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BIODEGRADATION OF DEADWOOD AND SAWDUST BY WHITE-ROT FUNGI ISOLATED FROM LAGOS AND OGUN STATES, SOUTHWEST NIGERIA

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ABSTRACT

Two varieties of Deadwood, Soil and Sawdust samples recovered from Ojo L.G.A., Lagos and Onigbongbo, Ado-Odo/Ota L.G.A., Ogun state via aseptic procedures were subjected to microbiological analysis over a period of 3 weeks. Heterotrophic fungal and bacterial population found on soil, deadwood and sawdust were isolated, thereafter biochemical characterization of the microbial isolates were conducted following standard and conventional methods. The RB 38 dye decolourization assay was used as the scientific evidence for identification of microbial isolates with cellulolytic enzymes. The fungal cellulolytic isolates were: Actinomycetes sp., Aspergillus fumigatus, Fusarium solani, and Aspergillus flavus. A gram positive bacterial isolate found to be consistent in the two varieties of samples used for this study was identified as Streptomyces ceolicolor , it decolourized RB 38 dye from blue to green. Future studies using molecular methods is expected to unravel the identities of other bacterial isolates.

Keywords: Biodegradation, Deadwood, Lignocellulose, Lignin, Sawdust, White-Rot Fungi

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1. INTRODUCTION

Wood, lignified gramineous and other annual plants are generally called lignocellulose because they are composed of the three main natural polymers: cellulose, hemicelluloses and lignin Foust et al. (2008). Cellulose is considered to be one of the most abundant biopolymers on earth. It is the main constituent of wood, and approximately 40% of the dry weight of most wood species cell wall is cellulose Sjöstrom (1993). The structural integrity of cellulose is one of the main obstacles of enzymatic hydrolysis of cellulose. However, for the isolation of cellulose, harsh extraction methods involving sequential acid and alkaline treatments are usually employed. Fiber

aggregation caused by sample processing is found in isolated cellulose, this does not necessarily represent the cellulose structure. The detailed molecular structure of plant cell wall cellulose remains unknown Ding and Himmel (2008). Sawdust originates from plant materials and is composed of cellulose, hemicellulose and lignin, hence the term lignocellulose. Lignin is well known for resistance to microbial degradation because of its high molecular weight and presence of biologically-stable carbon - to - carbon ether linkages Elsalam and El-Hanafy (2009).

White-rot fungi are a heterogeneous group of fungi that usually belong to basidiomycetes, although there are ascomycetous fungi that cause pseudo-white rot (also designated as Soft-rot type II), such as fungi belonging to the family Xylariaceae Liers et al. (2006). Basidiomycetous White-rot and some related litter-decomposing fungi are the only organisms which are capable of mineralizing lignin efficiently Kirk and Cullen (1998), Hatakka (2001). However more than 90% of all wood-rotting basidiomycetes are of the white-rot type Gilbertson (1980).

White-rot fungi are more commonly found on angiosperm than on gymnosperm wood species in nature. Usually, syringyl (S) units of lignin are preferentially degraded, whereas guaiacyl (G) units are more resistant to degradation. Many white-rot fungi colonize cell lumina and cause cell wall erosion, eroded zones coalesce as decay progresses and large voids filled with mycelium are formed. This type of rot is known as non-selective or simultaneous rot Blanchette (1995). Some white-rot fungi degrade lignin in woody plant cell walls relatively to a higher extent than cellulose, and they are called selective white-rot fungi, and an example is Phellinus nigrolimitatus Blanchette (1995).

Lignocellulose is a renewable organic material and is the major structural component of all plants. Lignocellulose consists of three major components: cellulose, hemicellulose and lignin. In addition, small amounts of other materials such as ash, proteins and pectin can be found in lignocellulosic residues in varying degrees based on the plant source Sanchez (2009). Lignin, the most abundant aromatic biopolymer on Earth, is extremely recalcitrant to degradation by linking to both hemicellulose and cellulose, it creates a barrier to any solutions or enzymes and prevents the penetration of lignocellulolytic enzymes into the interior lignocellulosic structures. Some basidiomycete's white-rot fungi are able to degrade lignin efficiently using a combination of extracellular ligninolytic enzymes, organic acids, mediators and accessory enzymes. The ligninolytic enzymes includes phenol oxidase (laccase) and hemeperoxidases; Lignin Peroxidase (LIP), Manganese peroxidase (MnP) and versatile peroxidase (VP), Dashtban et al. (2010). Physical and chemical treatment methods or a combination of both exists for the breakdown of lignocellulosic compounds; however, these methods are costly and hazardous to the environment El-Hanafy et al. (2008), Chandra and Singh (2012). Presently, the biological treatment method is the promising alternative since it is environment- friendly. Microorganisms which include fungi, bacteria and actinomycetes have been reported to oxidative degrade lignin El-Hanafy et al. (2008), Chandra and Singh (2012).

The aim of this study was to obtain evidences for lignocellulose degradation by native microbial population from tropical soil environment, southwest Nigeria as well as identify potential microbial candidates for integration into bioenergy development process and sustainable development in the West Africa region.

2. MATERIALS AND METHODS

2.1 SAMPLE SOURCE

Fungal Mycelia on decayed wood material (Parkia biglobosa) and fruiting bodies were randomly collected aseptically from Lagos State University, Ojo L.G.A., Lagos State and Onigbongbo, Atan in Ado-Odo/Ota L.G.A., Ogun State.

2.2 SAMPLE COLLECTION

Three different samples (Soil, Deadwood (Parkia biglobosa) and Sawdust (Uapaca Heudelotii) (local name Akun) collected independently from three different places in Lagos metropolis namely; Iba Housing Estate, Volkswagen Saw mill, Iba New site; these samples were labelled Deadwood, Sawdust and Soil respectively.

Also, the same type of samples were collected from Atan-Onigbongbo, Ogun- state.

2.3 DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES OF COLLECTED SAMPLES

Aliquot of each sample was taken and ground into powdered form; 10g of Deadwood, 10g of Sawdust and 10g of Soil samples were ground into powdered form independently using mortar and pestle. The powdered form of each of the different samples were used to determine respectively the physico-chemical properties of the samples.

2.4 PREPARATION OF MEDIA

Standard and conventional methods were followed in the preparation of Starch-Casein agar, Nutrient agar (NA) and Potato dextrose Agar (PDA) (Gerhardt et al. (1981) ; Buraimoh et al. (2015 a) .

2.5 ISOLATION AND STORAGE OF ISOLATES

Powdered form of decomposing sawdust, deadwood and soil were serially diluted respectively using serial dilution technique. Aliquot (0.1ml) of each sample was inoculated on starch-casein agar, Nutrient agar (NA) and Potato dextrose agar (PDA) aseptically and incubated at room temperature (26$\pm$2ºC). Incubation for Starch-Casein agar and PDA was 3 - 5days, Nutrient agar was incubated for 48hrs Smith (1981), Gerhardt et al. (1981).

2.6 ISOLATION OF FUNGI

A sterile scalpel was used to cut the suspected region colonized by the fungal species and thereafter inoculated onto sterile PDA plates Liers et al. (2006). Fruiting bodies and mycelia of fungi were detached with sterile scalpel from the bark of deadwood and rinsed with sterile water before inoculation onto potato dextrose agar and incubated at room temperature 26± 2 ºC for 5 days. The media were prepared aseptically following conventional and standard procedures as well as autoclaved at 121ºC, 151b pressure for 15 minutes Smith (1981).

2.7 CULTURAL IDENTIFICATION

Mixed fungal cultures observed on the PDA and Starch - Casein media were repeatedly sub-cultured until pure cultures were obtained following standard and conventional methods Smith (1981).

3. BIOCHEMICAL CHARACTERIZATION OF MICROBIAL ISOLATES

3.1 DYE DECOLOURIZATION ASSAY

Decolourization assays were carried out using Sabouraud dextrose agar (SDA) and Potato dextrose agar containing reactive blue 38 (RB38) dye and reactive black 5 (RB5) dye at two concentrations (75 and 150mg/l) at room temperature. The decolourization of dye indicated the presence of ligninolytic enzyme system in the inoculants Osono (2007), Barrasa et al. (2009).

3.2 MICROSCOPIC EXAMINATION

Binocular microscope was used to determine the presence of spores, hyphae, and clamp connections confirming presence of Basidiomycetes using ($\times$ 40) objective lens Smith (1981), Boer and Wal (2008).

3.3 DETERMINATION OF CELLULOLYTIC PROPERTY OF THE ISOLATES

Pure cultures obtained from serial-dilution of powdered samples were both aseptically and independently streaked on each sterile filter paper impregnated with RB 38 (reactive Blue) placed on the surface of starch-casein agar and Nutrient agar (NA). Thereafter Fungal isolates and bacterial isolates were stored on starch – casein agar and NA respectively Camarero et al. (1999), Buraimoh et al. (2015b).

3.4 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATES

The pure fungal isolates were thereafter observed using $\times$ 40 objective lens and bacterial isolates with $\times$ 100 objective lens of Microscope while biochemical tests were done with both the fungal and bacterial isolates. Biochemical tests include; cata-

lase test, oxidase test, Nitrate Reduction test, Starch hydrolysis test, Casein hydrolysis test Smith (1981), Gerhardt et al. (1981).

4. RESULTS

Table 1 **Mean Value of Physic-Chemical Analysis of Soil, Deadwood and Sawdust Samples**

Parameter	Soil	Deadwood	Sawdust
pH	6.94	6.01	6.09
N (%)	4.92	0.09	1.04
TOC (%)	3.59	2.02	0.06
$N0_3^-$	4.92	0.06	0.86
$P0_4^{3-}$	-	0.98	0.74
NH_4^+	-	-	-

Table 2 **Mean count from enumeration of isolates from both nutrient and starch casein agar**

	cfu/g	cfu/g	
Plates 10^5	PDA Soil	PDA Sawdust	Nutrient Agar(cfu/g)
Plate A	15	10	91
Plate B	12	06	101
Plate C	10	07	120
Plate A	19	10	188
Plate B	25	10	207
Plate C	27	12	192
Plate A	31	19	TNTC
Plate B	34	22	TNTC
Plate C	29	17	289
Mean Count	22.44	12.55	198.66

Key:
TNTC- Too numerous to count

Table 3 **Morphological & Biochemical tests on Bacterial Isolates**

Plat	*Grain Reaction*	Starch Hydrolysis	Cellulose Hydrolysis	Catalase Reaction	Oxidase Reaction	Casein Hydrolysis	Pigmenta	Probable Identity
A	+	+	+	+	+	+	*Creamy White*	*Streptomyces sp.*
B	+	+	+	+	+	+	*Creamy White*	*Streptomyces sp.*
C	+	+	+	+	+	+	*Creamy White*	*Streptomyces sp.*

Table 4 Morphological & Biochemical tests of Fungal Isolates

Plat	Starch Hydrol-ysis	Cellulose Hydroly-sis	Oxidase Reac-tion	Catalase Reac-tion	Casein Hydrol-ysis	Appearance on Starch-Casein Agar	Proba-ble Identity
A	+	+	-	-	+	Black	*Actino-mycetes sp.*
B	+	+	+	+	+	Deep Green	*Aspergillus flavus*
C	+	+	+	+	+	Deep Green	*A. flavus.*

Table 5 Morphological & Biochemical Characteristics of microbial Isolates from Soil

Plate	Starch Hydroly-sis	Cellulose Hydroly-sis	Oxidase Reac-tion	Catalase Reac-tion	Casein Hydrol-ysis	Appea on starch casein agar	Appearan on PDA	Proba-ble Identity
A	+	+	-	+	+	Black	Black.	*Actinomycetes sp.*
B	+	+	-	-	-	Blue	Light Blue	*Aspergillus. Fumiga-tus*
C	+	+	-	+	-	Deep Blue	Blue	*Fusar-ium solanii*
A	+	+	-	-	+	Black	Black	*Actinomycetes sp.*
B	+	+	+	-	+	Black	Black	*Actinomycetes*

Table 6 Mean Count of Fungal Isolates from Lagos and Ogun States Composite Samples

Fungal Isolates	Lagos	Ogun	Percentage (%) Occurrence
Aspergillus niger	29	16	28.3
Aspergillus flavus	15	22	23.27
Fusarium sp.	9	2	6.92
Penicillium sp.	11	8	11.95
Gliocladium sp.	6	18	15.09
Saccharomyces cerevisiae	5	9	8.81
Rhizopus sp.	3	6	5.66

5. DISCUSSION

The determination of physico-chemical properties of soil, Deadwood and sawdust from Lagos and Ogun states revealed both the level of acidity and nutrient in these three habitats of lignocellulosic fungi. Organic matter was more in the soil than either the Deadwood or sawdust. The NO_3^-nutrient was higher in the soil than found with both Deadwood and sawdust. Thus, suggesting that soil in the vicinity of the decomposing wood materials was richer in some nutrients. Although, PO_4^{3-}nutrient was not detectable due to its solubility and the fact that it is usually required in small concentration in the soil, consequently it's been absorbed by the wood (Table 1). The deadwood and sawdust degrading microorganisms were acidophiles. This was corroborated by the findings of Buraimoh et al. (2015 a). Morphological and biochemical analysis of both the fungal and bacterial isolates further revealed their identities (Tables 3, 4 and 5), this agreed with the findings of Blanchette (1995). Previously, it has been reported that there is a unique bacterial role in the functioning of lignocellulose - degrading basidiomycetes, the fungal extracellular enzymes provide low - molecular weight substances from the substrates which forms the basis for competition between the bacterial and fungal species, however, the occurrence of bacterial mycophagy has been observed. Basidiomycetes may benefit from nitrogen supply and detoxification of mycotoxic compounds by bacteria which enhances the proliferation of the white- rot fungi in deadwood. In wood degradation, basidiomycetes alter the environmental conditions to such an extent that bacterial species that survives rapid acidification, reactive oxygen species and toxic fungal secondary metabolites that results from the activities of basidiomycetes are regarded as having special properties Boer and Wal (2008), Tlaskal et al. (2021). Starch-Casein agar supplemented with RB38 dye was used as a selective medium for the isolation of lignocellulosic white - rot fungi. The study revealed mean lignocellulosic fungal population 12.3×10^{5cfu}/gfrom soil, 7.6×10^5 cfu/g from sawdust and104 $\times10^5$ cfu/g heterotrophic bacterial population from the soil (Table 2). Dye decolorization has been used as a simple assay for the identification of fungal ability to transform lignin. It is suggestive that these fungal isolates were able to produce ligninolytic peroxidases that empowers them to oxidize lignin Tocco et al. (2021) and Laccases produced by white - rot fungi. The dye discoloration in Starch-Casein medium supplemented with RB 38 provided the incontrovertible evidence of the capability of the isolates to degrade lignin both in the deadwood and sawdust samples. The white - rot fungal isolates obtained from the tropical environment in this study can be further integrated into wood recycling process for biofuels production as source of alternative energy. However, a bacterial species was observed to grow on both the starch-Casein medium supplemented with RB 38 and sterile filter paper soaked with the dye. This is suggestive of the capability of the bacterial strain to transform lignin. Future studies would unravel the identity of this bacterial isolate using molecular techniques.

6. CONCLUSION

This study revealed that lignin, the most recalcitrant natural polymer is not biodegraded only by white-rot fungi but that few bacterial species have the inherent capabilities for bioconversion of lignocellulose to other useful products which includes its use as biofuels which is an alternative source of energy.

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