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Isolation and screening of low-density polyethylene (LDPE) bags degrading bacteria from Addis Ababa municipal solid waste disposal site "Koshe"

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Abstract

Purpose This study aims to screen bacterial isolates from the Addis Ababa municipal solid waste dumping site (Koshe) for the biodegradation of low-density polyethylene bags and analyzes their efficiency in degrading plastic bags.

Methods In this study, low-density polyethylene bag-degrading bacteria were isolated from the Koshe municipal solid waste disposal area in Addis Ababa, Ethiopia. Screening of isolates for low-density polyethylene bag biodegradation was carried out using a clear zone method. Additionally, the efficiency of the isolates for low-density polyethylene biodegradation was evaluated using the weight loss method, scanning electron microscopy analysis, and Fourier transform infrared analysis. Finally, molecular identification of potential low-density polyethylene degrader bacterial isolates was done by 16S rDNA sequencing.

Results Isolates KS35, KS14, and KS119 resulted in significant weight loss of low-density polyethylene film (42.87 ± 1.91%, 37.2 ± 3.06%, and 23.87 ± 0.11% weight loss, respectively). These isolates were selected for further biodegradation study using scanning electron microscopy and Fourier transform infrared analysis. Scanning electron microscopy analysis shows the formation of pores, pits, and distortion of the plastic surface. Fourier transform infrared analysis indicates the appearance of new peaks at the surface of low-density polyethylene films. Phylogenetic analysis of the three potential bacterial isolates was also carried out, and the result indicates that the sequence of isolate KS35 had 99% similarity with sequences of *Methylobacterium radiotolerans* MN525302. Isolate KS119 had 100% similarity with *Methylobacterium fujisawaense* KT720189, and the sequence of isolate KS14 had 99% similarity with species of *Lysinibacillus fusiformis*.

Conclusions Weight loss, scanning electron microscopy analysis, and Fourier transform infrared analysis results show that isolates KS35, KS14, and KS119 have high potential in degrading low-density polyethylene bags.

Keywords Bacterial isolates, LDPE, Scanning electron microscopy, Fourier transform infrared spectroscopy, Biodegradation

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Background

Polyethylene is one of the most abundant commercially produced synthetic plastic materials. It is a polymer of ethylene, CH2-CH2, having the formula (−CH2-CH2-) n, where "n" is the number of carbon atoms (Sandhu and Shakya 2019). Among the polyethylene family, LDPE (low-density polyethylene) accounts for 60% of the total production of plastic bags, and it is the major component of municipal solid waste (Gajendiran et al. 2017).

Due to its high molecular weight, long carbon chain backbone, three-dimensional structures, hydrophobic nature, and lack of functional groups recognizable by microbial enzyme systems, LDPE is very resistant to biodegradation (Chiellini et al. 2003). Under normal conditions, the mineralization of LDPE takes more than ten decades (Otake et al. 1995). The extensive usage of LDPE is a severe environmental threat to terrestrial and marine ecosystems. Plastic waste disposed of into the environment entangles the animal or bird's body, thereby causing the mortality of the organism (Kumar and Raut 2015). It also blocks the sewage system and creates a breeding ground for mosquitoes. Improper disposal of plastic waste can result in lost revenue from tourism by deteriorating the natural beauty of the environment (Muthukumar and Veerappapillai 2015). Plastic materials dumped into the earth prevent the production and mobilization of nutrients in the soil (Gharahi and Zamani-Ahmadmahmoodi 2022). Some plastic products cause human health problems by causing immune and enzyme disorders, hormonal disruption, and even infertility and are carcinogenic (Koteswararao et al. 2014).

Different countries have adopted a range of approaches to discourage the use of plastic bags. In Ethiopia, Environmental Protection Authority has cited a proclamation prohibiting granting permits for the company manufacturing or importing non-biodegradable plastic bags with a thickness of less than 0.03 mm (Gazeta 2007). However, due to poor awareness of society, and the lack of a strong regulation system, different types of plastic bags are produced in large quantities and improperly disposed of.

Different methods are practiced for the management of plastic waste. These include recycling, incineration, biodegradation, and dumping in a landfill. Moreover, biodegradation is an environmentally sound full and cost-effective method of plastic waste management (Kumar and Raut 2015). Recent reports on discovering certain fungi and bacteria that degrade synthetic plastics have received scientific attention. Bacterial species associated with the degradation of LDPE bags include *Bacillus cereus* (Raut et al. 2015), *Pseudomonas knackmussii*, *Pseudomonas aeruginosa* (Hou et al. 2022), and *Streptococcus* species (Das and Kumar 2015). Polyethylene bags could be degraded by some fungal species such as *Aspergillus niger*, *Aspergillus flavus* (Deepika and Jaya 2015), and *Aspergillus versicolor* (Gajendiran et al. 2016). Biodegradation of LDPE film using *Bacillus amyloliquefaciens* has been reported by Das and Kumar (2015). They incubated LDPE film with two strains of *Bacillus amyloliquefaciens* for 60 days and obtained a 16% weight loss of film. The incubation of polyethylene film with *Pseudomonas* species, *A. niger*, *A. flavus*, and *Streptomyces* species for 6 months revealed the reduction in molecular weight of LDPE film by 24.22 ± 0.01%, 26.17 ± 0.05%, 16.45 ± 0.01%, and 46.7 ± 0.01%, respectively (Lee et al. 1991). Gajendiran et al. (2016) identified *Aspergillus clavatus* as polyethylene-degrading fungi with 35% weight loss of films after 90 days of incubation.

Even though many researchers have reported bacterial and fungal degradation of LDPE, significant degradation of LDPE wastes for environmental applications has not yet been achieved (Montazer et al. 2020). This study aimed to screen indigenous bacterial isolates that have the potential to degrade LDPE from plastic harboring municipal solid waste soil samples.

Materials and methods

Sample collection and substrate preparation

Soil samples were collected from Koshe solid waste disposal area in Addis Ababa, Ethiopia. The site is located in the Kolfe Keraniyo sub-city southeastern part of Addis Ababa (Fig. 1). This area serves as a solid waste disposal site for over 50 years. A total of 15 samples were collected from different sites randomly using closed sterile containers and transported to the laboratory in the ice box. The samples were homogenized and stored at 4 °C until used. LDPE granules were collected from Ethiopia Plastic Factory and used for enriching LDPE-degrader bacterial isolates after being prepared in powder form. The powder was prepared by immersing LDPE granules in xylene and boiling them for 15 min (Bhatia et al. 2014). The powder was washed with 95% ethanol, dried overnight in a hot air oven at 50 °C, and stored at room temperature for further use. Low-density polyethylene films required for the biodegradation study were purchased from a local market and prepared by cutting into 1.5 cm × 1.5 cm size pieces.

Culture enrichment and isolation of LDPE-degrading bacteria

Culture enrichment was performed to isolate bacteria that use LDPE as the sole source of carbon. The culture was enriched by suspending 1 g of the soil sample in 50 ml of sterile saline water and incubating it in a rotary shaker at 120 rpm for 4 h. Then, 5 ml of soil suspension was transferred into a 250 ml Erlenmeyer flask containing 100 ml of sterile mineral salt broth (1g/L K_2HPO_4,

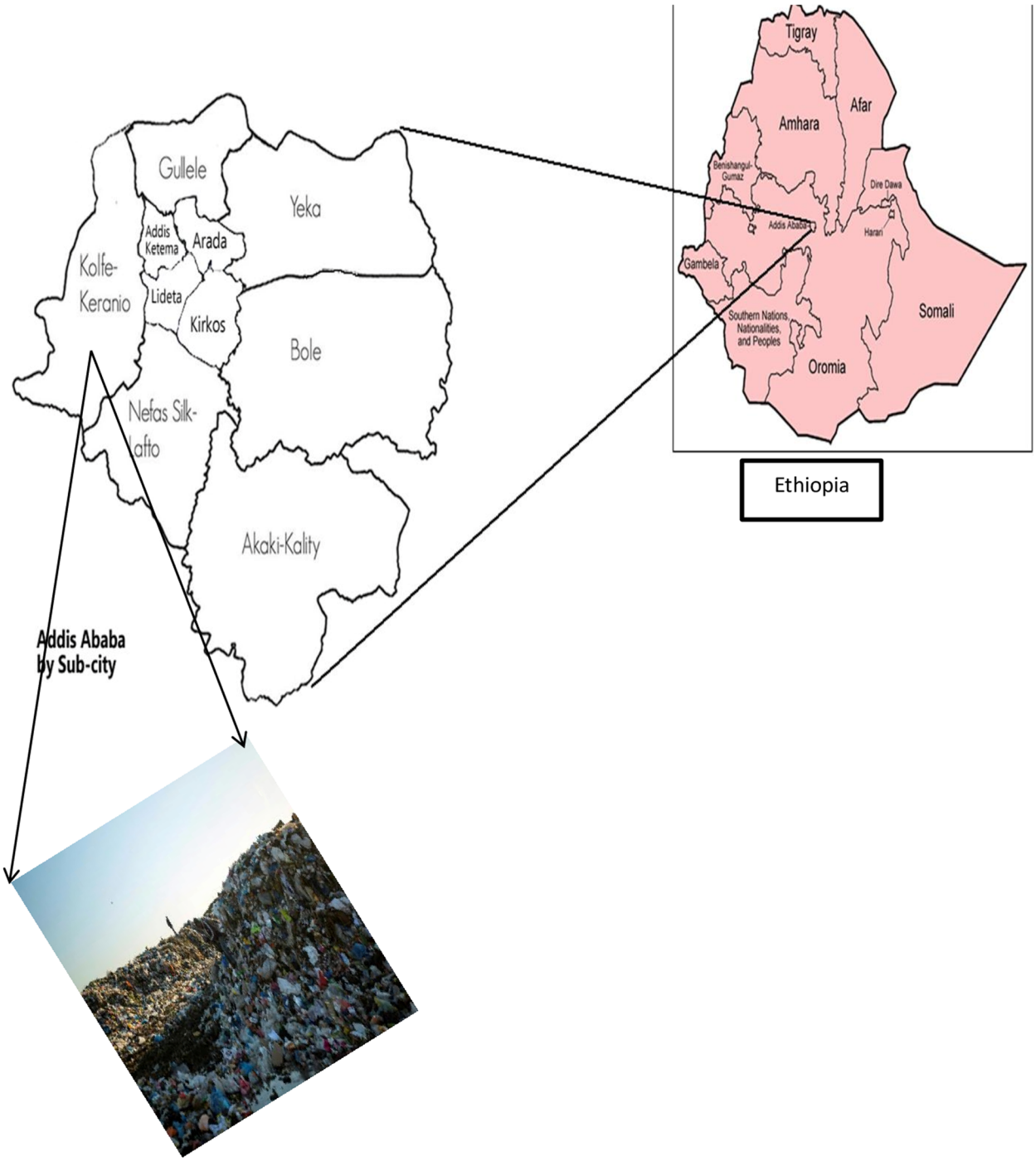

Fig. 1 Location and map of Koshe dumping site

0.2 g/L KH_2PO_4, 1 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4.7H_2O$, 1 g/L NaCl, 0.01 g/L $FeSO_4.7H_2O$, 0.002 g/L $CaCl_2.2H_2O$, 0.001 g/L $MnSO_4.7H_2O$, 0.001 g/L $CuSO_4.5H_2O$, 0.001 g/L $ZnSO_4.7H_2O$ and pH 7.0) and 0.2% (w/v) LDPE powder. All Erlenmeyer flasks were incubated in a shaker incubator at 35 °C and 120 rpm. After 1 week of growth, 5 ml of the enriched culture was transferred into 100 ml of freshly prepared mineral salt medium supplemented with 0.2% (w/v) LDPE powder. The third and fourth transfers were done successively under similar conditions. After four cycles of enrichment, 0.1 ml of serially diluted sample was spread on nutrient agar plates.

Isolated pure bacterial colonies were transferred into the nutrient broth and used for further study.

Screening of isolates for biodegradation of LDPE

Screening of bacterial isolates for LDPE degradation was carried out by the clear zone method. The synthetic medium required to determine clear zone formation around the colony was prepared by mixing polyethylene glycol (the soluble form of polyethylene) with a mineral salt medium at a concentration of 0.2% (w/v) and 15% (w/v) agar (Rosario and Baburaj 2017). The media was autoclaved at 121 °C, 15 lbs pressure for 15 min, and allowed to cool to 45 °C, and poured into sterile Petri plates. Once solidified, the isolated colonies grown on nutrient agar were inoculated using an inoculation loop and then incubated at 30 °C for 2 weeks. After 2 weeks of incubation, plates were stained with 0.1% Coomassie Brilliant Blue solution and destained to visualize a clear zone around the colony. Coomassie Brilliant Blue solution was prepared by dissolving 0.1% (w/v) of Coomassie Blue into 40% (v/v) methanol and 10% (v/v) acetic acid. The destaining solution was prepared by adding 40% (v/v) methanol into 10% (v/v) acetic acid. Agar plates were flooded with 0.1% solution of Coomassie Blue R-250 for 20 min. The solution of Coomassie Blue was poured off, and the plates were flooded with a destaining solution for 20 min. The bacteria producing a clear zone in a blue background are considered polyethylene degraders (Gupta et al. 2016).

Biodegradation studies

In the present study, untreated LDPE was used to analyze the biodegradation efficiency of bacterial isolates, while most early studies of microbial degradation of LDPE used pretreated films. A biodegradation test was performed using 0.2% (w/v) of LDPE films (1.5 × 1.5 cm) that had been dried overnight at 60 °C, weighed, disinfected (30 min in 70% ethanol), and air-dried for 15 min in laminar air flow chamber. The films (0.2 g) were aseptically added to Erlenmeyer flasks containing 100 ml of sterile mineral salt medium supplemented with 0.01% (w/v) of yeast extract. Each flask was inoculated with 1 ml of 24-h old culture grown in a nutrient broth medium. Then, cultures were incubated on a rotary shaker at 35 °C and 120 rpm for 60 days. Flask without inoculation served as a sterile control. The extent of biodegradation of LDPE film was determined after 60 days of incubation using the weight loss method, scanning electron microscope, and Fourier transforms infrared (FT-IR) analysis.

Determination of dry weight of the residual polymer

The percentage of weight loss was determined after 60 days of incubation on a rotary shaker (120 rpm) at 35 °C. To determine weight loss, residual LDPE films were collected and mixed with 2% (w/v) of aqueous sodium dodecyl sulfate (SDS). The mixture was incubated in a shaker incubator (120 rpm) for 4 h and then rinsed with distilled water to remove microbial film and residual medium. Finally, residual LDPE samples were collected on filter paper and dried overnight at 60 °C before being weighed. The weight loss was calculated and compared based on the following formula (Montazer et al. 2019):

$$\textbf{Weight loss}\ (\%) = \frac{(\textbf{Initial weight} - \textbf{Final weight})}{\textbf{Initial weight}} \times \mathbf{100}$$

Analysis of surface topography

The surface morphology of the LDPE film was analyzed after 60 days of incubation at 35 °C and 120 rpm through scanning electron microscopy (SEM) to check for any structural changes made by the activities of bacteria on LDPE film. The film was subjected to SEM analysis after washing with 2% (w/v) of aqueous sodium dodecyl sulfate and distilled water repeatedly through mild shaking for a few minutes. Additionally, the film was flushed with 70% ethanol to get a maximum surface to be exposed for visualization. A piece of air-dried film was placed on the sample holder, coated using a master coater, and then analyzed under a high-resolution scanning electron microscope (Gajendiran et al. 2016).

Fourier transform infrared (FT-IR) analysis

The structural change in the LDPE surface was investigated using the Fouriiier transform infrared (FT-IR) spectrometer. FT-IR analysis detects any change in the functional groups. Spectrum was recorded at 450–4000 wave numbers cm^{-1} for all LDPE samples (Gajendiran et al. 2016).

Sequencing and phylogenetic analysis

Total genomic DNA was isolated from the bacterial cultures grown for 24 h using the Bacterial Genomic DNA Purification Kit (GeneMark) following the manufacturer's instructions. The 16S rRNA gene was amplified using universal primers (forward primer (27F 5′-AGAGTT TGATCCTGGCTCAG-3′) and reverse primer (1492R 5′-GGTTACCTTGTTACGACTT-3′)) (Olukunle 2019). The PCR was performed on a Prime thermal cycler, UK, using Taq DNA polymerase. A total of 30 cycles of amplification were performed with template DNA. PCR reaction was performed as follows: denaturation at 94 °C for 4min, primer annealing at 56 °C for 1min, primer extension at 72 °C for 1 min, and final extension at 72 °C for

8 min. Finally, the PCR product was visualized through electrophoresis on a 1% agarose gel and sequenced using the Sanger sequencing technology (Applied Biosystems, India). Sequence analysis was done using the NCBI blast tool, and the best-matched organisms having valid names were recovered. The phylogenetic tree was constructed using the neighbor-joining method (MEGA version 11) after multiple sequence alignments with a bootstrap value of 1000 replicates. The 16S rRNA gene partial sequences are deposited in the NCBI database under accession numbers OK336096, OL315394, and OK465137.

Statistical analysis

Data were subjected to one-way ANOVA to observe the variation in weight loss among the bacterial isolates after 60-day incubation. Post hoc (Duncan) test ($p < 0.05$) was performed to determine the significance of the difference between bacterial isolates in reducing LDPE film weight after 60 days of incubation. Statistical analysis was done using the software SPSS version 16 (Awasthi et al. 2017).

Results

Isolation and screening of LDPE-degrading bacteria

Isolation of bacteria for biodegradation of LDPE was made after successive enrichment of culture using mineral salt broth supplemented with 0.2% of LDPE powder as a sole carbon source. Isolation of the bacteria was carried out on a nutrient agar medium, and a total of sixty bacterial isolates were obtained. Screening of bacterial isolates for LDPE degradation was carried out by the clear zone method. Out of 60 bacterial isolates obtained, fourteen isolates formed detectable clear zone around their colony (Fig. 2). Bacterial isolates were selected based on the diameter of the clear zone around their colony.

Biodegradation studies

Determination of dry weight of residual LDPE

We calculated the weight loss of the polythene strips. Out of fourteen bacterial isolates that form a clear zone around their colony, ten resulted in weight loss of LDPE films (Table 1). In our study, some isolates which form

Table 1 Percentage of weight loss of LDPE films (values are duplicates and expressed as mean ± standard deviation)

Isolates code	Initial weight (g)	Final weight (g)	Percentage of weight loss
KS119	0.2	0.1615	23.87 ± 0.11^{c}
KS114	0.2	0.197	1.25 ± 1.06^{e}
KS16	0.2	0.1999	0.025 ± 0.03536^{f}
KS17	0.2	0.1998	0.05 ± 0.07071^{f}
KS19	0.2	0.196	2.05 ± 1.48^{e}
KS35	0.2	0.1405	42.87 ± 1.91^{a}
KS116	0.2	0.176	13.31 ± 0.46^{d}
KS110	0.2	0.194	2.88 ± 3.36^{e}
KS117	0.2	0.199	0.35 ± 0.21^{e}
KS118	0.2	0.1999	0.025 ± 0.03536^{f}
KS15	0.2	0.198	0.75 ± 0.35^{e}
KS25	0.2	0.196	1.91 ± 2.42^{e}
KS26	0.2	0.1999	0.025 ± 0.03536^{f}
KS14	0.2	0.1465	37.2 ± 3.06^{b}
Control	0.2	0.2	0

Differences in means are indicated with lowercase letters. Means with same superscript letters are not significantly different, while means with different superscript letters are significantly different ($P < 0.05$)

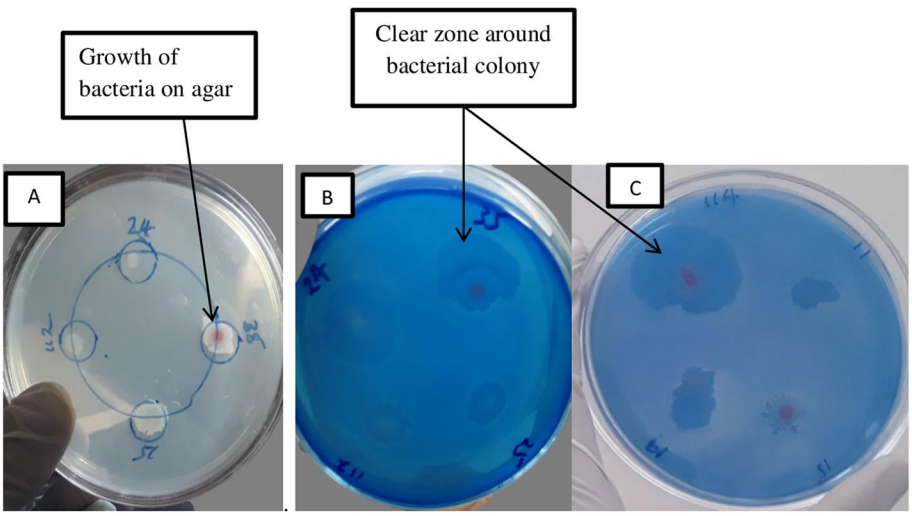

Fig. 2 Clear zone formed by isolates after 2 weeks incubation in mineral salt medium supplemented with 0.2% polyethylene glycol ("**A**" growth of bacteria on medium before staining with 0.1% Coomassie Brilliant Blue solution. "**B**" and "**C**" Clear zone around bacterial colony after staining with 0.1% Coomassie Brilliant Blue solution)

a clear zone around their colony did not show a significant change in the final weight of LDPE film. The maximum degradation was achieved by isolate KS35, followed by KS14 and KS119 (42.87 ± 1.91%, 37.12 ± 3.06%, and 23.87 ± 0.11%, respectively) after 60 days of incubation. Furthermore, strain KS35 and KS14 showed significant ($p < 0.005$) weight loss of LDPE film compared to other isolates. Three of them resulted in maximum weight loss (KS14, KS119, and KS35) were selected as potential LDPE degraders for further SEM and FT-IR analysis.

Scanning electron microscopic (SEM) analysis

Scanning electron microscopic analysis was carried out for three potential bacterial isolates (KS14, KS119, and KS35), which showed better activity during the biodegradation study using the weight loss method. Scanning electron micrograph showed various holes, cracks, pits, and irregularities on the LDPE film. The control film appeared with a smooth surface (Fig. 3).

Fourier transform infrared spectroscopy analysis

Oxidation or hydrolysis of LDPE by bacterial enzymes creates functional groups that improve the polymer hydrophilicity and degradability by microorganisms. In this study, the LDPE film biodegradation potential of the isolates was confirmed by FT-IR analysis. FT-IR analysis was used to detect the change in concentration of existing functional groups or the formation of new functional groups. FT-IR spectra of LDPE films after 60 days of incubation with selected bacterial isolates are shown in Fig. 4. The result shows that incubation of LDPE film with bacterial isolates has resulted in the generation of new functional groups, changes in the concentrations of existing functional groups, and disappearance of a

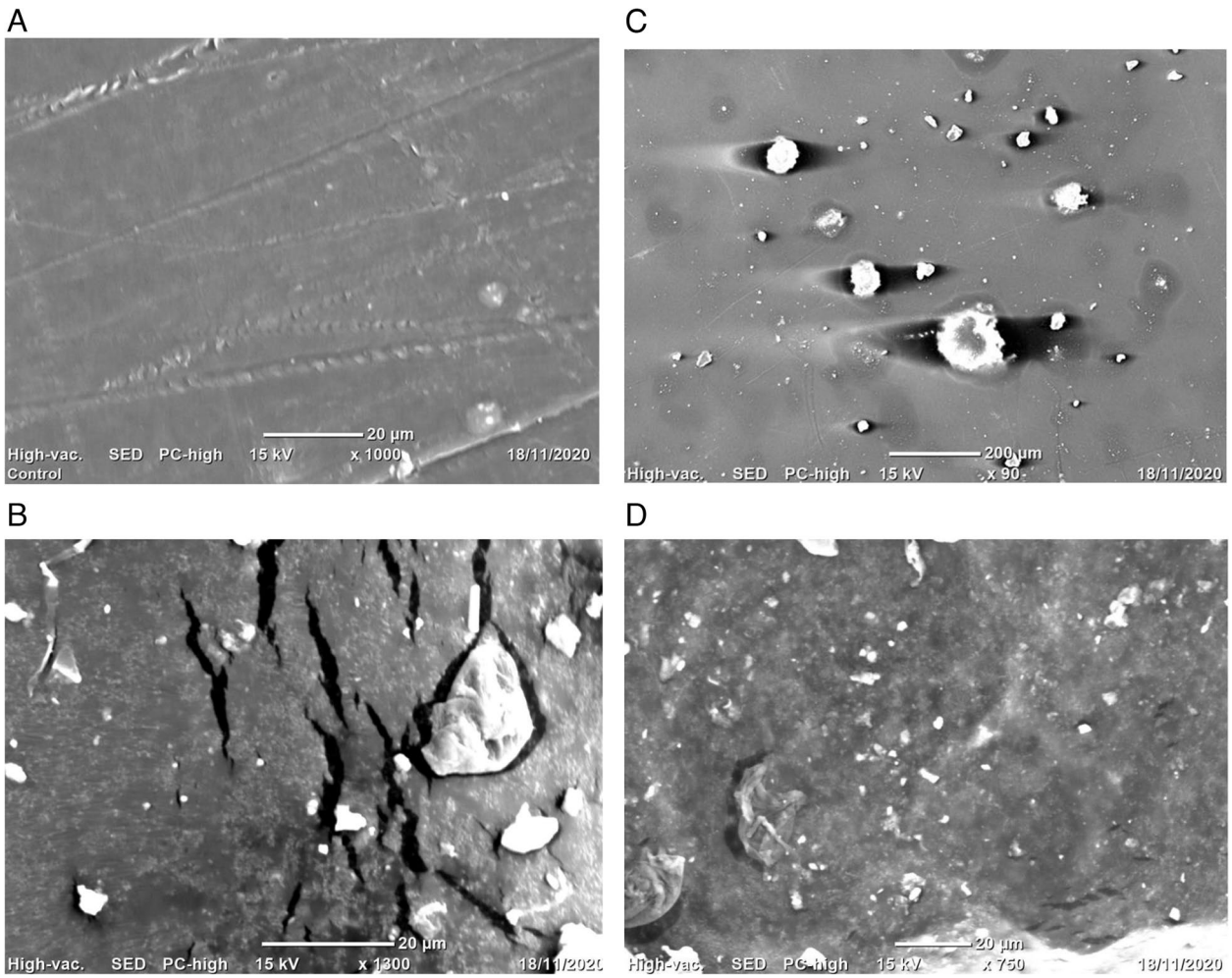

Fig. 3 Scanning electron micrograph of LDPE films after 60-day incubation with bacterial isolates showing surface disruption, holes, and wrinkles on the surface. **A** Control, **B** LDPE film after treatment with KS14, **C** LDPE film after treatment with KS119, and **D** LDPE film after treatment with KS35

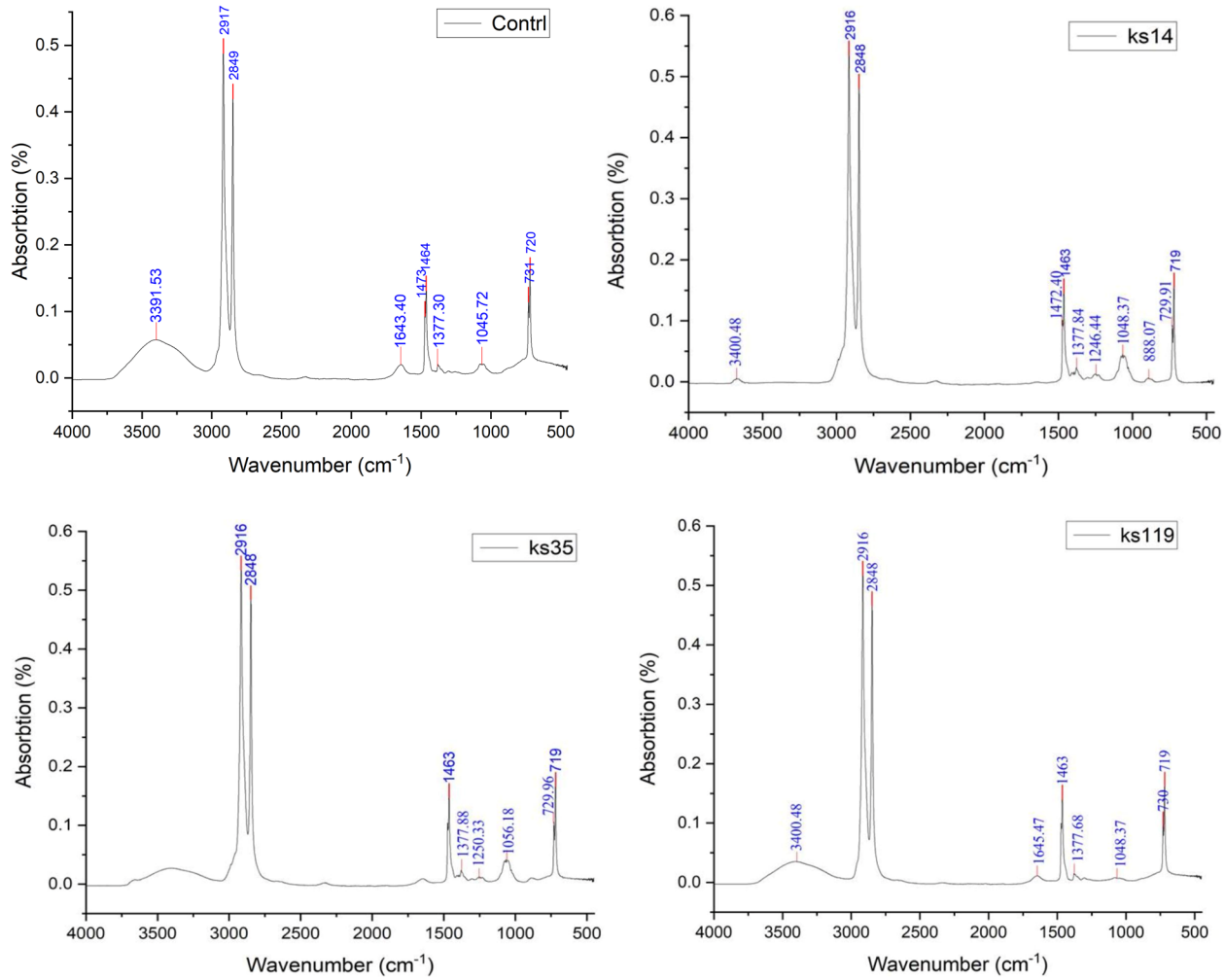

Fig. 4 FT-IR spectra of polyethylene sheet treated with bacterial inoculum and the control incubated at 35 °C for 60 days. Peak at 1643.78 cm^{-1} which represent C–C=C stretch of alkenes disappeared in all bacterial-treated LDPE film except KS 119-treated film. New peaks at 1250.9 cm^{-1} and 1246.44 cm−1 attribute to C–O stretch of ester's group

few functional groups at the surface of LDPE film either because of their consumption or production.

The FTIR spectrum of LDPE after treatment with KS14 and KS119 showed a decrease in wavelength number for O–H stretching of alcohol, and the band shifted from 3391.53 to 3401.04 cm^{-1} (O–H stretching of alcohol). The peak at 3391.53 cm^{-1}, 1473 cm^{-1} (C–H bend stretching vibration of alkanes), and 1643.40 (C=C stretching of alkenes) in KS35-treated LDPE film disappeared. Additionally, the peak at 1643.40 cm^{-1} (C=C stretching of alkenes) in KS14-treated film and peak at 1473 cm^{-1} in KS119-treated LDPE film disappeared due to the effective degradation of polyethylene film by bacterial isolates. A new peak appeared in KS35- and KS14-treated LDPE film at 1250.33 cm^{-1} and 1246.44 cm^{-1} wavelength numbers, respectively. Both peaks correspond to the C–O stretching of esters groups. The most prominent structural change was observed in the LDPE film treated with KS35 bacterial isolate.

Sequencing and phylogenetic analysis

PCR amplification of 16S rRNA gene was carried out, and the PCR products were sequenced using Sanger sequencing technology (Fig. 5). Sequences of the three bacterial isolates were compared against the sequences available in the NCBI, nr database using BLASTn. The sequence of isolate KS35 had 99% similarity with *Methylobacterium radiotolerans* MN525302. The sequences of isolate KS119 had 100% similarity with *Methylobacterium fujisawaense* KT720189, and isolate KS14 had 99% similarity with species of *Lysinibacillus fusiformis* (Fig. 6).

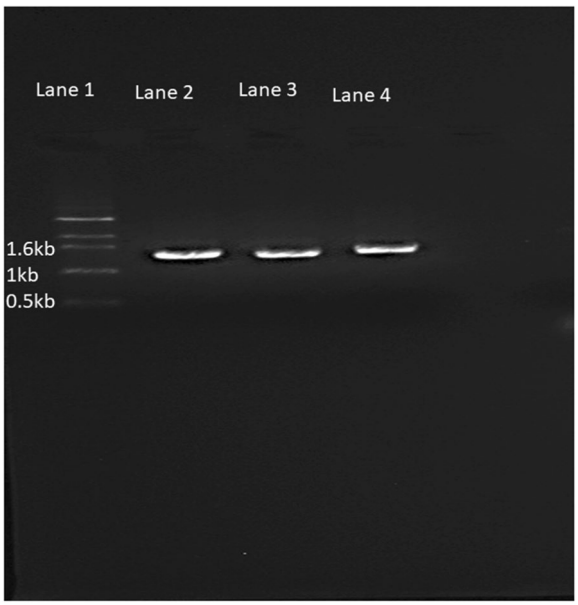

Fig. 5 PCR product analysis of 16S rRNA (1500bp) gene from LDPE-degrading bacteria, lane 1, 1kb DNA ladder; lane 2, KS 35; lane 3, KS 119; and lane 4, KS14

Discussion

Low-density polyethylene could degrade by different fungal and bacterial species isolated from various sources. Several researchers explored microbial populations inhabiting landfills (Muhonja et al. 2018), rhizosphere soil of mangroves (Sangale 2012), and marine water (Ambika 2014) for their polyethylene-degrading potential. Similarly, in this study, the Koshe solid waste disposal area in Addis Ababa, Ethiopia, was selected for isolation and screening of LDPE-degrading bacteria. In many LDPE biodegradation studies, the weight loss method was used to determine microbial consumption of polymers (Das and Kumar (2015), Jamil et al. (2017), and Gyung Yoon et al. (2012)).

In our study, the percentage of LDPE weight loss was calculated, and the highest value was recorded by isolates KS35 and KS14 (42.87 ± 1.91% and 37.2 ± 3.06%, respectively) (Table 1). This shows better degrading ability than the previously reported work by Kalia and Dhanya (2022), in which they have documented 4.38% (untreated) and 12.09% (xylene treated) LDPE films weight loss after 30 days of incubation with *Lysinibacillus fusiformis*. Maroof et al. (2021) also reported a comparatively low percentage of thermo-oxidized and UV-treated LDPE films weight loss (8.46 ± 0.3%) after 90 days of incubation with *B. siamensis*. Montazer et al. (2019) observed a percent

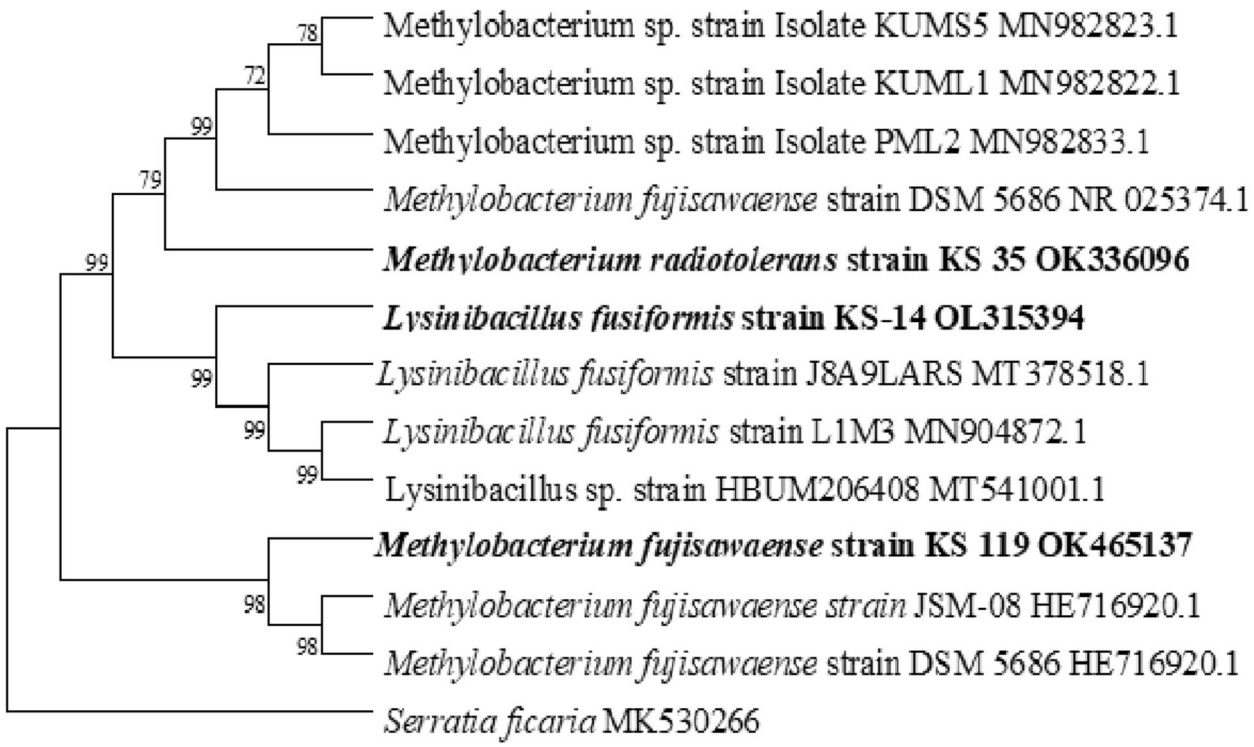

Fig. 6 Maximum likelihood phylogenetic tree of isolates based on 16S rRNA sequencing. The newly sequenced isolates are highlighted in bold. NCBI accession numbers of the respective sequences are noted behind the species names

decrease in LDPE (untreated and without additives) mass by 33.7% ± 1.2% for *C. necator* H16, which is comparable with our result. Gajendiran et al. (2017) also reported a 35% weight loss of LDPE films after 90 days of incubation with the fungi *Aspergillus clavatus*. A maximum decrease in weight loss (48.40%) of pre-treated LDPE films after 90 days of incubation with *C. lunata* SG1 in T-80 added medium was reported by Raut et al. (2015).

While weight loss provides solid evidence of polymer degradation, SEM analysis confirms the biodegradation ability of isolates by elucidating the change of the surface of LDPE films. Scanning electron microscopy analysis showed the deformation of the LDPE film and the formation of pits and holes after incubation of the film for 60 days with selected bacterial isolates (Fig. 3). In agreement with the present study, Das and Kumar (2015) reported that several cracks developed on the surface of LDPE film treated with the bacterial isolate *Bacillus amyloliquefaciens* BSM-1 after 60 days of incubation, whereas the control sample had an appearance of a smooth surface. Esmaeili et al. (2013) have also noticed surface erosion and the formation of pits and cavities on the surface of the LDPE samples after bacterial treatment. In a study by Yoon et al. (2012), SEM evidence confirmed that the smooth surface of the LDPE sheet became eroded as a result of the biodegradation of the polymer by *Pseudomonas* sp. E4. Incubation of LDPE films with *Aspergillus clavatus* strain JASK1 for 90 days also resulted in surface erosion, cracks, folding, and fungal colonization (Gajendiran et al. 2016).

In our present study, Fourier transform infrared spectral analysis was carried out to check the chemical degradation of polyethylene. The spectrum of LDPE films, incubated with the selected bacterial isolates, showed the appearance of new bands and the disappearance of existing bands due to bacterial activity. Analysis of the polyethylene spectral figures indicates the formation of new peaks at 1250.33 cm^{-1} and 1246.44 cm^{-1}, which corresponds to the C–O stretching of esters groups in KS35 and KS14, treated LDPE film, respectively. Functional groups such as an ester group, a carbonyl group, or an ether group are formed when a hydrogen atom on a long carbon-carbon bond is replaced by an oxygen atom (Ren et al. 2019). Alkane hydroxylases are the key enzymes mediating aerobic alkane degradation by hydroxylation of carbon–carbon bonds and the formation of primary or secondary alcohols (Montazer et al. 2020). In our study, the peak intensity of the band 1045.72 cm^{-1}, which corresponds to C–O of the ether group, increased. Ether is formed during the biodegradation of LDPE as a result of the epoxidation of alkenes by microbial alkene monooxygenase (Hou et al. 1979). A new peak was also observed at 888.07 (=C–H stretch of alkenes) in KS14-treated film (Fig. 4). A similar pattern of LDPE film spectra was reported by Gajendiran et al. (2017), where new peaks were observed at 1263.37, 1078.21 cm^{-1} (C–O stretch of ethers), and 987.55 cm^{-1} (=CH2 stretch of alkenes) after 90-day incubation of film with fungi *Aspergillus*. Muhonja et al. (2018) also analyzed the biodegradability of untreated LDPE using FT-IR and observed the formation of new peaks at 1700–1650 cm^{-1} and 1000–1100 cm^{-1}. The shifting, addition, and deletion of peaks indicate structural changes made by microbial activity (Bhatia et al. 2014).

Phylogenetic analysis of the three potential LDPE degrader bacterial isolates was performed using MEGA 11 software, and the result showed that they are closely related to *Methylobacterium radiotolerans* (KS35), *Lysinibacillus fusiformis* (KS14), and *Methylobacterium fujisawaense* (KS119). Previously, these bacterial species were reported to involve in the biodegradation of LDPE and other hydrocarbons. Montazer et al. (2021) have reported biodegradation of low-density polyethylene by *Lysinibacillus fusiformis* species isolated from larvae of the greater wax moth, *Galleria mellonella*. Lysinibacillus species isolated from dumpsites were also identified as effective polyethylene degraders (Muhonja et al. 2018). Kalia and Dhanya (2022) reported that *Lysinibacillus fusiformis* had xylenes treated and untreated LDPE degradation potential. Photolo et al. (2021) isolated *M. radiotolerans* that can detoxify heavy metals and promote plant growth from municipal solid waste. Nzila et al. (2016) also reported that *M. radiotolerans* isolated from soil contaminated with petroleum products was able to use naphthalene as the sole source of carbon, and this bacterial strain grows efficiently in the presence of ethanol. Degradation of LDPE by microorganisms had known for several years, and there is no report on the biodegradation of LDPE by *Methylobacterium radiotolerans* and *Methylobacterium fujisawaense* so far. This is the first experimental report on LDPE utilization as a carbon source under laboratory conditions by showing the effective ability of *Methylobacterium radiotolerans* and *Methylobacterium fujisawaense*.

The present work indicates that soil bacteria from solid waste dump sites show great efficacy in degrading virgin polyethylene. Previously, many researchers evaluated the biodegradability of LDPE after abiotic pretreatment. Abiotic pretreatment such as UV irradiation, chemical oxidation, and thermal treatment was employed to facilitate the microbial degradation of the polymer (Yoon et al. 2012). In the present study, untreated LDPE film was used for the biodegradation study, and promising results were obtained. A further effort to improve this degrading capacity through the

assessment of optimum conditions for microbial activity is necessary. Pre-treatment of polyethylene with environmentally friendly substances could also be adopted as a means to enhance polyethylene biodegradation so that this concept can be applied commercially and on a larger scale.

Conclusions

The present study aimed to screen and analyze the LDPE bag-degrading ability of bacteria isolated from the Koshe waste disposal area. The result from biodegradation studies (weight loss percentage, surface analysis using SEM, and FTIR analysis) reveals that isolates KS35, KS14, and KS119 which are closely related to *Methylobacterium radiotolerans*, *Lysinibacillus fusiformis*, and *Methylobacterium fujisawaense*, respectively, have high ability to degrade LDPE bags.

Abbreviations

LDPE	Lower density polyethylene
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscopic

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Authors' contributions

ZM is the main investigator and performed the laboratory bacterial isolation, screening, and analysis of lower-density polyethylene biodegradation and was a major contributor to writing the manuscript. MT conceived the idea of the study, led the project, and edited the manuscript. NT performed interpretation of Fourier transform infrared spectroscopy analysis results and managed the research activities of the study. The authors read and approved the final manuscript.

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Availability of data and materials

16S rRNA gene sequences supporting the results of this article are available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers OK336096, OL315394, and OK465137. All other data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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