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Sustainable Removal of Nanoplastics: Exploiting the Lipolytic Activity of *Pseudomonas aeruginosa* O6 Isolated from Mariout Wetland, Egypt

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ABSTRACT

The increasing problem of micro-nano plastic pollution in Egypt's coastal areas is a global threat to marine ecosystems. This study investigated an environmentally friendly bioremediation approach to tackle this issue, specifically looking at the identification and application of lipolytic bacteria found in plastic-polluted regions for breaking down nanoplastics. One of the seven bacterial strains examined showed an exceptional efficacy in degrading nanoplastics. This strain was identified as *Pseudomonas aeruginosa* using 16S rRNA gene sequencing and was recorded in the NCBI database with an accession number of PP087224. The strain was analyzed using a p-nitrophenyl palmitate assay to quantify its lipase production when exposed to different nanoplastics, such as polyethylene, polystyrene, and polyethylene terephthalate. The results showed notable differences in enzyme activity depending on the polymer type. The strain exhibited the highest lipase activity with polyethylene (142± 2U/ µL), followed by polystyrene (83± 1.4U/ µL), and the lowest activity was observed with polyethylene terephthalate (22± 2U/ µL) compared to the control. The study showed that the bacterial reaction to nanoplastic pollution differs depending on the polymer type. Scanning electron microscopy verified a 97% decrease in nanoplastic sizes and chemical structural changes. This was validated by Fourier transform infrared spectroscopy (FTIR) and X-ray differential (XRD) analysis, which showed modifications in the polymer's chemical structure and crystallinity. The research introduced a viable approach for nanoplastic remediation using particular bacterial strain and their enzymes, providing a new solution to the urgent problem of marine nanoplastic pollution utilizing the capabilities of *Pseudomonas aeruginosa O6*.

INTRODUCTION

The significant rise in worldwide plastic production, exceeding 360 million tons in 2018, and predictions suggesting a possible triple increase by 2050 have led to a crucial issue in plastic pollution **(Su *et al.*, 2022)**. This growing problem presents significant dangers to worldwide ecosystems, impacting non-living and living elements, highlighting

the crucial need for successful pollution control methods **(Ncube *et al.*, 2021)**. Each year, about 4.8 to 12.8 million metric tons of plastic waste enter the oceans due to poor waste management techniques, as emphasized by **Jambeck *et al.* (2015)**. This surge of plastic not only harms marine ecosystems but also impacts human well-being.

The impact on marine wildlife is profound, with instances of ingestion and entanglement leading to deteriorated health and compromised feeding and reproductive functions **(Staffieri *et al.*, 2019)**. Plastics' buoyancy allows them to be easily transported across long distances, bringing non-native and invasive species that endanger marine biodiversity. The ultraviolet (UV) radiation and other environmental variables break down plastics into smaller pieces called microplastics and nanoplastics. Particles of different sizes and shapes contribute to pollution, which creates additional ecological concerns **(Anand *et al.*, 2023)**. Various research investigations have shown that tiny plastic particles, specifically nanoplastics less than 1.0µm, have adverse effects on important commercial fish species such as the Nile tilapia **(Mattsson *et al.*, 2015; Yee *et al.*, 2021)**. Detrimental effects include compromised immune systems **(Hamed *et al.*, 2020, 2021)**, organ damage, and a severe imbalance of gut microbiota homeostasis **(Wu *et al.*, 2024)**. Thus, isolating microorganisms with substantial capability to degrade different nanoplastics at the nanoscale is urgent.

Within this context, Mariout Wetland in Alexandria, northern Egypt, stands out as a crucial area for research primarily due to its diverse microbial populations. The populations have adjusted to increased industrial and agricultural runoff, supporting a robust microbial ecosystem essential for preserving ecological equilibrium and water quality **(Farouk *et al.*, 2020)**. Profound research by **Yosef *et al.* (2022)** has shown unique functional gene families bacteria of polluted lakes, indicating an advanced ability for biodegradation. *Pseudomonas* bacteria, known for their lipolytic activity, are pivotal in breaking down nanoplastics. The microorganisms produce lipase enzymes that effectively break down ester bonds in plastics into smaller, easily degraded fragments, providing a promising and sustainable solution for reducing plastic pollution, particularly at the nanoplastic scale **(Kanmani *et al.*, 2015)**.

Despite extensive research into the distribution, absorption, fate, and effects of microplastics, as well as methods to remove them **(Wong *et al.*, 2020; Bhatt *et al.*, 2021)**, there is still a notable lack of knowledge regarding the removal of nanoplastics, especially those smaller than 1.0µm. Research on both biological and non-biological approaches to microplastic degradation has advanced. Microorganisms like algae, fungi, and bacteria have been identified as affordable and environmentally friendly solutions **(Qin *et al.*, 2021; Chen *et al.*, 2022; Cholewinski *et al.*, 2022)**. The lack of study on nanoplastic breakdown highlights a significant gap in our knowledge of how they interact with microbial organisms and the resulting enzymatic processes.

This study aimed to bridge this gap by focusing on the biodegradation of various nanoplastics down to 400nm, utilizing microbes from polluted areas, and examining their impact on lipolytic activity. Such investigation is a crucial step toward mitigating the

environmental impact of nanoplastics and enhancing bioremediation methods, ultimately contributing to global sustainability efforts.

MATERIALS AND METHODS

1. Plastic material

Polystyrene (PS), polyethylene terephthalate (PET), and polyethylene (PE) beads were purchased from (LanXess, China), with densities of 0.9, 1.38 and 0.915g/ cm^3, respectively. The average diameter of each plastic pellets was 4mm with colorless regular shape. Prior to the degradation study, the particles were sterilized by soaking them in 70% ethanol in a glass Petri dish and then dried for 16 hours in an air oven at 55°C.

2. Preparation and characterization of nanoplastic

This study involved the preparation of three distinct nanoplastic waste products using specific methods as follows (Fig. 1):

The polystyrene nanoplastics were prepared using a modified nanoprecipitation method according to **de Sousa Cunha *et al.* (2021)**, which involved the dissolution of 100mg of polystyrene pellets in 10mL of ethyl acetate 99% (Piochem, Egypt) with magnetic stirring for 12 hours. The dissolved solution was gradually added to an ethyl alcohol 99.9% (Piochem, Egypt) solution (volumetric ratio 1:40) under vortexing. Subsequent ultrasonication for 2 hours and centrifugation separated the formed nanoparticles, which were then dried at 60°C for 12 hours in a forced convection oven. The production of polyethylene nanoplastics involved the emulsification-solvent evaporation method with customizations **(Zokaei *et al.* 2023)**, where 1.0g of PE pellets was dissolved in 10mL of xylene (Piochem, Egypt) at 100°C for 30 minutes. Rapid cooling was achieved by adding icy deionized water, followed by sonication for 1.0 hour. The resulting emulsion underwent filtration, centrifugation, and washing with distilled water before being dried at 60°C for 12 hours. Preparation of PET nanoplastics followed a protocol established by **Rodríguez-Hernández *et al.* (2019)**, where PET microbeads were immersed in a strong trifluoroacetic acid (TFA) (99%, Sigma Adrich, Germany) solution at 50°C and allowed to rest overnight. Afterward, the solution was subjected to precipitation using a diluted aqueous solution of TFA 10%. This was followed by centrifugation and resuspension in a solution containing 0.5% SDS. Ultrasonication was employed for particle dispersion, followed by settling and recovery of the nanosized PET particles. The particles were then washed and dried at 60°C for 12 hours.

Fig. 1. Preparation of the three nanoplastic waste (above) macrosize (below) nanosize. (PS: Polystyrene, PET: Polyethylene terephthalate, and PE: Polyethylene)

3. Sample collection

The sediment sample was collected from the coastal region of Marriout Wetland, Alexandria, Egypt (GPS coordinate: 31°02'00.4"N 29°45'23.5"E), in August 2022. The sample was collected in sterile bags, and transported to the laboratory. Sediment samples were kept at 4°C for further work.

4. Culture media

The liquid carbon-free basal media (LCFBM) were used for the plastic degrading assays. It was prepared by dissolving 0.7g of K_2HPO_4, 1.0g of NH_4NO_3, 0.7g of KH_2PO_4, 5mg of NaCl, 2mg of $FeSO_4.7H_2O$, 2mg of $ZnSO_4.7H_2O$, 0.7g of $MgSO_4.7H_2O$, and 1.0mg of $MnSO_4.H_2O$ in 1L of deionized water. For subculture and preservation, Luria-Bertani (LB) media (HiMedia, India) was used and prepared according to the manufacturer's instructions. Whenever needed, 17g of agar (BandV, Italy) were added to solidify the previously mentioned media. For serial dilution experiment, 0.9% saline solution was prepared by dissolving 0.9g NaCl (Nasr.co, Egypt) in 100mL deionized water. Each media, buffers and solution were autoclaved (Diahan Scientific Co., Ltd., Korea) at optimal conditions.

5. Isolation of the nanoplastic degrading bacteria

Nanoplastic-degrading bacteria were isolated using a modified standard procedure in carbon-free basal media (CFBM) supplemented with a mixture of three nanoplastics as the sole carbon source **(Ekram *et al.*, 2020)**. To enhance emulsification, 0.005% SDS was added to the media to ensure colloidal and homogeneous dispersion of nanoplastic particles. Soil was mixed with 0.9% saline water in a conical flask, thoroughly shaken, and incubated for 4 hours to allow sedimentation. An aliquot (1.0ml) of the supernatant was serially diluted, and 100µL of each dilution was spread on the surface of nanoplastic agar media for primary screening, followed by three days incubation period at 37°C. The obtained isolates were further cultured on nanoplastic liquid media for secondary screening. The growth of isolated bacteria and the reduction in media turbidity were considered positive results. The mutant *Escherichia coli* strain DH5α served as the negative control, while liquid carbon-free basal media were used as the positive control. The experiment was conducted in triplicate.

6. Lipolytic activity assays

The nanoplastic-degrading strains were first tested for lipase production activity with Tween 80. A positive result was seen when a white precipitate formed around the extract and the rhodamine B assay was also performed, where a fluorescent halo around the colony indicated a positive result using well plate agar method according to **Nawani *et al.* (2006)**. Promising strains identified in the primary screening were then selected for quantification using a p-nitrophenyl palmitate (pNPP) assay, as described by **Rehman *et al.* (2017)**. A 100-µL sample of the enzyme extract was added to a 900-µL reaction mixture with 3mg of pNPP dissolved in 1mL of isopropanol. This mixture was made in 9mL of 50mM Tris-HCl buffer (pH 8.0) with 100µL of Triton X-100 and 10mg of arabic gum. A UV-Vis double beam spectrophotometer detected pNPP release at 410nm then incubated at 37°C till the appearance of the yellow color. The equation below was used to compute enzyme activity units.

Lipase activity = (Abs × standard factor*) / Time (min) × Enzyme amount (mL).

*The measurements were modified using the standard factor (1/ slope) of the standard curve. It was measured in international units (UI), where one unit equals the amount of enzyme that releases 1.0µmol of pNPP per minute at 25°C **(Rehman *et al.*, 2017)**.

7. Identification of bacteria

The genomic DNA of the nanoplastic-degrading strain was manually extracted following the method described by **Maloy (1990)**. Amplification of PCR products was performed using (2H) My Taq HS Red Mix (Bioline, BIO-25048). Bi-directional sequencing of the sample was conducted using a sequencer from Macrogen, Korea. Sequences were compared to sequence data obtained from the GenBank NCBI, a public data source. The phylogenetic analysis was executed using the MEGA 11 computational tool.

8. Biodegradation assays

8.1. Quantification of the nanoplastics

The study employed a modified version of the cloud point extraction (CPE) method, originally described by **Zhou *et al.* (2019)**, to concentrate nanoplastics. A 15mL centrifuge tube was filled with either the sample solution, followed by the addition of 30µL of 10% TX-100 aqueous solution and 100µL of 1.0 mol $MgSO_4$ solution sequentially. The solution was agitated and incubated undisturbed in a water bath at 45°C for 15 minutes. It was then centrifuged at 1509.3 ×g at 4°C for 10 minutes to separate a phase enriched with surfactant. Subsequently, the sample was transferred to a tiny glass tube, and the nanoplastic-rich TX-100 phase was subjected to further thermal digestion at 190°C for 3 hours to remove TX-100. The obtained particles were weighted according to the following equation

Weight loss (%) = (initial weight – final weight) / initial weight) × 100.

8.2. Particle count analysis

According to **Mukhanov *et al.* (2019)**, the nanoplastics were examined using an inverted microscope (IM) to determine the particle count and size variations. First, 50µl of the nanoplastics was taken directly from the liquid media and spread to a glass slide and dried. After calibrating the scale, all samples were examined under the inverted microscope. Using an inverted Nikon Eclipse TS100-F microscope coupled with a camera (Ikegami ICD-848P), digital images of all fields of view were captured in transmitted light (all objects appeared as silhouettes). The ImageJ software (https://imagej.net/ij/) was utilized to analyze the particle count in the obtained high-resolution images.

Particle count loss (%) = (initial particle number – final particle number) / initial particle number) × 100.

8.3. Dynamic light scattering

The mean diameter and heterogeneity of the obtained nanoplastics were measured using Nanosizer instrument (S 90, Malvern, United Kingdom) with a reading angle of 90° and a wavelength of 633nm at 25°C. Nanoplastic particles were filtered via 0.22µm syringe filter to eliminate bacterial contamination. To disperse any aggregates prior to subsequent examination, a sonicator equipment (Qsonica, LLC) was utilized to sonicate the suspension **(de Sousa Cunha *et al.*, 2021)**.

8.4. Scanning electron microscope (SEM)

The surface topography and properties of the three nanoplastics were analyzed using a Philips-X LP30 SEM both before and after biological degradation. Using the extraction process mentioned earlier, the nanoplastic waste was subjected to vapor fixation in a sealed container at 25ºC for one day. Afterward, the samples were coated with gold using BAL-TEC-SCDOOS.

8.5. ATR-FTIR and XRD analysis

The infrared absorption spectra were acquired utilizing a Satellite 5000 infrared spectrophotometer (FTIR, Bruker, USA), with air serving as the reference and a spectral range of 400– 4000cm^{-1}. Vibration spectra were obtained using attenuated total reflection (ATR) mode of FTIR with a resolution of 4cm^{-1}. These measurements detected the changes in functional groups on the three nanoplastic wastes. The FTIR spectra obtained were graphed using Origin PRO 2022b. The crystallinity of both treated and untreated samples was evaluated using an X-ray diffractometer. X-ray diffraction patterns were generated with a wide-angle operation setting, covering an angular range from 10 to 100° with a scan increment of 0.02°. The X-ray source was powered by a current of 40 milliamperes (mA) and a voltage of 40 kilovolts (kV), producing X-rays with a wavelength of 1.5418. All analyses were performed at an ambient temperature following standard procedures. The resulting XRD spectra were plotted using Origin PRO 2022b.

9. Molecular docking study

The molecular docking was conducted using Auto Dock software 4.2v **(Morris *et al.,* 2009)**. The 3D structures of the target enzyme *Pseudomonas aeruginosa* lipase were obtained from the protein data bank with PDB ID: 1EX9. The PS, PET, and PE structures were sketched and minimized using Avogadro software **(Hanwell *et al.,* 2012)**. The structures of the target enzyme were prepared before the docking process using AutoDockTools 1.5.6. (https://autodock.scripps.edu/download-autodock4/). Additionally, water and cp-crystalized ligands were removed. Furthermore, the missing atoms were added, and Kollman charges were assigned. The residues of the amino acid active site were determined according to the previous literature **(Nardini *et al.,* 2000)**, and then the receptors were converted to PDBQT format. The protein structure was kept rigid during the docking process, while the ligand bonds were rotatable to optimize the polymer structures. Discovery Studio Visualizer was used for docking analysis and visualization of the result (https://discover.3ds.com/discovery-studio-visualizer-download).

RESULTS

1. Characterization of nanoplastics

The produced nanoplastics were proven to be on the nano scale using dynamic light scattering and SEM pictures, showing average sizes of 210nm for PS, 400nm for PET, and 450nm for PE. The FTIR examination indicated no changes in the chemical composition between the original plastic pellets and the produced nanoplastics. These descriptions made them suitable for use in additional tests.

2. Isolation and identification of nanoplastic degrading bacteria

After four days of incubation, seven different bacterial colonies were grown on the carbon-free basal agar media supplemented with a mixture of three nanoplastics, while no

bacteria were found in the control group. The results showed that the bacterial colonies on the nanoplastic plates could potentially degrade three types of nanoplastics combined. In this study, the seven morphologically different bacterial isolates showed a distinguished behavior when isolated on the LB or CFB agar media. Furthermore, cultured on LB liquid media, they demonstrated different pigment production activities. The UV light images showed that almost all isolates lost their pigmentation when cultured on carbon-free media supplemented with a mixture of microplastics. The previous results demonstrated that nutrient source is determinative in pigment production in plastic-degrading bacteria. On the contrary, the isolates cultured on the LB agar media showed a pigment variation from brownish-green to blue-green pigmentations. Moreover, the isolates cultured on microplastic selective media showed a bluish-green fluorescence, except for the isolate O6, which showed partial pigmentation and lack of fluorescence emission.

Five bacterial isolates with promising plastic degradation ability and lipolytic activity were molecularly identified using the 16S rRNA Sanger sequencing. All the obtained isolates were found to belong to genus *Pseudomonas*. Each bacterial sequence was deposited in the NCBI GenBank under accession numbers. The isolate O5 showed 98.4% similarity with *Pseudomonas* sp. while isolate O6 showed 100% identity with Pseudomonas aeruginosa, other bacterial strains O2, O3, O7 were found similar to *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas otitidis*, respectively. The phylogenetic analysis confirmed the identity of tested strains with its compartments in the GenBank (Fig. 2).

3. Estimation of lipase production

The Rhodamine B method was used to test the lipolytic activity, as shown in Fig. (3a). It was clear from the bright halo around the *Pseudomonas* colonies in isolate O6 that lipase enzymes were detected in the primary screening. The Tween 80 precipitation test (Fig. 3b) showed that isolate O6 had lipase activity confirming the primary screening. The next step was to do a quantitative study of these two strains. The pH measurements were estimated for the promising strain O6. The results showed that pH has increased from 7± 0.2 to 8± 2. In addition, the lipolytic activity in the production media (olive oil) was tested over time, and the results showed that the optimum production of lipase enzyme after 24h at pH= 7 and the activity for the isolate were 23± 4U/ mL.

The lipolytic activity of *Pseudomonas aeruginosa* strain O6 was investigated using various carbon sources, including nanoplastics and traditional carbon compounds. The strain exhibited lipolytic activity toward the three nanoplastics tested. The lipolytic activities observed were 145± 2U/ mL for LDPE, 83± 4U/ mL for PS microplastics, and 22± 2U/ mL for PET microplastics (Fig. 3c). Interestingly, strain O-6 also demonstrated lipolytic activity when olive oil was the sole carbon source, with a measured activity of 20± 2U/ mL. However, the lipolytic activity decreased to 4± 1.5U/ mL when glucose was the sole carbon source. The results show that *Pseudomonas aeruginosa* strain O-6 is good at breaking down the three types of nanoplastics that were tested.

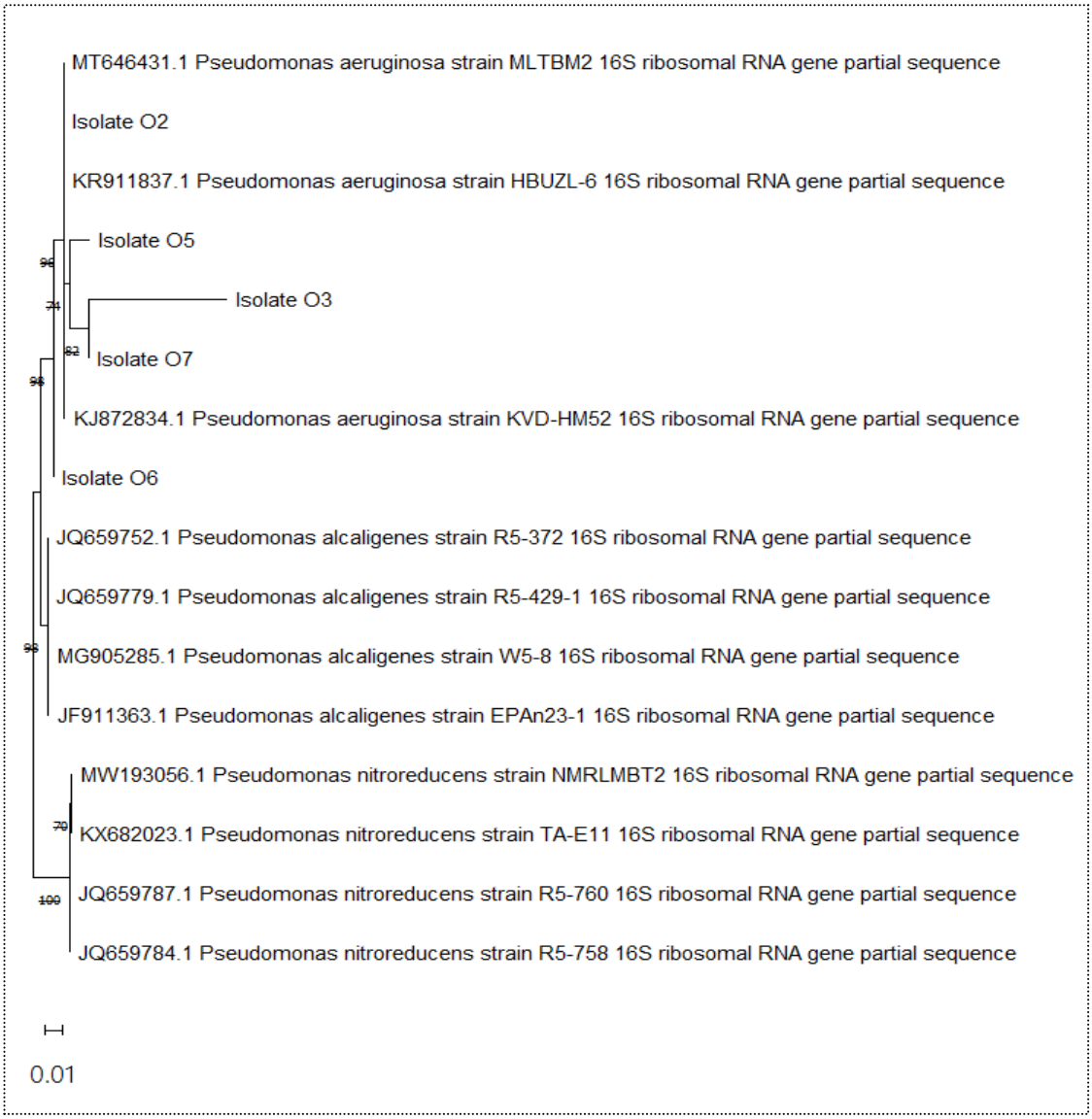


Fig. 2. Neighbor-joining tree to study the evolutional distance among other strains in the NCBI. The distance was computed using the Tamura-Nei method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown below the branches. It was conducted using MEGA 11

4. Particle count and size analysis

Particle counts were measured in the control group (untreated) and the test group (treated). A prominent reduction in the particle size and count was observed in the cell-free suspension. After four days of incubation with *Pseudomonas aeruginosa* O-6, the particle counts in the PS nanoplastics group dropped from 1.4×10^{7} to 2.1×10^{5} particles/ mL. This is a 98.5% drop in the particle count. Similarly, PET nanoplastics showed a 90.5% reduction in the particle count from 1.8×10^{5} to 1.7×10^{4} particles/ mL. Moreover, LDPE reduced the

particle diameter and count by 94%. This confirmatory test showed the rapid reduction in the number of nanoplastic particles and the random deformations in the plastic particles, as illustrated in Fig. (4c). The results indicated that the recently identified *Pseudomonas aeruginosa* O-6 strain possesses diverse polymer biodegradation properties. The particle count technique was confirmed by measuring the particle size over time using dynamic light scattering. The results showed that the particle diameters showed a 71, 76.5, and 69.2% reduction in particle size of the nanoplastic, as shown in **Fig. (4a).**

5. Quantification of the nanoplastics

The cloud point extraction method collected the leftover submicron plastics in the water-based media. The weight loss method was also used to measure how much nanoplastic was removed with the help of microorganisms. Researchers checked how well polyethylene, polystyrene, and polyethylene terephthalate nanoplastics broke down by finding out how much weight they lost after incubating with *Pseudomonas aeruginosa* for four days. The *Pseudomonas aeruginosa* strain O-6 was very good at breaking down PS, PET, and PE nanoplastics, with breakdown rates of 70% for PS, 49.8% for PET, and 40% for PE. A significance difference between the starting and ending weights indicated that the bacteria used PET, PE, and PS as the sole carbon sources (Fig. 4b). The control group had no bacteria and no decay rates, and nanoplastics were still in the culture media.

6. SEM analysis of the nanoplastic

An analysis was conducted on three types of nanoplastics that underwent microbial treatment to determine the extent of particle shape and size reduction. The nano-PET particles were spherical and 97% smaller than they used to be. The image displays PET nanoplastics with an average diameter of 550nm in irregular shapes (Fig. 5). After being treated with *Pseudomonas aeruginosa* O6, the *Pseudomonas* strain leads to the formation of nanoplastics that are smooth and round. Similarly, the untreated polystyrene nanoplastics exhibited a spherical shape with a non-uniform size, measuring around 210± 15nm in diameter. On the other hand, the treatment groups made nanoscale polystyrene particles with an average diameter of 15± 5nm. This means the polystyrene particles were 93% smaller. In the untreated group, the LDPE particles in the polyethylene nanoplastics had an average diameter of 3µm. After adding microbes, the polyethylene particles showed rod-shaped nanoparticles about 180± 10nm in diameter at their most significant point (Fig. 5).

7. FTIR and XRD analysis

The ATR/FT-IR study of polystyrene nanoplastics showed (Fig. 6a) varied absorptions. Absorptions at 1450, 1550, and 1600cm^{-1} indicated aromatic C=C bonds in both PS nanoplastics treated with *Pseudomonas aeruginosa* O6 and the control group, while multiple absorptions around 1000cm^{-1}. Stretching decreased and an absorption peaked at 1715- 1500cm^{-1} indicate carbonyl (-C=O) groups during PS breakdown. The nanoplastic

treated with *Pseudomonas* sp. also showed a wide absorbance of 3600cm^{-1}, indicating O-H stretching. In addition, polystyrene phenyl rings deformed at the 700- 900cm^{-1} range. Polyethylene nanoplastics formed carbonyl groups (-C=O) with a peak of 1720- 1750cm^{-1} (Fig. 6b). New peaks or changes at 1000- 1300cm^{-1} indicate alcohol and ether groups. The peak at 1370- 1470cm^{-1}, linked with alkyl C-H bending, also decreased. The peaks observed at 2800- 3000cm^{-1} correspond to aliphatic C-H stretching vibrations. PET microplastic analysis indicated a 1720- 1740cm^{-1} peak, indicating carbonyl groups and ester stretching (-C=O) (Fig. 6c). A further signal at 1730- 1750cm^{-1} indicates ester carbonyl groups. Furthermore, the 3300- 3500cm^{-1} reduction indicates ester bond hydrolysis in PET structure. The 680- 900cm^{-1} peaks also showed partial alteration, suggesting a structural shift in the PET polymer's aromatic ring.

The primary objective of the XRD analysis (Fig. 6d, e, f) in this study was to discern morphological alterations in the tested polymer structures. The XRD spectra revealed changes in the crystalline structure of PS and LDPE following treatment with the *Pseudomonas* strain across all three regions. However, the alteration in the crystalline structure of PET was less pronounced than the changes observed in the morphological structure of PS and LDPE.

8. Molecular docking analysis

Molecular docking is a valuable tool to predict the binding nature of different compounds with target proteins. Table (1) provides the binding energy of the best-docked pose of the studied ligands within the active site of the target enzyme. The docking results revealed that polystyrene demonstrates a notable binding energy of -8.2kcal/ mol upon interaction with the Pseudomonas aeruginosa lipase enzyme. This interaction involves the formation of π-Alkyl bonds with Met16, Leu17, and Leu252 residues. Additionally, polystyrene engages in π-Sigma and π-π bonds with Ile142 and Phe214, respectively. Moreover, a π-cation bond is formed with the His251 amino acid, as depicted in Fig. (7). In contrast, PET was observed to conform well to the active site of the target enzyme with an energy value of -5.6kcal/ mol. Strong conventional hydrogen bonds were identified between polyethylene terephthalate and Met16, Ser82, Ser112, Ala115, and His251 at distances of 2.76, 2.08, 2.19, 3.07, and 2.33Å, respectively. These interactions contribute to the stable binding of polyethylene terephthalate with the lipase enzyme. Furthermore, π-Sigma and π-Sulfur bonds are formed with the side chain of Leu231 and Met16 residues, respectively. Additionally, polyethylene, another ligand under consideration, exhibited a docking score of -4.2kcal/ mol and interacted with the lipase enzyme. It occupies the hydrophobic region of the enzyme by establishing hydrophobic interactions with Met16, Pro108, Ala115, Leu118, Leu131, Val135, Leu159, Leu162, and Leu231. These findings shed light on the diverse binding modes and energies associated with polystyrene, polyethylene terephthalate, and polyethylene in their interactions with the *Pseudomonas aeruginosa* lipase enzyme (Fig. 6) Additionally, Fourier transform infrared spectroscopy was conducted on the three

nanoplastics (a) PS, (b) PE, and (c) PET, and X-ray crystallography was performed on the three nanoplastics (d) PS, (e) PE, and (f) PET before and after treatment (PS: Polystyrene, PET: Polyethylene terephthalate, and PE: Polyethylene).

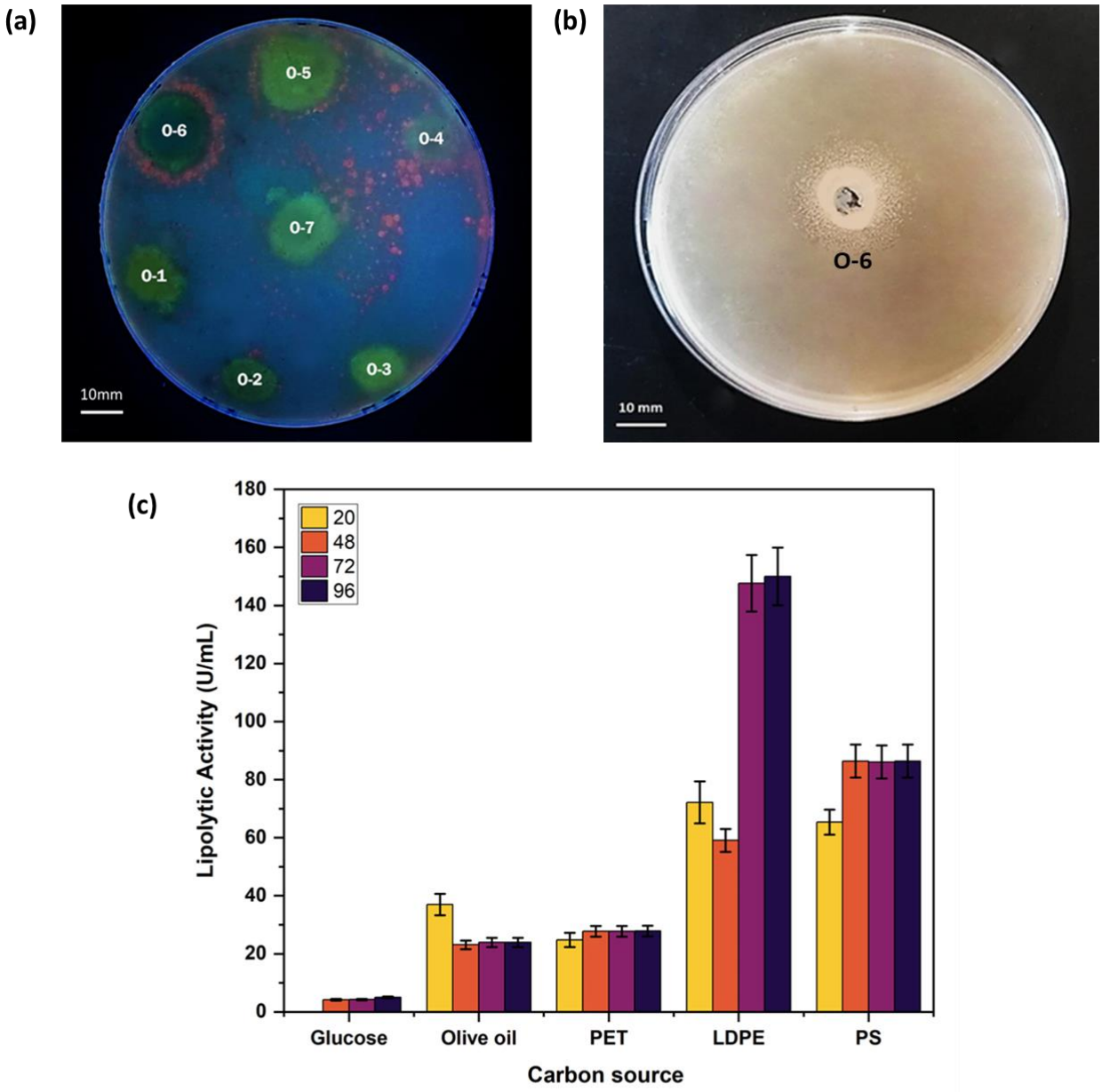


Fig. 3. Lipolytic activity of plastic degrading isolates **(a)** on rhodamine B, and **(b)** Tween 80 precipitation. **(c)** The lipolytic activity of isolate O-6 against three nanoplastics: PET, PE, PS, in addition to olive oil, and glucose as the sole carbon source after 20, 48, 72, 96h

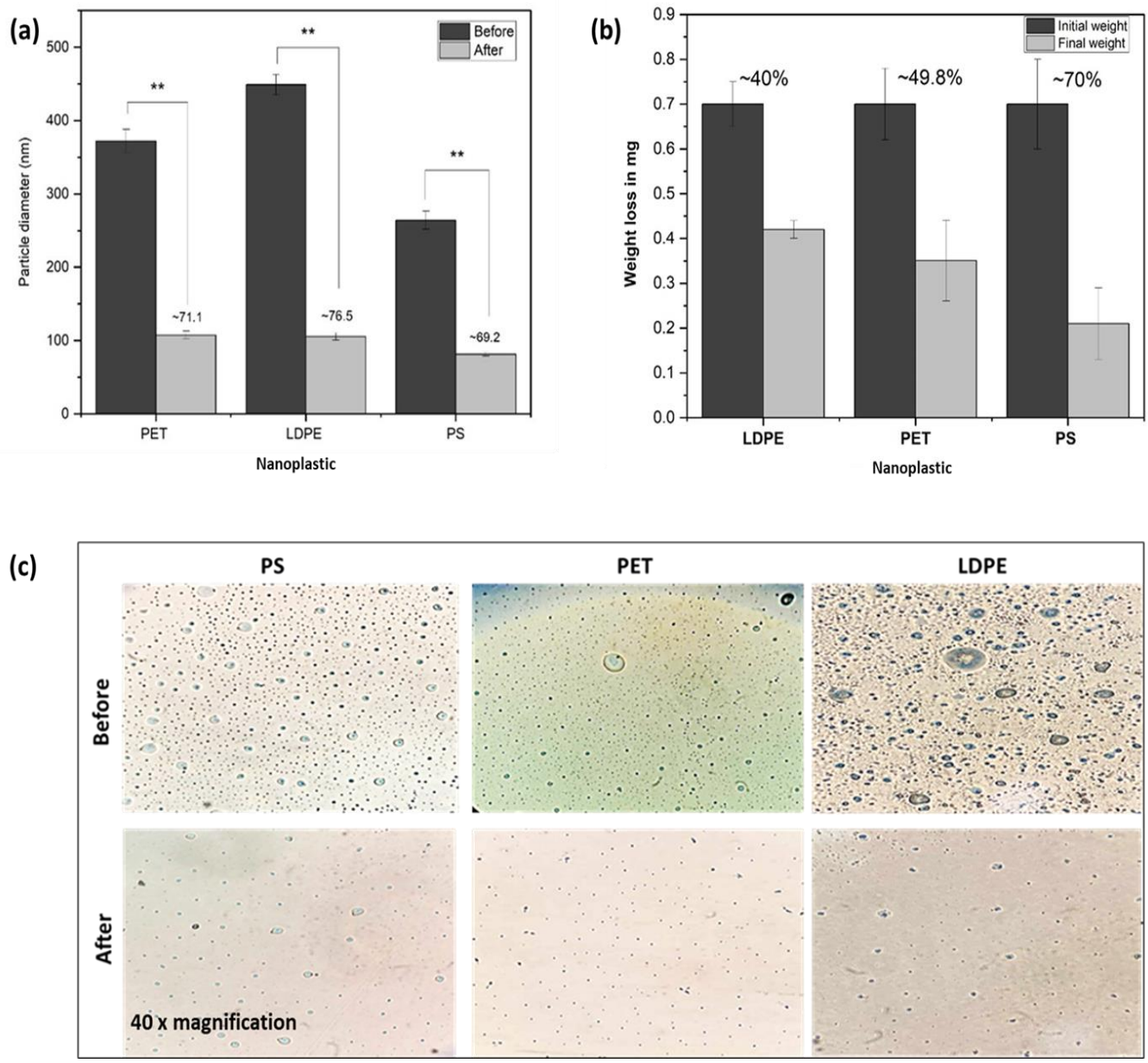


Fig. 4. Variation in the nanoplastic particles before and after treatment with *Pseudomonas aeruginosa* O6; **(a)** Particle size analysis using DLS, **(b)** Weight loss analysis, and **(c)** Particle count analysis using inverted microscope (magnification 40×)

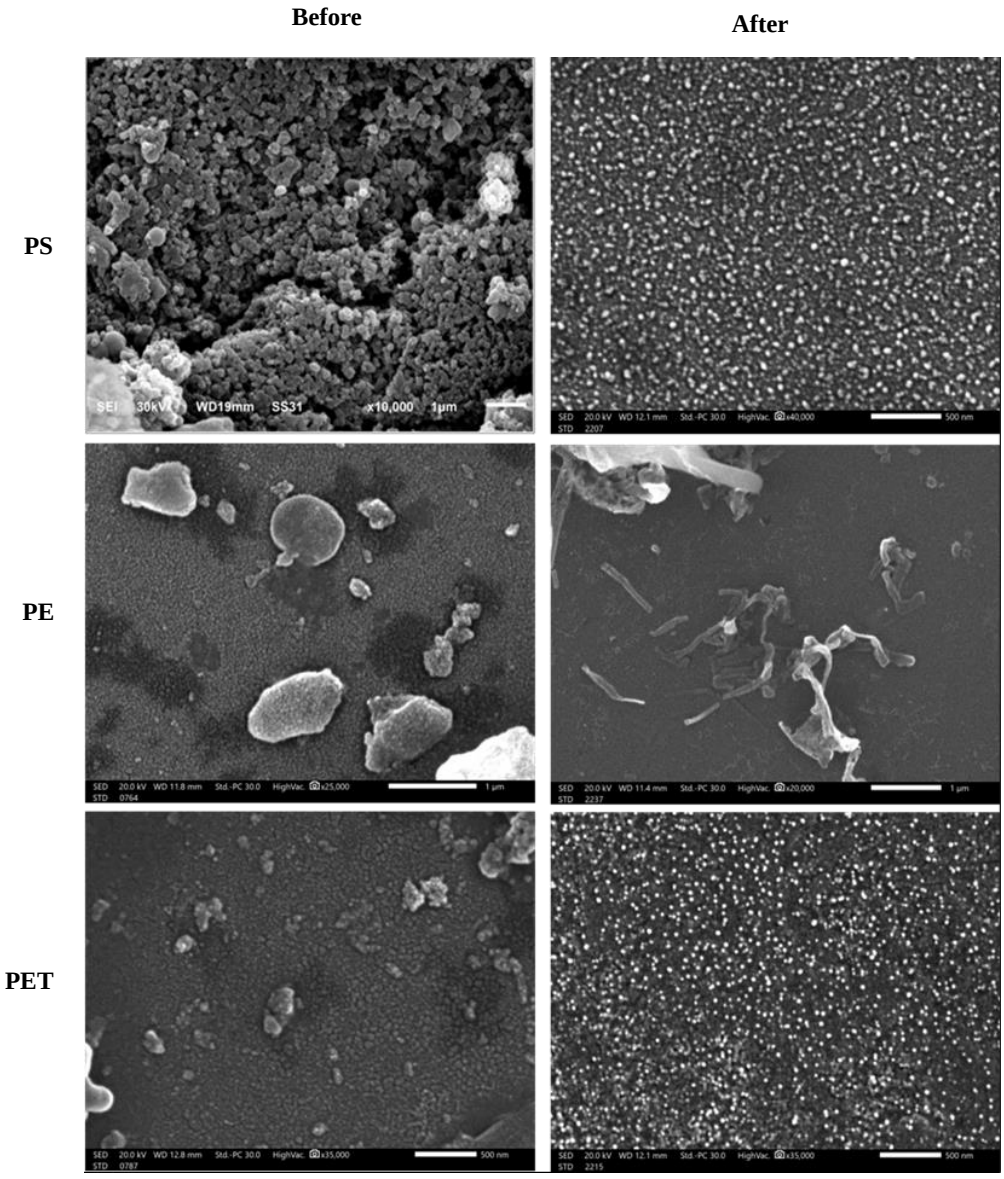


Fig. 5. Scanning electron microscope images of the three nanoplastics before (left) and after (right) treatment with *Pseudomonas aeruginosa* O6 (scale bars 1.0µm and 500nm)

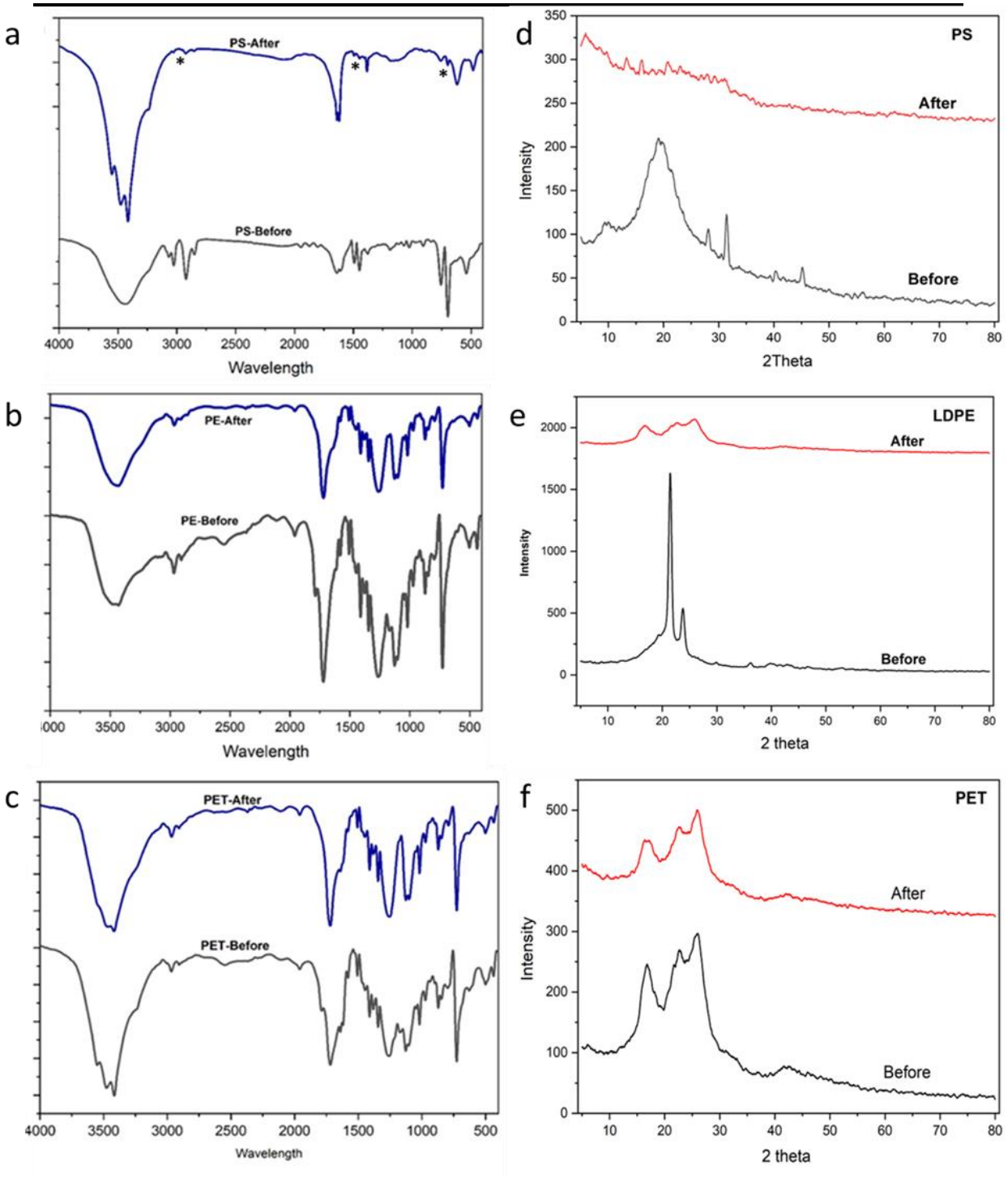
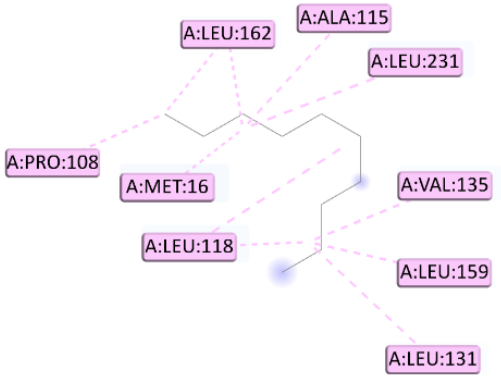
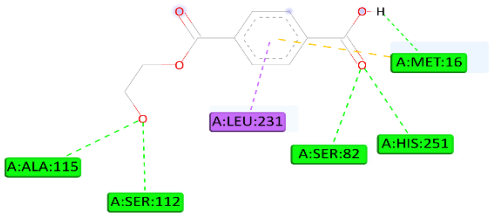
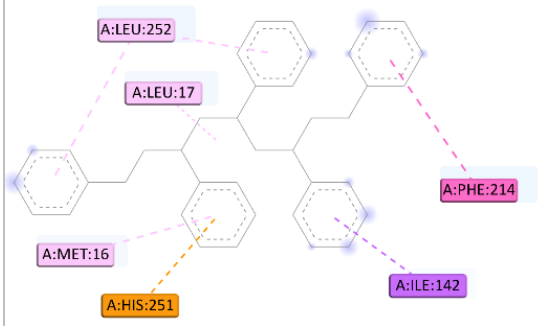


Fig. 6. FTIR and XRD analysis to **(a)** Polysytrene, **(b)** Polyethylene, and **(c)** Polyethylene ephthalate

Table 1. Binding energy and docking features obtained from ligand-protein docking

Compound	Protein	PDB ID	Binding score (kcal\mol)	2D interaction plot
Polyethylene (PE)	*Pseudomonas Aeruginosa* (Lipase)	1EX9	-4.2	
Polyethylene terephthalate (PET)			-5.6	
Polystyrene (PS)			-8.2	

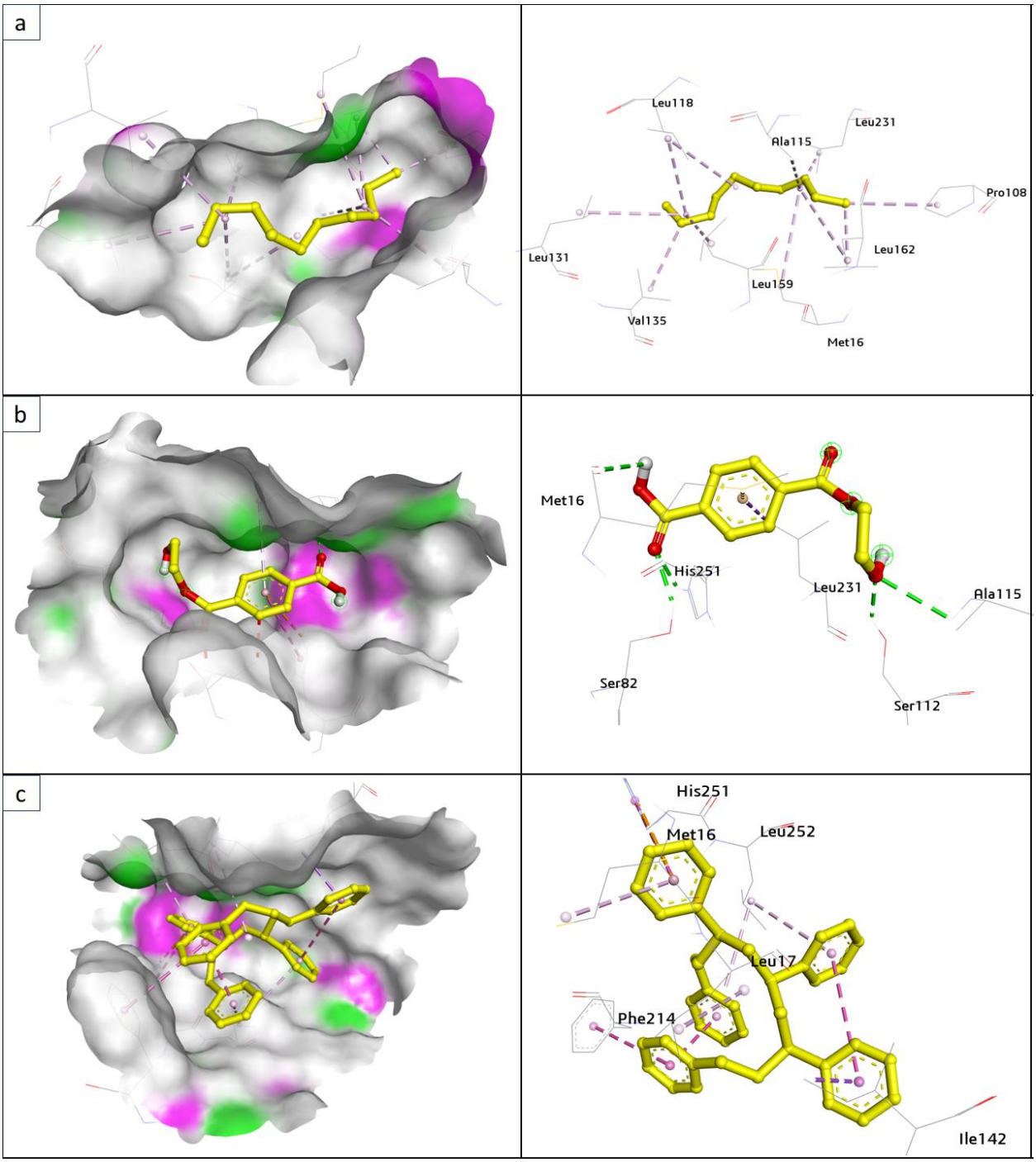


Fig. 7. Molecular docking of **(a)** Polyethylene, **(b)** Polyethylene terephthalate, and **(c)** Polystyrene, within the binding pocket of the lipase enzyme

DISCUSSION

This study aimed to address a significant gap in microplastic research by examining the biodegradation of nanoplastics, as small as 400nm, using microbial communities from polluted areas. Studying their impact on lipolytic activity is crucial for enhancing bioremediation techniques. These efforts will play a crucial role in lessening the environmental effects of nanoplastics and strengthening the advancement of worldwide sustainability. This research is among the first to investigate the intricate connection between microbial enzyme activity and nanoplastic biodegradation.

Seven bacterial strains were extracted from Lake Mariout silt in Egypt on the nanoplastic agar media. Three types of nanoplastics were added to carbon-free basal agar media to cultivate these strains. Following selection, the colonies were screened in carbon-free basal media with 10mg/ L nanoplastics. At dosages over 10mg/ L, nanoplastics showed an antibacterial activity, pigmentation, and fluorescence changes. Previous studies have revealed that nanoparticles greatly change bacterial behavior. There is a growing need for further research investigating nanoplastics' impact on gene regulation, in particular bacterial species, taking into consideration size, concentration, and type. Based on concentration, **Liu *et al.* (2013)** showed that iron nanoparticles may promote or inhibit bacterial growth and biosurfactant synthesis. **Zhang *et al.* (2020)** observed that cationic functional groups on nanoparticle surfaces may affect bacteria survival and membrane interaction.

Five of seven strains grew on liquid media, but only one had the maximum clarity. The strain was then tested for its lipolytic activity. The initial screening utilizing Tween 80 and Rhodamine B tests showed promising lipolytic activity for this strain. Sanger sequencing and 16S rRNA gene analysis identified it as *Pseudomonas aeruginosa with* 100% identity with the NCBI Genbank sequences. Several lipase-producing bacteria may degrade plastic. Lipases from *Lactobacillus* species successfully destroyed synthetic polyester poly (ε-caprolactone) (PCL) in 2017, according to **Khan *et al.* (2017)**. **Lee and Kim (2010)** found that *Pseudomonas aeruginosa* PBSA-2 degrades poly (butylene succinate-co-butylene adipate) using lipase. **Tan *et al.* (2021)** found an extracellular lipase from *Amycolatopsis mediterannei* that could break down plastic, indicating the necessity for lipase enzyme in the bioremediation research.

In this study, we explored the secretion of lipase enzymes by bacterial strains in response to treatments with nanoplastic particles of PE, PET, and PS. The lipolytic activity of the selected strain was quantified using pNPP to test the effect of three nanoplastics on the lipase production.

The pNPP assay revealed differential effects of nanoplastics on lipase production. This part of the study aimed to assess how strain O6, known for their lipase-producing capabilities, could degrade different nanoplastics and influence lipase function. These

insights contribute to a better understanding of the enzymatic degradation of nanoplastics and open potential avenues for plastic waste management.

High lipolytic activity was detected even though PS and LDPE do not contain ester linkages, which are normally necessary to activate lipase activity. The breakdown of these polymers might begin with the formation of intermediate metabolites, which could be caused by lipases. In order to make polystyrene nanoplastics more amenable to further enzymatic or microbial breakdown, lipases may come into contact with their surface and cause physical changes or alterations. Subsequent biodegradation studies using methods, such as FTIR, XRD, and SEM corroborate this theory. The first breakdown of polystyrene and polyethylene may also be greatly influenced by the cooperative activity of lipases with other enzymes, such as oxidative enzymes like laccases and peroxidases. A more effective degradation process for these polymers may be possible as a result of this synergistic relationship. Several studies have shown that nanomaterials have the potential to increase lipase's activity and stability. Both the lipase activity and thermal stability of self-assembled nanogels were significantly improved, according to studies conducted by **Sawada and Akiyoshi (2010)**. Several biomacromolecules may increase the efficacy and longevity of fixed lipase, according to studies conducted by **Huang *et al.* (2009)** on nanofibrous membrane surface modification. A heat-resistant lipase enzyme might be stimulated by nonionic detergents, according to research by **Guncheva *et al.* (2007)**. Immobilizing lipase on nanofibrous membranes improved enzyme stability and activity, according to **Zhu and Sun (2012)**. Nanoplastics, a kind of nanomaterial, may have a similar stimulating effect on bacterial lipase production, according to these findings.

Bacterial lipase degradation of nanoplastics was the focus of this investigation. To evaluate the changes in particle size and shape, SEM examination and dynamic light scattering (DLS) were used. Various nanoplastics showed a notable shrinkage, and the enzymatic breakdown process was thought to work by diverse processes. Multiple studies have shown that environmental stressors, such as UV irradiation and mechanical stress, may lead to the formation of smaller microplastic particles, confirming previous findings that microplastic particles undergo size reduction during biodegradation. **Weinstein *et al.* (2016)** and **Paço *et al.* (2017)** breakdown and environmental remediation of plastic pollutants. The ability of the marine fungus *Zalerion maritimum* to reduce the size and weight of polyethylene microplastics was shown by **Paço *et al.* (2017)**. It seems from the results that biodegradation, particularly when affected by environmental conditions and microbial activity, might lead to smaller microplastic particles.

The ATR/FT-IR investigation of PS nanoplastics showed that saturated C-C vibrations and aromatic C=C bonds change spectral absorptions, indicating aromatic ring biodegradation. New peaks with carbonyl groups and alcohols suggest intermediate PS breakdown. Lipase hydrolytic enzymes may lower microplastic media pH and exhibit

characteristic absorption peaks in the range of 700- 900cm^{-1}. Studies have shown that *Pseudomonas* species like *Pseudomonas aeruginosa* and *Pseudomonas citronellolis* can degrade plastics like PS and PVC by forming carbonyl groups and reducing average molecular weight. Fourier-transform-infrared spectroscopy (FT-IR) has confirmed biodegradation-induced plastic structural changes **(Kim *et al.*, 2020)**. *Pseudomonas* and *Bacillus* species may also biodegrade PET plastic **(Roberts *et al.*, 2020)**. Low-density polyethylene (LDPE) microplastics showed carbonyl group formation around 1720-1750cm^{-1}, indicating oxidation or oxygen-containing functional groups during biodegradation. FTIR research showed that *Pseudomonas* species like *P. putida* S3A may break down polyethylene **(Al-Jailawi *et al.*, 2015)**. *Pseudomonas* bacteria from sewage sludge degrade natural and manufactured polyethylene well, according to polyethylene a biodegradation research **(Nanda & Sahu, 2010)**.

Muhonja *et al.* (2018) found novel hydrocarbon degradation functional groups in biodegradation using FTIR. The research emphasizes *Pseudomonas* species' polyethylene-breaking potential, which has major environmental and waste management consequences. PET nanoplastics had comparable carbonyl group morphologies, suggesting lipases degrade PET polymer, but less effectively than LDPE and PS, underlining polymer biodegradation enzymatic complexity. *P. putida* S3A, *P. aeruginosa*, and a mixture of these and *Bacillus* species have been shown to degrade PET. Fourier transform infrared (FTIR) can assess biodegradation. This approach has been used to confirm *P. putida* S3A's degradation of polyethylene **(Al-Jailawi *et al.*, 2015)** and assess *P. aeruginosa's* PET biodegradation **(Rajandas *et al.*, 2012)**.

According to research by **Vague *et al.* (2019)**, polymers including PS, PET, and PE may undergo significant structural changes when exposed to *Pseudomonas* bacteria. Concerns about the biodegradability of ester-bonded polymers are raised by the noticeable morphological changes in PS and LDPE **(Vague *et al.*, 2019)**. Crystalline polyester, according to research by **Focarete *et al.* (1998)**, improves the enzymatic hydrolysis by *Pseudomonas* depolymerase A in a-PHB blends. Weight loss, morphological deformations, and spectroscopic changes have been observed when *Pseudomonas* bacteria break down LDPE films **(Kyaw *et al.*, 2012)**.

CONCLUSION

This investigation highlighted the significant problem of micro-nanoplastic pollution in Egypt's coastal areas, indicating a broader global concern about the health of oceanic ecosystems. This study isolated two lipolytic bacteria strains that can efficiently degrade nanoplastic pollutants through sustainable bioremediation strategies. The strains, identified as *Pseudomonas aeruginosa* using 16S rRNA gene sequencing and listed with NCBI accession number PP087224, showed promise in combating nanoplastic pollution. The

study demonstrated a variation in lipase enzyme activity among bacteria, with strain O6 exhibiting high activity levels against specific polymers. This variation highlights the specificity of bacterial responses to various nanoplastic types. The current study provided a strong evidence of the strains' significant impact on reducing nanoplastic particle sizes by up to 97% and altering chemical structure and crystallinity, as shown through FTIR spectroscopy and XRD analysis. The study emphasizes the efficacy of using *Pseudomonas aeruginosa* for nanoplastic degradation and showcases the broader application of microbial bioremediation in purifying marine environments from nanoplastic pollutants. This study outlined the potential to transform nanoplastic waste into valuable enzymes, offering environmental remediation and waste management.

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