Section A-Research paper

INFLUENCE AND ROLE OF THE WAXWORM MICROBIOME IN POLYETHYLENE DECOMPOSITION

Nazirov Muhammad-Latif, Khalilov Ilkhom*, Kobilov Fazliddin, Mardonov Ikrom

Department of Molecular biology, Institute of Microbiology of Academy of Science of Uzbekistan, Tashkent, Uzbekistan

Nazirov Muhammad-Latif.,
base doctoral student, Institute of Microbiology,
Academy of Sciences of Republic of the Uzbekistan, Uzbekistan, Tashkent
Khalilov Ilkhom.,
PhD of Biological Sciences, senior researcher, Institute of Microbiology,
Academy of Sciences of Republic of the Uzbekistan, Uzbekistan, Tashkent
Kobilov Fazliddin.,
base doctoral student, Institute of Microbiology,
Academy of Sciences of Republic of the Uzbekistan, Uzbekistan, Tashkent
Mardonov Ikrom.,
Junior researcher, Institute of Microbiology,
Academy of Sciences of Republic of the Uzbekistan, Uzbekistan, Tashkent

*Correspondence author`s email: ilkhom2002@yahoo.com, Tel: +99890189-35-00.

Abstract

10 bacterial isolates were isolated from waxworm larvae fed on polyethylene. Among isolated bacteria, *Pseudomonas* spp.4, *Pseudomonas* spp.2, *Pseudomonas* spp.1, and *Pseudomonas* spp.3 isolates decreased the PE weight by 0.05%, 0.34%, 0.53%, and 0.8%, respectively. According to FTIR analysis, *Pseudomonas* spp. 2, *Pseudomonas* spp.1, and *Pseudomonas* spp.3 isolates produce hydroxyl and carboxyl groups as a result of PE degradation. A PE sample left outdoors in the sun absorbed PE more actively than the bacterial isolates used in the experiment, with a decrease in mass and the formation of hydroxyl, ether, and carboxyl groups. Weak degradation of PE by bacterial isolates is unlikely to affect the long-term survival of waxworm larvae. The uptake of PE by the larva can be enhanced by the action of enzymes in the digestive system of waxworm larvae.

Keywords: Polyethylene (PE), biodegradation, pre-treatment, Moth worms, bacteria.

Introduction

The global plastics industry is rising at a rate of 4.8% per year and is predicted to reach $215 billion by 2024 (Goldstein 2021). Polyethylene (PE) is the most widely used synthetic polymer due to its properties such as high hydrophobicity, chemical resistance, electrical insulation, and low cost (Restrepo-Flórez et al., 2014). Currently, several kinds of polyethylene are used in the world. PE is a chemically and biologically inert polymer. Although polyethylene is durable due to its inertness and durability, it is one of the main sources of environmental pollution due to its inability to biodegrade. The main problem is related to the biodegradation of PE and the disposal of PE waste, which have not yet been resolved.

In recent years, microorganisms isolated from soil, the aquatic environment, and composts have been studied using living organisms during the decomposition of polyethylene (Albertsson AC et al., 1995; Nanda S & Sahu SS 2010; Jamil Suu et al., 2017; Balasubramanian V et al., 2010; Lobelle D & Cunliffe M 2011; Mahalakshmi V et al., 2012). In addition, scientific studies have shown that the larvae of *Plodia interpunctella*, *Galleria mellonella*, *Tenebrio molitor*, *and Zophobas atratus* can chew and digest plastic (Yang J et al., 2014; Bombelli P, Howe CJ, Bertocchini F 2017; Yang Y et al., 2015; Peng BY et al., 2020). It has been studied how insect larvae break down thin-film plastic bags and turn them into ethylene glycol. Due to the similarity of the chemical structures of beeswax and PE, the worm *G. mellonella* is responsible for the degradation of PE under the influence of biochemical metabolic mechanisms during the decomposition of beeswax (Kong HG et al., 2019). It is important to note that beeswax is a natural substance composed of palmitic and other long-chain aliphatic alcohols and fatty acids. Similarly, due to the long carbon chain of PE, about 100 pieces of *Galleria mellonella* worms have been studied to digest 92 mg of PE in 12 hr (Bombelli P et al., 2017).

Recent studies have shown that the main activity in PE degradation is due to the association of microorganisms in the insect microbiome (Ren L et al., 2019) or that degradation is carried out by enzymes secreted from the stomach of the insect. Peng B. Yu, and according to other authors, when LDPE (low-density polyethylene) and EPS (expanded plastic) were fed to larvae of *Zophobas atratus* and a gel permeation chromatographic analysis was performed, the process of depolymerization/biodegradation of LDPE and EPS were observed. But when the larvae were fed the antibiotic gentamicin and killed the microorganisms in the stomach, they found that the weight average molecular weight (Mw) and number average molecular weight (Mn) of LDPE and EPS did not change. Summarizing the results of the study, they analyzed the role of the microbiome in the biodegradation of LDPE and EPS. In addition, some authors have studied the degradation of PE (821 kDa) by *G.mellonella* larvae treated with a deuterium-labeled isotope (Peng BY et al., 2020).

Nevertheless, the isotope was not detected in the tissues of larvae that received deuterium PE for 72 hr or 19 days. Possibly, PE microparticles were observed in the gastrointestinal tract of the larvae. Simultaneously, these authors found that PE with a molecular weight of 641 kDa weakly biodegrades within 24 hr, and isotopic PE particles are present in the excrement of larvae. They concluded that *G.mellonella* larvae may be involved in the biodegradation of HDPE (high-density polyethylene), but not necessarily in metabolism. It seems that the main biodegrading organism for plastics of different compositions are enzymes of the gastrointestinal tract of an insect or microbiome, but meanwhile, the participation of both in cooperation in the metabolism of PE is still debatable (Réjasse A et al., 2022). Therefore, the purpose of the research is to determine the PE biodegradation properties of bacterial strains isolated from the stomach of *G.mellonella* larvae.

Materials and methods

Polyethylene film

The polyethylene film was cut into 30 mm x 30 mm for incubation on agar plates. For incubation in a liquid medium, it is cut into 100 mg and weighed on an analytical balance. PE is sterilized in 75% ethanol and dried in a laminar box.

Wax moth larvae

In the experiment, *G. mellonella* larvae (2-3 years old) were used. Wax moth larvae were brought from beekeeping farms in Uzbekistan. The larvae were fed PE for 14 days. However, to isolate bacteria from larvae, worms fed for 10 days of ET were used.

Medium

A medium using PE as the sole carbon source was prepared by slightly modifying the ASTM G22 standard (ASTM International 1996). 900 ml of modified LPEM2: the medium is prepared as follows: $(NH_4)_2SO_4$ - 1.0 g; $MgSO_4.7H_2O$-0.7 g; trace element - 1 ml; (NaCl - 0.5 g; $ZnSO_4.7H_2O$ - 0.2 g; Fe EDDHA - 0.2 g; $CuSO_4$ x $5H_2O$ - 0.2 g; $MnSO_4.H_2O$ - 0.1 g for the preparation of 100 ml of trace elements); pH-6.5

KH_2PO_4 -0.7 g per 100 ml of phosphate buffer; K_2HPO_4-0.7g pH-6.5

LPEM2 medium (900 ml) and phosphate buffer (100 ml) was added after autoclaving and cooling.

1000 ml of liquid nutrient medium LB Broth (Miller) is prepared as follows: yeast extract - 10 g; tryptone bacteriological-10 g; NaCl -5 g; pH -7.0.

1000 ml of liquid broth Chapekdox nutrient medium is prepared as follows: sucrose - 30.0 g; $NaNO_3$-3.0 g; K_2HPO_4-1.0 g; $MgSO_4$-0.5 g; KCl-0.5 g; Fe EDDHA-0.002 g; pH -7.0. 15 g of agar was added to 1000 ml of the liquid medium to prepare a solid medium. All media were autoclaved at 121°C for 20 min.

Isolation of microorganisms that decompose polyethylene

100 pieces of *Galleria mellonela* (3–4-year-old larvae) were placed in 75% ethanol, sterilized for 1 min, and washed with sterile distilled water. Then, using a scalpel, the larvae were divided into 3-4 parts and placed in a sterile mortar with a volume of 200 ml, to which 10 ml of sterile distilled water was added. Carefully ground homogenate was taken with a pestle and planted in a dense nutrient medium LPEM2 as an equivalent material. Cultures grown as single colonies on nutrient media were identified by microscopic analysis of bacterial genera using a Bergey bacterial detector.

Screening of strains degrading PE

100 µl of the homogenate was added to 10 ml of LPEM2 medium (1% sterile PE) and grown at 30°C, 200 rpm for 60 days. After 60 days, agar was inoculated into dishes containing LB broth (Miller). Incubated in a thermostat at 30°C for 1-3 days. Bacterial samples were planted in a liquid LB Broth (Miller) medium and made incubation for 12 hr at 30 °C. The cells were then centrifuged at 12,000 rpm and pelleted. Sterile water was used to remove the residual medium and resuspended the cell. 500 µl of the suspension was poured into plates with LPEM2 solid growth medium and covered with sterile polyethylene film (40 mm x 40 mm). As a control, PE was placed in a dish with a solid medium without bacteria. Each experiment was repeated 3 times. All samples were grown in a thermostat at 30 °C for 30 days. Bacterial strains forming a biofilm on the surface of PE were selected (pictured after 3 days).

Screening for biodegradation in liquid nutrient media

To determine the biodegradation of PE, its weight was measured, and a change in molecular weight and the appearance of metabolite products soluble in LPEM. 40 ml of LPEM raw material is placed in a 150 ml Erlenmeyer flask. Pieces of PE (100 mg) and 10 ml of bacterial suspension were cultivated (in parallel, LPEM2 was placed in the same way). A tube containing 40 ml of LPEM and PE (100 mg) food and containing no bacteria was used as a control. For each bacterium, three repeated experiments were performed. Bacterial and non-bacterial containers were kept in a shaker (220 rpm) at 30 °C for 30 days. After 30 days, PE pieces were washed with 1% SDS and incubated on a shaker for 4 hr to remove adhering bacteria. Then, washed in dis. H2O and dried at 60 °C for 12 hr and the weight of the samples was measured 5 times. Fragments of Ketene PE are tested on the IKA-Fure analyzer.

FT-IR (Fourier-transform infrared spectroscopy) spectroscopy

IR spectroscopic studies were carried out on an IR-Fure Inventio-S (Bruker, Germany) with a frustrated total internal reflection attachment in the range from 400 to 4000 cm-1.

Genomic DNA isolation and 16S rRNA gene PCR amplification

To identify molecular genetic identification of *Pseudomonas* strains, genomic DNA isolated from four types of *Pseudomanas* by modified Marmur method (Francisco Salvà-Serra et al., 2018).Universal oligonucleotide primers of bacterial 16S rRNA gene were used for PCR amplification: 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Lane, D. J., 1991). PCR amplification of DNA samples isolated from bacterial strains was carried out in the GenPak® PCR MasterMix kit. In this case, the reaction was prepared in a total volume of 20 µl, consisting of 10 µl of Dilution, 8.2 µl of double-distilled water, 0.4 µl of primer (27F and 1492R), and 1 µl of DNA samples. PCR amplification optimization initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 40 seconds, primer annealing at 55°C for 40 seconds, elongation at 70°C for 90 seconds, final elongation at 70°C for 7 minutes, during repeated in 35 cycles. Electrophoresis detected amplicons on a 1.5% agarose gel stained with ethidium bromide (Fig 3). PCR fragments were purified using a Qiagen gel extraction kit. The amount of PCR products purified from the gel and measured on the Nanodrop was corrected to 20 ng. The samples were sequenced using the BigDye Terminator v.3.1 cycle sequencing kit and the Applied Biosystems® Genetic Analyzers, 3130 series sequencer (Thermo Fischer Scientific, Russia).

Results

Isolation of microorganisms that decompose polyethylene

In the experiment, 100 pieces of *Galleria mellonela* (3–4-year-old larvae) were fasted for 24 hr after being fed with beeswax for 3 days. We found the larvae have been fed LDPE film for 10 days and had 17% PE uptake (the film formed holes). At the same time, wax moth larvae did not increase their mass and activity when eating PE. It was noted that the lack of behavior and the ability to eat decreased (Fig 1). Ten strains of bacteria were isolated from the gastrointestinal tract of the larvae (Fig 2). It is important to note that up to 14 days of feeding on PE, 80% of the death of larvae was observed. We analyzed that 20% of the larvae became pupae with reduced PE uptake.

Fig.1. PE appearance after Wax moth larvae have eaten for a week. B - sterilized larvae in 75% ethanol

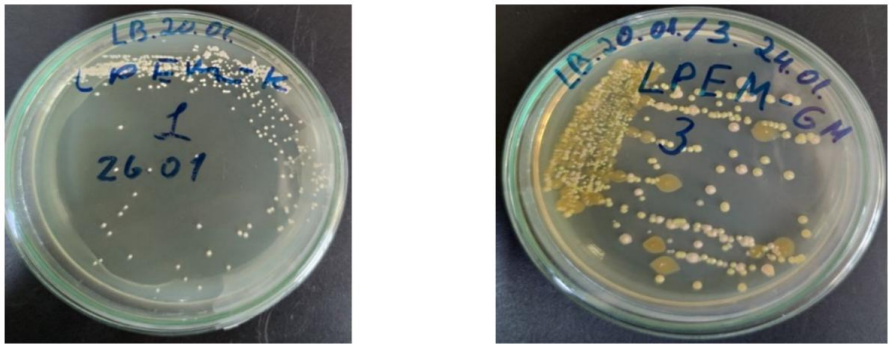

Fig.2. Colonies germinated within 3 days after inoculation of the homogenate onto LB plates

We grew Waxwing larvae fed PE for 10 days on LPEM2 solid medium. We determined the generation of bacterial isolates grown in the culture medium in a Bergi bacterial detector. Simultaneously, we observed 3 strains of *Bacillus*, 4 strains of *Pseudomonas*, 2 strains of *Rhodoccoccus*, and 1 strain of *Escherichia coli* by a Bergi bacterial detector.

Screening for biodegradation in liquid nutrient media

PE weight was measured to determine the biodegradation, change in molecular weight, and appearance of metabolite products soluble in LPEM medium. Therefore, at the beginning of our experiment, we studied the change in the mass of PE under the influence of isolates (Nanda S & Sahu SS 2010; Hadad D et al., 2005). For this, we incubated bacterial isolates in a liquid LPEM2 medium for 30 days and measured their weight five times (Table).

Table 1

PE uptake of isolated isolates

Number of isolates	100 mg weight PE						% change in PE
	M1	M2	M3	M4	M5	M (average)	
Bacillus sp. 1	99,8	100	100,05	100,05	100,05	99,99	-0,01%
Rodococcus sp. 1	99,75	99,8	99,85	99,85	99,9	99,83	-0,17 %
Pseudomonas 4	100	99,95	99,95	100,15	99,7	99,95	-0,05%
Escherichia coli	100,15	100,15	100,05	100,05	100,1	100,1	+0,1%
Pseudomonas 2	99,4	99,75	99,7	99,7	99,75	99,66	-0,34%
Bacillus sp. 3	100	100,05	100,05	100,05	100,1	100,05	+0,05%
Pseudomonas 1	99,45	99,55	99,5	99,35	99,5	99,47	-0,53%
Rodococcus sp.2	99,95	99,95	99,85	99,85	99,8	99,88	-0,12%
Pseudomonas 3	99,35	99,2	99,05	99,15	99,25	99,2	-0,8%
Bacillus sp. 2	100,25	100,05	100,15	100	100,35	100,16	+0,16%
Control action of UV	96,8	96,2	95,9	96,4	96,7	96,06	-3,94%
Control	100	100	100	100	100	100	0%

Our results showed that all bacteria related to the genus *Pseudomonas* caused a decrease in the weight of PE. For example, bacterial isolates of *Pseudomonas spp.* 4, *Pseudomonas spp.* 2, *Pseudomonas spp.* 1, and *Pseudomonas spp.* 3 reduced the PE weight by 0.05%, 0.34%, 0.53%, and 0.8%, respectively. Similarly, under the influence of bacterial isolates belonging to the genus *Rhodococcus*, i.e. *Rhodococcus spp.* 1 and *Rhodococcus spp.* 2, the weight reduction of PE was 0.17% and 0.12%, respectively. We found that *Bacillus spp.* 1 isolated the reduced weight of PE to 0.01%, but the amount of PE incubated with *Bacillus spp.* 2, *Bacillus spp. 1*, and *Escherichia coli* bacteria, on the contrary, increased from the initial weight. This may be because metabolites of bacterial isolates are absorbed by PE. As a control, we found that the mass of PE placed in the LPEM2 medium did not change within 30 days. In our experiment, PE left in the sun for 180 days was used as a second control. As a result, it was found that the mass of PE under the action of UV sun decreased by 3.94% compared to the mass of PE grown with bacteria. The results showed that although under the action of bacteria, a small reduction in the mass of PE was observed, the effect of UV light and temperature (average temperature 39 °C) affected the active decomposition of PE.

FTIR analysis

Usually, as our result of the PE decomposition, radicals with oxidation products are formed on its surface, and according to the results of FTIR spectrometry, hydroxyl groups (3500–3100 cm–1), ether groups (1.113 cm–1), carboxyl groups (1.647–1.716 cm –1 (Fig 6)). FTIR analysis of bacterial isolates cultured with PE for 30 days showed that bacterial isolates of *Pseudomonas spp.* 2, *Pseudomonas spp.* 1, and *Pseudomonas spp.* 3 produce carboxyl groups in the spectral range of 1647-1716 cm -1. In addition, we found *Pseudomonas spp.* 2, *Pseudomonas spp.* 1, and *Pseudomonas spp.* 3 isolates were biodegraded PE with the formation of hydroxyl groups in the FTIR range of 3500–3100 cm–1, although in small amounts. In other bacterial isolates used in the experiment, the formation of organic compounds as a result of PE degradation was not observed. It is important to note that during the degradation of PE under the action of solar UV light, higher hydroxyl groups, ester groups, and carboxyl groups are formed than in bacteria (in the spectral range 3500–3100 cm–1, 1.647–1.716 cm–1 and 1.113 cm–1).

Molecular-genetic analysis by 16S rRNA gene of polyethylene-degrading active strains.

Recently, the phylogenetic position of bacteria has been identified through the determination of 16S rRNA gene sequences. Because most 16S rRNA genes make up about 1,500 base pairs, some branches of the gene as domains (Archaea, Eucaea, and Bacteria) reveal broad characteristics of phylogenetic groups, while other branches of the gene reveal narrower characteristics of groups, such as type, class, and genus.

A similarity of 97% of the 16S rRNA gene sequence gives information about the genus of the bacteria, while a similarity above 99% gives information about the species (Bosshard, P. P et al., 2003).

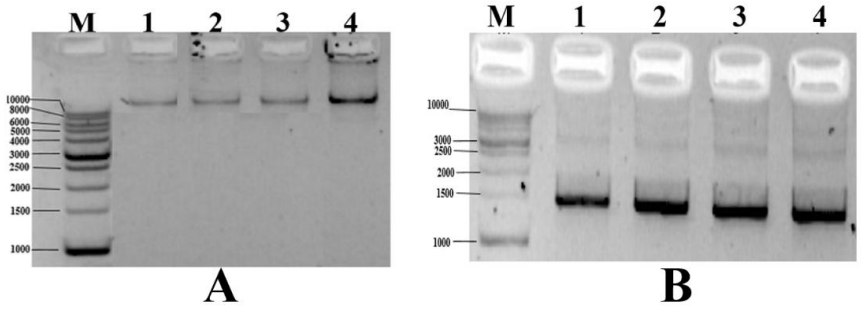

Fig.3. Genomic DNA of *P. stutzeri*, *P. fluorescens*, *P. putida*, and *P. aeruginosa* strains (A), and PCR product of 16S rRNA gene of these strains (B). M-1kb DNA marker, 1- *P. stutzeri*, 2- *P. fluorescens*, 3- *P. putida*, 4- *P.aeruginosa*.

As shown in Fig 3-A, high-quality and pure genomic DNA was isolated from *P. stutzeri*, *P. fluorescens*, *P. putida*, and *P. aeruginosa* strains using the modified Marmur method. The isolated DNA strand was subjected to PCR using specific primers, and it was observed that the PCR product of 1500 bp of the 16S rRNA gene of these four strains was formed (Fig. 3-B).

Amplicons were purified, concentrated to the desired concentration, and sequenced to determine the nucleotide sequence. When the PCR product of the 16S rRNA gene of these four strains was sequenced, 1452 pairs of nucleotides were found in *P. stutzeri* strain, 1422 pairs in *P. fluorescens* strain, 1400 pairs in *P. putida* and 1409 pairs in *P. aeruginosa* strain.

The obtained nucleotide sequences were checked by comparison in the Clustal Omega online program to determine the resulting mutations. When the DNA sequences were compared, 72 mutual mutations were found (Fig 4).

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P_stutzeri_Uzb      TGATCCTGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGA 60
P_aeruginosa_Uzb    ----------------------------------------CTAAATGCAGTCGAGCGGATGA          22
P_putida_Uzb        -----------------------------------------TGCAGTCGAGCGGATGA       17
P_fluorescens_Uzb   ----------------------------------GCGGCTACCATGCAGTCGAGCGGATGA       27
                                                     **********

P_stutzeri_Uzb      ATGGAGCTTGCTCCATGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGG 120
P_aeruginosa_Uzb                 CGGGAGCTTGCTCCTTGATTCAGCGGCGGACGCGTGAGTAATGCCTAGGAATCTGCCTGG
  82
P_putida_Uzb        CGGGAGCTTGCTCCTTGATTCAGCGGCGGA-CGGTGAGTAATGCCTAGGAATCTGCCTGG  76
P_fluorescens_Uzb                CGGGAGCTTGCTCCTTGATTCAGCGGCGGACGCGTGAGTAATGCCTAGGAATCTGCCTGG
  87
                 ********** ********** ***********************

P_stutzeri_Uzb      TAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGATAGC180
P_aeruginosa_Uzb                 TAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGC
  142
P_putida_Uzb                     TAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGC
  136
P_fluorescens_Uzb                TAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGC
  147
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P_stutzeri_Uzb      AGGCGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAG 240
P_aeruginosa_Uzb                  AGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGG
  202
P_putida_Uzb        AGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGG 196
P_fluorescens_Uzb                 AGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGG
  207
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P_stutzeri_Uzb      GTAATGGCTCACCAAGGCGACGATCCGAAACTGGTCTGAGAGGATGATCAGTCACACTGG 300
P_aeruginosa_Uzb                  GTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGG
  262
P_putida_Uzb        GTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGG 256
P_fluorescens_Uzb                 GTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGG
  267
            **************************** *********************************

P_stutzeri_Uzb                    AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGCGGAATATTGGACAATGGGC
  360
P_aeruginosa_Uzb                  AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGC
  322
P_putida_Uzb                      AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGC
  316
P_fluorescens_Uzb                 AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGC
  327
            ****************************************** ********************
P_stutzeri_Uzb      GAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT 420
P_aeruginosa_Uzb                  GAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT
  382
P_putida_Uzb        GAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT 376
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P_fluorescens_Uzb                GAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT
387
                                 ************************************************************
P_stutzeri_Uzb       AAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAA 480
P_aeruginosa_Uzb                 AAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCGACAGAATAA
442
P_putida_Uzb         AAGTTGGGAGGAAGGGCAGTAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAA 436
P_fluorescens_Uzb                AAGTTGGGAGGAAGCGCAGTAAGCTAATACCTTGCTGTTTTGACGTTACCGACAGAATAA
447
                                 ************** ******** ************************************

P_stutzeri_Uzb       GCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGA 540
P_aeruginosa_Uzb                 GCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGA
502
P_putida_Uzb         GCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGA 496
P_fluorescens_Uzb                GCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGA
507
                                 *************  **********************  **********************

P_stutzeri_Uzb       ATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCT  600
P_aeruginosa_Uzb                 ATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCT
562
P_putida_Uzb         ATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCT 556
P_fluorescens_Uzb                ATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCT
567
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P_stutzeri_Uzb       CAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGCAGAGCGTGGTGGAATTT 660
P_aeruginosa_Uzb                 CAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTT
622
P_putida_Uzb                     CAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTT
616
P_fluorescens_Uzb                CAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGGAATTT
627
                                 ****************************** ********** ** **** ************

P_stutzeri_Uzb                   CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACC
720
P_aeruginosa_Uzb                 CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACC
682
P_putida_Uzb                     CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACC
676
P_fluorescens_Uzb                CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACC
687
                                 ************************************************************

P_stutzeri_Uzb                   TGGGCTAATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
780
P_aeruginosa_Uzb                 TGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
742
P_putida_Uzb                     TGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
736
P_fluorescens_Uzb                TGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
747
                                 *** ** ******************************************************

P_stutzeri_Uzb       GTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGC  840
P_aeruginosa_Uzb                 GTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCATGAAATTTACTGGCCGC
802
P_putida_Uzb         GTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGC  796
P_fluorescens_Uzb                GTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGC
807
                                 ************************ ************ **** *** ** *    * ***

P_stutzeri_Uzb       -ACTAACGCATTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA 899
P_aeruginosa_Uzb                 AGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
862
P_putida_Uzb                     AGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
856
P_fluorescens_Uzb                AGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAA
867
                                 ************** *********************************************

P_stutzeri_Uzb                   TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
959
P_aeruginosa_Uzb                 TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGAGAAGAAG
922
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P_putida_Uzb                TTGACGGCGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
  916
P_fluorescens_Uzb           TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
  927
                    ******* ********************************************** ******

P_stutzeri_Uzb      CTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC  1019
P_aeruginosa_Uzb            CTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
  982
P_putida_Uzb        CTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC  976
P_fluorescens_Uzb           CTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
  987
                    ************************************************************

P_stutzeri_Uzb      TGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG  1079
P_aeruginosa_Uzb            TGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
  1042
P_putida_Uzb        TGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG  1036
P_fluorescens_Uzb           TGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGCGTTAAGTCCCG
  1047
                    ********************************************** ***********

P_stutzeri_Uzb      TAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTAATGGTGGGCACTCTAAGGAGAC  1139
P_aeruginosa_Uzb            TAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGAC
  1102
P_putida_Uzb        TAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGAC  1096
P_fluorescens_Uzb           TAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCACTCTAAGGAGAC
  1107
                    ************************************ **********************

P_stutzeri_Uzb      TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCC 1199
P_aeruginosa_Uzb            TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCC
  1162
P_putida_Uzb                TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCC
  1156
P_fluorescens_Uzb           TGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCC
  1167
                    ************************************************************

P_stutzeri_Uzb      TGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAGCT1259
P_aeruginosa_Uzb            TGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCT
  1222
P_putida_Uzb        TGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCT1216
P_fluorescens_Uzb           TGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCT
  1227
                    ********************************** ***************************

P_stutzeri_Uzb      AATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGG 1319
P_aeruginosa_Uzb            AATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGG
  1282
P_putida_Uzb        AATCTCACAATACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGG 1276
P_fluorescens_Uzb           AATCTCACAATACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGG
  1287
                    **** ** ** **************************************************

P_stutzeri_Uzb      AATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACA 1379
P_aeruginosa_Uzb            AATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACA
  1342
P_putida_Uzb        AATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACA 1336
P_fluorescens_Uzb           AATCGCTAGTAATCGCGAATCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACA
  1347
                    *************** ************* ******************************

P_stutzeri_Uzb      CCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAGTA--GCTAGTCTAACCTTCGCGC  1437
P_aeruginosa_Uzb            CCGCGCGTCACACCATGGGATGTGCGTTGCACCAGATGTTGCTTAGTCTAACCTTCGGGA
  1402
P_putida_Uzb        CCGCCCGTCACACCATGGGAGTGGTT-GCACC-AGAAGTA-GCTAGTCTAACCTTCGGGA  1393
P_fluorescens_Uzb   CCGCCCGTCACACCATGGGAGTGGGTTGCACC-AGAAGTA-GCTAGTCTAACCTTCGGAG 1405
                    **** ***************  *    *  *     ***************

P_stutzeri_Uzb      GGACGGTTACCACGG--           1452
P_aeruginosa_Uzb    GGACGGT----------  1409
P_putida_Uzb        GGACG------------          1398
P_fluorescens_Uzb   GACGGTACCACGGTGAT       1422
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Fig.4. Comparison of DNA nucleotide sequences of *Pseudomonas* strains with Clustal Omega online software

Then, when the 16S rRNA gene sequences of these strains were checked in the National Center for Biotechnology Information (NCBI) database, *P. putida* strain was 100% to *P. putida* bacteria, *P. fluorescens* strain was 100% to *P. fluorescens* bacteria, and *P. aeruginosa* strain was 100% *P.aeruginosa* bacteria and *P. stutzeri* strain was found to be 100% similar to *P. stutzeri* bacteria. Mega4 bioinformatics software was used to determine the phylogenetic tree of *P. stutzeri*, *P. fluorescens*, *P. putida*, and *P.aeruginosa* strains. A phylogenetic tree was constructed using the Maximum-likelihood statistical method using Mega4 bioinformatics software (Fig. 5).

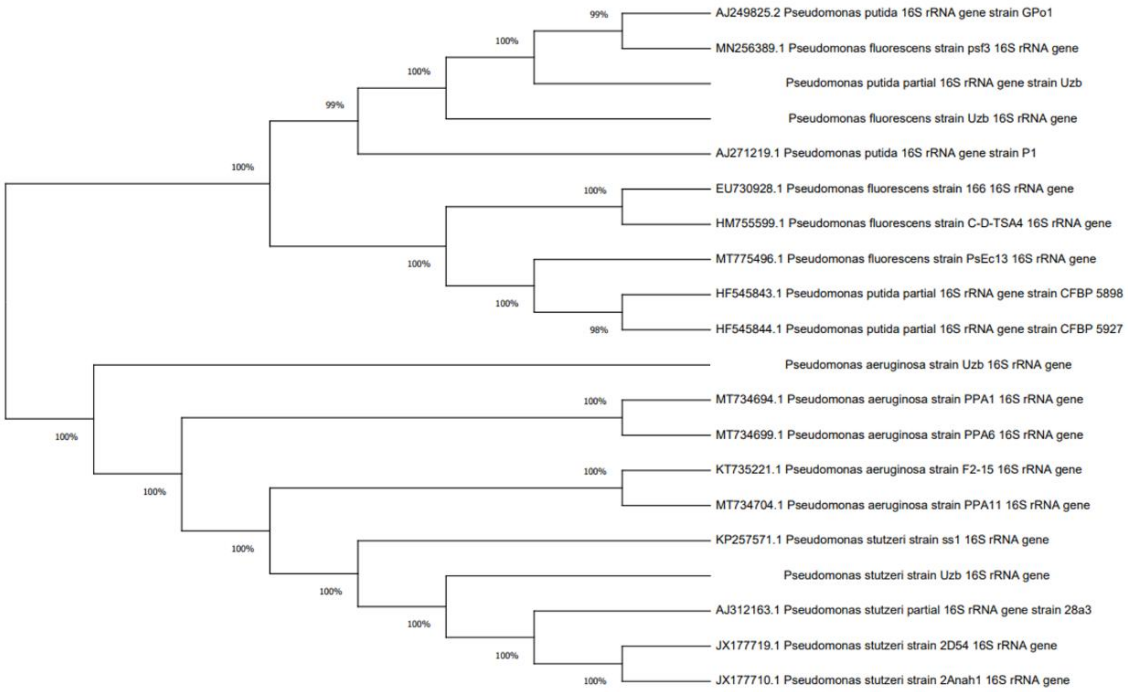

Fig. 5. Phylogenetic tree of *P. stutzeri*, *P. fluorescens*, *P. putida*, and *P. aeruginosa* strains using the Maximum-likelihood statistical method in the Mega4 bioinformatics program.

The phylogenetic tree showed that *P. fluorescens Uzb* and *P. putida Uzb* strains can be placed in the same cluster as *P. putida* and *P. fluorescens* strains in the NCBI database. In this case, the closeness of the nucleotide mutations of *P. fluorescens* Uzb strain 16S rRNA gene, and the similarity between *P. fluorescens Uzb* and *P. putida Uzb* was observed. Molecular genetic analysis of *P. stutzeri Uzb* and *P.aeruginosa Uzb* strains showed that they were placed in the species cluster with 100% similarity with *P. stutzeri* and *P.aeruginosa* strains in the NCBI database, and indeed *P. stutzeri Uzb* and *P.aeruginosa Uzb* strains stated that. Thus, the bacteria belonging to the genus *Pseudomonas*, identified with the Bergey bacterial identifier, were determined by the molecular-genetic method, and the types of bacterial strains were confirmed.

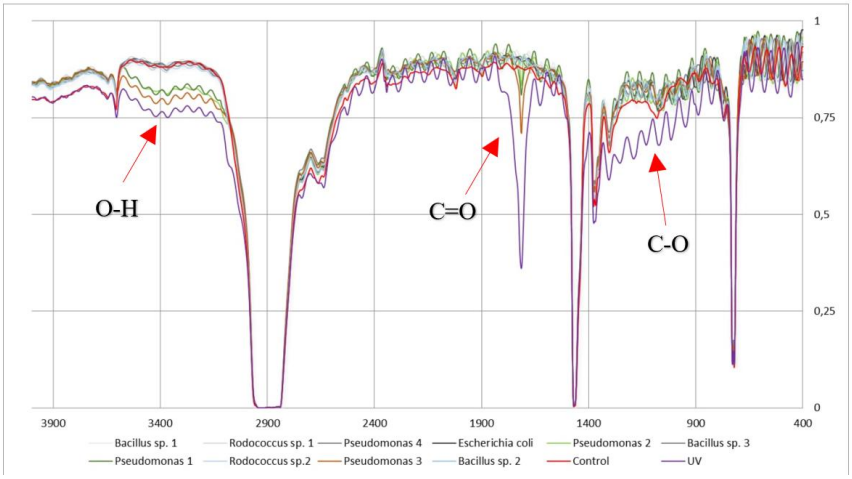

Fig. 6. FTIR analysis of bacterial isolates cultured with PE**.**

Discussion

Since polyethylene is one of the most common plastics in the world, its recycling has become a global problem today. Plastic polymers are widely used in agriculture, industry and our daily life due to their convenience and low cost. At the same time, pollution with plastic polymers, especially polyethylene (PE), has a negative impact on the environment, as well as on the health of animals and humans, because they decompose very slowly in natural conditions (Alabi OA et al., 2019). Although various methods of decomposing plastic waste are being tested, currently the safest method is the biodegradation method (Ghatge, S et al., 2020). To date, more than 30 different genera of microorganisms (bacteria and fungi) involved in plastic biodegradation have been identified. In addition, insects such as *Plodia interpunctella*, *Achroia grisella*, *Galleria mellonella*, *Corcyra cephalonica*, *Zophobas atratus* and *Tenebrio molitor* have also been found to feed on plastic (Peng BY et al., 2020; Bilal, H et al., 2021). In particular, microorganisms isolated from the microbiome of PE-feeding insects have the ability to degrade PE. Currently, there is ongoing debate as to whether PE degradation occurs through the earthworm's digestive system or whether the microbiome also plays a role in PE biodegradation. Experiments conducted by us have shown that the bacterium isolates from Waxworms fed with PE for 10 days were observed to break down PE although very few isolates. This indicates that the weak degradation of PE by this isolate does not support the long-term

digestion of the waxworm larva. Also, while there is mutualism between waxworm and microbiota in beeswax absorption, in our experiment it was observed that waxworm cannot feed on PE for a long time. When isolates from the wax microbiome actively biodegraded PE, the waxworm would not have died after 14 days but would have survived longer. During the first 10 days, the absorption of PE by the larvae was carried out under the influence of enzymes in the gastrointestinal system of the waxworm larvae, and the increase in the amount of ethylene glycol formed during the biodegradation of PE may have caused the death of the waxworm larvae. This research work will continue tomorrow. It is also known that these bacteria have resistance to heavy metals and bioremediation properties of soils contaminated with heavy metals (Usmonkulova et al., 2022; Usmonkulova et all., 2023)

Conclusion.

Bacteria isolated from the waxworm microbiome were found to belong to the genera *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Escherichia*. The isolated bacteria were studied to absorb PE at a low level. FTIR results showed that these bacteria were more likely to degrade PE to form carboxyl groups and relatively fewer hydroxyl groups. Bacteria have been shown to degrade PE and contribute little to the earthworm diet. Among bacteria, it was analyzed that bacteria belonging to the genus *Pseudomonas* are more biodegradable than bacteria related to other species in degrading PE.

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Conflict of interest

A statement about an article's members to disclose any conflicts of interest.

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Author`s orcid:

Nazirov Muhammad-Latif - https://orcid.org/0009-0007-7757-1644

Xalilov Ilkhom - https://orcid.org/0009-0003-5524-3309

Kobilov Fazliddin – https://orcid.org/0000-0003-4346-4545

Mardonov Ikrom – https://orcid.org/0009-0002-4976-731X