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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF AUTOCHTHONOUS CELLULOLYTIC MICROORGANISMS FROM THREE LANDFILL SITES IN LAGOS, SOUTHWEST NIGERIA

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Abstract: *This study was conducted to evaluate and identify native cellulolytic microorganisms to tropical sanitary landfill sites in Lagos, Southwest Nigeria as well as to provide insights into the potential of autochthonous microorganisms in solid waste management. Soil and leachate samples were obtained simultaneously (0 – 30 cm depth) at the same time from three waste dumpsites in Lagos, using aseptic procedures. Thereafter, composite soil samples and leachates were generated independently and transported to the laboratory for analysis. Microorganisms were isolated from both soil and leachate samples using the serial dilution technique on sterile nutrient agar (NA) and potato dextrose agar (PDA). Subsequently, cellulase-producing microbial species were identified using conventional and standard microbiological techniques as well as by cultivation on Starch-casein-agar. Pure cultures of isolates were inoculated on sterile filter paper placed on Starch - casein agar plate. Isolates were selected based on their metabolic capabilities to utilize the filter paper for growth. Screening for utilization of aromatic acids was carried out in 250 mL conical flasks containing composition: minimal agar medium (pH 7.2), 1.0 g/L aromatic acids (vanillic), 1.0 mL trace elements, phosphate buffer and Bromothymol blue as pH indicator. The DNA of some selected isolates with cellulolytic activity were extracted and sequenced using 16S rRNA sequencing, ITS, and bioinformatics tools. Consequently, among the bacterial species, Bacillus sp. had the highest cellulose degradative ability and was the most prevalent (50%) in occurrence among bacterial species while Aspergillus sp. emerged as the most commonly occurring fungal isolate (35.7%). Data of selected sequenced cellulolytic isolates were deposited at NCBI GeneBank with Accession numbers: KP843680.1 (Vibrio tubiashii), MK748310.1 (Aspergillus aculeatinus), LC496490.1 (Aspergillus aculeatus), CP029751.1 (Staphylococcus aureus) and JX144699.1 (Bacillus mycoides). Environmental surveillance of these microorganisms with microbial synergistic capabilities, could transform solid waste management into a highly efficient biotechnological process that facilitates volume reduction, waste recycling and Bioenergy production.*
Keywords: *Biodegradation, cellulose, genomics, landfills, lignocellulose, solid waste, sustainable development*

1. Introduction

Solid wastes are unwanted substances dumped that may be reprocessed, eliminated by incineration or other methods of disposal [1]. Nigeria, a country with a population of over 180 million people [2], is among the producers of solid waste in Africa. A considerable amount of waste is generated from diverse sources, such as commercial, industrial, household, agricultural, and educational establishments [3, 4]. However, despite various policies and regulations in place, solid waste management remains a significant challenge for the authorities, stakeholders, and the general population. Lagos is the largest city in Nigeria and the most populous urban area on the African continent that generates the highest volume of solid wastes annually in Nigeria.

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Consequently, its waste disposal problems are yet to be resolved [5]. Landfilling is a commonly utilized method for the disposal, compaction, and elimination of solid wastes [6, 7]. However, despite the existence of advanced techniques such as waste reduction, recycling, and composting, landfill continues to be the primary waste disposal option globally, resulting in significant environmental hazards.

Landfill is still widely used due to its relatively lower cost and its potential for environmental benefits when designed and operated as bioreactors for harnessing biogas, microbial enzymes, and leachate production [8, 6]. The consequences of improperly managed solid waste include serving as a breeding ground for pests such as rats, flies, and mosquitoes, reducing society's usable land area, obstructing the free flow of traffic, and causing societal problems in residential areas [9]. Improperly managed waste can also negatively impact property values as well as result in acid rain, and cause aquifer contamination [10]. However, solid waste can serve as a source of nutrients for microbial growth that can be converted into stable end-products that are environment - friendly and useful when integrated through industrial enzymatic processes. These enzymes include cellulase, pectinase and amylase [8, 11]. Cellulose is a polymer of glucose bonded together through β-1, 4 glycosidic linkages. Therefore, the degradation of cellulose requires separate approaches involving enzymatic hydrolysis and other complex processes for the production and recovery of products [12]. Cellulase has been identified as one of the most effective enzymes for degrading cellulose, hemicellulose, and lignocellulosic materials, which has great industrial applications [13]. Cellulases are categorized into three primary groups: exoglucanases, endoglucanases, and β-glucosidases. Exoglucanases cleave the outermost glucose units of cellulose chains, while endoglucanases cleave β-1, 4-glycosidic bonds within the cellulose chains. β-Glucosidases are responsible for cleaving the final β-1,4 linkage of cellobiose or small polysaccharides. *In vitro*, complete cellulose degradation is achieved through the combined action of three groups of enzymes: Exo-1, 4 glucanase, Endo-β-1, 4 glucanase, and β-glucosidase. However, Exo-1, 4 glucanase initiates hydrolysis by cleaving the bond from the non-reducing end of cellulose, thereby generating cellobiose molecules. In contrast, β-glycosidase hydrolyzes cellobiose to complete its degradation process [14, 11]. Cellulose degradation is an inevitable biochemical process that occurs within landfills. These anoxic and saturated environments are of significant interest for the discovery of cellulases that can be integrated into various industrial processes [13]. Microorganisms, including bacteria and fungi, have been found to produce diverse range of cellulases for the degradation of cellulosic waste. The bacterial-produced cellulase appears to be affixed to the cell wall and demonstrates limited ability to hydrolyze untreated lignocellulosic materials. Consequently, the degradation of cellulosic biomass plays a vital role in the carbon cycle within the biosphere [15]. The biological conversion of solid waste to bio - resources could mitigate environmental pollution and public health hazards in urban areas, underscoring the importance of cellulase research [16]. The composition of said cellulase primarily consists of organic matter derived from cellulose, making it a readily available resource for the removal of municipal solid waste (MSW) using robust microorganisms [17, 13]. The utilization of lignocellulose biomass as an alternative source of renewable energy production via green

technology is crucial and this synergizes with sustainable development goal initiatives. Lignocellulolytic bacteria such as *Bacillus* sp. can breakdown biomass by producing hydrolytic enzymes which are important in the successful conversion of biomass or lignocellulosic material into renewable energy [18].

2. Materials and methods

2.1. Collection of soil and leachate samples

Soil and leachate samples were collected from three dumpsites under the control of the Lagos State Waste Management Authority (LAWMA) in Lagos, Nigeria. The Hotel-Soluos dumpsite at the Alimosho Local Government, Earth Care Compost at the Ikorodu Local Government and Ojualaro dumpsite at the Epe Local Government Area. These dumpsites were labeled as A, B, and C, respectively. The soil and leachate samples were collected from each dumpsite in the morning between 9.00-10.00 am and in the evening, at 4.00-5.00 pm. Soil samples were collected aseptically at depths of 0-30 cm, using a sterile soil auger (10 cm diameter). Leachate samples were collected in sterile glass bottles at the same depth. The soil samples were labeled AS, BS, and CS, whereas the leachate samples were labeled AL, BL, and CL, respectively. The samples were immediately transported to the laboratory for physicochemical and microbiological analysis. Physicochemical analysis of collected samples were conducted according to the methods described by Bareither et al. [19].

2.2. Isolation of microorganisms from soil and leachate samples

Soil and leachate samples were serially diluted to 10^{-5} dilutions. Approximately, 1 mL sample of diluent 10^{-5} was taken and inoculated in duplicates on sterile Nutrient Agar (NA) plates and Potato Dextrose Agar (PDA) plates simultaneously using the spread plate technique. This was followed by incubation at 28 ± 2 ℃ for 24 – 48 h for the NA plates and for 4 – 7 days for the PDA plates. These plates were examined for growth after incubation and the number of colonies were counted to determine the viable count of microorganisms in the samples. Representative colonies were purified by repeated streaking on NA and PDA plates. Colonial morphology, microscopic morphology, and biochemical tests were used to identify isolated bacteria and fungi. The identified strains were kept at a low temperature (4 ℃) on PDA and NA slants [20, 21].

2.3. Biochemical characterization

Biochemical characterization of bacterial isolates was done following the procedures listed in microbiological methods [20, 22]. Catalase test, Oxidase test, Indole test, Methyl red test, Voges-Proskauer test, Citrate Utilization test, Gelatin Hydrolysis test, Starch Hydrolysis test, Urease test, and Sugar Fermentation test were among the biochemical tests carried out.

2.4. Morphological examination offungal cells

The potential fungal spore head, mycelium color, and hypha characteristics of fungal isolates were examined under the light microscope. Young mycelia were cut from the periphery of each culture by using a sterile razor blade and placed on clean glass slides. The cut sections were stained with lactophenol cotton blue and examined under a microscope with a × 40 objective lenses [23, 24].

2.5. Screening for cellulolytic activity

Preliminary screening of the cellulose-degrading capability of the bacterial and fungal isolates were performed by inoculating their pure cultures on sterile filter paper placed on Starch-Casein agar

plates with the following composition: Casein, Potassium nitrate, Magnesium sulfate heptahydrate, Dipotassium phosphate, Sodium chloride, Calcium carbonate, Iron (II) sulphate, agar and distilled water at pH 7.0 - 7.2. Isolates were selected based on their ability to utilize the filter paper. Thereafter, screening for the utilization of aromatic acids was carried out in 250 mL conical flasks containing minimal agar medium with the following composition: minimal agar medium (pH 7.2), 1.0 g/L aromatic acids (vanillic), trace elements (1.0 mL), phosphate buffer, and pH indicator bromothymol blue. The catabolism of aromatic acids results in increased pH of the medium, which can be seen visually by a change in color from green
(pH 7.2) to blue (pH > 7.2) [25, 26].

2.6. DNA extraction and amplification of ITS Gene

DNA Extraction

Approximately 80 mg (wet weight) of fungal cells that had been re-suspended in up to 200µl of deionized water in a ZR BashingBead™ Lysis Tube was obtained. It was secured in a bead beater fitted with a 2.0 mL tube holder assembly (Scientific Industries Disruptor Genie™, Cat. No. S6001-2 from Zymo Research Corp.) and processed at the maximum speed for 5 min. Then, the ZR BashingBead™ Lysis Tube was centrifuged at ≥10,000 × g for 1 min. Approximately, 400 µL of supernatant was transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 rpm (7,000 × g) for 1 min. The base of the Zymo-Spin™ IV Spin Filter was snapped off before use. Thereafter, 1,200 µL of Fungal DNA Binding Buffer was added to the filtrate in the collection tube of step four. Approximately, 800 µL of the mixture from step five was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 × g for 1 min. The flow-through was discarded from the Collection Tube and step six was repeated. Then, 200 µL DNA Per-Wash Buffer was added to the Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 × g for 1 min.

Thereafter, 500 µL of Fungal DNA Wash Buffer was added to the Zymo-Spin™ IIC Column and centrifuged at 10,000 × g for 1 min. The Zymo-Spin™ IIC Column was transferred to a clean 1.5 mL micro-centrifuge tube and 100 µL DNA Elution Buffer was added directly to the column matrix. It was then centrifuged at 10,000 × g for 30 seconds to elute DNA. Bacterial isolate DNA was extracted following the Zymo research protocols [27, 28, 29]. The sequence information was analyzed using NCBI Blast.

2.7. Polymerase Chain Reaction (PCR)

Amplification of the ITS/16S rRNA Gene and Sequencing

PCR to amplify the ITS gene of the fungal isolates using the primer pair ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') was done. Solis Biodyne 5× HOT FIREPol Blend Master mix was used. The bacterial 16S rRNA gene isolates were amplified following the protocols of Qiagen [29]. PCR was performed in 25 µL of a reaction mixture, and the reaction concentration was brought down from 5× concentration to 1× concentration containing 1× Blend Master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25 pMol of each primer (BIOMERS, Germany) and 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne). Additional Taq DNA polymerase was incorporated into the reaction mixture to obtain a final concentration of 2.5 units of Taq DNA polymerase, proof-reading Enzyme, 2 µL of

the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in an Eppendorf Vapo Protect Thermal Cycler (Nexus Series U.S.A.) for an initial denaturation at 95 °C for 15 min, followed by 35 amplification cycles of 30 s at 95 °C, 1 min at 58 °C, and 1 min 30 s at 72 °C. This was followed by a final extension step for 10 min at 72 °C. The amplification product was separated on a 1.5% agarose gel, and electrophoresis was performed at 80V for 1 hour 30 min. After electrophoresis, DNA bands were visualized by Ethidium bromide staining and 100bp DNA ladder was used as the DNA molecular weight standard [27, 30, 28].

2.8. Data analysis

DNA sequence generated in this study were blasted against the ITS/16S rRNA-type strain database on NCBI. The threshold for identification was set according to Wang et al. [31], with modifications to adapt species with a high congeneric sequence divergence range. An isolate was assigned to the species level if the best matching reference species showed ≥85% homology and the next best matching reference species showed at least 0.8% less sequence homology. A strain or isolate was assigned to the genus level when there was 80% to 85% homology to the best-matching species, or when more than one sequence entry of several species from the same genus showed ≥90% homology.

For sequences with <90% homology to the best match, an assessment of the congeneric sequence divergence range was performed by blasting an arbitrarily chosen reference sequence of a type organism for the genus of interest against the ITS/16S rRNA gene sequence database. The sequence divergence observed in these BLAST hits was used to adjust the congeneric sequence divergence range, and this range was used to determine the plausibility of the identification of sequences with <95% homology to the blast's best match. Where homology fell below 90% and without the support of a congeneric sequence divergence value, identification was considered unsuccessful. Phylogeny was constructed to support the identification by ITS sequence using the Fast-Minimum Evolution algorithm deployed on NCBI and visualized on MEGA11 [32]. The counts of species and genera were used as estimates of species and genus diversity, respectively. In the estimation of species diversity, unidentified isolates were considered to be different from the identified species and were therefore counted as different species. In the estimation of genus diversity, as opposed to the treatment in the estimation of species diversity, unidentified isolates were removed from the analysis. Other descriptive and inferential statistics were carried out in R using the following R packages: base [33], reshape2 [34], dplyr [35], ggplot2 [36], ggpubr [37], qpcR [38], knitr [39, 40, 41]. Data wrangling was achieved using Reshape2 and dplyr, inferential statistics were carried out in R, and data were visualized using ggplot2, ggpubr, qpcR, and knitr.

3. Results

Soil samples from all dumpsites mean temperature ranged between 30 °C and 34 °C, which falls within the mesophilic temperature range (20 - 45 °C) same as reported by Lu et al. [42]. A total of fourteen microorganisms were isolated. However, ten of these isolates were bacterial species and four were fungal species. These bacterial isolates belonged to the genera: *Bacillus, Enterobacter, Pseudomonas, Staphylococcus, Streptococcus,* based on cultural as well as biochemical characterization [20, 23]. The colonial and macroscopic characterization of the fungal isolates [23] showed that they

belong to the genera *Penicillium, Aspergillus, Fusarium,* and *Trichoderma* (Table 2). Hotel - Soluos Igando dumpsite in Alimosho local government had the highest percentage occurrence of both bacterial and fungal species (41.67% and 42.86%, respectively) among the dumpsites (Fig. 1 and 2).

The percentage of occurrence of the bacterial and fungal isolates from the three dumpsites studied were recorded, with *Bacillus* sp. having the highest value (50%) among the bacterial isolates, while *Aspergillus* sp. ranked the highest among the fungal isolates (35.7%) (Table 3 and 4). This observation agreed with the findings of Holt [43], who reported that the relative abundance of strains of the *Bacillus* genus in refuse samples was not surprising since this ubiquitous genus was known to include cellulolytic species and they were commonly found in soil and plant litter as well as compost, where they played a major role in biodegradation and bio-conversion of macromolecules.

A total of eleven isolates out of the fourteen isolates showed cellulase production on primary screening using Starch - Casein agar (SCA) with sterile filter paper serving as the substrate and secondary screening for utilization of aromatic acids. Some of the bacterial isolates encountered in this study showed a significant degradation of the filter paper by utilizing the cellulose present in the SCA. Highest degradative activity was observed in *Bacillus licheniformis* and *Enterobacter aerogens. Bacillus subtilis, Bacillus sphaericus,* and *Streptococcus sp.* showed moderate degradative ability, while *Pseudomonas cepaciae*, *Staphylococcus albus*, *Bacillus coagulans*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* showed no cellulose degradative ability (Table 1, 5 and 6).

Fungal isolates encountered in this study showed significantly greater degradative ability than the bacterial isolates (Table 7 and 8).

This study showed that municipal solid waste (MSW) dumpsites are principal sources of cellulolytic microorganisms and these microorganisms are recommended as sources of cellulases which can be used for both industrial enzyme production and the management of cellulose-containing solid wastes. This suggested their potential application in industries such as textiles, laundry, detergents, pulp and paper, as well as solid waste management. Five cellulolytic microorganisms were selected from cultural and morphological examination for 16S rRNA/ITS gene sequence analysis. However, gene sequencing and microbiological analysis using (https://blast.ncbi.nlm.nih.gov/Blast.cgi) validated three bacterial cellulolytic microorganisms. Surprisingly, these isolates were observed to have profound cellulase activity using cultural techniques. They were *Vibrio tubiashii* (Accession number KP843680.1), *Bacillus mycoides* (Accession number JX144699.1) and *Staphylococcus aureus* (Accession number CP029751.1) while cellulolytic fungal isolates were *Aspergillus aculeatinus* (Accession number MK748310.1) and 85.11% identity with similar strain in the GenBank, and *Aspergillus aculeatus* (Accession number LC496490.1) with 98.51% identity with similar strain in the GenBank. Notably both were obtained from soil and leachate samples from Hotel-Soluos and Earthcare compost landfills respectively (Fig. 1 and 2). The gene sequences for *Staphylococcus aureus* (Accession number CP 029751.1) were too large for BLAST; the bioinformatics tool engaged. Genomic sequencing of these isolates from all the wastes dumpsites gave incontrovertible evidence of the identities of these isolates, using the 16S rRNA and

ITS for bacterial genome and fungal genome identification respectively. The dataset repository base for these isolates is (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
Furthermore, both *Aspergillus* strains recovered in this study possess common ancestral origin as seen in Fig. 3 and 4. Similarly, *Bacillus mycoides* had common ancestral origin with *Pseudomonas alcaligenes.* This evidenced evolutionary diversity among microorganisms within landfills (Fig. 6). Hence, it is important to note that both identification methods were essential in harmonious characterization of microbes as observed in this study. It is also noteworthy that extraction and purification of the cellulolytic microbes (bacterial and fungal isolates) is needed in further studies for exact quantification of the hydrolytic capacity of the isolates for industrial applications.
Correlation analysis for the two variables (L1 and L2) under consideration suggested a high level of relationship between the variables with a correlation value of (0.996). Testing for the significant relationship / independence of the two variables using T-paired sampled test, the result showed that the variables were not significantly related since the significant value of .300 is greater than *p*-value of 0.05.
Correlation analysis for the two variables (S1 and S2) under consideration suggested a high level of relationship between the variables with a correlation value of 0.997. Testing for the significant relationship or independence of the two variables using T-paired sampled test, the result showed that the variables were not significantly related since the significant value of .430 is greater than *p*-value of 0.05.

4. Discussion

The MSW dumpsites under investigation was found to accommodate diverse microbial community as well as their potential for the remediation of notorious and hazardous contaminants. This was corroborated by Dhiman *et al*. [44]. The physicochemical properties of the soil and leachate samples suggested that the landfill sites were rich in nutrients and that diverse microbial communities inhabited the landfill dump sites [44]. The relatively high concentration of SO_4^{2-} detected in both soil and leachate samples suggested the presence of acid rain in the immediate environment of the landfill sites. The detection of hydrocarbons in both leachate and soil samples indicated that waste automobile oils were discharged from both nearby industries and automobile workshops (Tables 9 and 10), thus raising public health and environmental quality concerns [45].
Consequently, microbial degradation of cellulose, hemicellulose, and lignin, which has been reported to represent 91% of the methane potential of fresh refuse according to Barlaz *et al*. [46], is a great reservoir for biogas production and power generation [18]. These bacterial populations in landfill environments are diverse, complex, and largely unexplored [47, 44]. This was corroborated by the diverse populations of microbes detected and isolated in this study. and the selected microbes were characterized using molecular techniques Thus, incontrovertibly synergistic interactions among autochthonous microbial populations in landfill sites contributed significantly to the process of hydrolysis of lignocellulosic polymer materials in natural environment [48] which inferentially suggested that combined action of these microbial enzymes; exoglucanase, endoglucanase and *β*-glucosidase were elicited by the microbial communities in the MSW to achieve mineralization of lignocellulosic materials [49]. However, the fungal species from this study showed more profound cellulolytic

Table 1.

Cultural morphology and biochemical characteristics of bacterial isolates

Isolate Code	Color	Gram Reaction	Cellular Morphology	Catalase	Oxidase	Urease Test	Indole Test	Methyl Red Test	Voges-Proskauer Test	Gelatin Hydrolysis	Starch Hydrolysis	Citrate Utilization Test	Glucose	Sucrose	Lactose	Probable Identity
AS1	Cream	−	R	+	−	−	−	−	−	+	+	+	+	+	−	*Bacillus licheniformis*
AS2	Cream	+	R	+	+	−	−	−	+	+	+	+	+	+	+	*Bacillus subtilis*
BS1	Cream	+	R	+	+	+	−	−	+	+	−	−	+	−	−	*Bacillus sphaericus*
BS2	Cream	−	R	+	+	−	−	−	−	+	−	−	+	+	−	*Pseudomonas cepaciae*
CS1	Cream	+	C	+	+	−	−	−	+	+	−	−	+	+	+	*Staphylococcus albus*
CS2	Cream	−	R	+	+	−	−	−	+	+	−	+	+	−	−	*Bacillus coagulans*
AL1	Cream	+	C	−	−	+	+	−	−	+	+	+	+	+	+	*Streptococcus*
AL2	Cream	+	R	+	+	−	−	−	+	+	+	+	+	+	+	*Bacillus subtilis*
BL1	Cream	−	R	+	−	−	−	−	−	+	+	+	+	+	−	*Bacillus licheniformis*
BL2	Green	−	R	+	+	−	−	−	−	+	−	+	+	−	−	*Pseudomonas aeruginosa*
CL1	Yellow	+	C	+	−	+	−	−	+	−	−	−	+	+	+	*Staphylococcus aureus*
CL2	White	−	R	+	−	−	−	−	+	−	−	+	+	+	+	*Enterobacter aerogenes*

KEY:

AL: Hotel-Solous Dumpsite Leachate Sample
AS: Hotel-Solous Dumpsite Soil Sample
BL: Earthcare Compost Leachate Sample
BS: Earthcare Compost Soil Sample
CL: Ojualaro Dumpsite Leachate Sample
CS: Ojualaro Dumpsite Soil Sample
C: Cocci
R: Rod
+: Positive
-: Negative

Table 2.

Colonial and microscopic characteristics of fungal isolates

Fungal isolates	Macro-culture (Colonial characteristics)	Microscopy (Morphological features)
Aspergillus niger	Black colonies	Non-septate conidiophores, Aerial hyphae
Fusarium sp.	Magenta pink colonies	Branched conidiophores, non-septate hyphae
Penicillium sp.	Blue green colonies	Branched conidiophores, septate hyphae
Trichoderma harzianium	White colonies	Branched conidiophores, septate hyphae

Table 3.

Occurrence of bacterial isolates in dumpsites

Isolated Organisms	AS	BS	CS	AL	BL	CL	Total Number of Isolates	% Occurrence
Bacillus	2	1	1	1	1	ND	6	50
Enterobacter	ND	ND	ND	ND	ND	1	1	8.3
Pseudomonas	ND	1	ND	ND	1	ND	2	16.7
Staphylococcus	ND	ND	1	ND	ND	1	2	16.7
Streptococcus	ND	ND	ND	ND	ND	ND	1	8.3

KEY: *%: Percentage*
ND: Not Detected
AS: Hotel-Solous Dumpsites Soil Sample
BS: Earthcare Compost Soil Sample
CS: Epe Dumpsite Soil Sample
AL: Hotel-Solous Dumpsite Leachate Sample
BL: Earthcare Compost Leachate Sample
CL: Epe Dumpsite Leachate Sample

Table 4.

Occurrence of fungal isolates in the three landfill sites

Isolated Organisms	AS	BS	CS	AL	BL	CL	Total Number of Isolates	% Occurrence
Aspergillus	1	1	ND	ND	1	2	5	35.7
Fusarium	ND	ND	1	1	ND	ND	2	14.3
Penicillium	1	ND	ND	ND	1	1	3	21.4
Trichoderma	2	ND	1	1	ND	ND	4	28.6

KEY:
%: Percentage
ND: Not Detected
AS: Hotel-Solous Dumpsite Soil Sample
BS: Earthcare Compost Soil Sample
CS: Epe Dumpsite Soil Sample
AL: *Hotel-Solous Dumpsite Leachate Sample*
BL: Earthcare Compost Leachate Sample
CL: *Epe Dumpsite Leachate Sample*

Table 5.

Cellulose degradative ability of bacterial isolates from the three landfill sites

Bacterial Isolates	Growth
AS1	++
AS2	++
BS1	+
BS2	+
CS1	_
CS2	+
AL1	_
BL1	++
BL2	+
CL1	_
CL2	+

KEY: ++*: Excellent growth*
+*: Moderate growth*
-*: No growth*
AS: Hotel-Solous Dumpsite Soil Sample
BS: Earthcare Compost Soil Sample
CS: Ojualaro Dumpsite Soil Sample
AL: Hotel-Solous Dumpsite Leachate Sample
BL: Earthcare Compost Leachate Sample
CL: Ojualaro Dumpsite Leachate Sample

Table 6.

Utilization of aromatic acids by bacterial isolates from the three landfill sites

Isolate Code	pH	Color Change
AS1	7.3	Green to Blue
AS2	7.2	Green to Blue
BS1	7.5	Green to Blue
BS2	7.2	Green to Blue
CS2	7.4	Green to Blue
BL1	7.3	Green to Blue
BL2	7.2	Green to Blue
CL2	7.3	Green to Blue

KEY: *AS: Hotel-Solous Dumpsites Soil Sample*
BS: Earth care Compost Soil Sample
AL: Hotel-Solous Dumpsite Leachate Sample
CL: Epe Dumpsite Leachate Sample
BL: Earth care Compost Leachate Sample
CS: Epe Dumpsite Soil Sample

Table 7.

Cellulose degradative ability of fungal isolates from the three landfill sites

Fungal Isolates	Growth
Aspergillus niger	++
Fusarium sp.	++
Penicillium sp.	–
Trichoderma harzanium	+

KEY: ++*: Excellent growth*
+: Moderate growth
-: No growth

Table 8.

Utilization of Aromatic Acids by the Fungal Isolates from the Three Landfill Sites

ISOLATED ORGANISMS	pH	COLOR CHANGE
Aspergillus sp.	7.3	Green to blue
Fusarium sp.	7.2	Green to blue
Trichoderma harzanium	7.3	Green to blue

Table 9.

Determination of Physiochemical Parameters of Composite Leachate Samples

PARAMETERS	L_1	L_2
Hydrocarbon (ppm)	0.04	0.02
NH_4-N (ppm)	0.03	0.04
NO_3- N (ppm)	27.75	24.17
PO_4^{3-} (ppm)	0.21	0.18
TOM (ppm)	6.50	5.60
$SO2_4$ (ppm)	145.00	85.00
DOC (ppm)	5.53	4.76
pH	7.5	6.5

KEY:
L_1: Morning Leachate Sample
L_2: Evening Leachate Sample
TOM: Total Organic Matter
DOC: Dissolved Organic Carbon

-Test for Table 9.

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	L1	24.0700	8	49.69805	17.57091
	L2	15.7838	8	29.06268	10.27521

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	L1 & L2	8	0.996	0.000

T Paired Samples Test									
		Paired Differences					t	df	Sig.
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	L1 - L2	8.28625	20.92819	7.39923	-9.21016	25.78266	1.120	7	0.300

Table 10.

Determination of Physiochemical Characteristics of the Composite Soil Samples From Waste Dumpsites

PARAMETERS	**S_1**	**S_2**
Hydrocarbon (mg/kg)	0.12	0.09
DOC (mg/kg)	16.15	7.23
NH_4-N (mg/kg)	0.04	0.05
NO_3-N (mg/kg)	2.13	3.04
PO_4^{3-} (mg/kg)	0.02	0.04
TOM	19.00	8.50
SO_4	205.90	333.19
pH	8.0	8.5

KEY: *L_1: Morning Leachate Sample;* *TOM: Total Organic Matter;*
L_2: Evening Leachate Sample; *DOC: Dissolved Organic Carbon*

.

T-Test for Table 10.

Paired Samples Statistics					
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	S1	31.4200	8	70.89978	25.06686
	S2	45.0800	8	116.47437	41.17991

Paired Samples Correlations				
		N	Correlation	Sig.
Pair 1	S1 & S2	8	0.997	0.000

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	S1 - S2	-13.6600	46.13780	16.31218	-52.23216	24.91216	-0.837	7	0.430

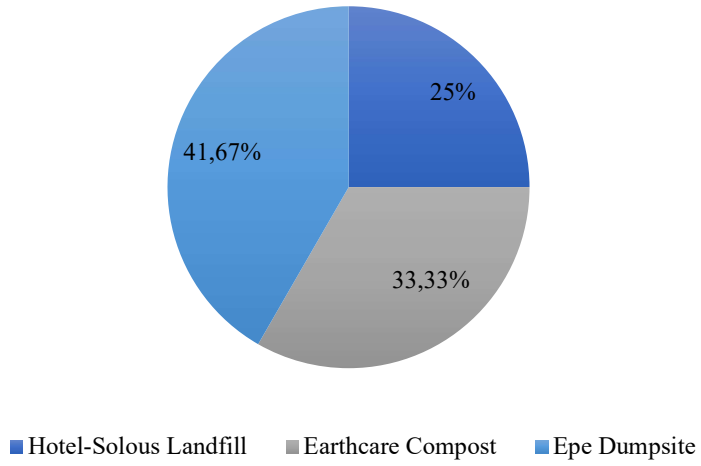

Fig. 1. The Percentage of Fungi Species Found in Dumpsites in Three Lagos State Local Government Areas

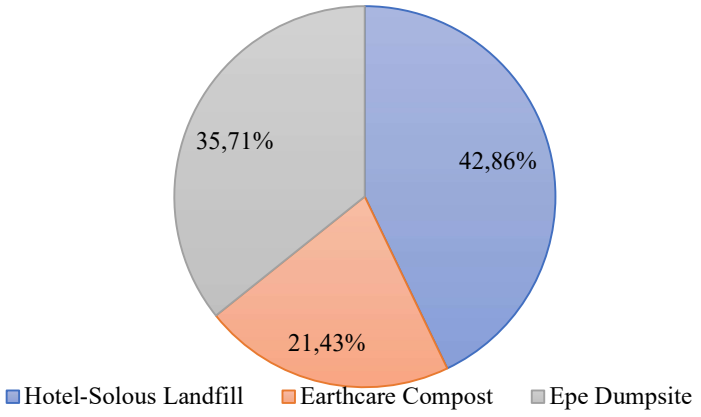

Fig. 2. The percentage of Bacteria Species found in Dumpsites in Three Lagos State Local Government Areas

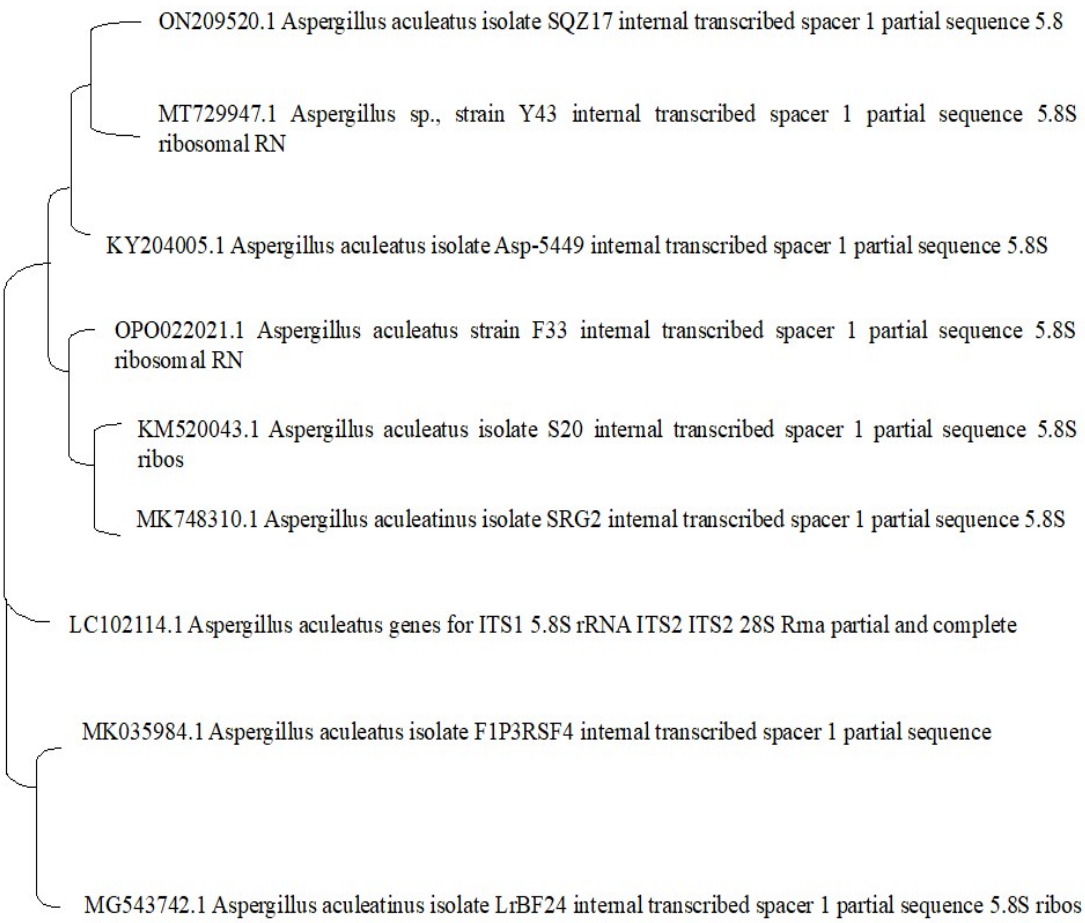

Fig. 3. **The phylogeny tree showing the ancestral closeness of *Aspergillus aculeatinus* (Accession number: MK748310.1) with other *Aspergillus sp*. of similar ancestors**

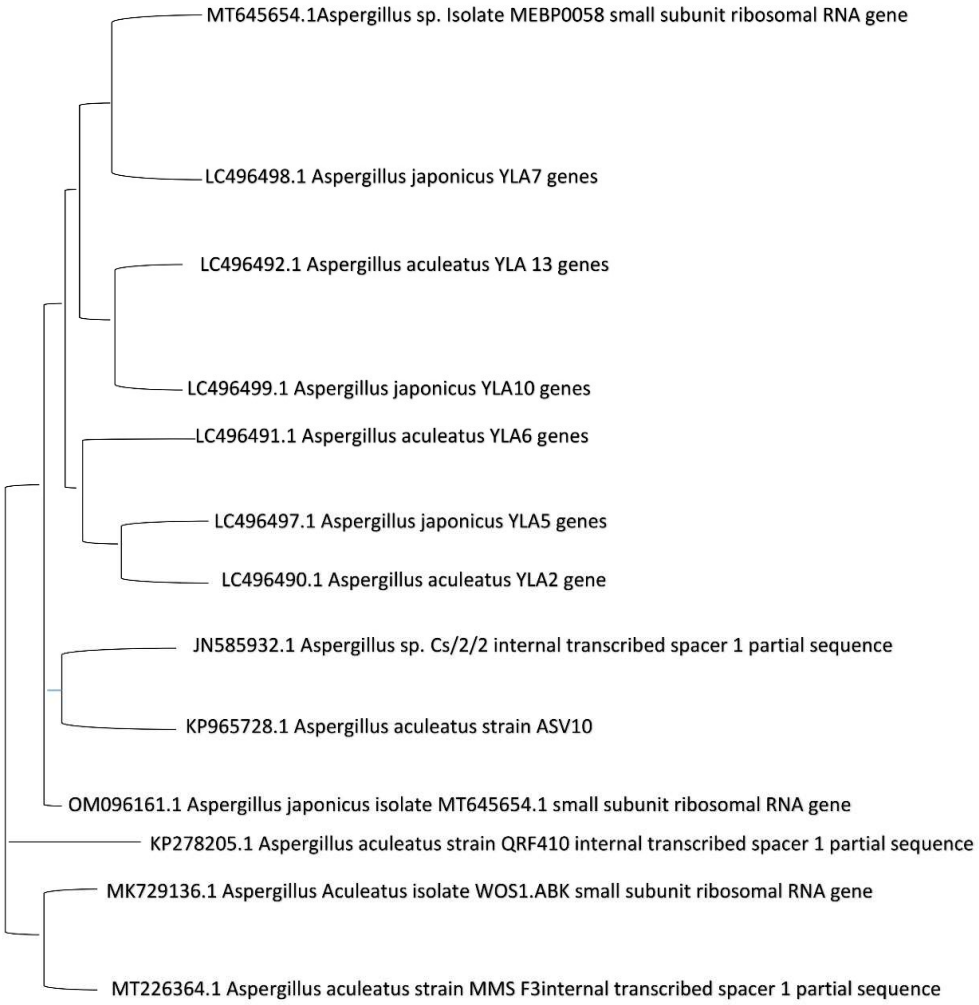

Fig. 4. The phylogeny tree showing the ancestral closeness of *Aspergillus aculeatus* (Accession Number: LC4966490.1) with other *Aspergillus* sp. of similar ancestors

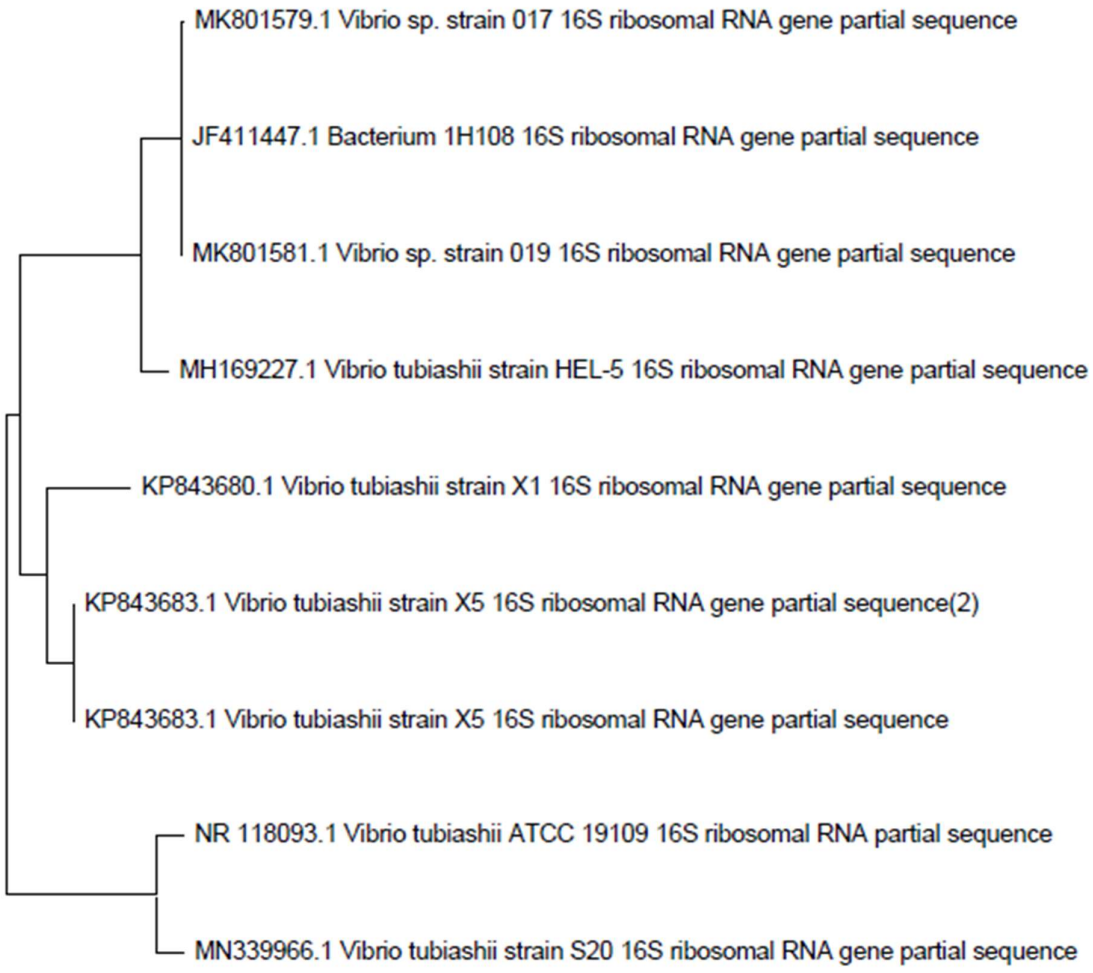

Fig. 5. The phylogeny tree showing the ancestral closeness of *Vibrio tubiashii* (Accession Number: KP843680.1) with other *Vibrio* sp. of similar ancestors

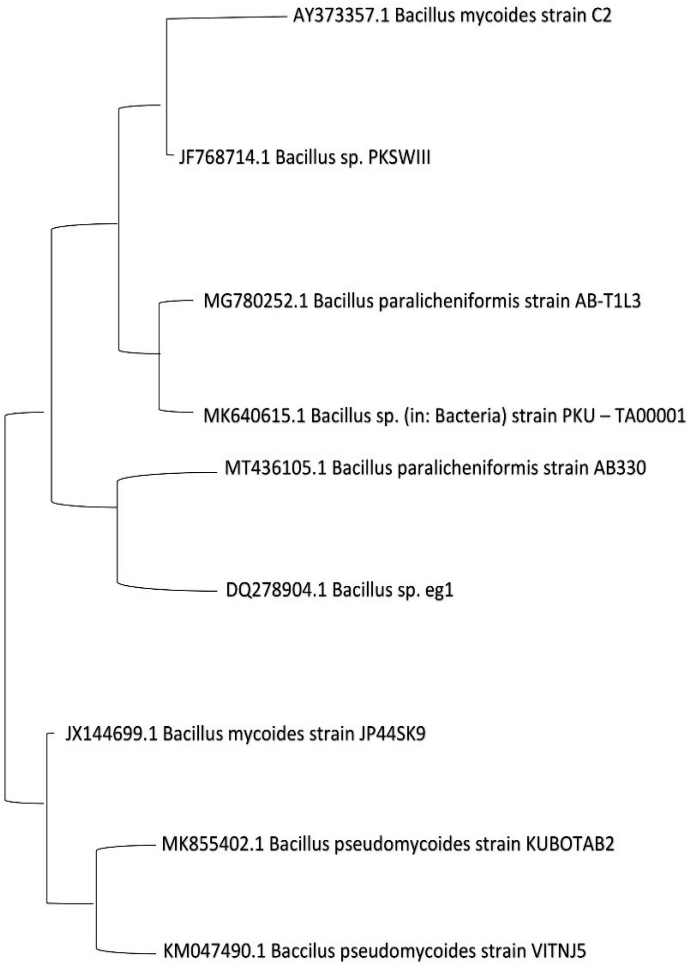

Fig. 6. The phylogeny tree showing the ancestral closeness of *Bacillus mycoides* (Accession Number: JX144699.1) with other *Bacillus* sp. of similar ancestors

activity than the bacterial species. This further established the fact that fungal populations were involved in the entire microbial biodegradation processes and chemical reactions followed by leachate and gas production, heat generation, waste compression and distortion that characterized the sanitary landfills [6, 50]. However, few fungal isolates have been selected for molecular characterization because of the paucity of funds. This result suggested that fungi are better cellulase producers than bacterial species, and this was corroborated by the findings of Guatam *et al*. [51], who discovered that many fungi capable of degrading cellulose synthesize large quantities of extracellular cellulase that are more efficient in depolymerizing cellulose substrates, unlike cellulase produced by bacteria, which appear to be bound to the cell wall and are unable to hydrolyze native lignocellulose preparation to any extent.

This study corroborated the findings of Co & Hug [13]. Although, bacterial populations have been reported with the capacity to produce hydrolytic enzymes for conversion of biomass or lignocellulosic material into renewable energy [18, 44]. Consequently, bio stimulation process has proved to be one of the effective means of remediation of organic pollutants in MSW as well as protection against soil pollution and public health hazards [52]. Groundwater is less likely to be contaminated by microorganisms than surface water [53] but when groundwater becomes contaminated due to the location of landfill site and its topography, it calls for public health concerns since freshwater supplies in developing nations is anchored on groundwater supplies. Groundwater quality subsequently determines its suitability for drinking and irrigational purposes [54]. Groundwater pollution and human health risks caused by leachate leakage has a worldwide environmental health consequence.

Although, it has been reported that setting the isolation distance between landfill sites and groundwater exploitation points would ameliorate the consequences of accidental leachate leakage into groundwater used for drinking water and bath purposes, the fact still remains that landfill microbial community has some pathogenic microbes [55]. The source of pathogenic microbes in MSW and groundwater has been traced to food wastes, pet faeces, absorbent products and biosolids. Food wastes contributed the greatest fecal coliforms [56]. The metagenomic community structure study of a landfill site in Asia detected presence of antibiotic-resistant genes diversity, pathogenic bacteria particularly *B. cereus* [57]. This corroborated the findings of the current study where pathogenic bacteria and fungi were isolated along with non-pathogenic microorganisms from the three landfills investigated, which suggested the existence of potential environmental health risks in landfills located near urban settlements.

5. Conclusions

Municipal landfills are engineered contaminated sites, which are effective and prevalent devices for the disposal of urban wastes, particularly in industrialized cities with fast-growing populations. Culture-based and 16S rRNA gene sequencing methods used in this study, identified the synergistic microbial species composition of three tropical landfill sites in a fast-growing city, Lagos. Cellulolytic microbes from both soil and leachates in this study harbor a variety of cellulases that can be further harnessed for bioconversion and production of industrial enzymes, as well as

serving as cheap natural resources for waste recycling to generate power through biogas production, solid waste volume reduction, and ameliorate public health risks, as well as to guarantee sustainable development. Landfills cited far away from human settlements would enhance public health safety and their integration into an alternative waste-to-energy program would both be environment - friendly and sustainable.

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