

Research Article

Isolation of plastic-degrading Gram-positive bacteria from Mosul Environment-Iraq

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Abstract

Plastic accumulation cause ecological problems effect on health, due to non-solubility of plastic in water, bacteria play role in degradation polymer, This study aimed to isolate and identify bacterial species which possessed capability in degrading polyethylene terephthalate (PET) represented by water bottle and low density polyethylene(LDPE) represented by shopping black bags, eleven soil samples were collected from soil polluted . After 4 months of incubation, 11 samples showed bacterial growth and were capable of using LDPE and PET as carbon sources, identified using the 16S rRNA sequencing technique. Six of them degraded PET were *Cytobacillus firmus* ZE14.iq , *Bacillus thaonhiensis* ZE17.iq , *Bacillus subtilis* ZE15.iq , *Micrococcus yunnanensis* ZE81.iq , *Ectobacillus funiculus* ZE19.iq, and *Peribacillus frigorigerans* ZE20.iq , while the other five isolates degraded LDPE were *Peribacillus frigorigerans* ZE13.iq , *Priestia megaterium* ZEV6.iq , *Paenibacillus lautus* ZE11.iq , *Bacillus subtilis* ZE16.iq , and *Bacillus subtilis* ZE12.iq. The degradation was detected using FTIR, GC-MS, weight loss, and SEM techniques; the FTIR spectrum showed variations in the intensity of functional groups. It formed a new chemical bond in the polymer , and the most potent ability to degrade (PET) was achieved by *Cytobacillus firmus* at 56%. In contrast, the highest percentage of weight loss for LDPE was 48% by *Bacillus subtilis*. GC-MS analysis revealed the formation of fatty acids, alcohols, Aldehydes, and other new compounds. SEM showed alterations in the physical properties of the PET surface, which degraded with bacterial treatment compared with the control plastic that was untreated with bacteria.

Keywords: Polyethylene terephthalate, Plastic degradation, Low density polyethylene, Gas Chromatography Mass Spectrometry (GCMS) analysis, Fourier-transform infrared spectroscopy (FTIR) , Scanning Electron Microscope (SEM)

INTRODUCTION

Plastic defined as organic polymer composed of a long carbon chain backbone linking large number of small repeated units that joined each other by strong covalent bonds formed by polymerization , a polymer which comes from two Greek words; poly meaning many and mer that meaning part, polymer is solid moldable versatile materials, several plastic produce from non-renewable petrochemicals derived from natural coal and fossil fuel . Plastics are divided into natural, semi-synthetic, synthetic, thermosetting, and thermoplastic (Faisal and ALSaffar, 2023).Durability, stability of polymer, easy manufacture and low cost ,these properties of plastic caused the use of polymer products yearly increased , plastic replaced paper and other product in all sectors of economy, agriculture, construction, tele-

communication, consumer goods, and packaging of food ,most form of plastic products are bags shop and water bottle that used in daily lives in world (Moshood *et al.*, 2022).

Utilize plastic materials improved quality of human life, but the lack of balance made plastic polymer causing big challenge for society, generate large volumes of plastic garbage, plastic waste effect on stability of ecosystem and people's health, uncontrolled utilize of plastic articles products lead to global environmental consequences (Hale *et al.*,2020). Polyethene waste has a high molecular weight and slowly degrades when left in the environment due to the loss of hydrolyzable bonds. Polyethene waste is a major source of pollution in modern society because natural degradation of plastic takes 20–100 years, depending on polymer type, molecular weight, and environmental conditions (Yang

et al., 2018). Several physical and chemical processing used to minimize harmful effects of plastic waste on ecosystem, but these processes very expensive, consuming high energy, and less effective, landfill plastics waste require large area (Chamas *et al.*, 2020), also when plastic burned released toxic gases into air, such as carbon monoxide, nitrous oxide, those gases carcinogenesis and cause lung diseases. These gases are also related to global warming. Trapped solar heat leads to a rise in the temperature of Earth's surface (Zahid *et al.*, 2024).

To reduce accumulation of waste plastic either via increase recycling rate, or transform waste into other less hazardous compound, friendly solution by natural breakdown different kinds of plastic polymer into its monomers by bio deterioration, and fragmentation, then re-synthesis to give new plastic polymer that easily biodegradable, natural biodegradation way protect our planet and keeps it clean, these monomer serve as nutrient for growth and energy source. (Biber *et al.*, 2019) .

Biological degrading the best application for plastic garbage remediation, more effective economical because consume low energy, and environment friendly with low carbon emission Natural deterioration of plastic garbage occur by action of organism and their active enzyme , those enzymes isolated from few microorganisms, including various genus of fungi, bacteria, and algae, these enzymes cleave ester bond within organic plastic polymers into monomers, , bacterial cell absorbed products during metabolism, and utilize plastic as energy source (Silva *et al.*, 2023). According to binding carbon atoms on chains of polymer, there are two kinds of polymer, first one is heteroatom polymer which have different atom such as hydrogen, nitrogen , oxygen ,sulfur, silicon that assemble around carbon atom on chain of polymer example : polyurethane (PUR) and polyethylene terephthalate (PET) that used in producing water bottle, While second kind is homoatom polymer contain only carbon bind carbon atom alone on chain of polymer , this polymer lack to polar function group ,therefor became resistant against degradation , example: polystyrene PS ,polypropylene PP, polyvinyl chloride PVC , and polyethylene PE which include high density polyethylene HDPE ,and low density polyethylene LDPE that used in producing shop bags . Plastics can be classified depending on the chemical structure of the polymer and its side chains. and various physical properties, such as high tensile strength and density (Lear *et al.*, 2021).

Plastics waste broken down on the land by sunlight into smaller units called micro-nano plastic MNPs , spoiled in air ,water and soil , MNPs serve as microbial niche , encourage genetic exchange and act as transferring hazardous pathogens and as a reservoir (Almashhadani and Qassim, 2025) , Ingested parts of

polyethylene waste by animal in aquatic accidentally may reach to liver ,brain, these toxic compounds bio accumulate influence on food chains for many organism when consume by humans cause changes on biogeochemical cycle, these units of polymer disrupt energy balance and cause danger factor for plant, animal , threatening aquatic life, stimulate inflammation, blockage in the intestines of fish and birds (Seeley *et al.*, 2020). Penetrate easily cell membranes, build up inside organ become cytotoxic, cause changes in weight of body, and cause tissue damage, also small size of plastics cause reduction of light transmission on the surface of sea, and eliminate photosynthetic of algae (Al-Hussayni *et al.*, 2023), Plastic garbage contaminates rivers, ocean, and groundwater, plastic waste accretion on the land reduces the availability of oxygen in soil, causes decrease of soil organisms, inhibits growth of plant and affects in soil fertility (Yuan *et al.*, 2020).

Natural deterioration of plastic garbage occurs through the action of organisms, which begin with the adhesion of microbes to the plastic surface and the formation of a biofilm. Microbial colonizers attack the plastic polymer chain because bacteria require a substrate for growth, yielding hydrophilic groups, and assimilate through a semipermeable membrane. Bacteria secret extracellular hydrolytic enzyme, that simplifies biodegradation long chain of polymer into short chain (Taher, Abdullah and AL Taie ,2024) Heteroatoms (S, N, and O) find in all structure of polyethylene terephthalate , which operate as sites for hydrolytic enzymatic reaction, breakage short chain of plastic polymer into fatty acids which have low molecular weight oxidize and decompose into biomass, water and CO₂. The hydrolytic enzymes comprise of lipases, esterases, depolymerases, and hydrolases which disrupt plastics polymer (Zantis *et al.*, 2021). Based on the above, the objective of the study was to identify phenotypically and genetically some bacterial strains isolated from soil for the degradation of polyethylene terephthalate and low-density polyethene.

MATERIALS AND METHODS

Media used for isolation

A specific medium was used to assess the ability of bacterial isolates to degrade polyethene as a carbon and energy source. Bushnell Hass broth composed of 1gm of K₂HPO₄, 0.02 gm of CaCl₂, 0.2gm of MgSO₄ .7H₂O, 1gm of FeSO₄, 1gm of NH₄NO₃, dissolved in 1000 ml of distilled water, pH adjusted at 7, distribute in 24 flasks, two of them used as controls, and each one contained 100ml of media. Those flasks were divided into two groups as follows: the first group included 12 flasks, each containing 1 g of local polyethene terephthalate, while the second group included 1 g of local low-density polyethene; one flask from each group

was used as a control. After that all flasks were autoclaved under 121°C and 1 atm pressure for 15 minutes (Hasan and ALSammak, 2024).

Samples collection and preparation

Eleven soil samples were collected from various locations in Mosul city from October to December 2024, including agricultural soil, landfills, and oil-polluted soil at a depth of 20 cm. After cleaning and purifying them, soil samples dried at room temperature, then 1g of each dried soil was added to 9 ml of sterile distilled water, to make serial dilutions, Then 100µl of 10⁻³ dilution was added to 22 flasks and the other two flasks used as control remain without addition soil samples, All flasks were incubated in shaking incubator at 28°C with 150 rpm for 4 months (Colaninno and Green,2021) .

Isolation of bacteria

After 4 months of incubation, 1 ml from each flask was used to make a serial dilution up to 10⁻³, and 0.1 ml of each dilution was spread on a nutrient agar plate and incubated at 28 °C for 1-7 days (Bakht *et al.*, 2020).

Identification of bacteria

Diagnosis of bacterial isolates depending on their phenotypic characteristics involved (colony's shape, color and Gram stain), and confirmed by using 16srRNA sequence (Al Omari, *et al.*,2024).

Molecular identification of bacteria species DNA extraction

DNA extraction for isolates using the kit provided by Geneaid company (Taiwan) (according to company instructions), with the addition of lysozyme to break down the cell wall first Measured concentration and purity of DNA extracted from isolates using a NanoDrop Spectrophotometer (Thermo Scientific), and Bacterial isolates identified using PCR and 16S rRNA gene sequencing with Universal Primers provided by Promega (Table 1) (Al Jarjary and Alsammak, 2023).

Sequence for 16S rRNA Gene

The sequence of all PCR product conducted in Macrogen, Korea. genetic congruence of 16S rRNA gene determined by using Basic Local Alignment Search Tool (BLAST) which is available in National Center Biotechnology Information (NCBI) (Salih and Saeed,2020).

Table 1. Primers used to amplify the 16S rRNA gene

Primer	Primer sequence (5' to 3')	Product size (bp)
27F	AGAGTTTGATCTGGCTCAG	1465
1492R	GGTTACCTTGTTACGACTT	

Fourier-transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (Bruker_ alpha , Germany) was used to determine changes of functional structural in plastic polymer , FTIR utilized after 4 months of incubation apart of cultures were centrifuged at 3000 rpm for 10 min and plastic pieces removed and washed with distilled water, plastic pieces dried at room temperature (25 ± 2 C), for 3 days, then recorded their infrared spectra on a FTIR spectrometer in the region 4000–400/cm, compared with control (sterile pieces of plastic which not treated with bacteria) (Bonifazi,2023).

Weight loss experiment

Plastic pieces removed from broth culture, washed with distilled water, dried at room temperature and weighted. The percentage of weight loss calculated by using the formula: (Lou,2020)

$$\text{weight loss} = (\text{initial weight} - \text{final weight}) / \text{initial weight} \times 100 \quad \text{Eq.1}$$

Gas Chromatography mass spectrometry (GC-MS)

10 ml of culture was centrifuged at 6500 rpm for 10 min to remove cell debris, and the supernatant was dried at room temperature for 3 days and used for GC–MS analysis, which is available at the Department of Environment and Water/Environmental Research Centre at Baghdad University (Oliveira, 2020).

Scanning electron microscope (SEM)

The dried pieces of degraded plastic were assessed using SEM and compared with the control (Nguyen, 2019).

Phylogenetic relationships of strains

To get an evolutionary relationship for bacteria strains through comparing the nucleotide sequence of gene 16s rRNA within program Mega 12 by using the Un-weighted Pair Group Method with Arithmetic mean UP-GMA, depending on (Tamura *et al.*, 2021).

RESULTS AND DISCUSSION

Bacterial growth

After 4 months of incubation, 11 samples in the present study showed bacterial growth and utilized LDPE and PET as sole carbon sources. Morphological and microscopic observations indicated that 11 bacterial isolates

were Gram-positive. These isolates were identified using molecular detection of the 16S rRNA sequence based on their ability to degrade. Six isolates had the capability to degrade polyethylene terephthalate PET, were identified molecularly and submitted to the National Center for Biotechnology Information (NCBI) and were given the accession number as in Table 2: *Cytobacillus firmus*, *Bacillus subtilis*, *Micrococcus yunnanensis*, *Ectobacillus funiculus*, *Bacillus thaonhiensis*, *Peribacillus frigiditolerans*, whereas the other five isolates, which had capability to degrade low-density polyethylene LDPE were identified as *Priestia megaterium*, *Paenibacillus lautus*, *Bacillus subtilis*, *Peribacillus frigiditolerans*, and *Bacillus subtilis*.

The results about the active role of *Bacillus* spp in deterioration of LDPE plastic is in agreement with Lalina (2021) and Ibiene (2013), the vital role of *Bacillus firmus* in decomposition PET in our study agree with the studies conducted by Jumaah (2017) in Anbar city/Iraq and Muhsin, *et al*, 2023) in Baghdad city and as in study of Al hajar *et al.* (2025) in Mosul city. While other bacterial genera and species have not been isolated previously in Mosul city, the role of *Micrococcus* spp. in the biodegradation of LDPE and PET plastic, as reported in the studies of Montazer (2018) and Gupta *et al.* (2022), has been demonstrated.

Weight loss

After 4 months of incubation, plastic pieces were weighed, and the results for gram-positive isolates showed that the highest degradation, determined by weight loss of polyethylene terephthalate, was 56% by *Cytobacillus firmus* as in Table 3, while the lower degradation was 40% by *Bacillus subtilis*. The highest degradation rate, 48%, was determined for low-density polyethylene by *Bacillus subtilis*, and the lowest, 38%,

Table 2. Bacteria isolates degraded polyethylene terephthalate(PET) and low density polyethylene (LDPE) with their accession number

NO	Bacteria strain	Accession number	Percentage of identification in NCBI
1	<i>Paenibacillus lautus</i> ZE11.iq	SUB 15298577	99%
2	<i>Bacillus subtilis</i> ZE12.iq	SUB 15296374	99%
3	<i>Peribacillus frigiditolerans</i> ZE13.iq	SUB 15292255	99%
4	<i>Cytobacillus firmus</i> ZE14.iq	SUB 15296358	99%
5	<i>Bacillus subtilis</i> ZE15.iq	SUB 15296369	99%
6	<i>Bacillus subtilis</i> ZE16.iq	SUB 15298578	100%
7	<i>Bacillus thaonhiensis</i> ZE17.iq	SUB 15296394	99%
8	<i>Ectobacillus funiculus</i> ZE19.iq	SUB 15296344	99%
9	<i>Peribacillus frigiditolerans</i> ZE20.iq	SUB 15292111	100%
10	<i>Micrococcus yunnanensis</i> ZE81.iq	SUB 15298579	100%
11	<i>Priestia megaterium</i> , ZEV6.iq	SUB 15296438	100%

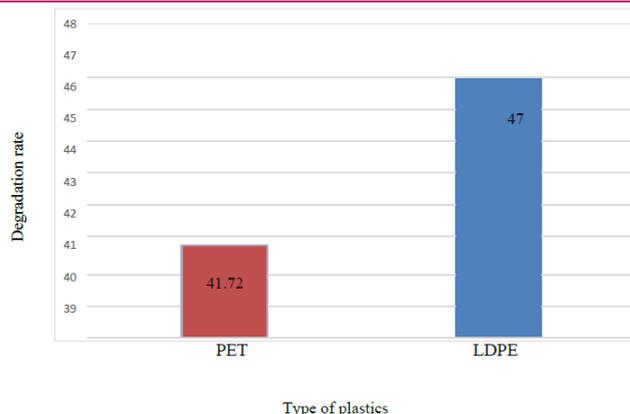


Fig.1 . Comparison of loss weight (%) of polymer by action of bacteria between polyethylene terephthalate (PET) and Low density polyethylene (LDPE)

was determined for low-density polyethylene by *Paenibacillus lautus*. LDPE degradation takes more time than PET, because integration of heteroatoms into the carbon chain makes PET hydrolyzable plastics which more sensitive than LDPE to degradation as in study of Zhang and Yan (2021), therefore the degradation rate of PET after treatment with Gram positive bacteria in our study more than degradation rate of LDPE, loss weight of plastic confirmed deterioration of plastic as in study conducted by Nadeem *et al.* (2021).

independent to investigate a significant comparison of loss weight between percentages of PET and LDPE factors included in this study; p-values < 0.05 were considered statistically significant, as in Fig. 1.

Fourier-transform infrared spectroscopy (FTIR)

Changes in the structure of PET and LDPE after 120 days treatment with bacteria confirmed by FTIR spectroscopy, results noticed the change in peaks

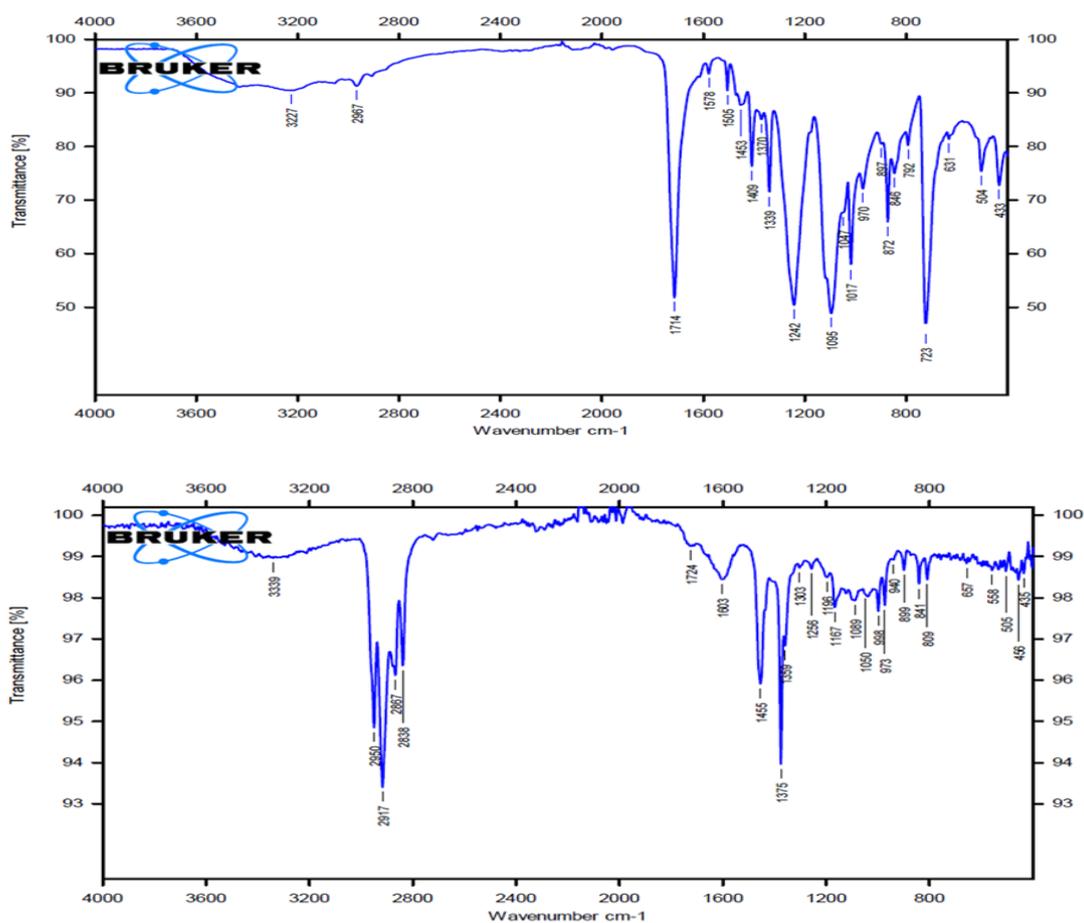
Table 3. Type of plastic degrading by bacteria and the percentage of weight loss

Bacteria	Type of plastic	Percentage of weight loss
<i>Cytobacillus firmus</i> ZE14 .iq	PET	56%
<i>Bacillus thaonhiensis</i> ZE17.iq	PET	54%
<i>Peribacillus frigitolerans</i> ZE20.iq	PET	50%
<i>Micrococcus yunnanensis</i> ZE81.iq	PET	45%
<i>Ectobacillus funiculus</i> ZE19.iq	PET	45%
<i>Bacillus subtilis</i> ZE15.iq	PET	40%

between the control and plastic samples with bacteria, as in Fig. 2. These changes include decrease in the absorption peak in main structure region and disintegration of PET chains into smaller pieces .these changes include decreased intensity of carbonyl ester , and increase intensity of carboxylic acid , which gave sign to the role of bacteria and their enzyme in cleaving long polymer chain , which indicate depolymerized process of polyethylene terephthalate PET occurred , also new chemical bond formed around 1700 cm⁻¹ , this new functional group contain oxygen and indicate to form ketone or carboxylic acid , these compound as a result of oxidative pro-

cess on PET chain , and changes involved decrease intensity of peak around 1100-1200cm⁻¹ indicate to break down of ester bond .

while changes in LDPE as in Fig. 3 include stretching at 2900 cm⁻¹, that refer to reduction of Alkyl group, also changes include formation new bond around 1000-1100 cm⁻¹, that confirmed that bacteria metabolized degradation products then linking them to form ether functional groups, as in the study of (Kirstein , 2021) that showed the capacity of bacteria to degrade low density polyethylene and use it as a source for their nutrition.

**Fig.2.** A) FTIR of PET polymer of control , B) FTIR of PET degrading by *Peribacillus frigitolerans*

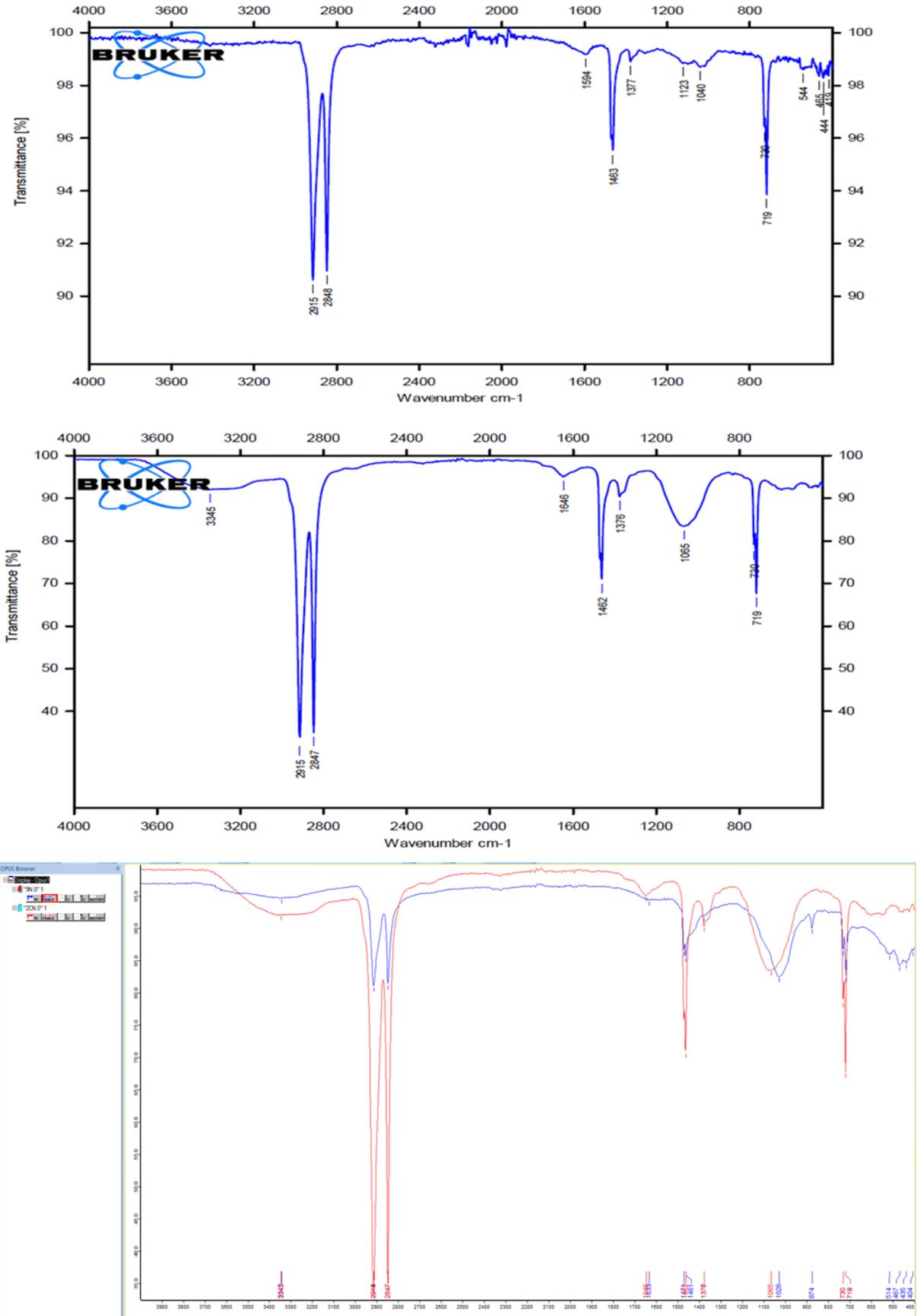


Fig.3.(A) FTIR of LDPE polymer of control , B) FTIR of LDPE degrading by *Bacillus subtilis* C) FTIR of LDPE polymer degrading by *Bacillus subtilis* , red color before treatment, blue color after treatment

Table 4. Compounds produced through biodegradation by bacteria using GC–MS analysis

Bacteria	Type of plastic	Compounds
<i>Cytobacillus firmus</i> ZE14.iq	PET	Dimethyl-hydroxy Propion aldehyde, ethyl, Propyn, Butyl acrylate , propenoic acid , hexen , Hydroxy pivalaldehyde, quinoxalinone, Ethynyl carbinol , Propynyl alcohol, tert-butyl ester
<i>Bacillus subtilis</i> ZE16.iq	LDPE	Pentanal, sulphuric acid dibutyl ester, propane , Acetoxy-methyl coumarin, Butyl acrylate, propenoic acid, Propyn, propionate, Valeric acid aldehyde, - dimethylpropane, Benzopyran
<i>Ectobacillus funiculus</i> ZE19.iq	PET	Oxirane, Butyl glycidyl ether, butoxy methyl-Propane , Propyloxiranemethanol, propane, Pentanol, pentyl alcohol, hydroxyproline, propyl ester, Amyl alcohol
<i>Peribacillus frigitolerans</i> ZE20.iq	PET	Hydroxyl-isopropoxy-propyl, indole--carboxylic acid methyl ester, methyl-benzo, Triphenyl benzotriazole

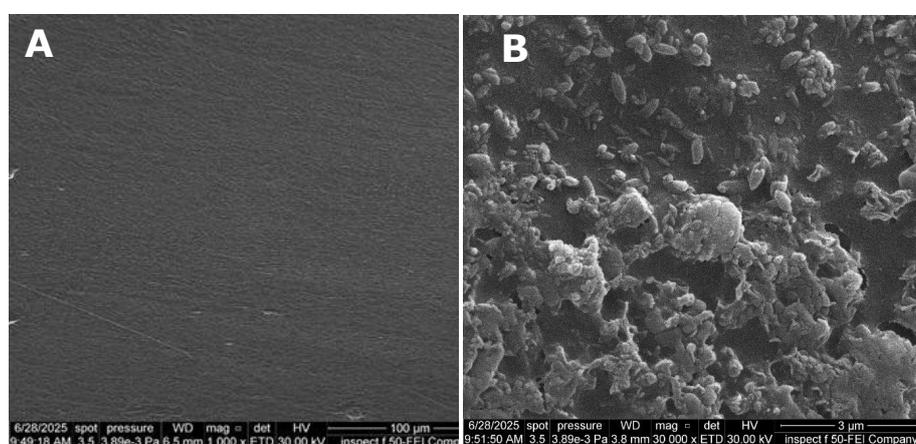


Fig.4. A) Image show SEM examination of untreated PET plastic , B) Image show SEM examination of treated PET plastic with bacteria

Gas chromatography coupled with mass spectrometry (GC-MS)

Results of GC-MS demonstrated that polyethene chains cleaved into smaller fragments during biodegradation, as evidenced by the formation of various carboxylic acids, as shown in Table (4) and compatible with results obtained by Jeon *et al.* (2021). The production of intermediate products such as fatty acids, aldehyde compounds, alcohol compounds, and small alkane compounds was consistent with the study by Gazi *et al.* (2019), which reported that these compounds are monomers produced by oxidative processes on polyethelene terephthalate and low-density polyethelene polymers.

Scanning electron microscope (SEM)

After 4 months of incubation, some PET pieces treated with Gram-positive bacteria were selected based on their weight loss and FTIR results for examination by SEM. The pieces of plastic compared with control (untreated plastic with bacteria) image showed smooth surface with non- porous as in Fig. 4A while the image in Fig. 4B noted cracked areas observed on the surface

of PET polymer , many big holes and grooves with different sizes spread in polymer. This indicates the role of enzymes secreted by bacteria in breaking the bonds in polymers and decomposing them, as in the study by Rani *et al.* (2022).

Phylogenetic relationships of strains

The species within the tree clustered into two groups, A and B The first group included Low GC Gram positive bacteria *Cytobacillus firmus* , *Bacillus thaonhiensis* , *Bacillus subtilis* , *Ectobacillus funiculus* , *Peribacillus frigitolerans* , *Priestia megaterium* , *Bacillus subtilis* , *Peribacillus frigitolerans* , *Bacillus subtilis* and *Paeni-bacillus lautus* at a similarity level 99.3%, while second group included High GC Gram positive bacteria *Micrococcus yunnanensis* at a similarity level 98.7%, as in Fig. 5.

Conclusion

Industrial activity is the actual cause of the accumulation of plastic garbage, which causes environmental pollution and the degradation of plastic polymers, which

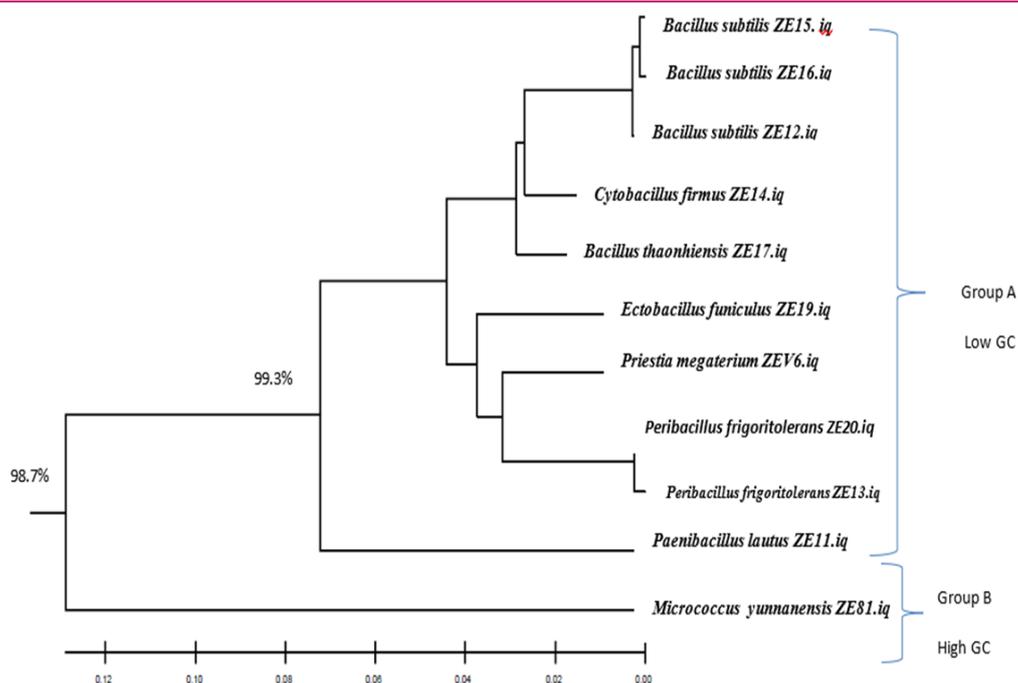


Fig.5 . Phylogenetic tree of bacteria strains under study

takes thousands of years. The awareness to the society when contaminated sites growing up, the study concluded many strain of Gram positive bacteria acted as safe choice for demolishing the plastic contaminants naturally by using soil bacteria that secret active enzyme in decomposition plastic waste and transform toxic waste to nontoxic substances, many strains of bacteria observed ability for degradation of PET and LDPE and used end product as only carbon source for their nutrition, loss weight of polyethylene terephthalate ,low density polyethylene , changes in tensile properties of chemical bonds of PET, LDPE and morphological changes on surface confirmed role of bacteria in decomposition. Polymer biodegradation addresses plastic waste accumulation and is considered a safe, effective, and economical technique.

Conflict of interest

The authors declare that they have no conflict of interest.

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