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Molecular characterisation of bacteria isolated from dump site at Gbogidi, Ilaro, Ogun State, Nigeria

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ABSTRACT: This study used both genetic and cultural methods to identify the bacterial species linked to dumpsite soil. Samples of soil were taken in Gbogigi, Ilaro, Ogun State. Both the Nutrient Agar method and the Serial Dilution approach were used to isolate the bacteria linked to the dumpsite soil. Using the Quick-DNA Fungal/Bacterial MiniPrepTM Kit, deoxyribonucleic acid (DNA) was extracted from pure cultures of bacterial isolates, and the concentration was measured with a Nanadrop 2000c Spectrophotometer. Using the universal primer pair 16SF and 16SR for bacteria, the isolated DNA's I6S rRNA gene was amplified using polymerase chain reaction (PCR). Utilizing an ABI3500 Genetic Analyzer, sequencing was done. In order to identify species and determine evolutionary trends, the sequences were aligned and compared with a subset of sequences available in the National Centre for Biotechnology Information (NCBI) database. The two types of bacteria that were found were un-cultured *macrococcus* sp. (KP131824.1) and *Bacillus pumilus* (KC844765.1). This study provided more information on the bacteria species associated with dumpsite soils and it has paved the way for the possibility of isolating species with degradation potentials and the degraded solid waste can be useful in agriculture as fertilizer.

Keywords: Bacteria, characterisation, dumpsite, isolation, molecular, Ogun State.

INTRODUCTION

Waste disposal has a huge environmental impact that can cause serious problems in some places, waste is being buried, and some waste will eventually rot, but not all, and in the process, it may smell or generate methane gas which is explosive and contributes to the greenhouse effect (Iyanyi *et al*., 2020). A dumpsite is a soil or land set aside for dumping wastes. The old or traditional method of disposal of waste which is similar to the landfill method of management of waste is the dumpsite (Iyanyi *et al*., 2020). The issues of the Municipal Solid Wastes are basically acute in developing countries because there is no transformation of the socio-economic standard of such countries with improvements in the way waste can be managed technologically (Iyanyi *et al*., 2020; Zhou *et al*., 2014). In some countries that are still developing, waste either solid or liquid is dumped on land or discharged into water bodies. Thus, microorganisms such as bacteria and fungi increase rapidly using the components of the waste materials as their nutrient source for growth and development, as well as degrading the organic materials in the waste (Iyanyi *et al*., 2020; Kumar *et al*., 2016). New strategies for the management of waste have to be employed due to the amount of waste generated in the fast-growing urban agglomerations, their hazards to human health and the environment as a whole, as well as the costs associated with urban waste systems management (Iyanyi *et al*., 2020; Afon, 2012).

Karen *et al*. (2015) and Iyanyi *et al*. (2020) reported that the health risk associated with those living within or close to a dumpsite as they are being exposed to environmental pollution likely air pollutants emitted by the waste causing health disorders such as lung cancer, respiratory diseases

and even deaths and children are most likely affected.

Microbes are essential to humans and other organisms because they are directly or indirectly related to fermentation, medicine, genetic engineering, food, industrial processes and many other fields of life. Molecular methods have provided more significant and reliable data and information than the traditional or conventional methods of identification of microorganisms (Iyanyi *et al*., 2020). A variety of detection methods or techniques have been developed and employed in order to provide more insight into the ecology of microorganisms over the last few decades (Iyanyi *et al.*, 2020; Zhou *et al.,* 2014; Roh *et al.,* 2010). To detect and characterize wider groups of organisms, highly conserved degenerate primers are designed, such as those used for amplifying 16S rRNA genes for bacteria and archaea (Fischer *et al*., 2016). Essential information has been gotten from several ecosystems that is vital to environment and human health (Alivisatos *et al*., 2015) using molecular approaches. This novel study was therefore tailored towards isolating and characterizing the bacteria associated with dumpsite soil using molecular tools. PCR amplicon sequencing of the 16S ribosomal RNA (rRNA) gene, which is the most conserved region in bacteria, was analysed and used as the basis for the characterization of the isolates.

MATERIALS AND METHODS

The study area

The soil sample was collected in the Gbogidi dump site that is located in Ilaro, Yewa South Local Government Area in the West of Ogun State Nigeria (latttude 6˚53' 20.44" N, longitude 3°00' 50.98" E) in the North of the Area (Figure 1). It has an area of 629 km^2 with a population of 46,999. The area has ten (10) wards: Ilaro 1, Ilaro 2, Ilaro 3, Iwoye, Idogo, Owode 1, Owode 2, Ilobi/Erinja, Oke-Odan and Ajilete. The daily temperature in Ilaro ranges between an average minimum of 23°C to a maximum of 34.2°C.

Sample collection

Surface and sub-surface of the soil samples were collected from five different points at a distance of 6 feet apart. Sub-surface samples were collected at a depth of 10 cm Gbogidi dump site and transferred into sterile zip lock bags and labelled as 'S' for the surface soil sample and 'Sf' for the sub-surface soil sample. Thereafter, the samples were transported to Federal Polytechnic, Ilaro Microbiology Laboratory for analysis. Similarly, samples for microbial characterisation were preserved at a low temperature of 4°C in the laboratory in other to avoid any changes and contamination of the samples.

Isolation and enumeration of bacteria

The spread plate and serial dilution methods described by Hartman (2011) were used to isolate the bacterium. Serial dilutions five times were made. Using the spread plate method, 0.1 ml of each of the 10^{-2}, 10^{-3}, and 10^{-4} dilutions were taken and aseptically inoculated into sterile, newly manufactured nutritional agar plates in duplicate. After a 24-hour incubation period at 37°C, the infected plates were checked for microbial growth. The physical traits of the various bacterial isolates were recognized, and discrete colonies were counted. Freshly made sterile nutrient agar was used to subculture and purify the isolated bacteria. For later usage, pure cultures of the bacteria were stored in agar slants.

Physicochemical characterization

The physicochemical parameters analysed were pH, moisture content, and water holding capacity, according to the method outlined by Mor *et al*. (2006).

Microbiological and biochemical characteristics of isolated bacteria (Gram staining)

Gram staining technique was conducted according to Belmekki *et al*. (2013).

Biochemical characterisation

Catalase and oxidase tests were conducted according to Khatoon *et al*. (2022)

Bacterial DNA extraction, amplification and sequencing

DNA extraction was carried out using the Zymo Quick DNA Fungal/Bacterial Mini prep kit technique, which was available at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and was slightly modified from the instructions provided by Iyanyi *et al*. (2019) and Iyanyi *et al.* (2020). The ratio of absorbance at 260 nm to that at 280 nm was used to determine the DNA's purity. Utilizing agarose gel electrophoresis, the DNA's quality can be determined. Utilizing bacterial universal primers for the 16S rRNA gene amplification, namely 16SF forward (5'GTGCCAGCAGCCGCGCTAA 3') and 16SR reverse (5' AGACCCGGGAACGTATTCAC 3'), rDNA fragments from the bacterial isolates were amplified via Polymerase Chain Reaction (PCR). 2.5µl of 10x PCR buffer, 1µl of 25mM $MgCl_2$, and 1ul of each forward and reverse primer made up the PCR cocktail mix. 1µl of

Figure 1. Gbogidi dump site.

DMSO, 2µl of 2.5mMDNTPs, 0.1µl of 5µ/µl Taq DNA polymerase, and 3µl of 10ng/µl DNA. The total reaction volume was made up to 25µl using 13.4µl Nuclease free water. The PCR cycling was initial denaturation at 94˚C for 5 minutes, followed by 36 cycles of denaturation at 94˚C for 30 seconds, annealing at 56˚C for 30 seconds and elongation at 72˚C for 45 seconds. Followed by an elongation step at 72˚C for 7 minutes and hold temperature at 10˚C. The GeneAmp PCR system (Applied Biosystems, France) was the PCR thermal cycler that was utilized. On a 1.5% agarose gel dyed with a safe view, the PCR products were visible. The PCR results were put onto a gel that had been prepared using 1X TrisBoris EDTA (Ethylene Diamine Tetra-acetic Acid) and agarose powder. For forty minutes, the electrophoresis was run at 100 volts. The gel was moved to a gel documentation system (Documentation microDOCTM, Cle Scientific Ltd., UK) following the run time. A UV transilluminator, a digital camera, and a gel holding area make up the gel documentation system. The digital camera recorded the amplification in the shape of bands when the UV transilluminator was shining on it. Thermo Fisher Scientific, Massachusetts, USA, used an ABI 3500 genetic analyzer to sequence amplified samples.

RESULTS AND DISCUSSION

Isolation and Identification

A total of n = 4 strains of bacteria were isolated and identified based on colonial morphology, microscopy, and biochemical characteristics. Among all, Gram-positive, Gram-negative, rod-shaped, and spherical-shaped bacterial strains were selected for further confirmatory tests. The molecular analysis further validated the bacterial strains (*KP131824.1* and *KC844765.1*) as *Uncultured maro coccus (KP131824.1)* and *Bacillus pumilus (KC844765.1).*

Physicochemical characterisation

The results of the physicochemical examination of the surface and subsurface soil samples collected from the dumpsite are shown in Table 1. The two soil samples for the surface and subsurface oil samples had pH values of 7.08 and 7.04, respectively, based on the data in Table 1. Significant ($p<0.05$) changes were observed between the two soil samples due to differences in moisture content and water-holding capacity. Subsurface soil had the highest moisture level, 14.80%, while surface soil had the lowest moisture content, 14.49%. In a similar vein, subsurface soil had the most significant ($p<0.05$) water-holding capacity at 45.90%, compared to 40.50% for surface soil. The values of the two soil samples revealed that they were somewhat alkaline. The pH concentration result was consistent with the findings of Shehu-alimi *et al*. (2020), who reported a pH range of 6.8, 7.10 and 7.21. The pH levels found in this study are within the WHO/FAO (2004) suggested range of 6.5 to 8.5. High pH levels may be a sign of nitrate losses and eventual water pollution. Increased leaching losses and inadequate application of ammonia fertilizers may be indicated by the propensity for soil acidification (Smith and Doran, 1996). The results of the moisture content and water holding capacity obtained in this study showed that high organic matter around dumpsites favours increased permeability, moisture content and water holding capacity. Ideriah *et al*. (2010) study indicated that dumpsites are rich in organic matter,

Table 1. Physicochemical characterisation of surface and sub-surface soil (dumpsite).

Parameters	Surface soil	Sub-surface soil (10cm)
pH	7.04[a]	7.08[a]
Moisture content (%)	14.49[b]	14.80[a]
Water holding capacity (%)	40.50[b]	45.90[a]

[ab]Means on the same row with different superscripts are significantly different at $p<0.05$.

Table 2. Colony features of isolated bacteria of surface soil.

Sample	Colony feature	Nature of colony
S1	White	Rod
S2	White	Rod
S3	Cream	Spherical
S4	White	Rod

Key: S1 - S4 = different growth isolates.

Table 3. Colony features of isolated bacteria of sub-surface soil.

Sample	Colony feature	Nature of colony
S1	Cream	Spherical
S2	Cream	Spherical
S3	White	Spherical
S4	White	Spherical

which is the source of nitrogen and phosphorus that enhances soil fertility and promotes plant growth.

Colony morphology and microscopic presentation of isolated bacteria

The different growth isolates (S1, S2, S3 and S4) of both soil samples were presented in Tables 2 to 5 and Figures 2 to 7 respectively. The colony feature of the growth isolated from surface soil at the dumpsite indicated white for isolates S1, S2 and S4 with their corresponding rod shape nature, while isolate S3 indicated cream colouration with a spherical shape (Table 2). Similarly, sub-surface soil isolates showed two cream colourations (S1 and S2) and two white colourations (S3 and S4) with all of them having the same shape (spherical) (Table 3). All the isolates from surface soil indicated the presence of gram-positive (+) bacteria with their corresponding shapes (Bacilli [rod]) for S1, S2 and S4 respectively, while only S3 indicated spherical (rod) shape structure (Table 4). On the other hand, only one isolate (S1) indicated the presence of gram-negative (-) bacteria, and the other three isolates

Table 4. Gram staining of surface soil.

Surface soil sample	Nature of gram	Shape
S1	+	Bacilli (Rod)
S2	+	Bacilli (Rod)
S3	+	Cocci (Spherical)
S4	+	Bacilli (Rod)

Key: + = Positive.

Table 5. Gram staining of sub-surface soil.

Subsurface soil sample	Nature of gram	Shape
S1	–	Cocci (Spherical)
S2	+	Cocci (Spherical)
S3	+	Cocci (Spherical)
S4	+	Cocci (Spherical)

Key: + = Positive,–= Negative.

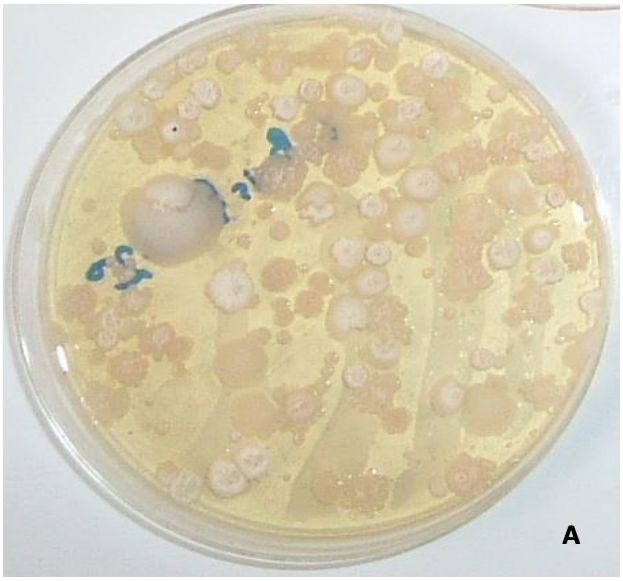
showed the presence of gram-positive (+) bacteria, with all the isolates having spherical (Cocci) shapes (Table 5). Most common bacteria isolated in this study are in tandem with the study of Sengupta *et al*. (2016) and Bhumbla *et al*. (2020) wherein the commonest organisms were Gram-positive cocci, gram-positive bacilli and gram-negative bacilli, respectively.

Biochemical characterization of bacterial isolates

The results of the biochemical tests conducted for surface and sub-surface soil isolates were presented in Tables 6 and 7. The results showed the presence of a positive (+) test for catalase and oxidase from all the isolates obtained from surface soil (Table 6). On the other hand, the results obtained from sub-surface soil isolates indicated a negative (-) test for both catalase and oxidase for the S1 isolate, while the rest isolates were positive (+) test for both catalase and oxidase (Table 7). A study conducted by Tariq *et al*. (2016) and Marimuthu *et al*. (2019 and Sadiqi *et al*. (2022 reported the same results of biochemical analysis. Isolated bacterial strains were partially characterized based on biochemical tests.

Molecular characterization of bacterial isolate

The results of the molecular characterization of the bacterial isolates after morphological differentiation and purification were molecularly characterized by DNA isolation, PCR amplification and sequencing of the 16s rRNA gene (Figure 8 and Table 8). Upon BLAST query of



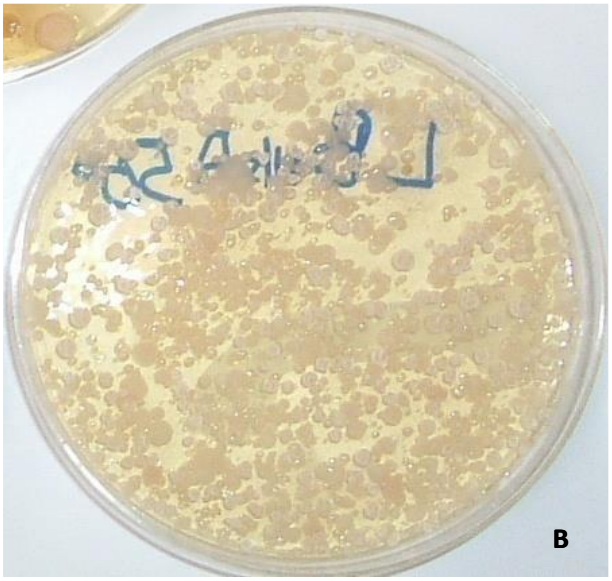


Figure 2. Microbial growth isolates obtained from dump site

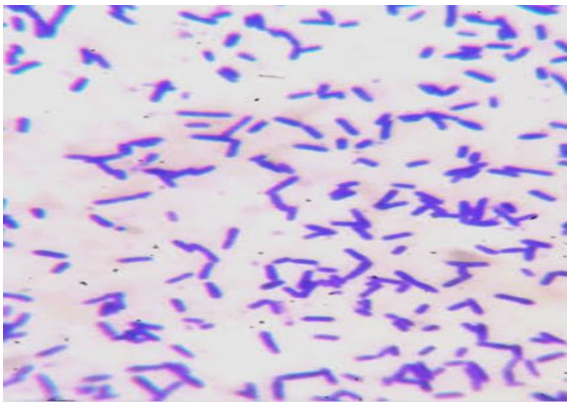


Figure 3. Gram-negative Bacilli.

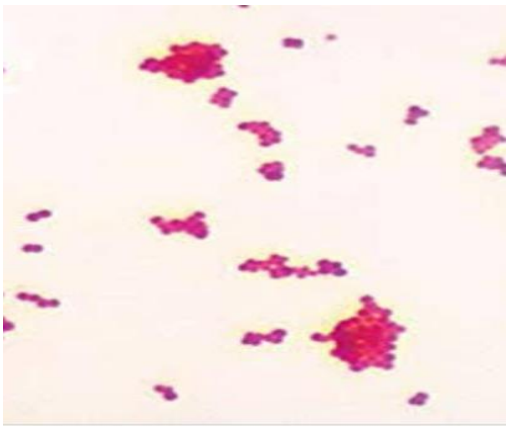


Figure 5. Gram-negative Cocci.

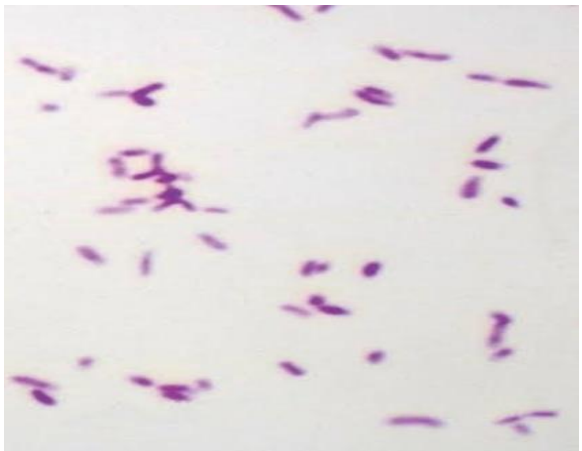


Figure 4. Gram-positive Bacilli.

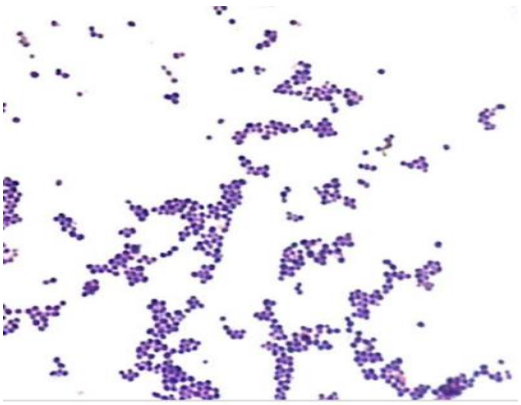


Figure 6. Gram-positive Cocci.

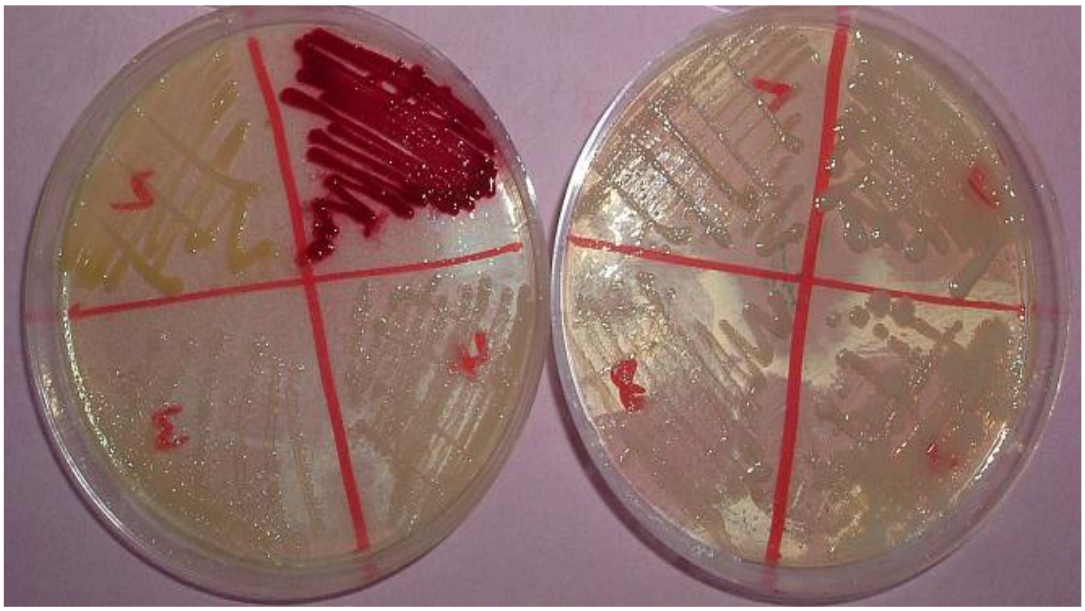


Figure 7. Sub-cultured Bacteria.

Table 6. Biochemical test of surface soil.

Sample	Catalase	Oxidase
S1	+	+
S2	+	+
S3	+	+
S4	+	+

Key: + = Positive.

Table 7. Biochemical test of sub-surface soil.

Sample	Catalase	Oxidase
S1	–	–
S2	+	+
S3	+	+
S4	+	+

Key: + = Positive, – = Negative.

surface soil sample molecular sequencing data, it was found to have 88.59% homologous identity with *Uncultured macro coccus* sp. *(KP131824.1)* and sub-surface soil sample molecular sequencing data were found to have 95.48% homologous identity with *Bacillus pumilus* sp. *(KC844765.1)* (Table 8). Only two bacterial species could be identified in this investigation using molecular techniques (PCR and Sanger sequencing). This approach to microbe identification is superior to the culture method, which focuses solely on the morphological and microscopic analysis of the isolates. Even so, there are drawbacks to PCR amplification and Sanger sequencing for identification. These methods are time-consuming and can only identify a restricted number of organisms because DNA extraction requires the clean cultivation of these organisms on media. However, a number of bacteria and other microbial communities of organisms can be detected using different molecular techniques such as shotgun metagenomics; however, these techniques are costly and necessitate intricate data analysis.

Table 8. 16s rRNA sequencing of bacteria.

Bacteria	16s r-RNA sequencing
Uncultured maro coccus (KP131824.1)	CGCGTCACTCCTAGCAGTGGATCTGGCTCAGGTGCGCTGGGACCCCCCCT AATAGTTTGCCCGGCCCGGGGGGGGGGGTTACCCCCCTTTAAAGGGTCCC GCGCGGGGGGCTTTGGGATCCTACTTTAAAAGCGGACCCACCGAACTTCG CATGAACCAATATTAAAAGACGGTTCTGCTGTCACTTATAGATGGACCCG CGGTTTATTAGCTAGTTGGTGAGGTAACGGGTCACCAAGGCGTCGATACA TATCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCA GTCTCCTACGGGAGGCAGCAGTAGGGATTCTTCCGCAATGGACGAAAGCC CGACGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACCC CGTTGTATGGGAAGAACAAGTATTTTTAGTAACTGAACGTCCTTGACGGT ACCTTACCAGAGAGGGGAGGGTATTCGGCGCCAGCAGCCGCGGTAATACG TAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGCGCGTAACGG CCCTCTTATGTCTGGTGTAAAGGGGTCGGCTAATCAGGGGATGGTGATTG GAATGGGGGAGACTTGAGTGGGGAGGAGGGGAGGGGAAGTCATGGGGGGC GGGGAAT
Bacillus pumilus (KC844765.1)	GTGTACATGTATTCACTCTTCTTGATGATCATGGCTCAGGTGCGCGTGTG TAGACACATCCTCTCCATATATAGTTAGCGGCGGACGCGGTGACTAACAC GTGCCCCCACCTAAAATGTAAGACTGGGATAACTCCGGGAAACCGGAGCT AATACCGGATAGTTCCTTGAACCGCATGGTTCAAGGATGAAAGACGGTTT CGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGT AATGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGC CACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGG GAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGA TGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCGA GAGTAACTGCTCGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT TATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGC CCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAG AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGG AGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGG AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCC GTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCA GCTAACGCATTAAGCACTCCGCCTGGGGGAGTACGGTCCGCAAGACTGAA ACTCAAAGGCAATTGGACGGGGGGCCCGGCACTAGCGGTGGAAGCATGTA GATCTAACTTCGAAGCCACCGTCGAAGAACCTTAACCAGGTCTTGAACAT CCTCTGACAACCCCTTAGAAGAATAGGGCTTTTCCTTCAGGGGAACAGAG

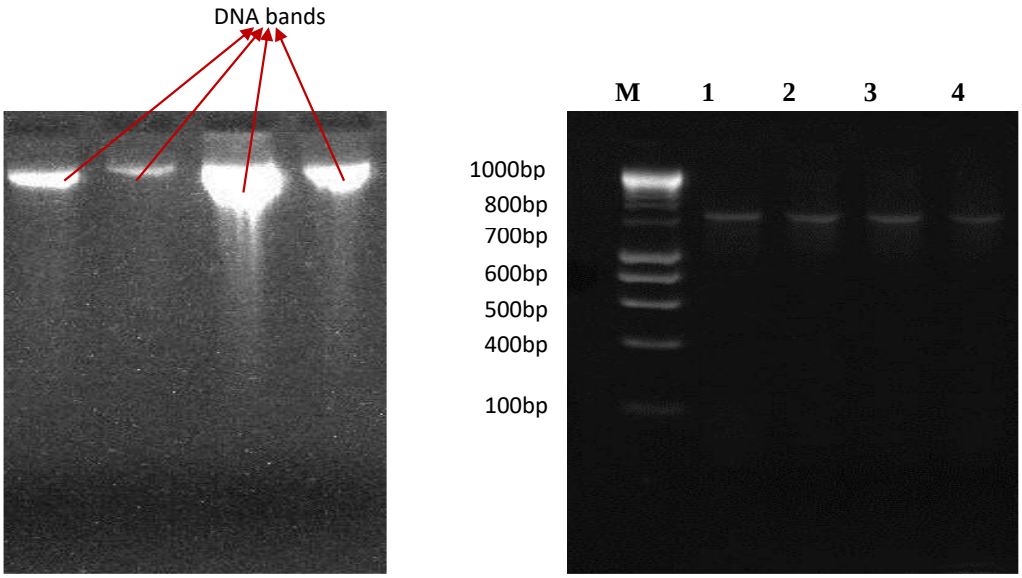


Figure 8. Gel electrophoresis of PCR amplification of 16S gene sequences M- 100bp DNA ladder.

Conclusion

Compared to conventional procedures, molecular techniques have shown to be more dependable. The 16S rRNA (ribosomonal RNA) gene was amplified, sequenced, and subjected to polymerase chain reaction for molecular characterisation in order to identify the bacterial isolates. This investigation characterized two bacterial isolates: *Bacillus pumilus* sp. (KC844765.1) and *Uncultured macrococcus* sp. (KP131824.1). It has been common practice to isolate these bacterial isolates from soil. The results of this study show that the physicochemical conditions at the Gbogidi dump site are not ideal for the survival of many bacterial species, which is important information for solid waste management. Indeed, a large number of harmless, biotechnologically useful bacteria can be found in dump sites. These can be exploited for financial gain by increasing agricultural yields, creating jobs, and improvement of agricultural yield.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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