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Investigation of the Solid Waste Degradation Potential and Molecular Identification of Bacteria Isolated from Municipal Solid Waste-Contaminated Soils in Uyo Metropolis, Akwa Ibom State, Nigeria

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Abstract

The soil is an important sink for waste disposal which has become a serious environmental challenge due to the associated impacts. This study was a comparative study of the bacterial communities across three municipal solid wastes (MSW)-contamianted soils with a pristine soil in Uyo, Akwa Ibom State, Nigeria; it further screened and molecularly identified the best MSW utilizers among the bacterial isolates obtained from culture-dependent analysis using an enrichment-based assay and Sanger sequencing, respectively. The viable counts from the Oron road site were significantly ($P < 0.05$) higher with $1.47 \times 10^8 \pm 1.3$ CFU g^{-1} at the 0-15cm depth and $1.21 \times 10^8 \pm 2.1$ CFU g^{-1} at the 15- 30 cm depth. The lowest counts were observed from Ikot Ekpene road site with $9.9 \times 10^7 \pm 0.8$ CFU g^{-1} at the 0-15 cm depth and $3.8 \times 10^7 \pm 2.0$ CFU g^{-1} at the 15-30 cm depth. In all, the pristine soil location had the lowest counts of $4.5 \times 10^4 \pm 0.3$ CFU g^{-1} at the 0-15 cm depth and $2.1 \times 10^4 \pm 0.3$ CFU g^{-1} at the 15-30 cm depth. The culture-based characterization identified the isolates as species from the genera Staphylococcus, Enterobacter, Pseudomonas, Escherichia, etc. with those of Bacillus having the highest occurrence. *Bacillus* sp., *Enterococcus* sp. and *Pseudomonas aeruginosa* were found across the dumpsites (100%). *Bacillus* sp. and *Pseudomonas aeruginosa* showed the strongest growth under the degradation-screening conditions and were identified as *Bacillus* sp. (PV810158.1) and *Pseudomonas aeruginosa (*PV810157.1), respectively by the 16S rRNA gene analysis. As indicated, these waste-contaminated soils harbor high bacterial population than the selected pristine soil and different species that are actively using the waste materials as energy and carbon sources for their growth and development. The municipal solid waste-utilization ability of the isolates makes them promising candidates for application in bioeconomy where they can be used for biological MSW treatment and bioremediation of MSW-contaminated soils.

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1.0 Introduction

Wastes describe substances that are discarded due to a perceived lack of utility or usefulness (Buragohain *et al*., 2020). It constitutes different types of which municipal solid waste (MSW) is one. Urban solid waste management is regarded as one of the most immediate problems (Meena et al., 2023). Approximately 2 billion tonnes of municipal solid waste are produced each year around the world and are indiscriminately disposed on lands (Nanda & Berruti, 2021). Improper waste management remains a major challenge, as a substantial portion of waste is deposited in open dumpsites (Eneh, 2025; Atofarati *et al.,* 2023). These dumpsites are common in many cities in developing countries, including Nigeria, and they can have significant impacts on residents and on ecological balance (Gebrekidan *et al*., 2024).Open dumpsite are areas where wastes are disposed in an uncontrolled manner. In these areas, wastes are deposited haphazardly (Remigios, 2010). The areas lack engineered controls: no leachate and landfill-gas management, and typically has limited operational measures that include user registration, control of tipping areas, and waste compaction. According to Gebrekidan *et al*. (2024) and Ajibade *et al.* (2021), open dumping threatens public and animal health, environmental sustainability, and economic development.

Municipal solid waste commonly referred to as garbage, refuse, rubbish, etc. describes solid wastes that are released from family units/households, businesses (shops, offices, markets, etc.) and institution (schools, hospitals, and other public places) (Afshan *et al*., 2023). It is heterogeneous in nature comprising biodegradable and non-biodegradable substances. The biodegradable portion is majorly domestic (kitchen and yard) waste, animal dung, agriculture waste, and more. The other portion is non-biodegradable and complex (Udoumoh *et al*., 2023). Typically, organic waste comprises of 50% cellulose, 15% lignin, 10% hemicellulose, 5% protein as well as starch, pectin and other soluble sugars (Chen *et al*., 2025). Much of this waste is left to decompose in open dumps attracting pests and other vermin and creating an environmental nuisance (Okey *et al*., 2025; Mahajan, 2023).

Microbes degrade the organics into mineral form, principally carbon dioxide (CO_2), by facilitated chemical oxidation (Chen *et al*., 2025) producing smell and other unattractive chaos (Afshan *et al*., 2023). This makes dumpsites breeding grounds for disease-carrying organisms; sources of air, water, and soil pollution; and contributors to greenhouse gas emissions (Njewa *et al.,* 2025; Iyanyi *et al.,* 2020; WHO, 2020). Different methods have been developed for waste disposal including physical, chemical and biological degradation. Biodegradation relies on natural agents such as microbial flora (used as bio-additives/inocula) and is often considered environmentally friendly (Singh, 2025). The inoculation of waste with microbial consortia can lower toxicity by releasing various secondary metabolites. In addition, these microorganisms facilitate the breakdown of complex waste into simpler forms. Reportedly, screening waste-dump sites can yield a wide variety of microbial flora with potential practical applications (Afshan et al., 2023). Microbial community structure can change when contaminants are introduced into an environment. These changes may include increased bacterial abundance, shifts in enzyme kinetics, and other physiological responses. Over time, microbial communities may adapt by enriching specific species capable of using the available substrates and/or tolerating the toxic effects of the contaminants (Uko *et al*., 2020). Certain microbial groups are well recognized for their role in biodegradation (Buragohain *et al*., 2020). Microbiological evaluation and biodegradation studies of municipal solid waste as reported by Pysarenko *et al.* (2025) and Antai *et al.* (2016) revealed the microbial ability to degrade these waste materials. Studies of

microbial characterization of open dumpsites revealed diverse microbial composition including E*scherichia coli*, *Bacillus* spp., *Klebsiella pneumoniae*, Staphyloccus aureus, Micrococcus species, Actinomyces Israeli, Enterobacter aerogenes, *Pseudomonas* spp., *Aspergillus niger, Penicillium* spp., Mucor species, *Rhizopus* spp. etc. as reported by Onuoha *et al*. (2026) and Ikon *et al*. (2022) among others in Nigeria. Reports of studies of MSW dumpsites in Uyo by Nicholas *et al*. (2026); Uchenna, *et al.* (2026); Udoumoh *et al*. (2023); and Nta et al. (2020) revealed poor impacts on the soil quality and the sites as reservoirs of microbial communities with functional capabilities particularly organic waste degradation.

Evaluation of microbial residents of dumpsites provides valuable information for environmental management and ecosystem remediation strategies (Selvarajan, *et al.,* 2022). Previous studies focused on the characterization and identification of microorganisms from MSW dumpsites using cultural-dependent methods (Uchenna, *et al*., 2026; Ikon, *et al*., 2022; Antai *et al.,* 2016). The microbial genomes hold a vast amount of information on evaluation. Molecular method is very valuable for the study of the microbial world including microbial identification (Udotong *et al.,* 2015). The 16S rRNA gene analysis enables resourceful investigation of the members of the microbial (Uko *et al.,* 2019). Information on the relationship between bacterial abundance, degradation potential, and molecular identity seems to be lacking on Oron road, Ukana Offot and Ikot Ekpene road MSW dumpsites in Uyo metropolis. This study investigated bacterial abundance and characterization using cultural-dependent methods, degradation potential and molecular identification of selected bacterial isolates across these three municipal solid waste-contaminated soils in Uyo using growth kinetics and 16S rRNA gene sequencing analysis. This study hypothesized that Oron road, Ukana Offot and Ikot Ekpene road dumpsites soils will have higher bacterial loads and the isolates will show greater MSW-degradation potential (determined form their higher growth kinetics/viable counts in the 72-h MSW test) than pristine/control soil.

2.0 Materials and Methods

2.1 Study area

This study was conducted in Uyo metropolis, Akwa Ibom State, Nigeria. Akwa Ibom State was created on 23rd September, 1987, with the promulgation of Decree 24 by General Ibrahim Badamosi Babangida, the military president of Nigeria. Uyo was chosen as the capital of Akwa-Ibom State because of its centrality and historical import. By the end of the 19th century, Uyo like most Nigerian communities was a rural place. However, beginning from the early twentieth century, urbanization began to take roots at Uyo. With the urban status, the city began to experience population growth in her urban centres, and some nearby rural areas which has resulted in various impacts on the environmental systems. Uyo has its boundaries in six local government areas namely, Ibiono/Itu (North), Uruan (East), Ibesikpo-Asutan and Etinan (South), and Abak (West) (Aniefiok-Ezemonye, 2023). Like other major cities in Nigeria, Uyo generates enormous municipal solid wastes that are dumped at different sites within the metropolis. These dumpsites are operated as open dumpsites. According to Okey *et al.* (2013), the average rate of municipal solid generation in Uyo is approximately 0.54 kg per capita per day, with a range between 0.49 and 0.60 kg/capita/day. This estimate reflects the growing urban population and increasing waste burden within the metropolis. According to Bassey *et al*., (2024) the municipal solid waste in Uyo is estimated at 1.34 kg per capita per day. Photographs of the study locations are below (Figure1 (A-D).

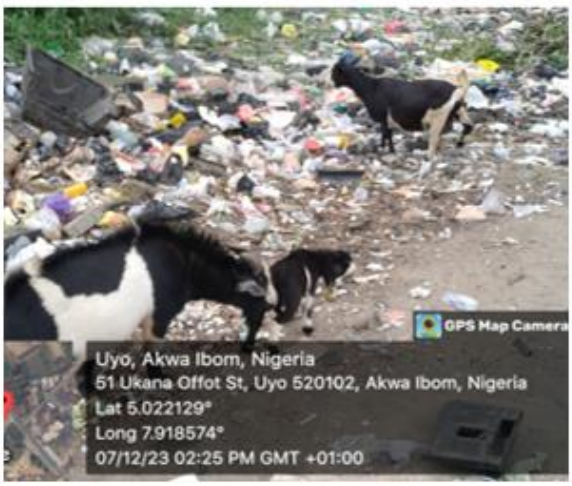
2.2 Sample collection

A total of 16 soil samples were collected for this study (Table 1). Fourteen (14) soil samples were collected from the three dumpsite designated at Oron road (Lat 5.030837° and Long 7.933541°), Ukana Offot

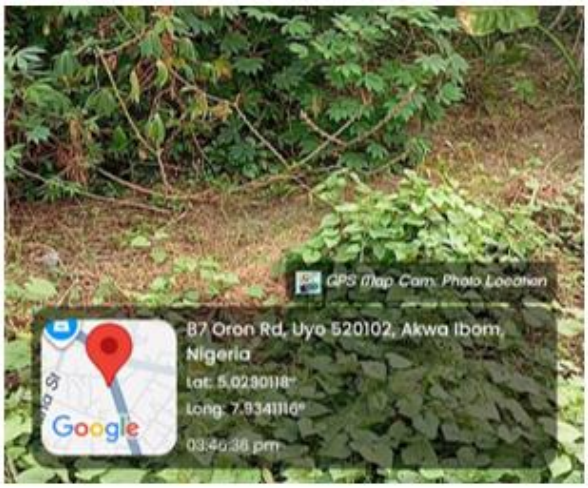
(Latitude 5.022129º and Longitude 7.918574º), Ikot Ekpene road (Latitude 5.043752º and Longitude 7.901984º) and 4 soil sample from the pristine soil which was a farmland located at Oron road (Latitude 5.0290118º and Longitude 7.9341116º). The samples were taken 20 m apart between two points within each location. Soil auger was used to collect the top soil (0 - 15 cm) and the subsoil (15 - 30 cm) from each location (2 samples from each soil depth making it 16 samples) into separate well-labeled sterile polythene bags and transported on ice-packed cooler to Microbiology laboratory, Akwa Ibom State University for microbiological analysis.

A: Oron Road

B: Ikot Ekpene Road



C: Ukana Offot Street



D: The Pristine Soil

Figure 1 (A-D): Photographs of Sampling Locations

Table 1: Sampling Design

S/N	Location	Coordinate	Depth (cm)	Number of (Independent) Samples
1	Oron Road	Lat 5.030837º and Long 7.933541º	0–15	2
			15–30	2
2	Ukana Offot	Lat 5.022129º and Long 7.918574º	0–15	2
			15–30	2
3	Ikot Ekpene Road	Lat 5.043752º and Long 7.901984º	0–15	2
			15–30	2
4	Pristine soil (Oron Road)	Lat. 5.0290118º and Long 7.9341116º)	0–15	2
			15–30	2
		Total		16

2.3 Enumeration and Isolation of Bacteria

Soil samples for the isolation of bacteria were prepared using serial dilution technique and inoculated using pour plating technique. Exactly 1 g of each soil sample was aseptically added to 9 mL of sterile normal saline (0.85 % NaCl) in a test tube, votexed to form 10^{-1} dilution factor under aseptic conditions. The soil sample was serially diluted by taking 1 mL from the 10^{-1} dilution factor into another test tube containing 9 ml of sterile normal saline to make 10^{-2} dilution factor. The same procedure was repeated up to 10^{-6} dilution factor. After the dilution, 1 ml aliquot from the dilutions 10^{-6} was inoculated into sterile Petri dishes in duplicates and about 15 mL of molten nutrient agar for total heterotrophic bacteria and eosin methylene blue agar for *E. coli* presence, were poured into the plates, swirled to mix, and incubated at 37 °C for 24 hours. Plates having countable colonies (30 – 300 colonies) were selected and the colonies enumerated and expressed as colony forming units per gram (CFU g^{-1}) of sample. Discrete colonies were purified by repeated sub-culturing on nutrient agar plate and pure cultures were preserved on agar slants in Bijou bottles in the refrigerator at 4 °C. The total heterotrophic bacterial count per location was calculated from the average of the duplicate plates and expressed as colony forming units per gram (cfu g^{-1}) of sample using the formula:

$$CFUg^{-1} = \frac{Number\ of\ colonies \times Dilution \times factor}{Volume\ of\ culture\ plated\ in\ milliliter\ (mL)}$$

2.4 Culture-based Characterization and Identification of the Bacterial Isolates

Pure cultures of bacteria were identified based on cultural characteristics, microscopic techniques and also enzymatic reactions as described by Ikot *et al*. (2026). The tests were catalase, methyl red, Voges-Proskauer, indole, oxidase, urease, sugar fermentation, etc. The characterized bacterial colonies were identified as described in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.5 Determination of the Degradation Potential of the Bacterial Isolates

The ability of the bacterial isolates from the MSW to utilize the waste (substrate) as a source of carbon and energy was assessed using an enrichment (waste - substrate) assay. An enrichment medium was prepared by supplementing mineral salt medium with MSW to support the growth of waste-degrading isolates. Ten milliliter of the mineral salt medium was dispensed into a sterile test tube. The organic portion of the MSW was obtained, air dried and homogenized to fine particles using mortar and pestle. Exactly 0.2 g of the prepared waste was added to the mineral salt medium in the test tube and sterilized at 121 ^{0}C for 15 minutes. The sterilized medium (15 mL) was dispensed into a sterile Petri dish and 0.1 mL of nutrient broth culture of bacterial isolate was inoculated. This inoculation procedure was repeated for all the isolates and incubated at 37 ^{0}C for 72 hrs in a shaker incubator. No control was added. Waste utilization and bacterial growth were assessed using viable counts (CFU) on nutrient agar by the standard spread plate technique. Using the densities derived from the viable counts, the number of generations (n), generation time (gt) and growth rate (gr) of the microbial load were calculated as follows (Ugwu *et al*., 2017):

i. Number of Generation (n) $= \frac{log\ b - log B}{Log 2}$

where B = bacterial count at zero time, b = bacterial count at the end of given period of time, Log = Logarithm to base 10.

ii. Generation time (gt) $= \frac{t}{n}$

where t = time that elapsed between b and B, and n = number of generation

iii. Growth rate (gr) $= \frac{n}{t}$

Cultures with high counts and high growth rates indicated the isolates' ability to utilize the MSW-derived compounds as a substrate.

2.6 Molecular Characterization and Identification of Isolates

Bacterial isolates were identified molecularly using Sanger sequencing method (Locher *et al*., 2022). The genomic DNA of the bacterial isolates was extracted using the Fungi/Bacteria

DNA Mini Prep Extraction Kit and procedure supplied by Inqaba, South Africa, and quantified using the Nanodrop 1000 spectrophotometer. PCR amplification of the 16S rRNA region of the extracted genomic DNA was carried out on a real-time thermocycler (ThermoFIsher, Model 9700, USA) with the universal primer sequences 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' (forward primer sequence) and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' (reverse primer sequence) and the amplicons viewed on 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The amplified PCR products were sequenced by the Sanger sequencing method at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria South Africa. Sequence chromatogram analysis was performed using FinchTV analysis software (version 1.4.0, Geospiza Inc.) and identified using BLASTN analysis and a neighbor-joining phylogenetic tree based on the sequences constructed using MEGA 12.0 software (Kumar *et al*., 2024). The sequences generated were deposited in the GeneBank under accession numbers PV810158.1 and PV810157.1.

2.7 Data analysis

Two-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics version 23 at the 5% significance level to compare the bacterial counts among the different dumpsites and control soils. Means were separated using Duncan's post hoc multiple comparison test and expressed as mean ± standard deviation (SD). A p-value < 0.05 was considered statistically significant.

3.0 Results and Discussion

3.1 Total Heterotrophic Bacterial Counts

The soil teems with microscopic life of bacteria, fungi, algae, protozoa and viruses (Chavhan and Wagh, 2013) that are involved in soil development and nutrient cycling (Mohammadi *et al*., 2011). Higher ($1.47 \times 10^8 \pm 1.3$ CFU g^{-1} and $1.21 \times 0^8 \pm 2.1$ CFU g^{-1} at 0-15 cm and 15-30 cm depth, respectively) and lower ($9.9 \times 10^7 \pm 0.8$ CFU g^{-1} and $3.8 \times 10^7 \pm 2.0$ CFU g^{-1}) bacterial counts were observed in the soil samples collected from the dumpsite at Oron road and Ikot Ekpene road, respectively (Table 2). The counts were significantly ($P < 0.05$) different among the locations and higher than those obtained from the pristine soil. These variations may be due to differences in the types of waste composition, microbial activities, and human influence occurring at the different dumpsites. Site inspection of the dumpsite at Oron road revealed large decomposable agricultural and food wastes than the other dumpsites with large volumes of non-decomposable materials including polyethylene and plastics, which may be the reason for the high bacterial loads from Oron road than others.

Table 2: Viable Bacterial Counts (CFU/g) obtained from the MSW-Contaminated and Control/Pristine Soil Samples collected in Uyo Metropolis

Sample Depth (cm)	Mean viable bacterial counts (CFU/g)			
	Ikot Ekpene Road	Oron Road	Ukana Offot Street	Control
0 - 15	$9.9x10^7 \pm 0.8^c$	$1.47x\ 10^8 \pm 1.3^a$	$1.32x\ 10^8 \pm 0.4^b$	$4.5x\ 10^4 \pm 0.3^d$
15 - 30	$3.8\ x\ 10^7 \pm 2.0^c$	$1.21x\ 10^8 \pm 2.1^a$	$1.03x\ 10^8 \pm 1.1^b$	$2.1x\ 10^4 \pm 0.3^d$

Data are means ± SD and the values with different superscript across the rows are statistically different at 5 % level of significance

The elevated bacterial counts observed at the Oron road dumpsite may also be explained by its proximity to a market area and the high population density of residents in the vicinity, which likely contributes substantial biowaste inputs. A soil which is rich in useable organic and decomposable materials encourages higher microbial activity and abundance of bacteria as

it supplies the necessary growth nutrients than the soil with lower organic matter (Pondei and Okeke, 2024). The lowest bacterial counts in the soil samples from Ikot Ekpene road dumpsite could be due to the location which is a little away from the residential areas, the kind of wastes, and possible toxicity of some of the waste's components on the bacterial community. According to Udochukwu and Yakubu (2022), certain components of municipal solid wastes are toxic to bacteria and can also inhibit microbial activities and metabolism in the soil, thereby leading to low microbial composition. Similarly, the low viable bacterial counts observed in the control/pristine location may be due to poor availability of useable organic nutrients for microbial growth in that location of the soil. Field observation indicated that the control location was a farmland under continuous cultivation and may be receiving inorganic/mineral fertilizers and pesticides for crop improvement and protection. According to Kumar (2021), these applications can be harmful to the soil microorganisms. In several studies, mineral fertilization has been found to reduce microbial diversity, including the plant-beneficial microbial taxa. Mineral fertilization strongly affects the number of microorganisms and the qualitative selection of entire communities of soil microorganisms (Dinca *et al.,* 2022). According to Kumar *et al.* (2026) and Dincă *et al.* (2022), synthetic fertilizers and herbicides alter soil microbial community and ultimately increase the negative environmental impacts of agriculture activities on soil microorganisms. These study observations are in agreement with Pondei and Okeke (2024) who evaluated the bacterial isolates of soils of different waste types in Yenagoa Central solid waste dumpsite, Nigeria and reported that the waste dumpsite soils had higher bacterial counts than the soil from the pristine location. These results however, differ from Udochukwu and Yakubu (2022), who reported lower bacterial counts in wastes dumpsites in some communities of Lagos Island than the garden soil used as control.

Soil depth is a fundamental factor that influence soil microbial communities and critical for soil formation (Hao *et al.,* 2021). The decrease in bacterial counts with increase in soil depth in this study is in consonant with Hao *et al. (*2021) who had previously reported decreased bacterial abundance, species richness, and diversity with the increase in soil depth. The higher soil compaction in deep soil layers reduces O_2 availability, limiting the growth of many microbial taxa (Obour *et al.,* 2017; Alaoui & Diserens, 2018). The observed higher bacterial counts at the topsoil (0 to 15 cm) layer may be due to the greater accumulation of organic compounds/nutrients in the topsoil, which has been reported to shape soil heterotrophic microbiota (Fierer, 2017; Hao *et al.,* 2021). It may also be attributed to the aerobic condition of the upper soil layer.

3.2 Characterization of the Bacteria Isolates

The morphological and biochemical characteristics of the bacterial isolates as presented on Table 3 revealed the presence of abundance rod-shaped bacteria with very few cocci. The isolates were identified as *Staphylococcus aureus*, *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Streptococcus* sp., *Enterococcus* sp., *Bacillus* species and *Pseudomonas aeruginosa* as presented on Table 2. The distribution of the isolates varied with the dumpsites. *Bacillus* sp., *Enterobacter* sp. and *Pseudomonas aeruginosa* were observed across the three dumpsites with 100% occurrence (Table 4). There was no *Staph. aureus*, *Klebsiella* sp. and *Streptococcus* sp. detected in Ikot Ekpene road; no *E. coli* in Ukana Offot and no *Streptococcus* sp. in the control location. *Staphylococcus aureus, Escherichia coli* and *Klebsiella* sp. showed 75 % occurrences. Similar bacterial species have also been reported from dumpsites by Ayo *et al*. (2026) and Enerijiofi and Ekhaise (2019). Enerijiofi and Ekhaise (2019) attributed the higher population of

Bacillus species from dumpsite soils to their high residence in the soil environments, their biodegradable capabilities, and spore structure, which increase their power of accumulation and survival in harsh environmental conditions. A significant ($P < 0.05$) percentage (85.0 %) was isolated from the solid waste dumpsites than the pristine soil location, and was abundant on the topsoils compared to the subsoils. This is for the fact that the MSW dumpsites have high organic matter contents, especially on the topsoil, which encourage the growth of bacteria according to Wemedo *et al*. (2020) and Pondei and Okeke (2024). These bacteria derive energy from their ability to metabolize the wastes as sources of nutrients (Dey *et al.,* 2023). Among the locations (Table 4), Ukana Offot street dumpsite had higher frequency of these bacteria, followed by Oron road, and then Ikot Ekpene road than the pristine soil.

3.3 Waste Degradation Potential of the Bacterial Isolates

Microorganisms are ubiquitous and versatile in activities (Iwatt *et al.,* 2026). Solid waste dump soils constitute numerous bacterial diversities according to Enerijiofi and Ekhaise, (2019). In this study, different metabolically active waste-utilizing bacterial community was observed in the waste-contaminated soil ecosystem (Figure 2) indicating bacterial species adaptation to life in solid waste-contaminated environment. In a 72-hour degradation assay, the isolates demonstrated the ability to utilize the MSW as substrate. Growth-kinetics analysis showed that the growth rate varied among the bacterial isolates, with some exhibiting marked increase during the test period (Figure 2). *Bacillus* sp. and *Pseudomonas aeruginosa* achieved the highest bacterial counts and growth rates indicating they were the best waste utilizers. These results imply robust biological activity and the potential of these isolates to contribute to MSW degradation. Soil microorganisms are majorly involved in soil nutrition through their role in organic matter degradation (Mohammadi *et al*., 2011). Microbial metabolism, by-products, and enabling environmental conditions are known to enhance degradation in the different ecosystems and habitats (Liu, 2023).

3.4 Molecular Identification of the Best Bacterial Waste Utilizers

Genetic-based methods provide information on the microbial identity. As phylogenetic markers, ribosomal sequences present in all organisms allow organisms to be distinguished on all phylogenetic levels (Udotong et al., 2015). Analysis of the 16S rRNA gene sequences of the two best waste utilizers, Bacillus sp. (isolate G1) and Pseudomonas aeruginosa, followed by evolutionary analysis, showed sequence homology and close relatedness to members of the genera Bacillus and Pseudomonas (Figures 2 and 3, respectively). The sequences were most closely related to Bacillus sp. MK134612.1 and Pseudomonas aeruginosa OQ826823.1. Pseudomonas aeruginosa and Bacillus sp. have been reported by Chhetri et al. (2022) and Dave and Das (2021) for their ability to effectively degrade waste materials. They are being considered to be effective agents of biotechnology owning to their metabolic versatility, extracellular enzyme production, biodegradation capabilities, biofilm formation, and ability to recycle nutrients (Savich & Novik, 2016). Findings from this study make these two confirmed bacterial isolates valuable microbial candidates for application in waste treatment and environmental bioremediation.

3.5 Phylogenetic Analysis of the Bacterial Isolates

The molecular identification results based on 16S rRNA gene sequencing were supported by the phylogenetic analyses (Table 5; Figures 3 and 4). The *Bacillus* isolate showed 100% query coverage and 99.24% sequence similarity with *Bacillus* sp. strain 13TM7 (GenBank accession MK134612.1).

Table 3: Morphological and Biochemical Characteristics of the Representative Bacteria Isolated from the MSW-Contaminated and Control/Pristine Soil Samples

Dumpsite	S/n	Isolate ID	Pigmentation	Size	Shape	Elevation	Margin	Opacity	Surface Texture	Gram Reaction	Cell Type	Biochemical Tests												Probable Organism
												Oxidase	Catalase	Coagulas	Citrate	Urease	MR	VP	Spore	Glucose	Fructose	Galactose	Manitol	
Oron Road	1	1A	Yellow	Medium	Circular	convex	Entire	Opaque	Smooth	+	Cocci clusters	-	+	+	+	+	+	+	-	+	+	+	+	*Staphylococcus aureus*
	2	1B	Cream	Large	Circular	Raise	lrregular	Opaque	Dull	+	Rod	-	+	-	+	-	-	+	+	+	+	-	-	*Bacillus* sp.
	3	1C	Cream	Large	circular	convex	Entire	Opaque	Smooth, glittering	-	Rod	-	+	-	+	-	-	+	-	+	+	+	+	*Enterobacter* sp.
	4	1D	Green-metallic Sheen	Medium	Circular	Convex	Entire	Opaque	Smooth/glittering	-	Rod	-	+	-	-	-	+	-	-	+	+	+	+	*Escherichia coli*
	5	1E	Cream	Medium	Circular	Raise	Entire	Opaque	Smooth, moist	-	Rod	-	+	-	+	+	-	+	-	+	+	+	+	*Klebsiella* sp.
	6	1F	Greenish blue	Medium	Irregular	Raise	Irregular	Translucent	Smooth, moist	-	Rod	+	+	-	+	-	-	-	-	-	+	-	+	*Pseudomonas* sp.
	7	1I	White	Small	Circular	Convex	Entire	Opaque	Smooth/moist	+	Cocci	-	-	-	-	-	-	+	-	+	+	+	-	*Streptococcus* sp.
Ukana Offot																								
	1	S1	Cream	Small	Circular	Convex	Entire	Opaque	Smooth/moist	+	Cocci	-	-	-	-	-	-	+	-	+	+	+	-	*Streptococcus* sp.
	2	S2	Yellow	Medium	Circular	Convex	Entire	Opaque	Smooth	+	Cocci	-	+	+	+	+	+	+	-	+	+	+	+	*Staphylococcus aureus*
	3	S3	Cream	Large	Circular	Convex	Entire	Opaque	Smooth/glittering	-	Rod	-	+	-	+	-	-	+	-	+	+	+	+	*Enterobacter* sp.
	4	S4	Cream	Medium	Irregular	Raise	Irregular	Opaque	Fuzzy/wrinkled	+	Rod	V	+	-	+	-	-	+	+	+	+	V	+	*Bacillus* sp.
	5	S5	Greenish blue	Medium	Circular	Raise	Irregular	Translucent	Smooth/moist	-	Rod	+	+	-	+	-	-	-	-	-	+	-	+	*Pseudomonas* sp.
	6	S8	White	Small	Circular	Convex	Entire	Translucent	Smooth/moist	+	Cocci	-	-	-	-	-	-	-	+	-	+	-	+	*Enterococcus* sp.
	7	S9	Cream	Small	Circular	Convex	Entire	Opaque	Smooth/moist	-	Rod	-	+	-	+	+	-	+	-	+	+	+	+	*Klebsiella* sp.
I																								

	1	V1	Green Metallic Sheen	Medium	Circular	Convex	Entire	Opaque	Smooth, glittering	-	Rod	-	+	-	-	-	+	-	-	+	+	+	+	*Escherichia coli*
	2	V2	Greenish blue	Medium	Circular	Convex	Irregular	Translucent	Smooth/ moist	-	Rod	+	+	-	+	-	-	-	-	-	+	-	+	*Pseudomonas* sp.
	3	V3	Cream	Medium	Circular	Raise	Entire	Opaque	Dull, dry	+	Rod	-	+	-	+	-	-	+	+	+	+	-	-	*Bacillus* sp.
	5	V5	Cream	Medium	Irregular	Raise	Entire	Transparent	Moist/glittering	-	Rod	-	+	-	+	-	-	+	-	+	+	+	+	*Enterobacter* sp.
Control/Pristine soil	1	C1	Yellow	Medium	Circular	Convex	Entire	Opaque	Smooth	+	Cocci	-	+	+	+	+	+	+	-	+	+	+	+	*Staphylococcus aureus*
	2	C2	Cream	Medium	Circular	Raise	Entire	Opaque	Dull, dry	+	Rod	-	+	-	+	-	-	+	+	+	+	-	-	*Bacillus* sp.
	3	C3	Green-metallic Sheen	Medium	Circular	Convex	Entire	Opaque	Smooth/glittering	-	Rod	-	+	-	-	-	+	-	-	+	+	+	+	*Escherichia coli*
	4	C4	Cream	Small	Circular	Convex	Entire	Opaque	Smooth/moist	-	Rod	-	+	-	+	+	-	+	-	+	+	+	+	*Klebsiella* sp.
	5	C5	Greenish blue	Medium	Circular	Raise	Irregular	Translucent	Smooth/moist	-	Rod	+	+	-	+	-	-	-	-	-	+	-	+	*Pseudomonas* sp.
	6	C6	Cream	Large	circular	convex	Entire	Opaque	Smooth, glittering	-	Rod	-	+	-	+	-	-	+	-	+	+	+	+	*Enterobacter* sp.

MR - Methyl red; VP - Voges Proskaur; + = positive; - = negative; V=Variable

Table 4: Frequency of Occurrence of the Bacterial Isolates from the Different MSW-Contaminated Soils

S/N	Bacterial Isolate	Oron Road	Ukana Offot	Ikot Ekpene Road	Control/Pristine soil	Occurrence (%)
1	*Staphylococcus aureus*	+	+	-	+	75
2	*Bacillus* sp.	+	+	+	+	100
3	*Enterobacter* sp.	+	+	+	+	75
4	*Escherichia coli*	+	-	+	+	75
5	*Klebsiella* sp.	+	+	-	+	75
6	*Pseudomonas* sp.	+	+	+	+	100
7	*Streptococcus* sp.	+	+	-	-	50

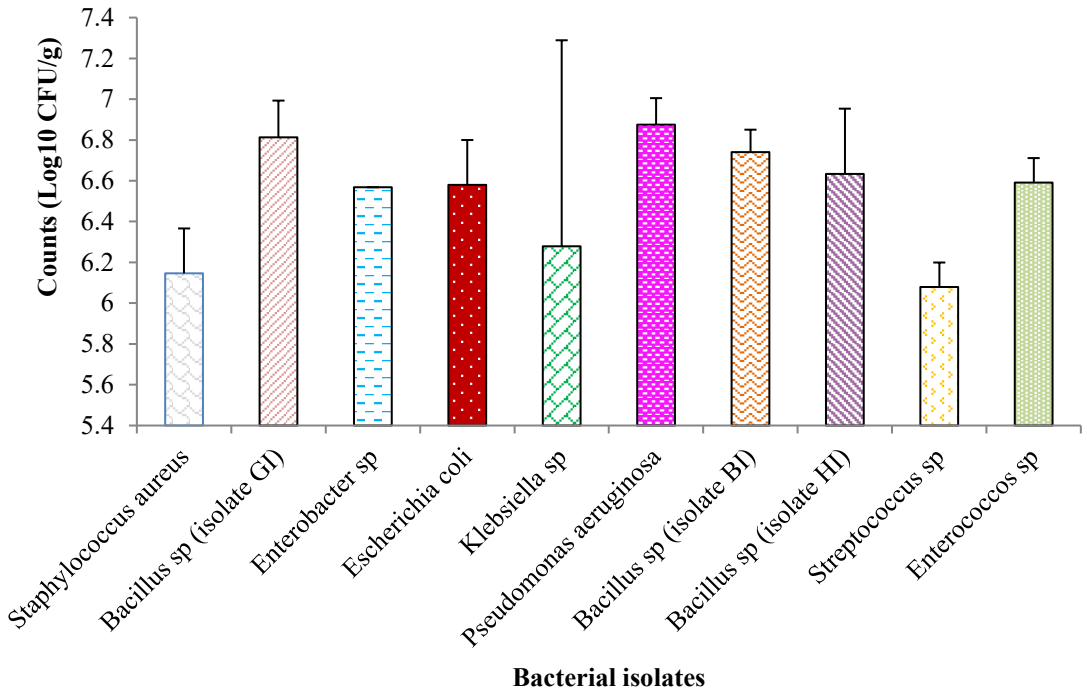


Figure 2: MSW Degradation Screening of the Bacterial Isolates (log_{10} CFU/g)

Consistent with the BLASTN result, the sequence obtained from the isolate (GenBank accession PV810158.1) clustered closely with *Bacillus* sp. strain 13TM7 in the neighbor-joining phylogenetic tree, with a high bootstrap support value of 99%. The isolate also occurred within the broader *Bacillus* lineage containing *Bacillus infantis* strain HQB51, which was supported by 90% bootstrap. The high sequence similarity and strong bootstrap support therefore provide molecular evidence for the close phylogenetic relationship of the isolate with the Bacillus group.

Similarly, the *Pseudomonas* isolate showed 100% query coverage and 99.78% sequence similarity with *Pseudomonas aeruginosa* strain MW585052 (GenBank accession OQ826823.1). In the phylogenetic analysis, the isolate sequence (GenBank accession PV810157.1) clustered with *Pseudomonas aeruginosa* strain F1, with 99% bootstrap support. The *Pseudomonas aeruginosa–Halopseudomonas xiamenensis* grouping was further supported by a bootstrap value of 98%, while the deeper node connecting this group with other *Pseudomonas* taxa had 90% bootstrap support. The agreement between the high BLASTN sequence similarity (99.78%) and the strong phylogenetic clustering supports the identification of the isolate as *Pseudomonas aeruginosa.*

The BLASTN sequence similarity, complete query coverage, and phylogenetic clustering with high bootstrap support were concordant for both isolates, providing additional support for their molecular identification. The phylogenetic analysis therefore complemented the sequence-based identification by demonstrating that the isolates clustered within their respective closely related bacterial lineages.

Table 5: Molecular Identification of the Bacteria Isolates

Isolate	Query coverage (%)	Closet blast Match	Percentage match	GenBank accession (This Study)	Organism in the Gene Bank
Bacillus sp.	100	MK134612.1	99.24	PV810158.1	*Bacillus* sp.
Pseudomonas sp.	100	OQ826823.1	99.78	PV810157.1	*Pseudomonas aeruginosa*

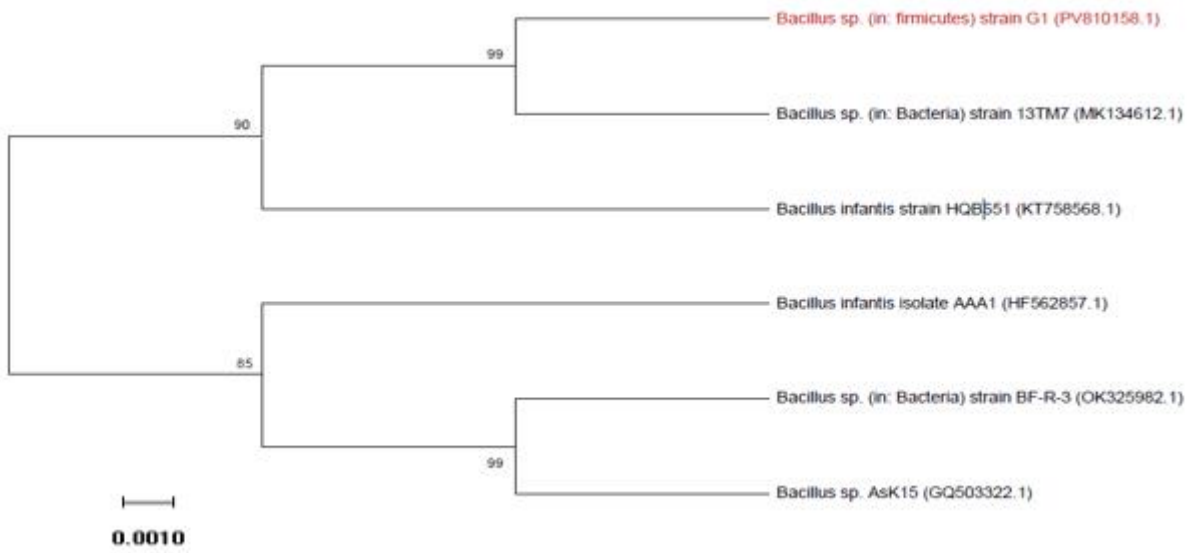


Figure 3: Evolutionary relationship of the *Bacillus* species with some closely related strains in GenBank. The numbers at the nodes indicate the percentage of replicate trees based on 1000 bootstrap. The scale bar corresponds to a 0.01 nucleotide base substitution per site

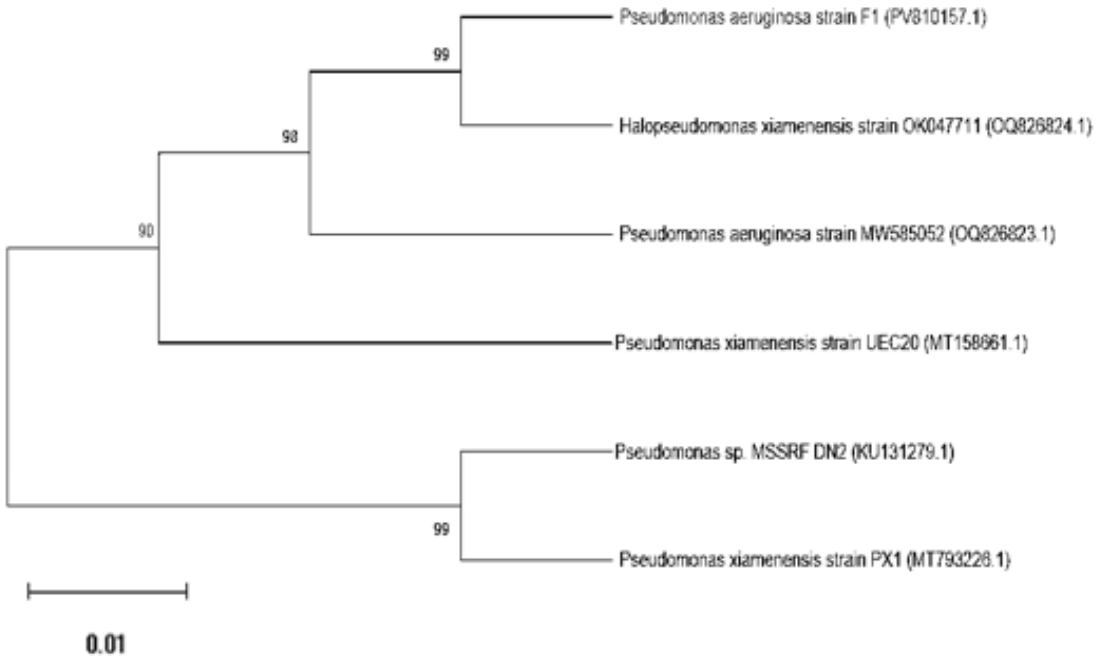


Figure 4: Evolutionary relationship of the *Pseudomonas aeruginosa* with some closely related strains in GenBank. The numbers at the nodes indicate the percentage of replicate trees based on 1000 bootstrap. The scale bar corresponds to a 0.01 nucleotide base substitution per site

4.0 Conclusion

Wastes are generated everyday. Inadequate disposal and management of these wastes create significant environmental concerns. Open municipal solid waste (MSW) dumpsites are prominent in some areas of major cities including Uyo. These sites harbor high densities and compositions of microbial communities with the potential for organic matter degradation. Some of the bacterial species detected from MSW-contaminated soil in this study, were *Staphylococcus aureus, Enterobacter sp, Escherichia coli, and Streptococcus sp*. The isolates responded positively to the MSW-utilization test with higher growth rates and other kinetics, resulting in greater bacterial abundance. The best waste-utilizers among theses were from the genera Bacillus and Pseudomonas. MSW-conatminated soils harbor rich metabolically active bacterial population that utilize organic waste as nutrient source. This findings provide a strong rationale for further investigation of the

bacterial isolates, *Bacillus sp*. and *Pseudomonas aeruginosa* in biodegradation studies and application in sustainable waste management.

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