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(RESEARCH ARTICLE)

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Biodegradation of low-density polyethylene (IDPE) by bacteria isolated from dump sites in some metropolitan cities in north central Nigeria

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Abstract

In this study, biodegradation of Low-Density Polyethylene (LDPE) by bacteria isolated from dump sites was evaluated in a liquid Basal Salts Medium. The bacteria, including *Psuedomonas aeruginosa, Bacillus megaterium, Providencia stuarti, Alcaligenes faecalis, Enterobacter hormaechei, Klebsiella pneumonia and Proteus vulgaris* were isolated from soil samples taken from municipal dump sites in some metropolitan cities in North Central Nigeria, namely, Abuja, Makurdi and Jos and screened for their ability to utilize LDPE using the clear zone method. 0.500 gram waste LPDE strips (1 cm x 5 cm) were placed into a 500 milliliter flask containing sterilized liquid medium at 30 °C and incubated in a rotary shaker for eight (8) weeks. Each bacterium was added to a separate flask. Biodegradation was measured by pH changes of the media and gravimetrically by weight loss of the waste LDPE strips two weekly during the incubation period. The results obtained showed a gradual decrease in the pH of the media originally set at 7.05 with incubation time for all isolated bacteria. *Psuedomonas aeruginosa* and *Providencia stuarti* recorded the highest weight loss of the LDPE strips after eight (8) weeks at 19.80±0.04 %, with a final pH of 3.75±0.01 and 19.20±0.42 %, and final pH 4.85±0.01 respectively followed by *Bacillus megaterium* at 13.40±0.10% and final pH of *3.95*±0.01. *Klebsiella pnuemonia* and *Proteus vulgaris* recorded the least gravimetric weight loss at 1.40±0.02 %, with a final pH of 4.75±0.01 and 0.80±0.01 %, pH 4.85±0.01 respectively. This work reveals that bacteria play a vital role in the degradation of low-density polyethylene waste in the natural environment. This can be applied to the development of commercial bioreactors in the future for the degradation of polyethylene wastes.

Keywords: LDPE strips: Degradation; Polyethylene wastes; Weeks; Dump sites

1. Introduction

Low- Density Polyethylene (LDPE) polymer with high hydrophobic level and high molecular weight is the main constituent of carrier bags (cellophane bags) [1] and are used extensively in packaging and other industrial and agricultural applications such as packing of foods, textiles, laboratory equipment and automotive components, among others. It is the most typically found non-degradable solid waste that has been recognized as a major threat to human and marine life [2,3]. The properties of polyethylene that makes it suitable for making various products, i.e. strong, durable and flexible, also makes it bad for the environment; thus LDPE wastes can take up to a thousand years in the environment before any form of degradation occurs [4].

Low-Density Polyethylene (LDPE) accounts for 60% of the total plastic production and the most commonly found solid waste [5, 6]. They are recalcitrant (resistant to microbial attack) and therefore remain more or less inert to degradation and deterioration for several years, leading to their accumulation in the environment [7]. As a result, low density polyethylene in the environment and their disposal evokes a big ecological issue [8].

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The current state of plastic (LDPE) bag waste pollution in Nigeria is alarming. Several environmental impacts including blockage of waterways, flooding and choking of animals, soils and mosaic litters of polyethylene wastes in the landscape requires urgent attention [9, 10]. There is need to explore microbial degradation of LDPE in the metropolis of North Central Nigeria, as it is a cheaper alternative to the more expensive and toxic alternatives of incineration and use of landfills which takes up land that should be used for crucial development urgently needed in this part of the world.

The aim of this study is to degrade Low-density polyethylene (LDPE wastes) using bacteria isolated from soil in dump sites of some parts of North Central Nigeria.

2. Material and methods

2.1. Chemicals and Reagents

Nutrient Agar for the isolation of bacteria consisted of the following in one liter of distilled water; Peptone 10.0 g, Sodium Chloride 5.0 g, Meat extract 10.0g and Agar 15.0g.

2.2. Nutrient Basal Media content:

The basal salts mineral media used contained the following elements (prepared in distilled water): 12.5g/l K_2HPO_4; 3.8/l KH_2PO_4; 1.0g/l $(NH_4)2SO_4$; 0.1g/l $MgSO_4.7H_2O$ and 5ml trace element solution contain each of the following elements (prepared in distilled water): 0.232g/l H_3BO_3;0.174g/l,$ZnSO_4.7H_2O$;0.116g/l$FeSO_4(NH_4)2SO_4.6H_2O$;0.096g/l$CoSO_4.7H_2O$;0.022g/l$(NH_4)6Mo7O_2.4H_2O$;8.0mg/l $CuSO_4.5H_2O$; 8.0mg/l$MnSO_4.4H_2O$

2.3. Sample Collection

Garbage soil samples from waste disposal sites were collected using the method of Anbuselvi, [1]. Soil samples were collected from three biggest garbage dump sites in each of the four cities under study. Soil sample were collected from top 10cm of the soil profile using a sterile spatula and placed in sterile sample bags and clearly labeled before taking to the laboratory. Also, waste LDPE polyethylene films (clear), from each site was also collected in separated sample bags, labelled and transported to the laboratory.

2.4. Isolation and characterization of bacteria

A method described by Anbuselvi, [1] was used in isolation of the bacteria. One gram of soil was transferred into a conical flask containing 99ml of sterile range solution (for easy dissolution of the sand), shaken and serially diluted. Pour plate method was used for the isolation of bacteria using nutrient agar for each dilution. The plates were incubated at 30^oC for 24hours. The developed colonies were sub-cultured repeatedly to get pure colonies and then preserved in a slant at 4 ^oC.

The bacterial strains were identified macroscopically by examining colony morphology, surface pigment, shape and size on nutrient agar plates.

Microscopic examination including gram's staining was used to study the staining behavior, shape and cell arrangement.

Motility test was performed, and biochemical tests were carried out such as catalase, gelatin, hydrolysis, triple sugar, Indole, methyl red, VP, starch, and citrate tests following the Bergey's Manual of Determinative Bacteriology (2000).

2.5. Waste Polyethylene bag preparation and Culture condition.

A method described by Kyaw & Champakalakshmi, [11] was used in preparing waste polyethylene. Polyethylene films were collected from three sites randomly selected from dump sites in each of the four capital cities chosen for this study in North Central Nigeria. These were cut into (5 cm X 1 cm) strips and then washed first with tap water to remove all debris and soil particles. Then, they were washed with 70% ethanol for 30 minutes, washed with distilled water and subsequently dried in incubator at 60 ^oC before exposure to the bacterial isolates earlier identified. Inoculation and incubation was carried out under aseptic condition.

2.6. Pretreatment and Preparation of Low Density Polyethene Powder

The method described by Das and Kumar [12] was used. LDPE films were cut into small pieces (2 cm strips). Each strip was dipped in xylene and boiled for 15 minutes (until the plastic strip dissolved). It was cooled until it was palm bearable

and then crushed with a blender at 3,000 rpm. This was left to evaporate the xylene, and then washed with ethanol to remove any xylene residues. It was dried in hot air oven at 60 ^{o}C overnight and stored at room temperature for further use.

2.7. Screening of LDPE utilizing bacteria

This was carried out using the clear zone method described by Usha *et al*., [13], Anbuselvi, [1]. LDPE powder (3 %) was added to a basal salt mineral medium at a final concentration of 0.1 % (w/v) respectively. The mixture was sonicated for 1 hour at 121 ^{o}C and pressure of 15 psi for 20 minutes.

About 15ml of sterilized medium was poured before cooling in each plate. The isolated microbes were inoculated into the polyethylene containing agar plates and then incubated at 25-30^{o}C for 2-4 weeks. The organisms producing zone of clearance around their colonies were characterized and selected for biodegradation tests.

2.8. Characterization of PE degrading bacterial isolates

The taxonomic identification of the bacterial isolates capable of degrading PE, including biochemical characterization and PCR amplification of the 16S rRNA was carried out using the method of Munhonja *et al*., [14]. The partial nucleated sequence of the 16S rRNA from each isolate was determined (using ABI system 3730 XL) and was deposited in the NCBI database under Gene bank with an accession number.

2.9. Measurement of pH Changes

Using the methods of Arutchielvi, [15] the pH of the basal mineral media inoculated with bacterial isolates were monitored using a pH meter at 2, 4, 6, and 8 weeks' incubation to ascertain microbial activity and biodegradation of the LDPE strips.

2.10. Biodegradation measurement- Weight loss method

The polyethylene films after exposure to each of the bacterial isolates were evaluated for weight loss using the methods of Hadad *et al*., [16]. They were washed thoroughly with 2 % (v/v) aqueous Sodium Dodecyl Sulphate (SDS) solution for 4 hours. The strips were dried at 60 ^{o}C overnight in an incubator and placed on a filter paper before weighing with a microbalance; the percentage weight loss was determined using the following formula:

$$\text{Weight loss (\%)} = \text{initial weight} - \text{final weight}/\text{initial weight} \times 100.$$

2.11. Data Analysis

All analysis was conducted in triplicate and analyzed using Microsoft Excel Windows 10 program and Smith Statistical Package (SSP) version 3.1, with significance determined at 95% interval. Results are presented as means ± standard error of the mean.

3. Results

3.1. Morphological and biochemical characterization of bacteria

The cultural, morphology and biochemical characteristics of the bacteria isolated from dump sites soil is as shown in Table 1. Seven different genus were identified based on colony morphology, gram staining and biochemical tests (Table 1). The different strains were *Psuedomonas sp (1). Psuedomonas sp. (2), Bacillus sp., Alcaligenes sp., Klebsiella sp., Providencia sp., Acinetobacter sp., and Enterobacter sp*

Table 1 Cultural, Morphological and Biochemical characteristics of Bacteria isolated from dump sites from parts of North Central Nigeria

Cultural / morphology / biochemical tests	**Bacteria -1**	**Bacteria - 2**	**Bacteria-3**	**Bacteria - 4**	**Bacteria - 5**	**Bacteria - 6**	**Bacteria - 7**	**Bacteria -8**
Gram stain / cell shape	+ Rods (Chain)	- Rods	- Rods	- Rods	- Rods	- Coccobacilli	- Rods	- Rods
Motility test	+	+	+	-	+	-	+	+
Catalase test	+	+	+	+	-	+	+	+
Gelatin hydrolysis test	+	-	-	-	-	-	?	?
Indole test	-	-	-	-	+	-	-	+
Methyl Red test	-	-	?	-	?	-	-	+
Starch Hydrolysis test	+	-	-	+	?	?	?	?
Citrate utilization test	-	-	+	+	+	+	+	+
Voges- Proskauer test	+	-	?	-	-	-	+	_
Inference/ Bacterial genus	*Bacillus sp.*	*Pseudomonas sp*.	*Alcaligenes sp*.	*Klebsiella sp*.	*Providencia sp.*	*Acinetobacter sp.*	*Enterobacter sp.*	*Proteus sp.*

3.2. Molecular characterization of bacteria

The Agarose gel electrophoresis of the 16S rRNA gene bands and sizes (1500 bp) is as shown in plates 1, 2 and 3 respectively.

The Phylogenetic relationship (evolutionary distance) of the bacterial isolates based on 16S rRNA gene nucleotide sequences are shown in the phylogenetic trees (Figures 1, 2 and 3). Numbers at the nodes indicate bootstrap values from the neighbor-joining analysis of the sampled data sets. The bars represent sequence divergence.

3.3. Screening of bacteria for LDPE utilization

Table 2 Screening for polyethylene utilization by different isolated bacteria species

Bacterial	**Zone of clearance (mm)**
Pseudomonas aeruginosa1	11.01±0.14
Bacillus megaterium	15.06±0.16
Providencia stuarti	16.25±0.55
Alcaligenes faecalis	15.30±1.02
Acinetobacter venetianus	12.98±1.11
Enterobacter hormaechei	9.04±0.75
Klebsiella pneumonia	6.65±0.02
Proteus vulgaris	7.13±01

Table 2 shows the zone of clearance on nutrient agar by different bacterial isolates showing utilization of LDPE as sole source of carbon were Pseudomonas aeruginosa1 had 11.01±0.14 mm, Bacillus megaterium had 15.6±0.16mm, Providencia stuarti with 16.25±0.55mm, Alcalagenes faecalis15.30±1.02mm, Acinetobacter venetianus 12.98±1.1mm, Enterobacter hormaechei 9.04±0.75mm, Klebsiella pneumonia 6.65±0.02mm, and Pseudomonas aeruginosa2 15.56±0.22mm respectively

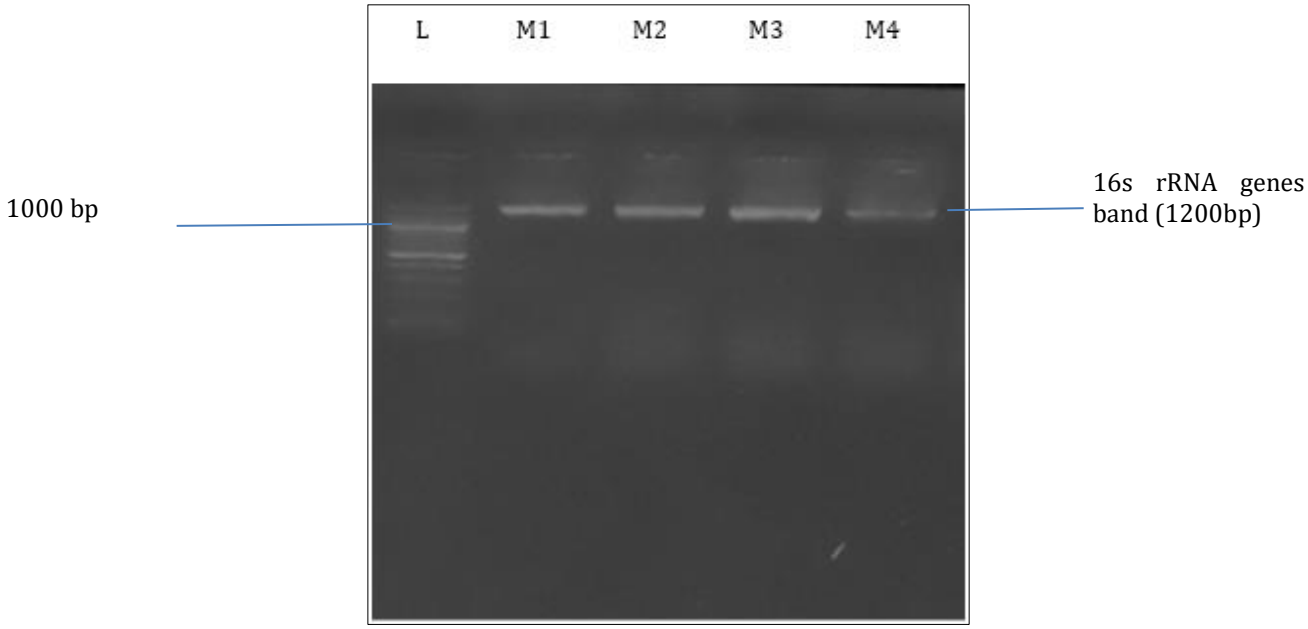

Figure 1 Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates from Makurdi (M). Lanes M1-M4 represent the 16SrRNA gene bands (1500bp), lane L represents the 100bp molecular ladder

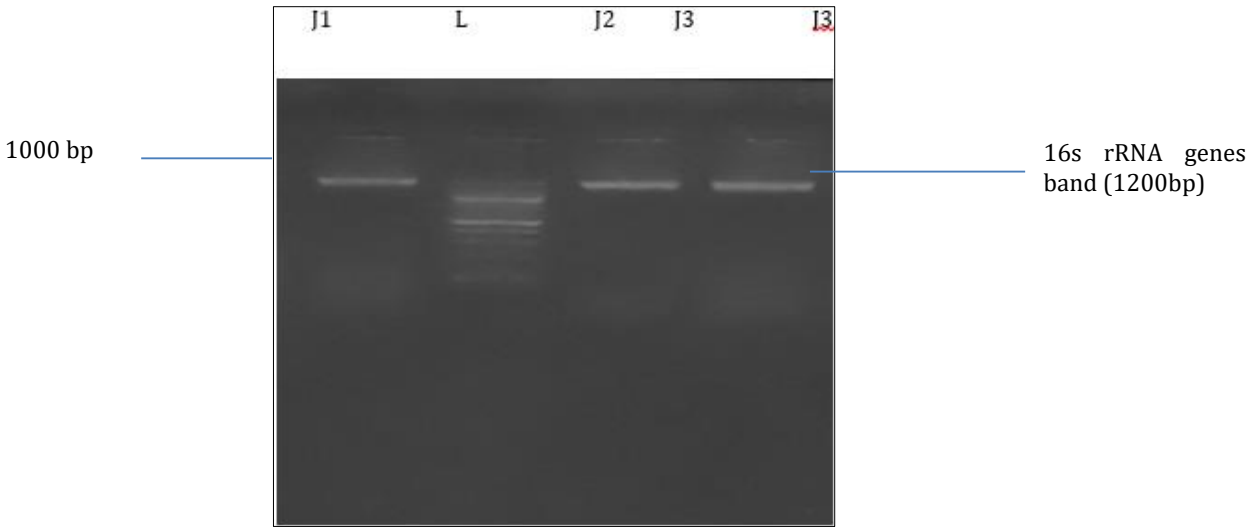

Figure 2 Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates from Jos(J). Lanes J1, J2, J3 represent the 16SrRNA gene bands (1500bp), lane L represents the 1000bp molecular ladder

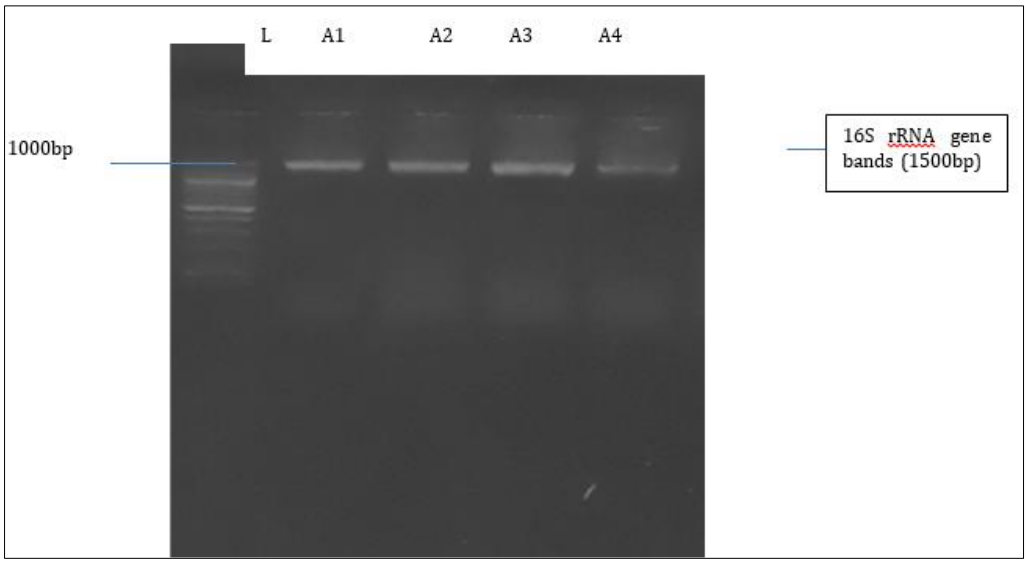

Figure 3 Agarose gel electrophoresis of the 16S rRNA gene of some selected bacterial isolates. Lanes A1-A4 represent the 16SrRNA gene bands (1500bp), lane L represents the 1000bp molecular ladder

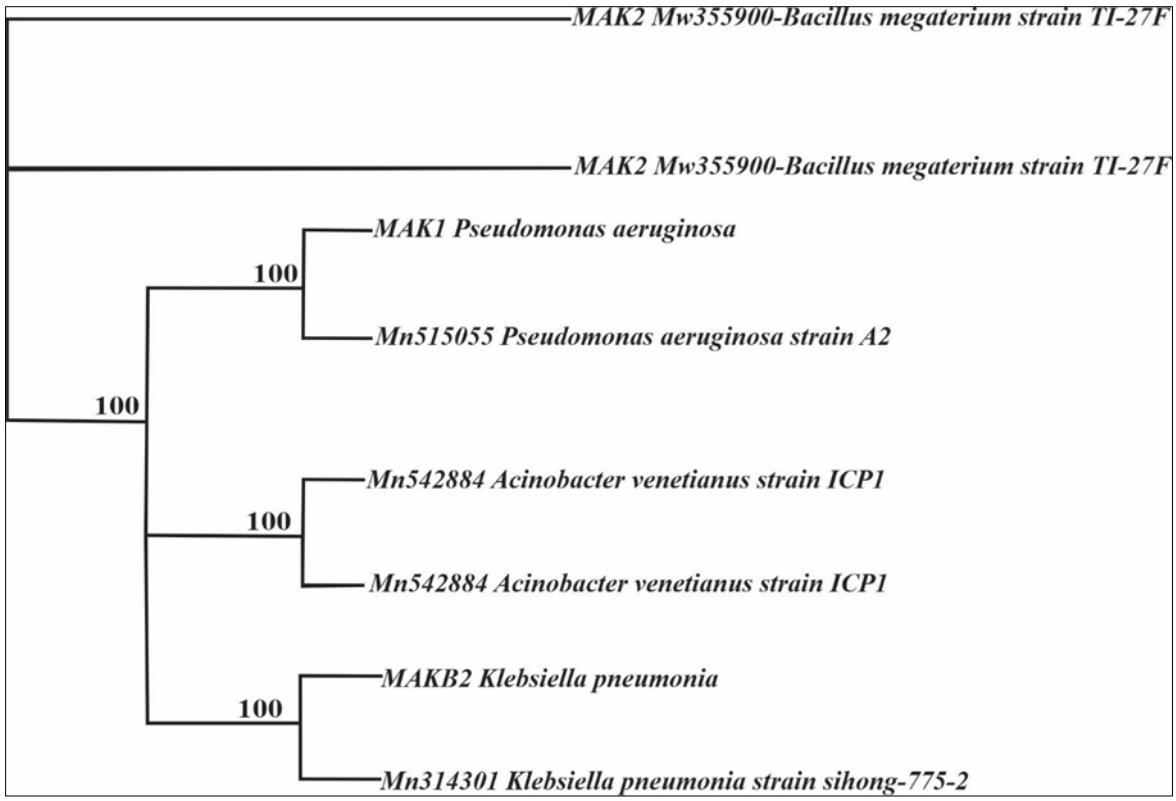

Figure 4 Phylogenetic relationship of bacterial isolates from Makurdi based on 16S rRNA gene nucleotide sequences. Numbers at the nodes indicate bootstrap values from the neighbor-joining analysis of the sampled data sets. Bar represents sequence divergence

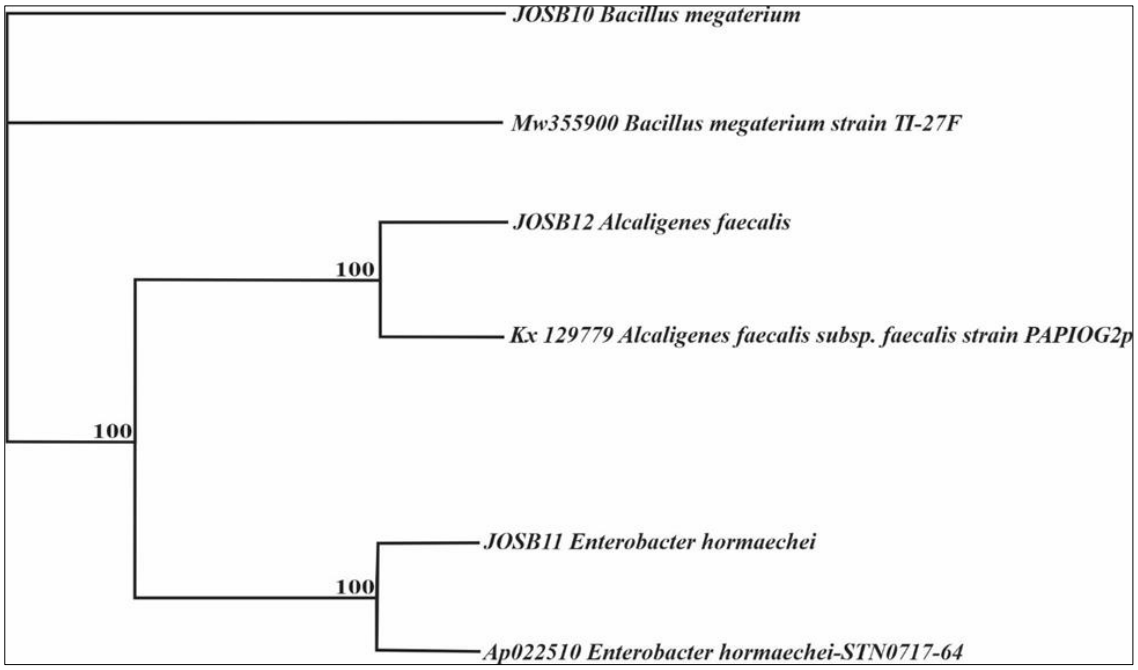

Figure 5 Phylogenetic relationship of bacterial isolates from Jos based on 16S rRNA gene nucleotide sequences. Numbers at the nodes indicate bootstrap values from the neighbor-joining analysis of the sampled data sets. Bar represents sequence divergence

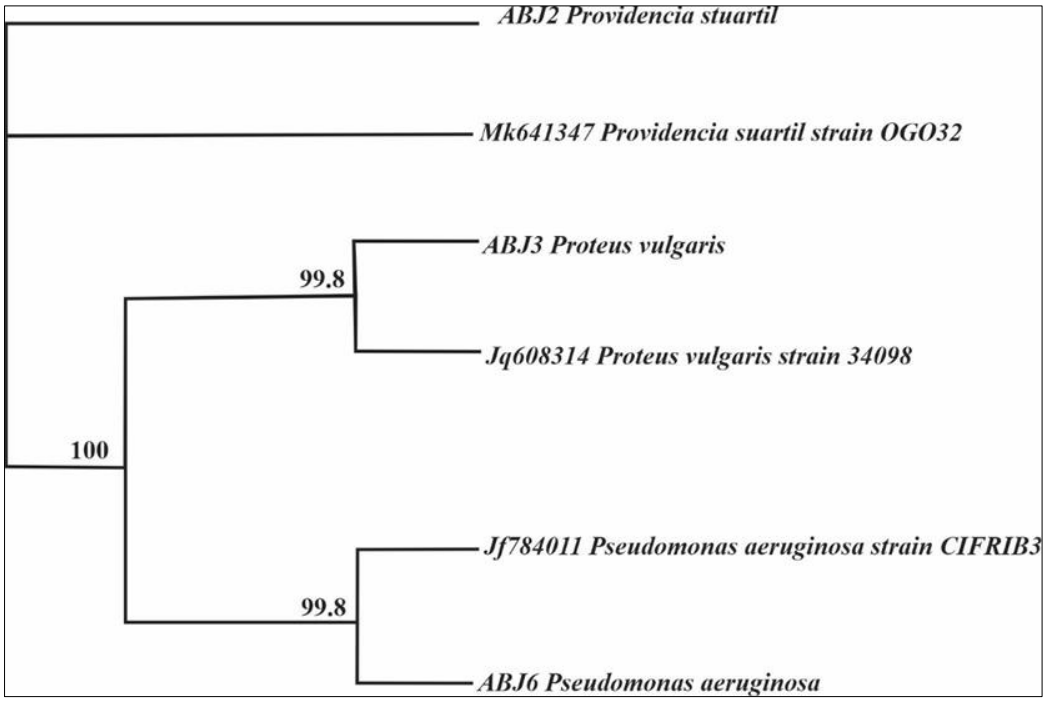

Figure 6 Phylogenetic relationship of bacterial isolates from Abuja based on 16S rRNA gene nucleotide sequences. Numbers at the nodes indicate bootstrap values from the neighbor-joining analysis of the sampled data sets. Bar represents sequence divergence

3.4. pH changes of incubation media

The changes in the pH of the media (originally set at 7.05±0.02) incubated with bacterial isolates after eight weeks ranges from 3.75± 0.01 to 4.85±0.01 with all the isolates causing PH reduction in the media. The highest reduction in

pH was observed for *Pseudomonas aeruginosa 1* (3.75+0.01) and *Psuedomonas. Aeruginosa 2* (3.78±0.01) followed by and *Bacillus megaterium* (3.95±0.01) when compared with the control as shown in Figure 4.

Table 3 Changes in pH of Media over Time of Incubation with Bacterial Isolates

Bacteria	**Initial**	**pH changes over time (weeks)**			
		2	**4**	**6**	**8**
Control	7.05±0.02	7.05±0.02	7.05±0.02	7.05±0.02	7.05±0.02
Pseudomonas aeruginosa 1	7.05±0.02	6.02±0.05	5.35±0.01	4.81±0.03	3.75±0.01
Pseudomonas aeruginosa 2	7.05±0.02	6.05±0.05	5.55±0.01	4.85±0.03	3.78±0.01
Bacillus megaterium	7.05±0.02	6.06±0.05	5.80±0.01	4.90±0.03	3.95±0.01
Providencia stuarti	7.05±0.02	6.09±0.05	5.95±0.01	5.95±0.03	4.85±0.01
Alcaligenes faecalis	7.05±0.02	7.02±0.05	6.88±0.01	5.90±0.03	4.51±0.01
Enterobacter hormaechei	7.05±0.02	6.08±0.05	5.91±0.01	5.60±0.03	4.55±0.01
Klebsiella pneumonia	7.05±0.02	7.03±0.05	6.85±0.01	5.80±0.03	4.75±0.01
Proteus vulgaris	7.05±0.02	6.09±0.05	5.85±0.01	5.50±0.03	4.85±0.01

Results show average of three readings per isolate after 8 weeks of incubation

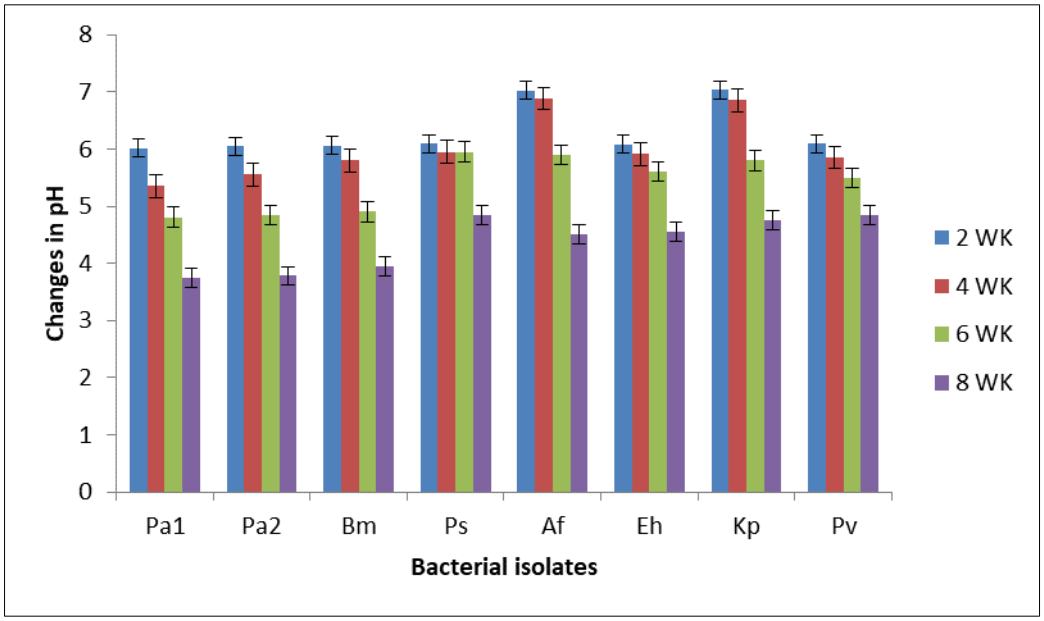

Figure 7 Changes in pH of media over time of incubation with bacterial isolates

3.5. Weight loss measurements

The percentage weight reduction of LDPE waste by bacterial isolates between 2-8 weeks of exposure were within the range of 0.0-19.80±0.04% and the highest percentage reduction was at 8 weeks duration for *Psuedomonas aeruginosa* (Pa1: 19.80±0.04%), *Psuedomonas aeruginosa* (Pa2: 19.40±0.08%) and *Providencia staurti* (19.20±0.42%)but low at 2-6 weeks durations for *Klebsiella pneumoniae* with percentage weight reductions ranging between 0.60±0.17% – 1.40±0.02% and after 2-8 weeks duration for *Proteus vulgaris* with percentage weight reductions rangingrom 0.0-0.80±0.00% as shown in Figure 6 .

Table 4 Biodegradation of Low –Density Polyethylene waste films by Bacterial Isolates

Bacteria	**Initial weight of LDPE strip(g)**	**Percentage weight loss of LDPE films over time (weeks) (%)**			
		2	**4**	**6**	**8**
Control	0.500	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Pseudomonas aeruginosa1	0.500	9.60±0.18	12.80±0.41	14.20±0.09	19.80±0.04
Pseudomonas aeruginosa2	0.500	11.00±0.10	13.20±0.30	18.80±0.01	19.40±0.08
Bacillus megaterium	0.500	5.40±0.20	11.60±0.61	13.20±0.04	13.40±0.10
Providencia stuarti	0.500	6.60±0.11	8.20±0.41	17.40±0.001	19.20±0.42
Alcaligenes faecalis	0.500	6.20±0.12	6.80±0.12	7.60±0.21	8.00±0.81
Enterobacter hormaechei	0.500	3.60±0.21	5.40±0.01	5.60±0.11	5.80±0.31
Klebsiella pneumonia	0.500	0.60±0.17	1.20±0.17	1.40±0.19	1.40±0.02
Proteus vulgaris	0.500	0.00±0.00	0.60±0.00	0.60±0.00	0.80±0.00

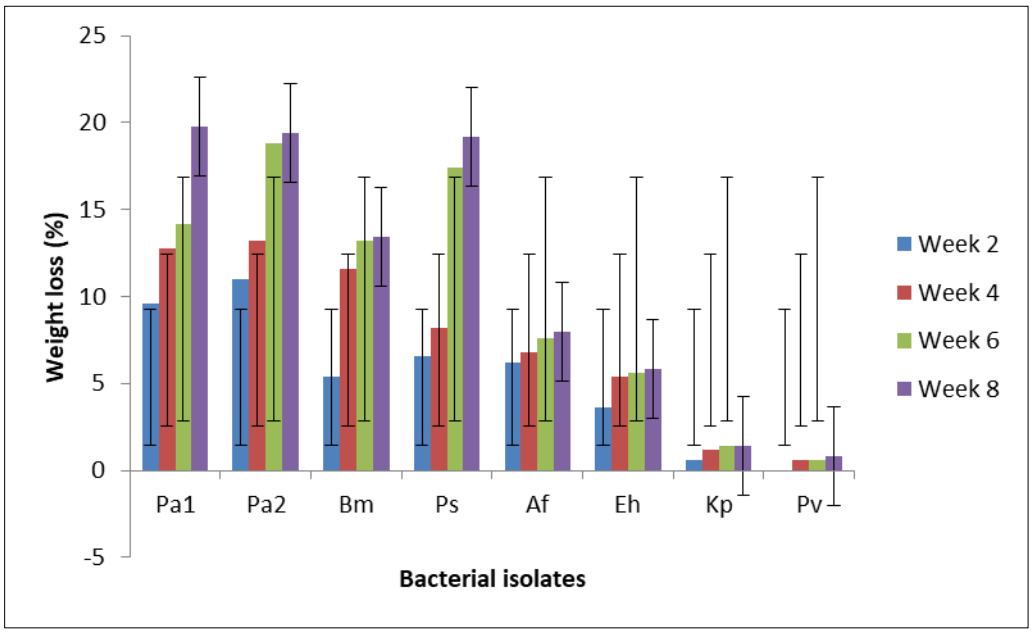

Figure 8 Percentage weight loss of waste LDPE films degraded by Bacteria isolated from dump sites in parts of North Central Nigeria

4. Discussion

The molecular identification of isolates shows evolutionary distance between the bacterial isolates such as *Providencia stuarti, Alcaligenes faecalis, Acinetobacter venetianus, Enterobacter hormaechei, Bacillus megaterium, Proteus vulgaris* and *Pseudomonas aeruginosa*; this is similar to earlier studies by Krueger *et al.,* [17] which reported high frequency of occurrence of different bacteria from dump sites using morphological, biochemical and molecular techniques as employed in this study.

Following screening for utilization of LDPE using the clearance zone method [18], *Pseudomonas aeruginosa* (1 & 2), *Bacillus megaterium, Providencia stuarti, Alcaligenes feacalis, Enterobacter hormaechei, Klebsiella pneumonia* and *Proteus vulgaris* were found to use LDPE as a sole carbon source. These were selected for further biodegradation studies.

Monitoring pH changes of the basal mineral medium used in incubating the bacterial isolates and waste LDPE films, it was observed that there was a general reduction in pH during the eight-week incubation period for all the bacteria selected for biodegradation studies. Initial pH was 7.05 ± 0.02 for the incubating mineral salt medium for the bacterial isolates, however after 8-week incubation, the lowest reduction in pH was observed for the *Pseudomonas aeruginosa* 1 measured as 3.75 ± 0.01 (Table 4).

This reduction in pH shows that the culture was still metabolically active and that the LDPE was utilized for its growth. The reduction in pH not only affirms the consumption of LDPE film as the sole source of carbon [12, 15, 19], it also confirms that microorganisms secrete a variety of intra and extracellular enzymes into the media which might be responsible for the degradation of the polymer [20]. During the polymer degradation process, complex polymers such as LDPE are first broken down into short chains or monomers by exoenzymes that are small enough to permeate through cell walls to be utilized as carbon and energy sources by a process of depolymerization [3]. The concomitant decrease in pH with incubation period for biodegradation might not be unconnected with the fact that degradation of hydrocarbon compounds such as low-density polyethylene usually leads to the production of organic acids that invariably leads to lowering pH of the media [21, 22, 23].

Biodegradation was measured at different intervals after incubating LDPE film with the eight bacterial at 37 ^{0}C. In all cases, there was time dependent weight loss of the starting LDPE over time of incubation.

After eight weeks of incubation, weight loss of LDPE strips by bacteria ranged from 0.8 % for Proteus vulgaris to 19.8 % for *Pseudomonas aeruginosa* I. There was no weight loss observed in control experiment, confirming the action of the bacterial isolates. Among the eight bacterial isolates used for the biodegradation experiments, Pseudomonas aeruginosa 1 and *Psuedomonas*. *aeruginosa* 2, *Providencia stuarti*, and *Bacillus megaterium* were found to be the most effective in degradation of LDPE after 8 weeks of incubation. This is in line with results obtained from previous studies by Deepika and Jaya, [18] which reported that Pseudomonas species have significant plastic degradation capacity, degrading up to 24.2 % of plastic polymer within a period of 6 months. Similarly, Kyaw *et al.,* [24] studied the biodegradation of LDPE by Pseudomonas species and reported that after 120 days of incubation, the percentage weight reduction was 20% in *Pseudomonas aeruginosa* (PAO1), 11 % in *Pseudomonas aeruginosa* (ATCC) strain, 9% in *Pseudomonas putida* and 11.3 % in *Pseudomonas syringae* strain. Badrimarayanan, [25] also reported that *Pseudomonas alcaligenes* exhibited significant polyethene degradation ability.

In a similar study by Ojiego *et al.,* [26], it was reported that 6 of the bacterial isolates from dump sites in Abuja, Nigeria investigated for plastic degradation, only two genera, *Providencia* spp. and *Proteus* spp. were found to be the best degraders of plastic materials under controlled conditions. Wanjohi *et al.,* (2018) also reported biodegradation activities of *Providencia* sp. and *Proteus* sp., however the rate of degradation attributed to these bacterial species in this study was higher than that reported by Ojiego et al., [26]. These variations may be attributed to the differences in the bacterial species / strain and in the molecular weights of the plastics used in the biodegradation studies.

According to Ru *et al*., [27], molecular weight of the polymer / plastic materials generally affects their physical properties such as solubility and surface areas, which in turn determine the rates of biodegradation and valorization by microorganisms. In a different study by Asmita *et al.,* [28], it was found that *Bacillus subtilis* and *Pseudomonas aeruginosa* were identified as potential biodegraders of the polyethylene terephthalate (PET) and Polystyrene (PS) which are important plastic materials. These results are similar to that obtained for *Bacillus* and *Pseudomonas* species isolated in this study.

Biodegradation of plastic materials occurs through the activities of specie-specific microbial enzymes [27]. Recently, Mohanan *et al*., [29] and Shilpa & 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 microbes release special extracellular enzymes, which adsorbs to the polymer surface stepwise followed by hydroperoxidation and then hydrolytic cleavage until mineralization occurs. Only microbes that possess these enzymes and in the presence of optimum environmental conditions and nutrient substrates, can efficiently breakdown the plastic polymers [26]. Reduction in weight may also be due to the consumption of LDPE film as a sole carbon source by the bacterial isolates, confirming these organisms' capacity to degrade LDPE.

5. Conclusion

This study indicates that naturally growing soil microbes such as bacteria from dump sites in metropolitan cities of North Central Nigeria show great capacity to utilize Low-Density Polyethene (LDPE) at different degrees. The highest reduction in weight of LDPE waste films (degradation activity) was obtained after 8 weeks for all bacterial isolates, with the highest weight loss recorded fo*r Pseudomonas aeruginosa* 1 (19.80±0.04%), while the least weight reduction (degradation activity) was recorded for *Proteus vulgaris (0.80±0.00%).* Further investigation on the metabolic pathways, enzymatic reactions and metabolites would contribute to greater understanding of the exact mechanisms of biodegradation by these bacterial strains which should help to develop in situ process of LDPE biodegradation in order to make it commercially viable.

Compliance with ethical standards

Disclosure of conflict of interest

The author declared no conflict of interest exist.

Authors Contributions

This study was conducted in collaboration of all authors. All authors read and approved the final version of the manuscript.

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