

Analysis of Decomposing Wood Wastes in the Lagos Lagoon using Terminal-Restriction Fragment Length Polymorphism and Illumina Sequencing Platform

O. M. Buraimoh^{1,2}, G. A. Adewumi^{1*}, N. M. Akinyemi¹, O. O. Amund¹, M. O. Ilori¹, F. C. Michel Jr.²



¹Department of Microbiology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.

²Department of Food, Agricultural and Biological Engineering, The Ohio State University
Wooster, OH 44691, USA.



ABSTRACT: Indiscriminate disposal of wood shavings and sawdust into the Lagos lagoon usually constitute environmental hazard and varying degree of threats to marine biotic communities. In this study we applied terminal-restriction fragment length polymorphism (T-RFLP) and illumina sequencing of 16S ribosomal RNA gene, to describe the microbial ecology of decomposing wood wastes in the Lagos lagoon, Nigeria. The terminal restriction fragments (TRFs) generated corresponds to over 100 bacterial genera and numerous uncultured bacterial clones. Phylogenetic analysis of the TRFs of 16S rRNA gene sequences and cultured bacterial sequences established genetic relatedness and divergent relationships. T-RFLP in combination with illumina sequencing platform identified bacterial species (*Acinetobacter* sp., *Clostridium* sp., *Planctomyces* sp., *Escherichia coli*, *Pantoea agglomerans*, *Dyella japonica*, *Ochrobactrum* sp., *Prevotella* sp., *Runella slithyformis*, *Enterobacter aerogenes*, *Gordonia amicalis*, *Klebsiella* sp., *Thalassospira* sp. and *Serratia* sp.) not previously accounted for in culture-dependent analysis. Bacterial strains such as *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Simiduia agarivorans*, *Microbulbifer* sp. and *Cellulomonas fimi* further identified in illumina sequences were found to be involved in the biodegradation of lignin and lignin derivatives. Hence, understanding of the diverse autochthonous microbiota of decomposing wood wastes in the Lagos lagoon is essential in the development of effective biotechnology programme, to tackling the menace of sawdust pollution.

KEYWORDS: Wood wastes, Biotic communities, Microbial diversity, Amplicon sequencing, Culture-independent techniques.

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I. INTRODUCTION

The tropical Lagos lagoon is a shallow (maximum depth of about 5 m) elongated body of water, open and tidal (between 0.3 and 1.3 m), with surrounding submerged ridges and reefs (Sogbanmu *et al.*, 2016). It is the largest, most complex and impacted lagoon system in West Africa and Gulf of Guinea that receives tributaries from rivers such as Yewa, Ogun, Ona and Osun; stretches from Cotonou, Republic of Benin, extends to the bay of Niger Delta in Nigeria, and discharges into the South Atlantic Ocean through the lagoon harbour (Amaeze *et al.*, 2015; Obi *et al.*, 2016; Nkwoji *et al.*, 2020). In terms of geographical description the Lagos lagoon is strategically located within most densely urban and metropolitan areas of Lagos State, where various human engagements like fish farming, sand mining, shipping activities, canoe transportation and saw milling take place (Ajagbe *et al.*, 2012).

All these activities create anthropogenic wastes, resulting from refuse dumping, sewage disposal, domestic, municipal and industrial wastes, including organic and inorganic pollutants, which threatens the continuous existence of diverse biotic communities (Eruola *et al.*, 2011; Elliot and Elliot, 2013). Of particular interest is the haphazard dumping of

wood shavings and sawdust at the Ebute-Meta axis of the lagoon, which accumulates over time and naturally decompose *in situ* owing to the activities of autochthonous microorganisms that inherently colonize them. However, little is known about the microbial community structure, diversity, taxa dynamics and phylogenetic identities of these decomposing woods.

Efficient decaying of wood shavings and wastes is dependent on the wood constituents, properties and inhabiting microbiota (Kielak *et al.*, 2016). Wood wastes are generally composed of cellulose, hemicelluloses and lignin in varying percentages, and are regularly released into the ecosystems of the Lagos lagoon from the saw milling activities. Deterioration of these lignocellulose wastes is mainly achieved by complex microbial processes, involving both bacterial and fungal communities (Valášková *et al.*, 2009). Fungal degradation of wood wastes, as a biological pre-treatment process, enhances break down of the structural cell wall components and removal of lignin from lignocellulosic biomass, to aid easy penetration of other polysaccharides, such as cellulose and hemicelluloses (Owens *et al.*, 1994; Kainthola *et al.*, 2021). There are reports on the enzymatic degradation of cellulose and lignocelluloses biomass of wood wastes in the Lagos lagoon and other environments by fungal

*Corresponding author: gadewumi@unilag.edu.ng

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species, namely *Aspergillus flavus*, *A. niger*, *A. giganteus*, *Cladosporium oxysporum* and *Trichoderma aureoviride* (Akpata, 1980; Chinedu, 2007; Mtiu and Nakamura, 2008; Shweta, 2011). Majorly, laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, feruloyl esterase, aryl-alcohol oxidase, quinone reductases, lipases, xylanase and catechol 2, 3-dioxygenase lignolytic enzymes are involved in the catabolic breakdown of these compounds (Ozer *et al.*, 2020; Kumar and Chandra, 2020). Also, bacterial strains capable of causing decay of cellulose and lignin have been documented. Previously, we reported the characterization of lignocellulolytic *Streptomyces*, *Bacillus* and *Paenibacillus* species associated with decomposing wood residues in the Lagos lagoon (Buraimoh *et al.*, 2015). Elsewhere, bacterial catabolism of lignin, lignin-derived fragments and lignin-related aromatic compounds have also been reported (Zimmermann, 1990; Masai *et al.*, 2007; Ahmad *et al.*, 2010; Ventorino *et al.*, 2015; Montella *et al.*, 2017).

Evidence of synergistic interactions between bacteria and fungi, co-inhabiting decomposing wood wastes are available. For example, bacteria can exchange intermediate organic carbon sources and lignin decomposition derivatives, mainly aromatic compounds, provided by fungal species with nutrients such as iron, nitrogen and vitamins, for effective and efficient wood wastes degradation (Brunner and Kimmins, 2003; Hoppe *et al.*, 2014). To understand the reason behind this coexistence in a typical wood dump site in the Lagos lagoon, it is important to analyse the microbial community profile, to gain insight into the phylogenetic relatedness and evolutionary relationship of interacting microbial strains.

Cultivation-independent molecular techniques, which directly characterize the microbiota present in a particular habitat, by examining the entire genomes without need for culturing or isolation, is gaining wide scientific recognition in the field of microbial ecology (Handelsman, 2004; Rissenfeld *et al.*, 2004; Cocolin *et al.*, 2013). These methods have been increasingly used to determine the composition of complex microbial populations by analysing the total DNA or RNA derived from mixed diverse ecosystems, such as soil, marine water, lagoon sediments, lignocellulose and cellulolytic wastes, and fermented food products (Pearce *et al.*, 2012; Adewumi *et al.*, 2013; Staley *et al.*, 2013; Romi *et al.*, 2015; Obi *et al.*, 2016; Cai *et al.*, 2018; Kanokratana *et al.*, 2018). Advancement in high-throughput DNA sequencing has delivered an entirely new strategy; next generation sequencing (NGS), which employs massively parallel approaches to produce millions of short DNA sequence reads in a single run at cheaper cost (Chi, 2008; Quail *et al.*, 2012). These deep sequencing platforms have facilitated the understanding of the microbial ecology, richness, species distribution and phylogenetic taxa of decaying wood wastes (van der Lelie *et al.*, 2012; Ottosson *et al.*, 2015; Kielak *et al.*, 2016; Yang *et al.*, 2016; Poulsen *et al.*, 2022). However, there is dearth of information on the application of these techniques to profiling the microbiota of wood dump sites in the Lagos lagoon, Nigeria. Hence, in this study, we combined terminal-restriction fragment length polymorphism (T-RFLP) with illumina sequencing, two culture-independent molecular

biology assessment methods, to generate in-depth data on the microbial community structure, distribution and diversity of decomposing wood wastes in the Lagos lagoon; this would assist to support efforts geared towards developing relevant biotechnological approaches in tackling the menace of sawdust pollutants in the environment, in addition to discouraging indiscriminate dumping of wood shavings and wastes.

II. MATERIALS AND METHODS

A. Site Description and Samples Collection

Decomposing wood shavings and sawdust from eight (08) locations at a depth of 20 cm each were obtained from Oko Baba sawmill, at the Ebute-Meta axis of the Lagos lagoon (Co-ordinates: N 6° 29' 21.8"; E 003 °, 23' 29.3") in sterile containers. They were transported immediately into the laboratory in refrigerated boxes, stored at -80 °C and analyzed within 24 h of sampling.

B. Total DNA Extraction, Quantification and Purity Determination

Total community DNA was extracted from a composite wood waste, consisting of wood sawdust, collected from eight (08) locations at different points within the lagoon. Ten grams of the decomposing wood wastes was homogenized in 90 mL sterile 0.1 M potassium phosphate buffer saline (Calbiochem, San Diego, CA, USA) in a sterile stomacher bag (Seward, London, UK), using Stomacher 400 circulator (Seward, London, UK) at 250 rpm for 3 min. The homogenate was allowed to stand for *ca.* 30 min, after which 30 mL was transferred to sterile 50 mL Falcon tube (Falcon, Beckton Dickinson, Sparks, MD, USA). This was centrifuged (Sigma 6K 15, Sartorius, Germany) at 5,000 x *g* for 10 min at 4 °C. The supernatant was discarded, and wood pellets washed twice with sterile deionized water. DNA was extracted from the pellets using the Ultraclean Soil DNA kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, the wood pellets were suspended in 750 µL Lysis Solution, containing 5 mg/mL lysozyme (L-7651, Sigma, Steinheim, Germany) and 20 µL of 5U/µL mutanolysin (M9901, Sigma, Israel) in the Bashing Bead Lysis Tube; incubated at 37 °C for 1 h, with intermittent mixing. This was thereafter centrifuged at ≥ 10,000 x *g* for 1 min at room temperature, after which 400 µL of the supernatant was added to the Spin Filter in a collection tube and centrifuged at 7,000 x *g* for 1 min. DNA binding buffer was added to the filtrate in the collection tube, and the resulting mixture transferred to the Spin Column.

DNA Wash Buffer was added to the Spin Column in a collection tube and centrifuged at 10,000 x *g* for 1 min. Finally, the Spin Column was transferred to a clean 1.5 mL microcentrifuge tube and 100 µL DNA Elution Buffer added directly to the column matrix, incubated for 2 min and then centrifuged at 10,000 x *g* for 1 min for DNA elution. DNA obtained was spectrophotometrically quantified and purity checked at 260 nm using 2000ND NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

C. Polymerase Chain Reaction (PCR) Amplification of 16S Ribosomal RNA Gene and T-RFLP

DNA sample was subjected to 16S ribosomal RNA gene PCR amplification using 3 fluorescent labeled forward primers ActF (CGCGGCCTATCAGCTTGTTG), 8F (AGAGTTTGATCCTCGCTCAG) and 11F (GTTTGATCMTTCGCTCAG), including 1 reverse primer 907R (CCGTCAATTCMTTTRAGTTT), each at 0.8 μ M (IDT, Coralville, USA). The 25 μ L reaction mixture contained 50 ng template DNA, 1X PCR buffer with 1.5 mM MgCl₂ (P2192, Sigma-Aldrich), 200 μ M each of the dNTPs (Sigma-Aldrich) and 1.2 U Taq DNA polymerase (D1806, Sigma-Aldrich) and 18.4 μ L sterile deionized water. Amplification was performed in a thermal cycler (BIO-RAD, iCycler, USA), with initial DNA denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 65 °C for 1 min and extension at 72 °C for 30 s. The PCR was completed with a final extension temperature of 72 °C for 7 min. For T-RFLP analysis, amplicons fluorescently labeled were purified using the GenElute Gel Extraction kit (NA1111, Sigma-Aldrich), and digested with HhaI, MspI and RsaI (Promega, Madison, WI, USA) for 12 h at 37 °C, which was followed by deactivation of 20 min at 65 °C.

Fluorescently labeled terminal restriction fragments (TRFs) were analysed using capillary electrophoretic (CE) genetic analysis system facility of the Ohio Agricultural Research and Development Centre, Ohio, USA (CEQ) (CEQ 8800, Beckman Coulter, Inc.), to estimate their peak sizes and also to determine nucleotide sequences. TRFs sequences were compared with those deposited in RDP (R10, U27) 1, 519, 357 16S bacterial ribosomal RNA (<http://mica.ibest.uidaho.edu/pat.php>) to obtain closest known bacterial identities (Shyu *et al.*, 2007). Pairwise and multiple alignments of these sequences including those of related bacterial species were carried out using ClustalW 2.0.12 (Thompson *et al.*, 1994), according to the Kimura two-parameter model (Kimura, 1980). Phylogenetic and molecular evolutionary analyses were conducted using MEGA7 software (Kumar *et al.*, 2016). The statistical reliability of the tree was evaluated by bootstrap analysis of 1000 replicates (Felsenstein, 1985).

D. Barcoded Illumina MiSeq Sequencing

DNA sample of the decomposing wood wastes was amplified by targeting the V4-V5 small sub-unit (SSU) of the eubacterial 16S ribosomal RNA gene amplicon, using the barcoded-primer approach to multiplex paired-end illumina sequencing as previously described (Caporaso *et al.*, 2010). The 25 μ L PCR reaction volume consisted of 25 ng template DNA, 1X Phusion HF reaction buffer (New England Biolabs, Ipswich, MA, USA), 2.5 mM MgCl₂ (Sigma-Aldrich), 0.8 μ M each of 515F (5'-adapter GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-adapter GGACTACHVGGGTWTCTAAT-3'), 200 μ M each dNTP and 0.5 U Phusion Hot Start flex DNA polymerase (New England Biolabs, Ipswich, MA, USA). The 5' end of the forward and reverse primers were barcoded, each with 8-bp illumina overhang adapter sequences, to enable sample multiplexing and sorting of mixed sequence reads.

Amplification programs were as follows: 95 °C for 5 min; 26 cycles of 95 °C for 15 s, 55 °C for 30s and 72 °C for 30s; with a final extension of 72 °C for 5 min. PCR products were analyzed in 1.5 % (w/v) agarose gel electrophoresis for size determination, and subsequently purified using QIAquick gel extraction kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. In brief, equal volume of Membrane Binding Solution was added to the PCR amplified products. This was transferred to the Minicolumn assembly, and incubated at room temperature for 1 min. After this centrifugation was carried out at 16,000 x g for 1 min; flow-through discarded, Minicolumn reinsert into the Collection Tube, and Membrane Wash Solution added. This was further centrifuged at 16,000 x g for 1 min, and the Collection Tube emptied. The Minicolumn was carefully transferred to a clean 1.5 mL microcentrifuge tube, and 50 μ L nuclease-free water added. Incubation was carried out at room temperature for 1 min, and final centrifugation at 16,000 x g for 1 min, to collect the purified DNA. Preparation of the V4-V5 library from the pooled amplicon sample and sequencing on illumina MiSeq platform (Illumina, San Diego, CA, USA) were conducted by a commercial service provider (Inqaba Biotechnical Ltd., Hatfield, Pretoria, South Africa).

E. Bio-Informatic Processing and Analysis of Illumina Sequences

The paired-end raw illumina fast sequence files obtained from the sequencing facility were de-multiplexed, adapter sequences removed and quality-checked using FastQC software version 0.11.5 (Babraham Institute, United Kingdom). Subsequently, de-multiplexed paired-end reads were filtered of substitution, ambiguous bases, and chimera errors checking and removal. Quality-filtered paired-end sequence reads were then analyzed in the Quantitative insights into Microbial Ecology (version 2) (QIIME 2) software (Bolyen *et al.*, 2019) by using the DADA2 denoiser (Callahan *et al.*, 2016) to obtain amplicon sequence variants (ASVs). Bacteria taxonomic assignment of the sequences were clustered into operational taxonomic units (OTUs) according to a 97 % identity cut-off using the QIIME workflow script.

III. RESULTS AND DISCUSSION

A. Analysis of Bacterial Diversity of Decomposing Wood Communities using T-RFLP

DNA extracted from the decomposing wood wastes and shavings were found to be suitable for PCR amplification in terms of quantity and purity; this is a critical step, shown to be essential in previous microbial ecosystems profiling (Di Pinto *et al.*, 2007; Santosh *et al.*, 2016). Expectedly, PCR amplified 16S ribosomal RNA gene of ActF and 907R primers gave band size of 700 bp, while combination of 8F or 11F and 907R primers yielded product size of 900 bp (Figures 1 and 2). Fluorescently labeled PCR amplicons, which were digested with HhaI, MspI and RsaI restriction endonucleases generated more than four thousand TRFs, whereas MspI produced the highest TRFs. Weidner *et al.* (1996) earlier observed variation in the number of TRFs based on the restriction digestion potential of restriction endonucleases. TRFs nucleotide sequences compared with those deposited in RDP database

revealed 107 bacterial genera and more than 3,000 uncultured microbiota in the decomposing wood community. A compilation of the identities of the major bacterial genera and species is shown in Table 1, with the predicted and observed values of TRFs.

Further analyses of selected sequences obtained from the decomposing wood wastes, together with bacterial sequences in a previous study (Buraimoh *et al.*, 2015), aided the construction of a phylogenetic tree as shown in Figure 3.

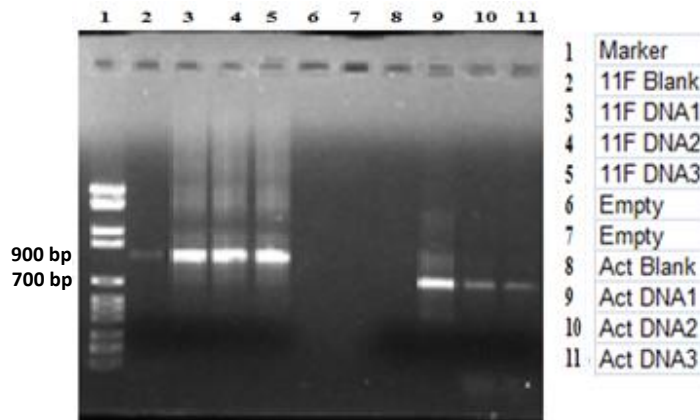


Figure 1: T-RFLP agarose gel electrophoresis of amplified 16S rRNA gene of bacterial wood community using ActF and 907R primers (700 bp), and 11F and 907R primers (900 bp).

The three different groups clustered separately on the basis of species, establishing divergence, evolutionary and clonal relationships. Group I consists of *Streptomyces goraiensis* (AF170913), a strain from the total DNA of decomposing wood wastes; it formed a sub-group of 85 % bootstrap support with *S. albus* DOB (KF977551), *S. coelicolor* COB (KF977550), *S. albogriseolus* AOB (KF977548) and *S. pseudogriseolus* EOB (KF977552) strains cultured from degrading lignocellulosic sawdust residues of the Lagos lagoon (Buraimoh *et al.*, 2015). Other bacterial species, such as *Acinetobacter* sp., *P. agglomerans*, *D. japonica*, *Ochrobactrum* sp., *Prevotella* sp., *R. slithyformis*, *Clostridium* sp., *Ornithinococcus* sp. and uncultured bacteria clones, not previously reported during culture-dependent analysis, were also found in this group.

These findings further confirmed the inherent prejudices and inadequacies of classical microbiological methods, based on plating techniques, which hitherto had been criticized because only culturable microorganisms are isolated while others whose growth requirements are not readily accessible are missed out (Rantsiou and Cocolin, 2006). It was previously demonstrated that about 25-50 % of the active microbial community cannot be cultured in vitro using laboratory media (Ampe *et al.*, 1999). These Viable but Non-Culturable (VNC) cells may be responsible for efficient biodegradation of decomposing wood wastes and shavings in situ in the Lagos lagoon; however, this has to be investigated in further empirical studies after successful culturing of their

Table 1: Identities of major bacterial species based on Terminal Restriction Fragments (TRFs) nucleotide sequences of decomposing wood wastes community in the Lagos lagoon.

16S ribosomal RNA gene closest known relative	Predicted value	Observed value	Abundance	Accession number [†]
<i>Dyella japonica</i>	479	478.71	0.000015	AB110496
<i>Planctomyces</i> sp.	95	94.43	0.000947	AB015527
<i>Dietzia</i> sp.	80	80.94	0.00002	DQ060378
<i>Onchrobacterium</i> sp.	106	106.56	0.000268	AY662685
<i>Odysella</i> sp.	120	120.27	0.000039	EU305573
Uncultured <i>Verrucomirobia bacterium</i>	82	81.11	0.000359	GQ350123
<i>Bacillus</i> sp.	100	100.99	0.0000169	EF165014
<i>Streptomyces goraiensis</i>	59	59.93	0.012874	AF170913
<i>Paenibacillus harenae</i>	481	478.71	0.115582	AY839867
Uncultured <i>bacteroides</i>	94	94.01	0.000051	DQ656210
<i>Clostridium</i> sp.	116	116.69	0.000257	DQ218319
<i>Prevotella</i> sp.	481	478.71	0.115582	AY158021
<i>Bradyrhizobium</i> sp.	106	106.56	0.000268	FT555225
<i>Acinetobacter</i> sp.	70	70.33	0.000192	GQ284532
<i>Ornithinococcus</i> sp.	71	70.33	0.000192	AB188221
<i>Shewanella</i> sp.	90	82.2	0.001651	AY573039
<i>Psychroserpens burtonensis</i>	91	90.15	0.001761	AY771723
<i>Treponema pectinovorum</i>	94	94.01	0.000051	AF302940
<i>Pantoea agglomerans</i>	102	101.01	0.000169	EF569226
<i>Escherichia coli</i>	106	106.6	0.000157	GQ456068
<i>Nitrospira</i> sp.	107	106.6	0.000157	HM224507
<i>Cronobacter sakazakii</i>	120	120.59	0.00039	HM069215
<i>Enterobacter aerogenes</i>	120	120.59	0.00039	HM475271
<i>Serratia</i> sp.	121	120.59	0.00039	EJ667500
<i>Micromonospora</i> sp.	139	138.39	0.001696	AB609325
<i>Thalassospira</i> sp.	180	180.52	0.000111	EU907920
<i>Klebsiella</i> sp.	180	180.52	0.000111	EU435307
<i>Roseatelesde polymerans</i>	488	487.06	0.069499	AB495143
<i>Gordonia amicalis</i>	80	80.94	0.00002	EF424581
<i>Tsukamurella</i> sp.	80	80.94	0.00002	EU181255
<i>Kribbella</i> sp.	80	80.94	0.00002	EU080972
<i>Pedobacter</i> sp.	95	94.43	0.000947	AJ551152

[†]Accession no. of closest relative organisms of nucleotide sequences found in RDP database (R10, U27) 1, 519, 357 16S bacterial ribosomal RNA (<http://mica.ibest.uidaho.edu/pat.php>).

species in the laboratory. Hence, studies involving polyphasic strategy for thorough investigation of microbial contents of various ecosystems have been documented (Romi *et al.*, 2015; Greppi *et al.*, 2013; Johnston *et al.*, 2016). Group II, though is a sub-cluster of Group I; however, the low bootstrap support of 16 % in this group is an indication of a relatively independent divergent and evolutionary traceability between *Streptomyces* and *Bacillus*. The group consists of *Bacillus megaterium* NOB (KF977554), *Bacillus* sp. OOB (KF977555), *Paenibacillus* sp. ROB (KF977556), *Bacillus* sp. (EF165014) and *Paenibacillus harenae* (AY839867), all clustering together, indicating common ancestral origin and adaptation to the Lagos lagoon. Group III comprises *B. bataviensis* FOB (KF977553) and *S. aureus* BOB (KF977549), both of which were also cultured from decomposing lignocelluloses sawdust residues.

B. Taxonomic Richness, Distribution and Diversity of Decomposing Wood Wastes Microbiota Based on Illumina Sequencing

Total community DNA of decaying wood wastes amplified and sequenced on *illumina MiSeq* platform, revealed 23 phyla, 36 classes, 43 orders, 43 families and numerous taxa genera and species. Top on the phyla were Proteobacteria (8,170 reads; 56.3 %), Actinobacteria (2,572 reads; 17.7 %), Bacteroidetes (1,403 reads; 9.7 %), Firmicutes (840 reads; 5.8 %) and Planctomycetes (669 reads; 4.6 %) (Figure 4(a)). Alphaproteobacteria (4,072 reads; 28.1 %) and Gammaproteobacteria (3,674 reads; 25.3 %) were the dominant classes, both in the phylum Proteobacteria, followed by class Actinobacteria (2,572 reads; 17.7 %), Sphingobacteriia (499 reads; 3.4 %), Flavobacteriia (472 reads; 3.3 %), Clostridia (444 reads; 3.1 %), Planctomycetia (442 reads; 3.0 %), Bacteroidia (380 reads; 2.6 %), Deltaproteobacteria (377 reads; 2.6 %), and then Bacilli (275 reads; 1.9 %) (Figure 4(b)). The phyla Actinobacteria, Bacteroidetes and Planctomycetes were represented by Actinobacteria, Bacteroidetes and Planctomycetia classes, respectively. The major orders include Actinomycetales (12.6 %; class Actinobacteria), Alteromonadales (12.6 %; class Gammaproteobacteria), Rhizobiales (11.8 %; class Alphaproteobacteria), Rhodobacterales (8.1 %; class Alphaproteobacteria), Oceanospirillales (5.4 %; class Gammaproteobacteria), Acidimicrobiales (3.9 %; class Acidimicrobiia), Sphingomonadales (3.5 %; class Alphaproteobacteria), Gammaproteobacteria_incertae_sedis (3.5 %; class unknown), Sphingobacteriales (3.4 %; class Sphingobacteriia), Flavobacteriales (3.3 %; class Flavobacteriia), Rhodospirillales (3.1 %; class Alphaproteobacteria) and Planctomycetales (3.0 %; class Planctomycetia) (Figure 4(c)). Notable families in the sequence reads are Alteromonadaceae (8.6 %; order Alteromonadales), Rhodobacteraceae (8.1 %; order Rhodobacterales), Idiomarinaceae (3.7 %; order Alteromonadales), Nocardiaceae (3.6 %; order Actinomycetales), Rhodobiaceae (3.5 %; order Rhizobiales), Hyphomicrobiaceae (3.3 %; order Rhizobiales), Flavobacteriaceae (3.2 %; order Flavobacteriales), Rhodospirillaceae (3.0 %; order Rhodospirillales) and

Planctomycetaceae (3.0 %; order Planctomycetales), apart from the unknown families, which constitute 8.2 % (Figure 4(d)). Meanwhile, three genera are dominantly present, which comprise *Marinobacter* (4.9 %; family Alteromonadaceae), *Gordonia* (3.5 %; family Theaceae) and *Idiomarina* (3.5 %; family Idiomarinaceae) (Figure 4(e)).

It is interesting to note that various affiliations of microbial taxa identified in this study have been reported in diverse wood-decomposing ecosystems, with assignable ecological functions. For instance, Firmicutes, Xanthomonadales (family; Xanthomonadaceae) and Pseudomonadales belong to the class Gammaproteobacteria; Rhodospirillales, Burkholderiales belonging to the class Betaproteobacteria, and some genera of Actinobacteria, were found dominant at different stages of pine-wood decomposition (Kielak *et al.*, 2016), and in other wood decomposing environments, including beech wood and wood sawdust (Folman *et al.*, 2008; Zhang *et al.*, 2008; Valášková *et al.*, 2009). *Pseudomonas* and *Luteibacter*, two genera of the order Pseudomonadales were also dominantly present in freshly cut pine wood decaying chips (Noll *et al.*, 2010). Acidobacteria, though less represented in our phyla, its adaptation and subsequent colonization of wood-decaying environments is well-documented (Sait *et al.*, 2006; Jones *et al.*, 2009; Valášková *et al.*, 2009; Yamada *et al.*, 2014). According to Covino *et al.* (2016) Gram negative Proteobacteria (alpha, beta, gamma and delta-proteobacteria) dominantly occupied the bacterial composition of creosote-treated co-composting wood community, based on next-generation sequencing (NGS). Other less represented bacterial phyla were Bacteroidetes, Firmicutes and Actinobacteria. van der Lelie *et al.* (2012) identified the phyla Firmicutes, Proteobacteria and Bacteroidetes as the most abundant among the diverse community microbiota under anaerobic conditions of poplar wood biomass degradation. Similarly, Actinobacteria, Bacteroidetes, Chloroflexi, Firmicutes and Planctomycetes, including the orders, Actinomycetales and Caulobacterales (genus: *Phenylobacterium*) were reported to be bacterial indicator for cellulose metabolism, an important component of lignin and wood waste residues (Schellenberger *et al.*, 2010; Verastegui *et al.*, 2014). *Phenylobacterium immobile* was specifically found to remove toxic aromatic compounds from wood wastes (Lingens *et al.*, 1985). Bugg *et al.* (2011) identified lignin-degrading bacterial strains in the classes Actinobacteria, alpha and gamma-proteobacteria. In the present study we observed Cyanobacteria forming a minor portion of our phyla sequence reads; this was also noticed in the pyrotag data of an anaerobic microbial community of decomposing poplar wood chips (van der Lelie *et al.*, 2012).

As previously mentioned the microbial community structure of wood decomposing wastes in the Lagos lagoon consists of numerous uncultured and cultured microbial species, including *R. flavefaciens*, *F. succinogenes*, *S. agarivorans*, *Streptomyces albogriseolus*, *Microbulbifer* sp. and *C. fimi*. *R. flavefaciens* and *C. fimi* are cellulolytic bacteria in the phyla Firmicutes and Actinobacteria, respectively. These microorganisms have been reported to play important roles in the degradation of plant polymers-cellulose and

hemicelluloses (Fontes and Gilbert, 2010). *F. succinogenes*, a Gram-negative, anaerobic bacterium, possesses complex hydrolytic enzymes, such as glucanases, xylanases, glycosyl hydrolase, which are involved in the degradation of lignin and lignin carbohydrate complexes (Jun *et al.*, 2007). *S. agarivorans* is a Gram-negative, chemoheterotrophic, marine bacterium, capable of degrading hetero-polysaccharide compounds, such as cellulose, agar, alginate and chitin (Kislyuk *et al.*, 2010). Coniferyl alcohol, one of the major precursors of lignin was found completely degraded by autochthonous *S. albogriseolus* strain isolated from decaying wood residues in the Lagos lagoon ecosystem (Buraimoh *et al.*, 2017). Gram-negative gamma-proteobacterium, *Microbulbifer hydrolyticus* has been found capable of degrading lignin and lignin-like polymers (Lim *et al.*, 2006). In addition to these hydrocarbon-degrading bacterial strains, namely *G. terrae*, *G. namibiensis*, *Cobetia* sp., *Polymorphum gilvum*, *Algiphilus aromaticivorans* and *Lutimaribacter litoralis*, inhabiting soil, sea water and marine sediments were also found among the illumina sequences representing the microbiota structure of decomposing wood wastes in the Lagos lagoon.

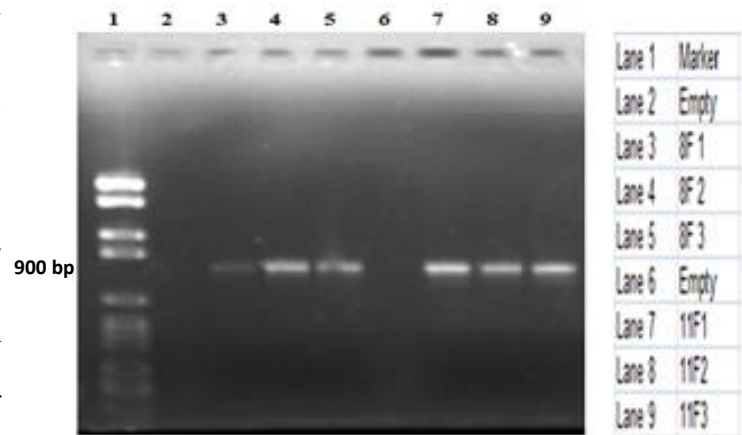


Figure 2: T-RFLP agarose gel electrophoresis of amplified 16S rRNA gene of bacterial wood community using 8F and 907R primers (900 bp).

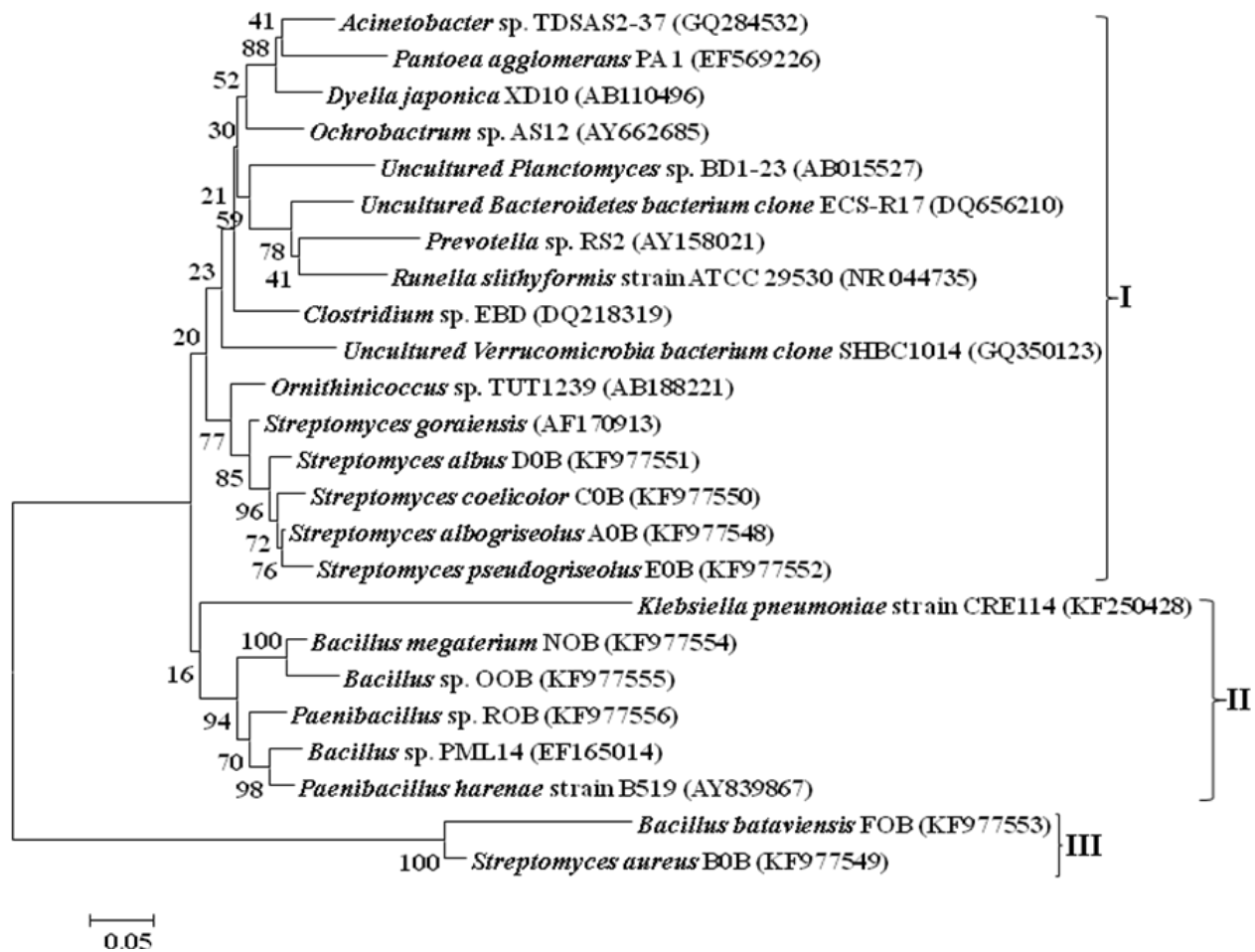


Figure 3: Phylogenetic tree of pairwise and multiple alignments of Terminal Restriction Fragments (TRFs) of 16S ribosomal RNA gene sequences of bacterial species and uncultured microbiota in the decomposing wood wastes community of the Lagos lagoon, together with bacterial sequences retrieved from GenBank database. Evolutionary history and distances were inferred and computed using neighbour-joining (Saitou and Nei, 1987) and the p-distance method (Nei and Kumar, 2000), respectively.

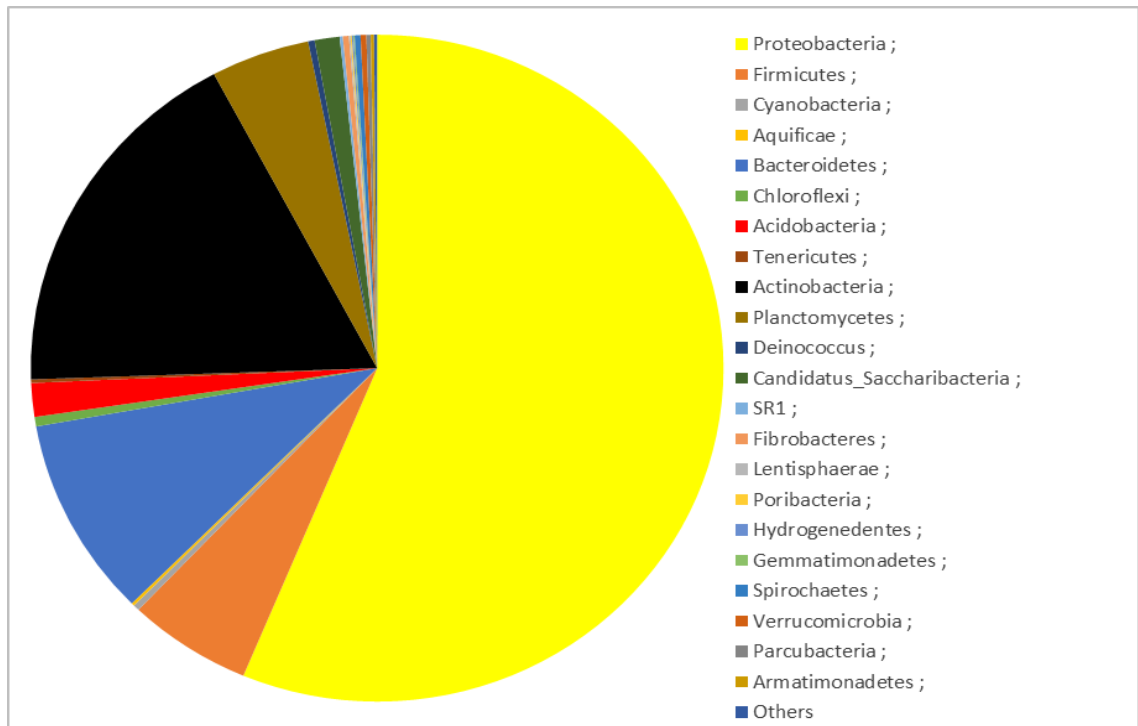


Figure 4(a): Taxonomic distribution based on Phylum of the total sequence reads obtained from illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria. Others are any OTUs below 0.1 (i.e. < 0.1).

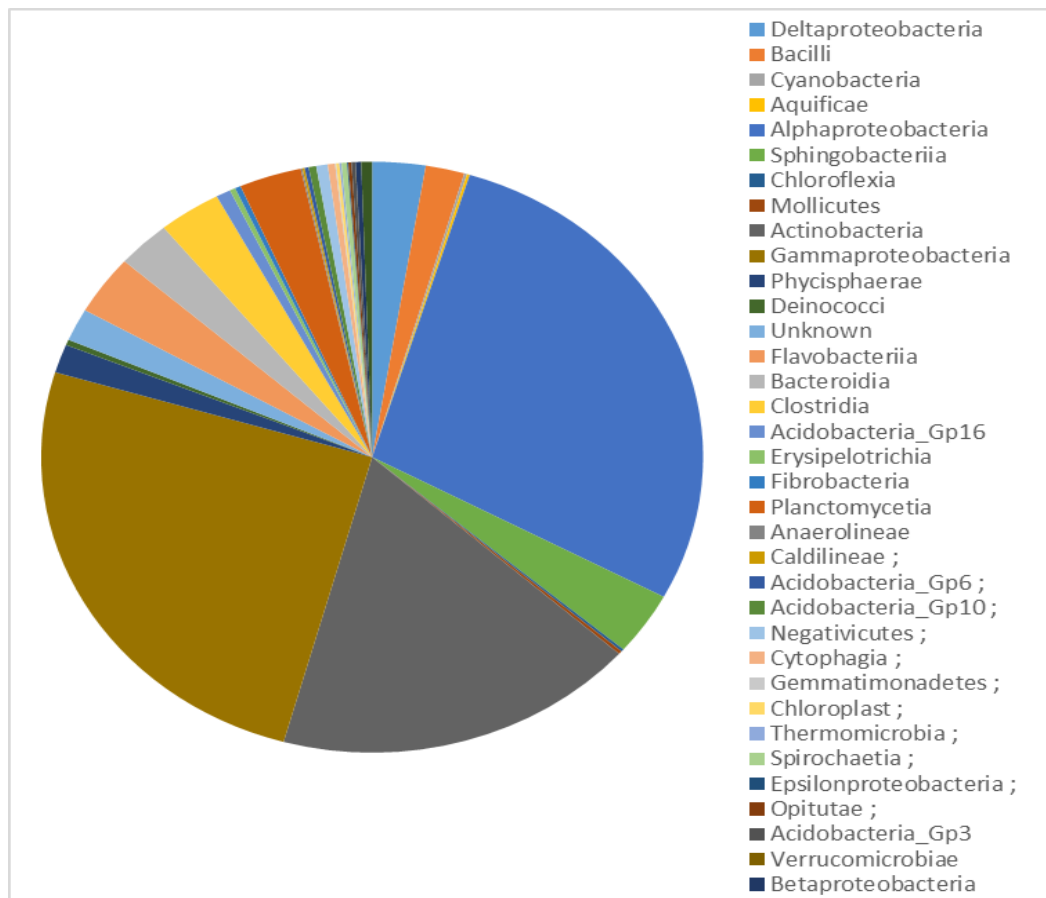


Figure 4(b): Taxonomic distribution based on Class of the total sequence reads obtained from illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria. Others are any OTUs below 0.1 (i.e. < 0.1).

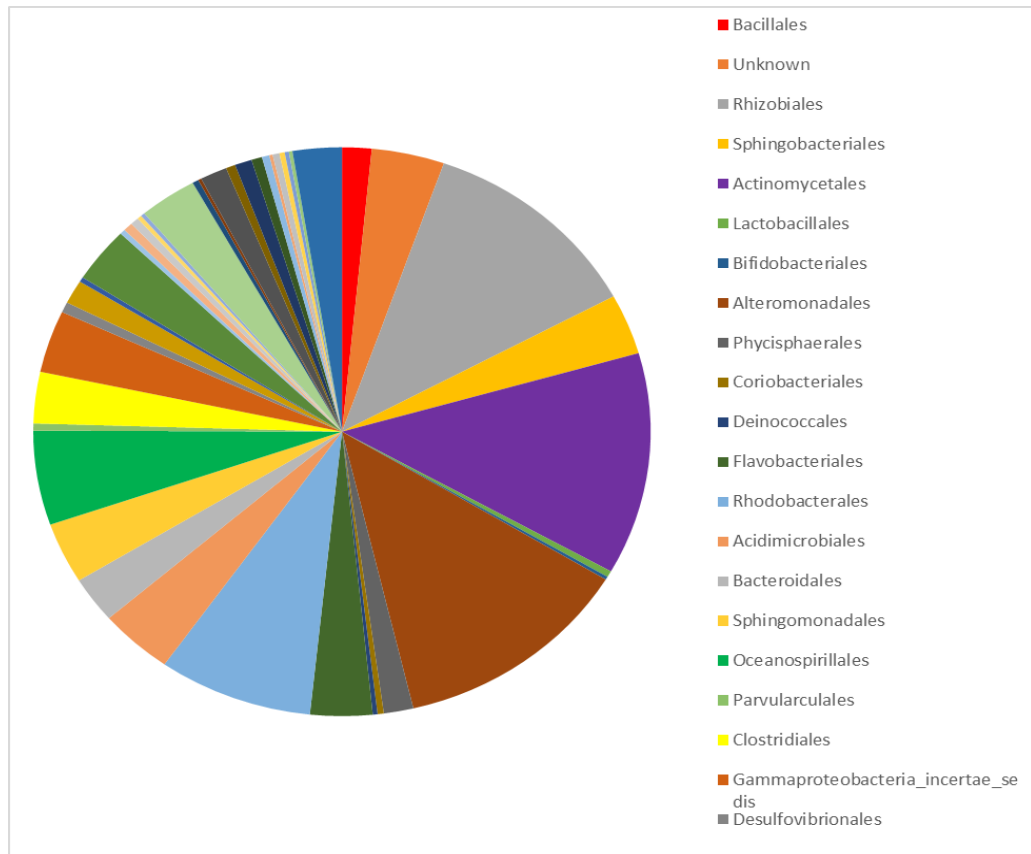


Figure 4(c): Taxonomic distribution based on Order of the total sequence reads obtained from illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria. Others are any OTUs less than or equal to 0.1 (i.e. ≤ 0.1).

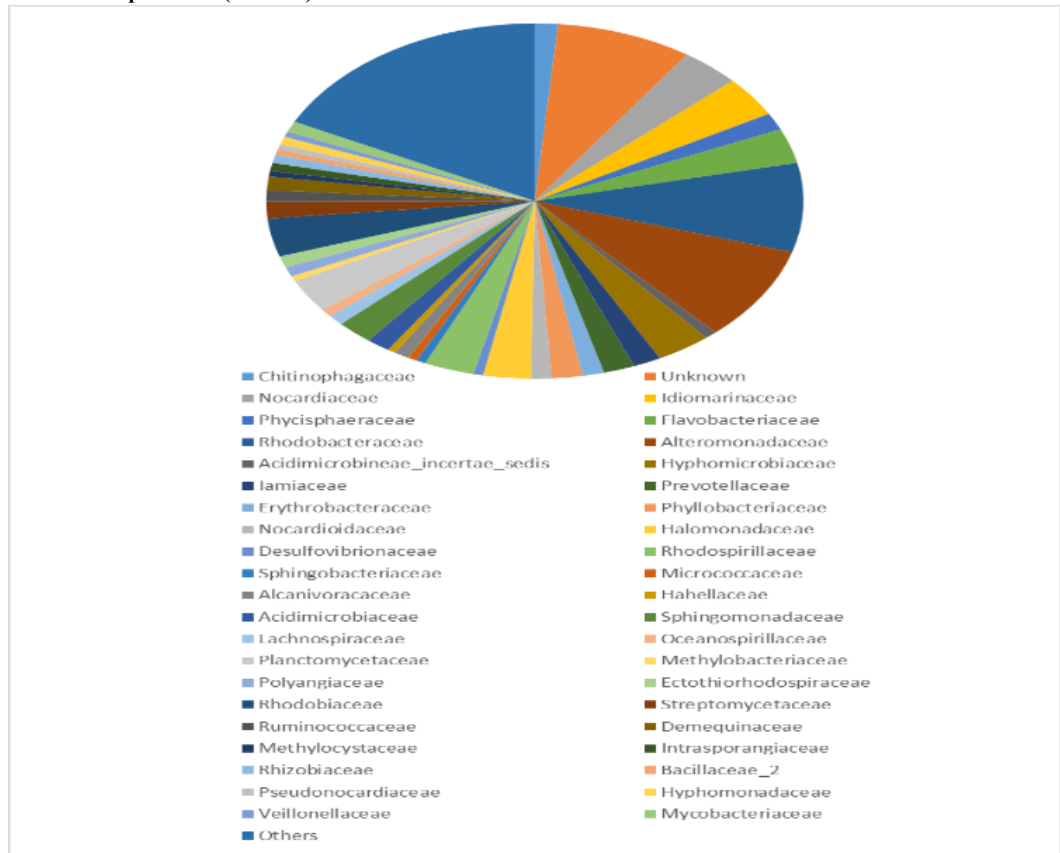


Figure 4(d): Taxonomic distribution based on Family of the total sequence reads obtained from illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria. Others are any OTUs less than or equal to 0.4 (i.e. ≤ 0.4).

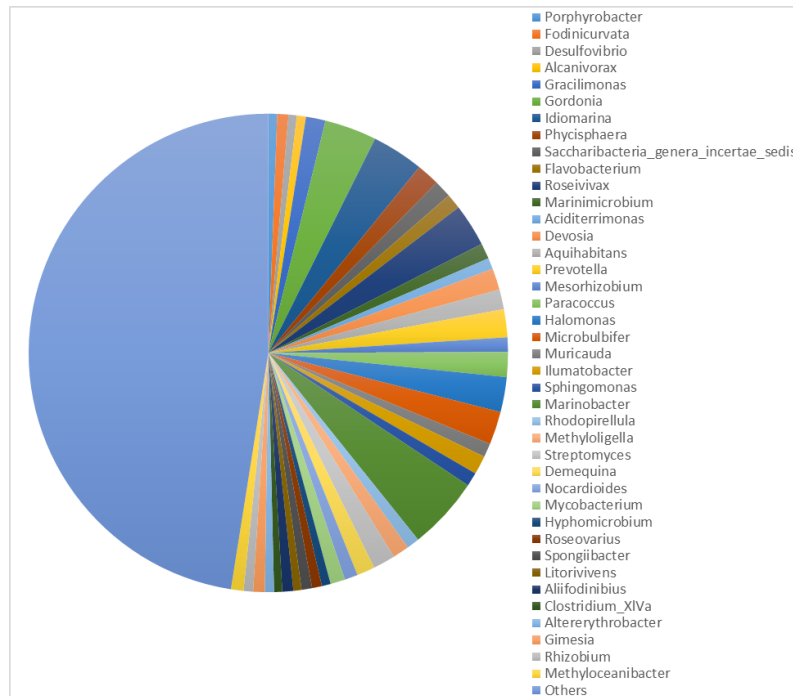


Figure 4(e): Taxonomic distribution based on Genus of the total sequence reads obtained from illumina sequencing of metagenomic DNA of decomposing wood wastes in the Lagos lagoon, Nigeria. Others are any OTUs less than or equal to 0.5 (i.e. ≤ 0.5).

IV. CONCLUSION

The combined use of T-RFLP and illumina sequencing has provided an unprecedented robust data on the complex microbial taxa structure and phylogenetic profile of naturally colonized decomposing wood wastes in the Lagos lagoon, revealing greater heterogeneity than earlier reported. The taxonomic identities and operational taxonomic unit (OTU) affiliations of the consortia microbiota may open a frontier needed for the development of efficient lignocellulose and wood wastes degradation process in the environment.

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AVAILABILITY OF DATA AND MATERIALS

Paired-end sequence reads from the present study have been deposited in the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information under the BioProject ID PRJNA560344 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA560344/>) and submission ID SUB6178128 (<https://submit.ncbi.nlm.nih.gov/subs/sra/SUB6178128/overview>).

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