

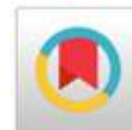


Biodegradation of plastic materials obtained from solid waste dumpsites in Nigeria, using native bacterial strains

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Abstract

Plastic packaging materials constitute a major potential environmental pollutant due to their slow degradation rates. This study aimed to isolate the plastic-degrading bacteria from the solid waste dumpsites of Abuja, Nigeria. Soil samples (n= 72) and plastic materials (bottles and bags) were collected from the dumpsites using soil augers and manual picking, respectively. Bacteriological analysis of the soil samples revealed the recovery of a total of 54 bacterial isolates, which were distributed among the genera of; *Proteus* sp. (33.3 %), *Providencia* sp. (29.63 %), *Pseudomonas* sp. (16.67 %), *Bacillus* sp. (9.26 %), *Micrococcus* sp. (5.56 %), *Escherichia coli* (1.85 %), *Enterobacter* sp. (1.85 %), and *Serratia* sp. (1.85 %). The bacterial isolates were inoculated into a series of shake flasks containing nutrient broth and pre-sterilized strips (1×1 cm) of plastic bags (0.05-0.0514 g) and plastic bottles (0.05-0.0529 g), and then incubated at 30 °C for 60 d to monitor their biodegradation using the weight loss method. The strips of bottles (0.58-49.00 %) were more susceptible to biodegradation than the plastic bags (0.78-15.40 %) after 60 d of incubation. The results demonstrated that about 6 of the bacterial isolates belong to the two genera of *Proteus* sp. and *Providencia* spp., and were considered the best bio-degraders. Molecular characterization of these potent isolates has identified them as *Proteus mirabilis* strain PPB3 (49.00 %), *Proteus mirabilis* strain UPMSD3 (32.07 %), *Proteus mirabilis* strain HH133 (20.41 %), *Proteus mirabilis* strain SSBIKEN (15.40 %), *Providencia vermicola* strain M4 (14.96 %), and *Providencia vermicola* strain 11 (12.20 %). These strains could be considered as potential biodegradation agents for the plastic materials that are prevalent in dumpsites.

Keywords: Biodegradation, Dumpsites plastic materials, *Proteus* sp., *Providencia* sp.



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1. Introduction

Over the years, humans have tried to device different utility materials made of ivory, paper, silk, plant leaves, cottons, rubber, and glasses among others, as packaging containers (Phil, 2019; Patil, 2018). Recently, science and technology have made plastic-derived packaging materials as one of the best substitutes, probably due to their toughness; resilience, flexibility, and light weight among others (Phil, 2019; Emmanuel-Akerele and Akinyemi, 2022). The commonest raw materials used for synthesis of the plastics are polymers of hydrocarbons (i.e., coal and natural gas), which are naturally resistant to degradation by the microorganisms, chemicals, and physical agents (Patil, 2018; Afreen *et al.*, 2020). The most recalcitrant synthetic types of plastics are the polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyethylene (PE) (Jambeck *et al.*, 2015). Most of the commonly used plastic packaging bags and bottles for food; drugs, water, research samples, and garbage containers, among others are made mainly from polyethylene and polyethylene terephthalate, respectively (Vona *et al.*, 1965). These are mostly polymers of low-density and high-density molecular weights, which are not readily biodegraded (Raziyafathima *et al.*, 2016). After usage, plastic wastes are indiscriminately discarded at open spaces, road sides, and waste bins, where they are ultimately transported to the solid waste dumpsites/ landfills, especially in some urban areas. Due to lack of efficient disposal and management mechanisms for the disposed plastic materials, they eventually accumulate over time in the ecosystem, causing terrestrial, aquatic, and atmospheric pollutions. Hence, there is a need for degradation or recycling of these plastics to reduce their abundances and deleterious effects in the environment.

Natural degradation of plastics has been found to take thousands of years, which is thus a very slow process that needs enhancement. An ecofriendly

technique that is being considered by the environmentalists involves the use of plastic-utilizing microbes or their products, to enhance the biodegradation process (Shilpa and Meena, 2022). Most studies in the past have focused on biodegradation of the different plastics materials, such as polyvinyl chloride, polypropylene, polystyrene, polyethylene terephthalate, and polyethylene (Raziyafathima *et al.*, 2016; Shilpa and Meena, 2022). Several scientists have identified more than 90 microbial genera, including *Bacillus*, *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, *Micrococcus*, *Galleria*, *Enterobacter*, *Proteus*, *Aeromonas*, *Comamonas*, *Delftia*, *Providencia*, *Stenotrophomonas*, *Fusarium*, and *Aspergillus*; among other bacteria and fungi, with efficient plastic biodegradation capacities (Mahdiyah and Mukti, 2013; Singh and Gupta, 2014; Gumbi *et al.*, 2019; Afreen *et al.*, 2020; Mohanan *et al.*, 2020; Shruthi *et al.*, 2020; Shilpa and Meena, 2022). For instance, two bacterial strains mainly; *B. licheniformis* and *Achromobacter xylosoxidans* have been successfully used to degrade the plastic wastes (PVC and PE) (Saeed *et al.*, 2022). Similarly, three bioplastic (polycaprolactone) degrading bacterial strains, including *B. megaterium*, *Alcaligenes aquatilis*, and *Shewanella haliotis* have been previously reported (Ariole and George-West, 2020). Other strains of bacteria and fungi have been considered as potential bioremediation agents for the plastic polymers (Shilpa and Meena, 2022).

Although, the poorly managed dumpsites are potential sources of pollutants to the environment; however, recent reports have shown that waste dumpsites can act as potential reservoirs for the recovery of potent bacterial spp. with diverse biotechnological and industrial potentials (Iheme *et al.*, 2017; Ayansina *et al.*, 2019; Nnolim *et al.*, 2020). Bacteria are the most ubiquitous and abundant microorganisms that can utilize both of the organic and inorganic materials as sources of nutrients and

energy ([Atashgahi *et al.*, 2018](#)). To survive in polluted environments, some of the native bacteria develop requisite enzymatic machineries to utilize the pollutants and release mineral nutrients needed for survival of the plants and different animals. A recent study conducted by [Dutta *et al.*, \(2021\)](#) reported that bioremediation applied in the polluted environments represented a relatively better waste management technique than the physicochemical methods, which are generally non-ecofriendly. Hence, it is pertinent to constantly monitor the bacterial communities of dumpsites with regard to exploiting them as bio-stimulants and/ or bio-augmentative agents in bioremediation of the public health pollutants. The current study was therefore undertaken to characterize the native plastic-degrading bacteria from dumpsites/landfills in Abuja, the capital city of Nigeria, which harbor diverse forms of plastic materials among other solid municipals, agricultural and industrial wastes.

2. Materials and methods

2.1. Study area

This study was carried out on selected solid waste dumpsites within Abuja, Nigeria (Fig. 1). Abuja land mass is approximately 7,300 km² with an estimated population of 2,238,800 ([Sawyer *et al.*, 2017](#)). Abuja is bounded to the East, West, South and North by Nassarawa, Niger, Kogi, and Kaduna States, respectively. As the capital city of Nigeria, Abuja is becoming highly populated as a result of the rural-urban migration. Consequently, several solid waste dumpsites were produced as a result of the expansion in constructions, institutions, agricultures, industries, and other anthropogenic activities. The six major solid waste sites employed were located within Abuja municipal, including Gosa, Abaji, Bwari, Gwagwalada, Kuje, and Kwali local governmental areas.

2.2. Samples collection

Soil samples (n= 72) were collected within bags from the solid waste dumpsites of Abaji; Bwari, Gosa, Gwagwalada, Kuje, and Kwali, using AMS soil augers

(Arts Manufacturing and Supply Inc. USA) at a depth between 0-45 cm. Low density plastic bags and bottles were also collected manually from the same dumpsites into sterile containers. All the samples were appropriately labeled and then transported promptly to the laboratory for further analysis.

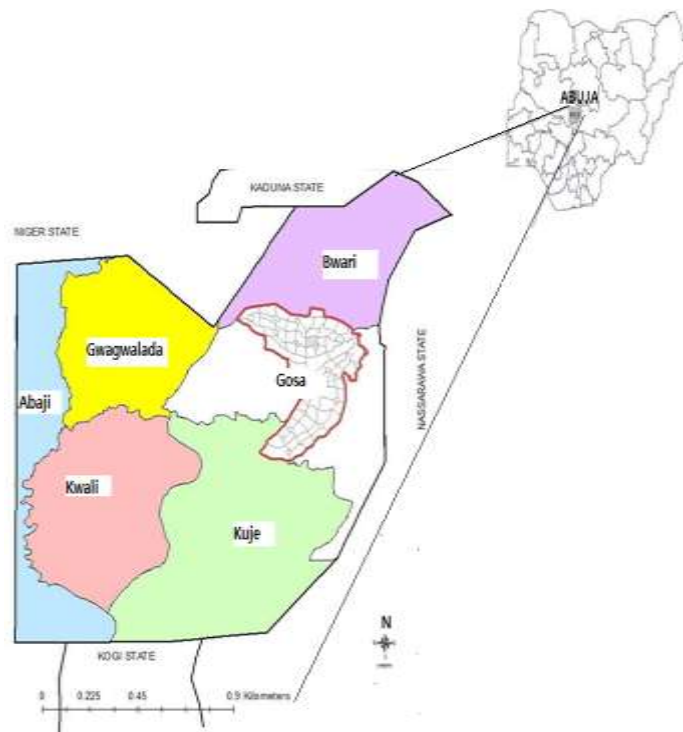


Fig. 1: Map of Nigeria showing Abuja and the six study areas ([Ezeamaka *et al.*, 2018](#))

2.3. Isolation and identification of bacteria from the dumpsite soil samples

Approximately 1 g of each soil sample was weighed and then dissolved in 9 ml of sterile distilled water before a serial dilution was made up to the dilution of 10¹⁰. An aliquot sample (0.1 ml) was withdrawn from the dilutions 10⁸-10¹⁰ and aseptically inoculated into the surface of nutrient agar (NA) (Oxoid, UK) Petri plates, and then spread uniformly using a sterile glass spreader ([Cheesebrough, 2000](#)). The plates were incubated at 30 °C for 24 - 48 h. All the plates were examined daily for bacterial growth, and the developing bacterial colonies were enumerated. Results were expressed as colony-

forming-units per gram of soil sample (cfu/ g). Pure cultures of the predominant bacterial colonies were identified phenotypically according to their colony morphologies, microscopic characteristics (i.e. Gram stain reaction, cell shape and arrangement), and biochemical assays, including catalase test; oxidase test, motility test, indole test, methyl red test, Voges Proskauer test, urease test, citrate test, nitrate reduction test, and fermentation assays of several sugars, including fructose lactose, sucrose, and mannitol ([Cowan and Steele, 1974](#); [Ogbulie *et al.*, 2001](#); [Holt *et al.*, 2002](#)).

2.4. Biodegradation of the plastic materials

All the representative bacterial isolates (n= 54) were used for the plastic biodegradation assay for periods of 15, 30, 45, and 60 d, using the method described by [Ogunbayo *et al.*, \(2019\)](#) with slight modifications. The collected plastics bags and bottles were cut into tiny square strips (1×1 cm) using a sterile pair of table scissors. They were cleaned with tap water and surface sterilized with 70 % (v/v) ethanol. Further washing was done with sterile dist. deionized water, 0.1 % mercuric chloride, and re-washed with dist. deionized water. They were then pre-weighed using an electronic metler beam balance (Mettler Toledo® MS303TS/00 Model, USA) to obtain initial weights of 0.05-0.0514 g, and 0.05-0.0529 g, respectively. The sterile pre-weighed plastic strips (n= 20) were aseptically transferred into series of 2 flasks (for each bacterial isolate) containing 100 ml of nutrient broth (NB) (Oxoid UK). Exactly 1.0 ml of standard suspension of 18 - 24 h broth culture of each bacterial culture, equivalent to 0.5 McFarland standards (10⁶ cells/ ml) was individually inoculated into the different NB flasks that contain the plastic material. Control flasks containing tiny square strips (1×1 cm) of the plastic bags and bottle without the bacterial suspensions were maintained along the test experimental set-up. All flasks were incubated at 30 °C and monitored for 15, 30, 45, and 60 d. After each incubation period, three strips of plastic bags and bottles were aseptically removed from each NB culture using a sterile forceps, washed thoroughly with tap

water, ethanol, and then with dist. water. The plastic strips were shade dried and then weighed for a final weight, which was then recorded. The same steps were repeated for all the treated samples in duplicates. The percentage of degradation (%) of each strip of bags and bottles by the tested bacteria was determined through calculating the % of weight loss of the plastic materials using the following formula of [Ogunbayo *et al.*, \(2019\)](#):

Weight loss of the plastic material (%) =

$$\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

2.5. Molecular characterization of the efficient bacterial plastic bio-degraders

The genomic DNA was extracted from the best bacterial plastic bio-degraders using AccuPrep Genomic DNA Extraction Kit K-30321, as described previously by [Oliwa-Stasiak *et al.*, \(2010\)](#) with slight modifications. Molecular identification of each bacterial isolate involved analysis of the extracted mitochondrial DNA fragment of 16S rRNA genes, using the Polymerase chain reaction (PCR). The primers used were; 16F-GTGCCAGCAGCCGCGCTAA, and 16R-AGACCCGGGAACGTATTAC (789 bp). The PCR cycling conditions used for DNA amplification were as follows; Initial denaturation: 5 min. at 94°C, 30 cycles of denaturation (94°C, 30 sec), annealing (56°C, 30 sec) and (72 °C, 90 sec), final elongation for 10 min. at 72 °C., and then chilled at 4 °C. The PCR products were visualized using ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated through comparison with the mobility of a hyper Ladder 1, which was ran alongside the experimental samples, in addition to a negative control. The PCR products (amplicons) were sequenced using a Genetic Analyzer 3130×1 sequencer (Applied Bio systems) according to the manufacturers' manual, whereas the used sequencing kit was that of Big Dye terminator 3.1 cycle sequencing kit.

Alignment of the sequences, editing of the untrimmed sequence, and estimation of the GC-contents were carried out using a Bio Edit (<http://www.mbio.ncsu.edu/BioEdit>). The partial DNA sequences of 16S rRNA genes were further aligned with MEGA-X. For comparison, the sequences were analyzed using the basic local alignment of sequences tool (BLAST, <http://www.ncbi.nlm.nih.gov/BLAST>).

2.6. Statistical analysis

Data were analyzed using Microsoft Excel Window 7, and results are presented in tables. Where necessary, the data obtained were statistically analyzed using Analysis of variance (ANOVA), and a *p*-value less than 0.05 was considered significant. Differences in means were analyzed using Duncan's Multiple Range Test.

3. Results and Discussion

3.1. Isolation and identification of the bacteria

Out of the 72 soil samples analyzed bacteriologically, a total of 54 bacterial isolates were recovered, and phenotypically characterized (Table 1). The genera of bacterial isolates with their percentage (%) of occurrences were; *Proteus* spp. (33.3 %), *Providencia* spp. (29.63 %), *Pseudomonas* spp. (16.67 %), *Bacillus* spp. (9.26 %), *Micrococcus* spp. (5.56 %), *E. coli* (1.85 %), *Enterobacter* spp. (1.85 %), and *Serratia* spp. (1.85 %), as presented in Table (2). Among these isolates, *Proteus* spp., *Providencia* spp., and *Pseudomonas* spp. were detected in all the solid waste dumpsites, whereas *Bacillus* spp. and *Micrococcus* spp. were obtained from at least three solid waste dumpsites. On the other hand, the least frequent bacterial isolates include *E. coli*, *Enterobacter* sp., and *Serratia* sp., which were recovered from Abaji, Bwari, and Gwagwalada dumpsite samples, respectively. Most of the bacterial isolates recovered in this study (i.e., *Proteus* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., *E. coli*, *Enterobacter* sp., and *Serratia* sp.) have been previously reported from solid waste dumpsites in Abia State University Teaching Hospital, Aba, Nigeria

([Ndimele et al., 2014](#)), Calabar, Cross River State, Nigeria ([Bassey et al., 2015](#)), Arusha, Tanzania ([Mwaikono et al., 2015](#)); Port Harcourt, Nigeria ([Williams and Hakam, 2016](#); [Okoronkwo and Okpokwasili, 2018](#)), Benin City, Nigeria ([Oviasogie et al., 2010](#); [Omusi et al., 2017](#); [Oshoma et al., 2017](#)), Abraka, Delta State, Nigeria ([Odum et al., 2020](#)), and Southern Assam, India ([Chandani et al., 2020](#)). Recently, a special strain of *Providencia* sp. (*P. rettgeri* AVR20) was recovered from the solid waste dumpsites of Madurai, Tamil Nadu, and India ([Anjanapriya et al., 2022](#)). This indicates that most of the recovered bacterial isolates are widely distributed in the solid waste impacted soils. The relatively high abundance of some of the bacterial isolates, including *Proteus* sp., *Providencia* sp., *Pseudomonas* sp., *Bacillus* sp., and *Micrococcus* sp. suggest that they possess higher abilities to breakdown and utilize the solid wastes. Previous studies have reported that the bacterial isolates obtained from the dumpsites were efficient degraders and utilizers of organic waste components, and they were used as sole sources of carbon and energy ([Emmanuel et al., 2017](#)).

3.2. Biodegradation of the plastic materials

Table (3) shows the plastic (strips of bags and bottles) biodegradation potentials of the bacterial isolates after 15, 30, 45, and 60 d of incubations at an ambient temperature (30 °C). According to results of this study, it was observed that 20 out of the 54 dumpsite bacterial isolates demonstrated varying degrees of degradation (%) of the used plastic materials. The % of degradation (weight losses) ranged from 0.02 -9.67 % (after 15 d of incubation), 0.10-17.02 % (after 30 d), 0.14-27.08 % (after 45 d), and 0.58-49.00 % (after 60 d) for the plastic bottles, and were; 0.04 -2.90 % (after 15 d), 0.12-4.78 % (after 30 d), 0.38-8.93 (after 45 d), and 0.79-15.40 (after 60 d) for the plastic packaging bags. Generally, the rate of weigh losses of the plastic bags and bottles increased with increasing the days of incubations. The highest incubation period (60 d) yielded the most significant biodegradation activities, which were recorded in terms of % of weight losses of the plastic materials.

For the strips of bottles, the most significant biodegraders of dumpsites (≥ 12 % weigh loss) after 60 d of incubation, include isolates from Gosa (*Proteus* sp. G10), Gwagwalada (*Proteus* sp. Gw₄), Bwari (*Proteus* sp. B₄), Abaji (*Providencia* sp. A₂), and Kwari (*Providencia* sp. Kw₂), with recorded weigh losses of 49.00 %, 32.07 %, 20.41 %, 14.96 %, and 12.20 %, respectively. Only one of the isolates (*Proteus* sp. A₄) significantly attacked the strips of plastic bags samples

yielding a weight loss of 15.40 %. Currently, the rate of plastic biodegradation (%) by the tested bacterial isolates suggested that most species of *Proteus* and *Providencia* were recorded as efficient plastic biodegraders. However, *Bacillus* spp., *Pseudomonas* spp., and the remaining species of *Proteus* and *Providencia* showed significantly ($p > 0.05$) low rates of plastic biodegradation.

Table 1: Phenotypic characterization of the bacterial isolates recovered from dumpsites of Abuja

Biochemical test	Isolates code							
	A	B	C	D	E	F	G	H
Grams reaction	(-) rods	(-) rods	(-) rods	(-) rods	(-) rods	(-) rods	(+) rods	(+) cocci
Catalase	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Oxidase	(-)	(+)	(-)	(-)	(-)	(-)	(+)	(+)
Motility	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)
Spore	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Indole	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(-)
Methyl red	(-)	(+)	(+)	(-)	(+)	(-)	(-)	(-)
Voges proskauer	(-)	(-)	(-)	(+)	(-)	(+)	(+)	(+)
Urease	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)
Citrate test	(+)	(+)	(+)	(+)	(-)	(+)	(+)	(-)
Nitrate reduction	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Glucose	(+) Ac	(+) Ac	(+) Ga	(+) Ac/Ga	(+) Ac/Ga	(+)Ac	(+)Ac	(+)Ac
Fructose	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Lactose	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(-)
Sucrose	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Mannitol	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Probable identity	<i>Pseudomonas</i> spp.	<i>Providencia</i> spp.	<i>Proteus</i> spp.	<i>Enterobacter</i> spp.	<i>Escherichia coli</i>	<i>Serratia</i> spp.	<i>Bacillus</i> spp.	Micrococcus spp.

Where; Ac: Acid production, Ac/Ga: Acid and gas production; (+): Positive; (-): Negative

Table 2: Distribution of the bacterial isolates within the solid waste dumpsites of Abuja

Solid Waste Dumpsites	<i>Pseudomonas</i> spp.	<i>Providencia</i> spp.	<i>Proteus</i> spp.	<i>Enterobacter</i> spp.	<i>Micrococcus</i> spp.	<i>Bacillus</i> spp.	<i>Escherichia coli</i>	<i>Serratia</i> spp.
Abaji	+	+	+	-	-	+	+	-
Bwari	+	+	+	+	-	-	-	-
Gosa	+	+	+	-	+	+	-	-
Gwagwalada	+	+	+	-	+	+	-	+
Kuje	+	+	+	-	-	+	-	-
Kwali	+	+	+	-	+	-	-	-
Total	9/54	16/54	18/54	1/54	3/54	5/54	1/54	1/54

Where; +: Detected, -: Not detected

Table 3: Percentage weights loss of plastic bags and bottles collected from solid waste dumpsites, Abuja, after treatment with the bacterial isolates

Isolate (Code)**	Wight loss of bottles strips (%)				Weight loss of bag strips (%)			
	15 d	30 d	45 d	60 d	15 d	30 d	45 d	60 d
<i>Pseudomonas</i> sp. (G ₁₂)	0.52	0.98	1.48	2.86	0.45	1.05	1.41	1.78
<i>Pseudomonas</i> sp. (Gw ₂)	0.89	1.42	2.01	4.63	1.19	2.05	3.67	7.98
<i>Bacillus</i> sp. (G ₃)	1.02	1.89	2.78	6.20	0.46	0.93	1.69	3.14
<i>Providencia</i> sp. (Ku ₄₃)	0.92	1.49	2.52	5.67	0.05	0.12	1.21	1.79
<i>Providencia</i> sp. (G ₄)	0.18	0.55	0.89	1.93	1.34	2.21	3.78	5.90
<i>Pseudomonas</i> sp. (B ₂)	0.02	0.08	0.14	0.58	1.02	1.98	3.34	5.90
<i>Proteus</i> sp. (Gw ₂)	0.02	0.15	0.93	1.56	0.67	0.88	1.07	2.18
<i>Providencia</i> sp. (Kw ₂)	2.56	4.78	7.82	12.20*	0.15	0.48	0.98	1.77
<i>Providencia</i> sp. (A ₂)	2.66	4.88	9.05	14.96*	0.55	0.78	1.09	2.38
<i>Pseudomonas</i> sp. (Kw ₃)	0.12	0.48	0.61	1.15	0.57	0.83	1.12	2.38
<i>Proteus</i> sp. (A ₄)	0.88	1.51	2.23	4.95	2.87	4.78	8.93	15.40*
<i>Pseudomonas</i> sp. (B ₃)	0.03	0.10	0.43	0.94	1.02	2.25	2.81	5.84
<i>Proteus</i> sp. (Gw ₁)	1.02	1.59	2.86	5.72	1.09	2.86	3.84	7.90
<i>Providencia</i> sp. (G ₁)	0.09	0.78	1.00	1.94	1.09	2.67	3.89	7.94
<i>Proteus</i> sp. (G ₁₀)	9.67	17.02	27.08	49.00*	0.04	0.15	0.38	0.79
<i>Pseudomonas</i> sp. (Kw ₁)	0.67	1.25	1.98	3.78	2.90	3.67	5.03	8.10
<i>Bacillus</i> sp. (A ₃)	0.78	1.56	2.20	4.50	0.07	0.15	0.78	1.20
<i>Proteus</i> sp. (Gw ₄)	5.02	11.68	17.24	32.07*	0.15	0.67	0.97	2.16
<i>Proteus</i> sp. (B ₄)	4.12	7.23	11.43	20.41*	0.10	0.55	0.89	2.15
<i>Bacillus</i> sp. (B ₁)	0.25	0.59	1.28	1.78	0.09	0.34	0.78	1.58
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p-value	0.000	0.000	0.001	0.004	0.002	0.000	0.000	0.000

Where; **Isolate codes from Gosa (G), Gwagwalada (Gw), Kuje (K), Kwari (Kw), Abaji (A), and Bwari (B). *Potential plastic bio-degraders (>12 % weight loss)

The present findings are in line with the previous study of [Wanjohi et al. \(2018\)](#) who reported that bacterial strains, including *B. anthracoides*, *P. cepacia*, *Proteus penneri*, *Providencia stuarti*, and *Providencia rettgeri*, possess varying degrees of biodegradation capacities of the plastic cups and polyethylene bags. However, the current rates of plastic biodegradation abilities by the different bacterial strains, especially by *Proteus* sp. and *Providencia* sp. were relatively higher than those reported by [Wanjohi et al., \(2018\)](#). These variations might be attributed to the differences in the bacterial species/ strains, and in molecular weights of the different plastics used in biodegradation. Generally, most of the *Proteus* sp. and *Providencia* sp. in this study bio-degraded the plastic bottles better than the plastic bags, suggesting that the plastic bottle samples

were more susceptible to biodegradation by the tested bacteria than the bag samples. According to [Ru et al., \(2020\)](#), molecular weights of the polymer/ plastic materials generally affect their physical properties, such as solubility and surface areas, which in turn determine the rates of bio-degradation and volarization by the microorganisms. Thus, the observed susceptibility of the plastic bottles over the plastic bags probably indicates that they possess relatively lower molecular weights, and thus were more amenable to microbial biodegradations than the plastic bags. This finding is in discordant with the previous studies conducted by [Wanjohi et al., \(2018\)](#); [Usman et al., \(2019\)](#), who reported relatively better biodegradation of the polyethylene bags than the plastic cups/ bottles. These variations might be due to the differences in the bacterial species and/or in molecular weights of the

plastic materials. According to [Artham and Doble, \(2008\)](#), the microorganisms respond differently to the various polymers, according to the microbial species and the polymers molecular weights.

Although, the populations of *Pseudomonas* sp. and *Bacillus* sp. and their rates of biodegradation of the plastic materials were significantly lower than those of *Proteus* sp. G₁₀, *Proteus* sp. Gw₄, *Proteus* sp. B₄, *Proteus* sp. A₄, *Providencia* sp. A₂, and *Providencia* sp. Kw₂; however, they generally displayed promising potentials in reducing the weight losses of the plastic bags (1.78 - 8.10 %), and the plastic bottles (0.58 – 4.68 %), as shown in Table (3). A previous study conducted by [Asmita *et al.*, \(2015\)](#) identified *B. subtilis* and *P. aeruginosa* as potential bio-degraders of the polyethylene terephthalate (PET) and polystyrene (PS), which were important classes of plastic materials, and thus supported the current findings. Similarly, *B. subtilis*, and *Pseudomonas* sp. were reported to bio-degrade the ground polyethylene bags and plastic bottles ([Usman *et al.*, 2019](#)). Biodegradation of the plastic materials occurs via the activities of specie-specific microbial enzymes ([Ru *et al.*, 2020](#)). Recently, [Mohanan *et al.*, \(2020\)](#); [Shilpa and Meena, \(2022\)](#) reported that upon microbial exposure to any plastic material, and depending on the molecular weights, chemical structure, and crystalline nature of this plastic, the microbe releases special extracellular enzymes, which adsorbs to the polymer surface followed by stepwise hydroperoxidation and then hydrolytic cleavage until mineralization occurs. Only microbes that possess these enzymes and in presence of the optimum environmental conditions and nutrient substrates, they can efficiently breakdown the plastic polymers. Hence, the efficient biodegradation capacities currently observed by the tested bacterial isolates, mainly; *Proteus* sp. G₁₀, *Proteus* sp. Gw₄, *Proteus* sp. B₄, *Proteus* sp. A₄, *Providencia* sp. A₂, and *Providencia* sp. Kw₂, suggest that these isolates possess the specific hydrolytic enzymes needed for adsorption and hydrolysis of the plastic materials. Several recent studies conducted by [Mohanan *et al.*, \(2020\)](#); [Ru *et al.*, \(2020\)](#); [Atanasova *et al.*, \(2021\)](#) have

attributed the observed variations in bio-degradation results to the differences in molecular weights, chemical structure and crystalline nature of the plastics, in addition to the microbial species and rates of the hydrolytic enzymes production.

3.3. Molecular characterization of the plastic bio-degrading bacteria

Current results of the 16S rRNA characterization of the selected six bacterial isolates (i.e., *Proteus* sp. G₁₀, *Proteus* sp. Gw₄, *Proteus* sp. B₄, *Proteus* sp. A₄, *Providencia* sp. A₂, and *Providencia* sp. Kw₂) revealed a similar characteristic molecular weight bands at 789 bp, indicating the relatedness of these bacterial species (Fig. 2).

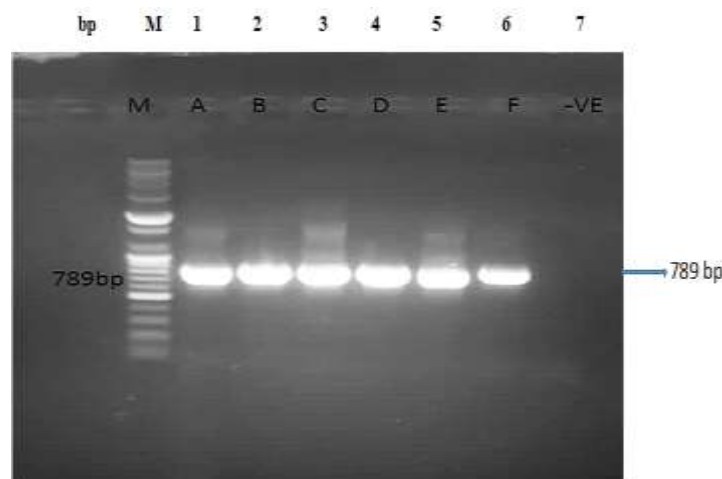


Fig. 2: Agarose gel electrophoresis showing characteristic bands recovered from 16S gene amplification using PCR assay

Where; Marker: 100 bp; Lane A: *Proteus mirabilis* B₄; Lane B: *Proteus mirabilis* G₁₀; Lane C: *Providencia vermicola* A₂; Lane D: *Proteus mirabilis* A₄; Lane E: *Proteus mirabilis* Gw₄; Lane F: *Providencia vermicola* Kw₂, and Lane -VE: Negative control

The selected 6 bacterial isolates belong to 4 strains of *Proteus mirabilis*, and 2 strains of *Providencia vermicola*. Results of their sequences showed 87.60 % -97.50 % pairwise identity to *Proteus mirabilis* strain PPB3, *Proteus mirabilis* strain UPMSD3, *Proteus*

mirabilis strain HH133, *Proteus mirabilis* strain SSBIKEN, *Providencia vermicola* strain M4, and *Providencia vermicola* strain 11 (Table 4). These currently isolated bacteria are novel strains with significant potentials for biodegradation of the plastic bottles and bags, which are chemically composed of polyethylene terephthalate (PET) and polyethylene (PE), respectively. Novel plastic-biodegrading bacterial strains, such as;

Enterobacter sp. bengaluru-btdsce01, *Enterobacter* sp. bengaluru-btdsce02, *Pantoea* sp. bengaluru-btdsce03 (Skariyachan et al., 2016), *B. wudalianchiensis*_UMT (2A), *P. aeruginosa*_UMT (Bakht et al., 2020), *B. tropicus* (MK318648) (Samanta et al., 2020), and *Providencia rettgeri* AVR20 (Anjanapriya et al., 2022), have been recovered from waste dumpsites of several previous studies, which is in line with results of the current study.

Table 4: Sequence analysis of *Proteus mirabilis* and *Providencia vermicola* strains

Dumpsite isolates	Pairwise identity (%)	NCBI Accession	16S rRNA confirmed strain
<i>Proteus mirabilis</i> G ₁₀	97.50 %	HM771658	<i>Proteus mirabilis</i> strain PPB3
<i>Proteus mirabilis</i> GW ₄	96.50 %	MH393635	<i>Proteus mirabilis</i> strain UPMSD3
<i>Proteus mirabilis</i> B ₄	87.60 %	HQ407305	<i>Proteus mirabilis</i> strain HH133
<i>Proteus mirabilis</i> A ₄	95.80 %	MH020182	<i>Proteus mirabilis</i> strain SSBIKEN
<i>Providencia vermicola</i> A ₂	92.60 %	MG815137	<i>Providencia vermicola</i> strain M4
<i>Providencia vermicola</i> Kw ₂	96.00 %	MH333229	<i>Providencia vermicola</i> strain 11

Conclusion

This study has shown that the native bacterial genera recovered from the solid waste dumpsites of Abuja were; *Proteus*; *Providencia*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *E. coli*, *Enterobacter*, and *Serratia* spp. Results showed that the potent biodegrading strains of the plastic bottles and bags, include *Proteus mirabilis* (*Proteus mirabilis* strain PPB3, *Proteus mirabilis* strain UPMSD3, *Proteus mirabilis* strain HH133, and *Proteus mirabilis* strain SSBIKEN), and *Providencia vermicola* (*Providencia vermicola* strain M4 and *Providencia vermicola* strain 11). These strains could be considered as potential biodegrading agents of the plastic bottles and bags, which are the most abundant plastic packaging waste materials within the dumpsites/ landfills. However, further study is required to characterize and purify the specific enzymes responsible for utilization of the plastic wastes, with a view to exploiting them as future biotechnological tools for plastics bio-remediation.

Conflict of interest

The authors declare that no competing interests exist.

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Ethical approval

Not applicable in this study.

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