. This was stirred very well using glass rod. Samples of the saw dust waste were serially diluted in 10 folds. A sterile pipette was used to transfer 1ml from it to the second tube and shaken to mix. 1ml was serially transferred from the first test tube to the second test tube and from the third to the fourth test tube up to the tenth test tube for each sample. The tubes were properly labelled to indicate the sample and dilution. They were covered with sterile plastic caps and used for total plate counts as in (Cheesbrough, 2002). With appropriate dilution, 1ml was cultured into sterile plates and agar poured on it employing the pour plate method. Plates were incubated at 37^{o}C for 24hours and counted after the expiration of the incubation period. The formulae below was used to calculate the bacteria load in each case.

TVC (Cfu /g) = 1/V × N × 1/D

V = Volume of inoculum

N = Numbers of colonies counted

D = Dilution factor

Enumeration of Cellulolytic Bacteria from Saw Dust

The cellulolytic medium according to (Cruickshank *et al.*, 1975) was used for the enumeration of the cellulolytic bacteria. It comprised $CaCO_3$, 2g; $MgSO_4.7H_2O$, 1g; K_2HPO_4, 1g; $(NH_4)\ 2SO_4$, 1g; cellulose powder, 5g and agar, 15g in 1L 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 this organisms. They were swirled to mix and allow to solidify. Enumeration of these organisms were performed after incubation at 30^{o}C for 48 hours. 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 identification.

Characterization and Identification of Bacterial Isolates

The bacterial isolates were characterized and identified after the Gram reaction and cell micro

morphology were studied.Other tests carried out were motility,spore formation,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 (Collins *et al*.,1989;Cheesbrough,2005;Adeoye,2007;Agwung-Fobellah and

Kemajou,2007;Ochei and Kolhatkar,2008).Bacterial identification was performed using the

keys provided in the Bergeys Manual of Determinative Bacteriology.

A total of ten (10) different samples were collected from different locations in the saw mill located in Ahiake,Umuahia,Nigeria.

Table 1 shows the bacteria isolated and their percentage occurrence. The bacteria isolates from sawdust are; *Pseudomonas* spp 21.8%, *Bacillus* spp 18.4%, *Escherichia coli* 12.6%, *Staphylococcus* spp 10.3%,*Klebsiella* spp 16.1%, *Micrococcus* spp 6.9% and *Cellulomonas* spp 13.8%. The bacterial genera isolated from sawdust waste showed that *Pseudomonas* spp had the highest occurrence of 21.8%, seconded by *Bacillus* spp of 18.4% while *Micrococcus* spp shows the lowest percentage of 6.9%.

Table 4.1 shows the total aerobic plate count range in the various sawdust waste sample location with each range from the highest bacterial load to the lowest, 2.33 x 10^{7} to 8.2 x 10^{6}Cfu /g while the coliform count range from 7.6 x 10^{6} to 1.7 x 10^{6}Cfu /g and the cellulolytic count ranged from 4.7 x 10^{6} to 2.6 x 10^{6} .

III. Results

Table 4.1: Bacteria isolated from sawdust and their percentage occurrence

Bacteria	No. of isolates	% occurrence
Escherichia coli	11	12.6
Staphylococcus spp	9	10.3
Klebsiella spp	14	16.1
Cellulomonas spp	12	13.8
Bacillus spp	16	18.4
Micrococcus spp	6	6.9
Pseudomonas spp	19	21.8

Table 4.2: Microbial (bacterial) count of saw dust waste samples

SAMPLE LOCATION	TAPC	CC	CLC
DS A	8.2×10^{6}	7.6×10^{6}	4.7×1^{6}
DS B	6.1×10^{6}	3.5×10^{6}	4.1×1^{6}
DS C	5.3×10^{6}	2.01×1^{6}	3.7×1^{6}
DS D	5.1×10^{6}	2.1×10^{6}	4.0×1^{6}
DS E	2.33×1^{7}	5.7×10^{6}	4.4×1^{6}
DS F	1.02×1^{7}	4.5×10^{6}	2.6×1^{6}
DS G	1.04×1^{7}	4.8×10^{6}	3.1×1^{6}
DS H	4.6×10^{6}	1.7×10^{6}	3.3×1^{6}
DS I	1.0×10^{7}	3.8×10^{6}	3.0×1^{6}
DS J	1.01×1^{7}	4.2×10^{6}	3.9×1^{6}

KEY:

DS = Dumpsite

TAPC = Total aerobic plate count

CC = Coliform count

CLC = Cellulolytic count

DISCUSSION

This study shows the isolation, identification and characterization of bacteria emanating from sawdust generated in Ahiake saw mill,Umuahia,Abia State.The bacteria isolated from the sawdust waste were *Escherichia coli*, *Staphylococcus* spp, *Klebsiella* spp, *Cellulomonas* spp, *Bacillus* spp,*Micrococcus* spp and *Pseudomonas* spp. This result is in line with the works of Eze *et al*., (2010);Williams and Dimbu, (2015) and Lennox *et al*.,(2010).

The presence of some bacterial species which include *Escherichia coli*, *Klebsiella* spp depicts the possibility of faecal contamination of the sawdust waste which could have been enhanced by unhygienic practices as well as poor sanitary conditions of the sample collection sites (Eze *et al*, 2010; Williams and Dimbu,2015).The presence of *Staphylococcus aureus* in the sawdust waste must have also been due to the poor sanitary condition of the site of collection of the samples (Eze *et al*;2010).

Bacterial species like *Pseudomonas* spp and *Micrococcus* spp are popular waste degraders (Eze and Okpokwasili, 2010; Eze and Ikeri,2010).The presence of these organisms should be of concern to avoid the outbreak of gastroenteritis (Eze *et al*, 2011). Cellulose, which is one of the main constituent of sawdust have been reported by (Hitchner and Leaderwood,1982), to be mainly degraded by cellulose enzyme. Cellulose is mainly produced by the enzyme,cellulase.This enzyme is produced by several microorganisms, bacteria inclusive. The bacteria cellulose is constitutively produced in the presence of cellulose (Jones and Lee, 2008).Good living and Yoshitoshi(2002) reported that the presence of bacteria in wood sawdust waste facilitates the degradation of the wood sawdust waste.It has been reported that when natural environment are contaminated with pollutant, the indigenous microbial communities are likely to contain microbial communities are likely to contain microbial populations of different taxonomic characteristics which are capable of degrading contaminating waste (Eze *et al*;2010). The high bacterial load facilitates the degradation of the sawdust waste in the environment.

CONCLUSION

From this study, bacteria species like *Escherichia coli* and *Klebsiella* spp which are seen as decomposers were isolated from the sawdust waste. Bacteria species which are known to have the ability to degrade cellulose, were also isolated from the sawdust samples. The high percentage occurrence of *Pseudomonas* spp and *Bacillus* spp, in the sawdust waste shows that these bacteria play leading roles in the degradation of the sawdust waste. *Micrococcus* spp, though present in low percentage in the sawdust waste is also believed to be a major organic waste degrader, sawdust waste inclusive. When saw dust waste are left untreated or

unprocessed they tend to harbor a high number of pathogenic bacteria as lucidly shown by the study.

Recommendation

This study recommends that sawdust waste should not be left unattended to as they are fertile breeding ground for the proliferation of pathogenic bacteria species, hence they should be reprocessed or disposed properly . Also, proper public education of the saw mill workers on the right way to handle sawdust waste generated in the saw mill is very expedient if the surrounding environment is to remain healthy and fit for the survival and existence of life.

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