Multistep Enzymatic Low-density Polyethylene Degradation via Phenylalanine Monooxygenase and Isocitrate Lyase in *Pseudomonas aeruginosa*

*Hong Rae Kim[1§], Ye Eun Lee[2§], Dong-Eun Suh[1], Donggeun Choi[1], Sukkyoo Lee[2]**

[1]Department of Research and Development, Repla Inc., Suwon 16679, Republic of Korea

[2]Department of Brain Sciences, Graduate School, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 42988, Republic of Korea

Corresponding author: E-mail: slee2012@dgist.ac.kr

Mailing address: Department of Brain Sciences, Daegu Gyeonbuk Institute of Science and Technology, Daegu 42988, Republic of Korea, Phone: (+82) 53-785-6611. Fax: (+82) 53-785-6109. E-mail: slee2012@dgist.ac.kr

§ Hong Rae Kim and Ye Eun Lee contributed equally to this paper.

Keywords: Polyethylene, Biodegradation, Enzymes, Oxidation, Depolymerization

Abstract

Plastics have become indispensable in modern industries; however, their resistance to natural degradation poses environmental challenges. Biological degradation technologies employing microorganisms offer promising solutions. Here, we analyzed the transcriptome and proteome of *Pseudomonas aeruginosa*, a plastic-degrading microorganism found in the gut of superworms, to identify the genes and enzymes upregulated during polyethylene degradation. Functional analyses of these upregulated genes and enzymes using the Kyoto Encyclopedia of Genes and Genomes and Gene Ontology databases revealed an increase in lipid and hydrophobic amino acid metabolism, suggesting their involvement in polyethylene degradation. Based on these analyses, we identified phenylalanine monooxygenase, which is capable of oxidizing plastics, and isocitrate lyase, which is involved in C-C bond cleavage. To investigate the involvement of these enzymes in polyethylene degradation, *phhA* and *aceA* were transformed into *Escherichia coli*, and the enzymes were produced and purified. The purified enzymes were then reacted with polyethylene and analyzed. The results revealed the formation of hydroxyl (-OH) and C-O groups on the polyethylene surface after treatment with phenylalanine monooxygenase, confirming its ability to oxidize polyethylene. Isocitrate lyase alone did not affect polyethylene production; however, when combined with phenylalanine monooxygenase, it contributed to a reduction in molecular weight. This suggests a two-stage process of polyethylene degradation involving oxidation and depolymerization that requires sequential action of multiple enzymes. Thus, we identified the enzymes involved in each stage and demonstrated the degradation ability of polyethylene using purified enzymes.

Synopsis: Polyethylene is the most commonly used plastic; however, its degradation mechanism remains unclear. This study revealed a two-step process involving oxidation and depolymerization during polyethylene degradation. Furthermore, enzymes involved in each stage were identified.

1. Introduction

Plastics are widely used in various industries because of their light weight, durability, and affordability. Despite the annual production of approximately 380 million tons of plastic, the recycling rate is approximately 9% [1]. Consequently, plastics introduced into the soil and oceans undergo degradation, leading to microplastic pollution [2]. Polyethylene accounts for 36% of the overall plastic composition and contributes significantly to plastic pollution [3].

Biological degradation of plastics has attracted growing interest. This process is performed at room temperature and pressure, making it environmentally friendly. Recent studies have highlighted the ability of microorganisms to degrade plastic [4, 5]. However, the efficiency of microbial degradation in addressing plastic pollution remains relatively low.

One of the challenges in the biological degradation of plastics lies in their exceptionally high molecular weight, which hinders their transport into cells. Generally, substances with molecular weights below 500 Da can be transported into cells, whereas commercially used polyethylene has a molecular weight of approximately 200,000 Da. Consequently, for microorganisms to break down polyethylene, they need to secrete extracellular polyethylene-degrading enzymes and transport the resulting degradation intermediates into the cell to serve as an energy source [6].

The degradation of polyethylene is challenging because of its exclusive composition of carbon and hydrogen, necessitating the involvement of multiple enzymes for effective degradation [7]. Initially, oxidoreductases act on polyethylene, initiating the formation of oxygen-containing functional groups. Subsequently, depolymerizing enzymes come into play in the second step, resulting in a reduction in molecular weight [8]. Microbial oxidases, such as alkane monooxygenase and cytochrome P450, are involved in initial oxidation [9, 10, 11]. Esterases, such as lipases, are presumed to be involved in the depolymerization process. Unlike polyester

plastics such as polyethylene terephthalate, polyethylene consists of C-C bonds, posing a challenge for depolymerization by esterases [8, 9]. Lyases, which can break bonds through mechanisms other than hydrolysis, can also cleave C-C bonds. Therefore, lyases have the potential to serve as depolymerization enzymes for polyethylene cleavage.

To enhance the effectiveness of plastic degradation, a comprehensive understanding of the mechanisms underlying plastic degradation and identification of pertinent enzymes are essential. Enhancing plastic degradation efficiency involves the discovery and optimization of enzymes involved in the process [12]. Recent studies have employed proteomic analysis to unravel microbial polyethylene metabolic pathways and their correlations with lipid metabolism [13, 14]. However, exploration of plastic-degrading enzymes using multiomics techniques, along with subsequent molecular biology approaches for expression and functional analyses, remains relatively limited.

In this study, we investigated *Pseudomonas aeruginosa*, which is known for its capacity to degrade polyethylene, with three main objectives: 1) to conduct a comprehensive analysis of the transcriptomics and proteomics of *P. aeruginosa* to identify candidate genes involved in plastic degradation.; 2) to produce and purify proteins from the selected genes using gene cloning; and 3) to assess the plastic degradation ability of the enzyme at the molecular level. Through these steps, we aimed to uncover the enzymes associated with polyethylene degradation and elucidate the mechanism of polyethylene degradation.

2. Materials and Methods

2.1 Polyethylene Degradation by *P. aeruginosa*

2.1.1. Cultivation of *P. aeruginosa* using polyethylene as the carbon source

P. aeruginosa was cultivated using low-density polyethylene (LDPE) as the carbon source. *P. aeruginosa* was inoculated into LB medium and cultured for 24 h in a shaking incubator (28 °C, 130 rpm). After centrifuging 1 mL of the culture (13000 rpm, 5 min), a cell pellet was obtained. The pellet was resuspended in 1 mL of liquid carbon-free basal medium (LCFBM) and subjected to additional centrifugation to eliminate residual nutrients. This washing procedure was repeated thrice. Subsequently, cells were cultured in 25 mL of LCFBM containing 0.4 g of LDPE (weight-average molecular weight [Mw] ~4,000 and number-average molecular weight [Mn] ~1,700, Sigma-Aldrich, MO) as the exclusive carbon source. Inoculation was performed at a 1/100 dilution based on an OD of 1.0. After a 7-day incubation period, the polyethylene degradation capability of *P. aeruginosa* was assessed.

2.1.2. Colony-forming units (CFU) counting

To verify microbial proliferation using LDPE as the carbon source, CFU were quantified. One milliliter of culture sample was combined with 9 mL of LCFBM. Following six consecutive dilutions of this solution, 100 µL was spread on LB agar plates and incubated for 24 h. CFU/mL was calculated by counting the number of colonies in the diluted solutions.

2.1.3. Weight-loss measurement

The dry weights of the plastics were measured to evaluate LDPE biodegradation. After cultivation, the plastics were collected using a cell strainer (pore size: 40 µm, SPL, Korea) and subjected to an overnight treatment with 2% SDS to eliminate microorganisms adhered to the plastic surface. Subsequently, the plastics were thoroughly washed twice with deionized (DI)

111 water and oven-dried at 60 °C for 24 h.

112 **2.1.4. SEM imaging**

113 To conduct SEM imaging and FT-IR, XPS, and contact angle analyses, plastic films are
114 required. The LDPE films (density: 0.93 g/cm^3) were cut into 2×2 cm pieces. Subsequently,
115 the films were subjected to the cultivation method outlined in Section 2.1.1. The surface
116 characteristics of the LDPE films were examined using field-emission SEM (Merlin Compact,
117 Zeiss, Germany). The films were securely affixed with carbon tape and coated with platinum,
118 and SEM images were captured. Biofilm formation and corrosion of the film surface were
119 evaluated using SEM to assess the biodegradation capabilities of the candidate plastic
120 degraders.

121 **2.1.5. ATR FT-IR spectroscopy**

122 The LDPE film was subjected to a 24-hour treatment with 2% SDS and washed with DI
123 water to eliminate the biofilm. To assess the changes in the chemical structures of the LDPE
124 films, an ATR FT-IR spectrometer (iS50, Thermo Fisher Scientific, MA, USA) was used to
125 examine alterations in the functional groups. The FT-IR analysis employed single-bounce
126 attenuated total reflection spectroscopy, covering wavenumbers from 400 to 4,000 cm^{-1}.

127 **2.1.6. X-ray photoelectron spectroscopy**

128 X-ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Electron, MA,) was used to
129 analyze the elements and binding energies on the surface of the LDPE film. The samples were
130 secured with carbon tape and subjected to measurements in the energy range of 276–300 eV
131 for the C1s scan and 0–1,350 eV for the survey scan.

132 **2.1.7. Contact angle analysis**

133 Contact angle analysis was conducted to assess changes in surface hydrophilicity resulting

from LDPE degradation. The film samples were prepared by fixing both edges of the LDPE films to a glass slide with cellophane tape. Approximately 15 µL of water was then applied to the surface using a contact angle analyzer (Phoenix 300, SEO, Suwon, Korea). Measurements were taken at three different locations and the average values were calculated

2.2. Transcriptomic Analysis of *P. aeruginosa* during Polyethylene Degradation

2.2.1 mRNA sequencing

To unravel the alterations in the transcriptome during polyethylene biodegradation, *P. aeruginosa* was cultured in minimal media with LDPE as the carbon source for 7 days, and subsequently, RNA sequencing was performed. The control group consisted of *P. aeruginosa* cultured in LB for the same duration of 7 days. Total RNA concentration was calculated using Quant-IT RiboGreen (Invitrogen, CA) and the integrity of the total RNA was assessed. Only high-quality RNA (RIN>7.0) was used for library construction. Libraries were prepared with 1 µg of total RNA using the Illumina TruSeq Stranded mRNA Sample Prep Kit (Illumina Inc., CA). Bacterial rRNA-depleted samples were generated using the NEBNext rRNA Depletion Kit (NEB, MA,) and a cDNA library was constructed. The cDNA library was quantified using KAPA Library Quantification kits (KAPA BIOSYSTEMS, MA) and qualified using TapeStation D1000 ScreenTape (Agilent Technologies, CA). Indexed libraries were subjected to paired-end (2×100 bp) sequencing using an Illumina NovaSeq system (Illumina Inc.) by Macrogen Inc. (Seoul, Korea).

2.2.2 mRNA-Seq data analysis

Raw reads were processed to remove low-quality and adapter sequences using Trimmomatic 0.38. The reads were aligned to *P. aeruginosa* PAO1 using Bowtie 1.1.2 [15]. The reference genome sequence of *P. aeruginosa* PAO1 (GCF_000006765.1) and annotation data were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/assembly/GCF_000006765.1/).

Aligned reads were assembled and the relative abundance of genes was estimated using HTSeq v0.10.0 [16]. Statistical analysis was performed to identify differentially expressed genes (DEGs) using the estimated abundance of each gene in the samples. Genes with one more than zeroed Read Count values in the samples were excluded. The filtered data were log2-transformed and subjected to TMM normalization. Statistical significance of the differential expression data was determined using exactTest (EdgeR) and fold change (p-value<0.05, $|\log2FC| \geq 1$). Functional annotation of DEGs was performed using DAVID (https://david.ncifcrf.gov/home.jsp) against public databases such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO). PCA was used to visualize the similarities among the samples.

2.3. Proteomic Analysis of *P. aeruginosa* during Polyethylene Degradation

2.3.1. Sample preparation for proteomics

To examine alterations in the proteome during polyethylene biodegradation, *P. aeruginosa* was cultured in minimal media with LDPE as the carbon source for 7 days, followed by LC-MS analysis. As a control group, *P. aeruginosa* cultured for the same duration of 7 days in LCFBM supplemented with 2% glucose was used to mitigate potential interference from extraneous proteins in the proteome data caused by LB medium.

Bacterial samples were suspended in lysis buffer (8 M urea, 0.1 M Tris-HCl buffer, pH 8.5) containing a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland) and sonicated. The protein concentration was quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). The filter-aided sample preparation method on a Microcon 30 K centrifugal filter device (Millipore, MA,) was used for protein digestion. Reduction with Tris(2-carboxyethyl)phosphine and alkylation with iodoacetic acid were followed by trypsin digestion. The resulting peptides were desalted and eluted on C18 spin columns.

2.3.2. LC-MS/MS proteomic analysis

The samples were analyzed using an UltiMate 3000 RSLC nano LC system (Thermo Fisher Scientific) coupled with a Q Exactive Plus mass spectrometer (Thermo Scientific). The peptides were loaded onto an Acclaim PepMap 100 Trap Column (Thermo Fisher Scientific) and subsequent peptide separation was performed using an Acclaim PepMap RSLC analytical column (Thermo Fisher Scientific). Mass spectra were acquired in the data-dependent mode with an automatic switch between full scan (m/z 400–2,000) and 10 data-dependent MS/MS scans.

MS/MS raw files of each analysis were searched using Proteome Discoverer™ software (ver. 2.5) against *P. aeruginosa* database downloaded from UniProt. MS/MS data were analyzed using peptide-spectrum match validation step and SEQUEST HT process. After the searching process, data with FDR below 0.01 and at least six peptides in length were selected. Functional annotation of differentially expressed proteins was performed using DAVID (https://david.ncifcrf.gov/home.jsp) against public databases such as the KEGG and GO. PCA was used to visualize the similarities among the samples.

2.4. Expression and Purification of Cloned Genes, *phhA* and *aceA*

2.4.1. Cloning of phenylalanine monooxygenase and isocitrate lyase genes

PCR was conducted using primers targeting *phhA*, which encodes phenylalanine monooxygenase (also known as phenylalanine hydroxylase [PAH]), and *aceA*, which encodes isocitrate lyase (ICL), in the genomic DNA of *P. aeruginosa* (Table S1). The gDNA served as the template for the first-round amplification, which included approximately ±100bp regions of the genes using phhA_F1, R1 and aceA_F1, R1 primers. Subsequently, the PCR product from the first round was used as a template for the second round of PCR, employing phhA_F2, R2 and aceA_F2, R2 primers to amplify the gene sequences. The PCR conditions included initial denaturation at 95 °C for 10 min, denaturation at 95 °C for 45 s, annealing at 47 °C for

30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplified *phhA* and *aceA* were purified and ligated into a pGEM-T-easy vector (Promega, WI). The vector containing each gene was digested with *Not*I and *EcoR*I, respectively, and ligated into the PET28b(+) vector (Sigma-Aldrich). The resulting plasmids were then transformed into *E. coli* DH5a cells and sequenced. The pET28b(+) vector containing each gene was extracted and transformed into *E. coli* BL21(DE3) cells for protein purification.

2.4.2. Expression and purification of phenylalanine monooxygenase and isocitrate lyase

Recombinant *E. coli* BL21(DE3) cells were cultured on LB medium containing kanamycin at 37 °C until reaching an OD600 of 0.6–0.8. Upon reaching the desired optical density, IPTG was added at a final concentration of 1 mM and the culture was further incubated for 6 h. The IPTG-induced bacterial culture was centrifuged (3000 rpm, 10 min) and the cell pellet was resuspended in 40 mL binding buffer. Sonication was performed for 25 min using an ultrasonic homogenizer and the cell lysates were centrifuged (9000 rpm, 30 min). Purification of phenylalanine monooxygenase was conducted according to the Ni Sepharose FF Agarose (Cat.LT22871, LifeTein, NJ) protocol (Table S2). The flow rate was controlled at 1 mL/min and the column was filled with 5 mL His Ni Superflow resin (Code.Z5660N, TaKaRa, Tokyo, Japan). A filter was inserted on top of the resin, and 15 mL of ddH2O was added to wash the column. Subsequently, 15 mL binding buffer, cell lysate, another 15 mL mL binding buffer, followed by 40 mM washing buffer, and 60 mM washing buffer were added sequentially. Finally, 10 mL of elution buffer was added, and 1 mL fractions were collected for SDS-PAGE analysis. ICL was purified using fast protein liquid chromatography. HisTrap HP columns (1 mL, GE Healthcare, IL) were used for purification, employing binding buffer (20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4) for elution, followed by elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4).

Proteins were analyzed following the protocol provided by the OneGel PAGE kit Plus (Biosolution, Seoul, Korea). A mixture of 40 μL of protein solution and 10 μL of 5× reductase buffer (containing SDS and DTT) was heated to 100 °C for denaturation. Next, 50 μL of the protein sample and 10 μL of Precision Plus Protein Dual Color standards marker (10–250 kDa, Bio-Rad, CA) were loaded onto the gel, which consisted of 12% acrylamide stacking and separating gels. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250. Subsequently, the gels were fixed in a solution of 50% methanol and 10% glacial acetic acid, stained, destained using a solution of 40% methanol and 10% glacial acetic acid, and stored in 5% glacial acetic acid solution. Contaminants, including imidazole, were removed via dialysis against 1× PBS (pH 7) by changing the buffer three times using high-retention seamless cellulose tubing (Sigma-Aldrich). The concentrations of purified PAH and ICL were determined using the Bradford assay.

2.5. Functional Analysis of Phenylalanine Monooxygenase and Isocitrate Lyase in Polyethylene Degradation

To analyze the biodegradation of the enzymes on the LDPE film, based on the experimental results from the thermal shift assay obtained under optimal expression conditions, the LDPE film (ET31-FM-000151, Goodfellow, UK, Mw~240,000 Da) were treated with enzymes at a concentration of 0.5 g/L, using 50 mM sodium phosphate buffer (pH 6) at 30 °C for 30 days.

2.5.1. Chemical analysis of LDPE degradation by enzymes

The LDPE film was treated with phenylalanine monooxygenase, ICL, and a mixture of the two enzymes in a glass vial containing 50 mM sodium phosphate buffer for 30 days. The LDPE film was subjected to a 4-hour treatment with 2% SDS and washed with DI water to remove the residual enzyme attached to the film.

The LDPE film was subjected to FTIR analysis to assess the chemical modifications

induced by phenylalanine monooxygenase. The potassium bromide pellet method was employed as follows: The LDPE film was dissolved in a solvent mixture containing 1,2,4-Trichlorobenzene and 0.015% butylated hydroxytoluene, and the solution was maintained in a glass vial at 160 °C for 10 min to achieve a final concentration of 2 mg/mL. Subsequently, the solvent was evaporated under nitrogen gas for 6 h. The LDPE film pellets were compressed at room temperature under a pressure of 6 MPa for 1 min using a 13 mm diameter pellet die and digital hydraulic presser (CrushIR, Pike Technologies, WI, USA). Pellets were created by blending 1–2 mg of each sample with 100 mg of potassium bromide. The analyses were performed using an FT-IR spectrometer (Thermo Fisher Scientific) in the transmission mode. Scans were conducted over the range of 4000 to 500 cm^{-1}.

The changes in the elemental composition of the plastic surface were examined by measuring the binding energy using X-ray photoelectron spectroscopy (Thermo Scientific). The samples were secured with carbon tape and subjected to measurements in the energy range 276–300 eV for the C1s scan.

Thermogravimetric (TGA) analysis was performed to assess the thermal stability of degraded LDPE. Approximately 10 mg of the sample was heated from 50 to 600 °C at a rate of 10 °C/min. The analysis was conducted under nitrogen at a flow rate of 60 mL/min using a TGA analyzer (TA Instruments, DE).

2.5.2. Analysis of LDPE depolymerization by enzymes

Mw and Mn were determined using high-temperature gel permeation chromatography. The LDPE film was dissolved in a mixed solvent of TCB and 0.04% BHT to 1.5 mg/mL and the mixture was filtered through a 0.26 μm stainless steel mesh. The mixtures (300 μL) were injected into a GPC operating at an eluent flow rate of 1.0 mL/min and a temperature of 160 °C (EcoSEC HLC-8421 GPC/HT, Tosoh Bioscience, Tokyo, Japan).

3. Results and Discussion

3.1. Polyethylene Degrading Bacteria, *P. aeruginosa*

P. aeruginosa is widely recognized for its remarkable ability to degrade plastic [17]. *P. aeruginosa* proliferated using LDPE as the sole carbon source in minimal medium. The initially inoculated *P. aeruginosa* at a concentration of 4.93×10^5 CFU/mL underwent a 100-fold increase, reaching 5.27×10^7 CFU/mL by Day 4. On Day 7, the cell count reached saturation (Table 1). Subsequently, transcriptome and proteome analyses were conducted after cultivating *P. aeruginosa* for one week. As *P. aeruginosa* proliferated, the culture medium became turbid, and 2.14% of LDPE was degraded over the course of 7 days (Figure 1a, 1b). SEM imaging was conducted to inspect the LDPE film surface, revealing a smooth surface in the control sample, whereas the *P. aeruginosa*-cultivated sample exhibited surface corrosion (Figure 1b).

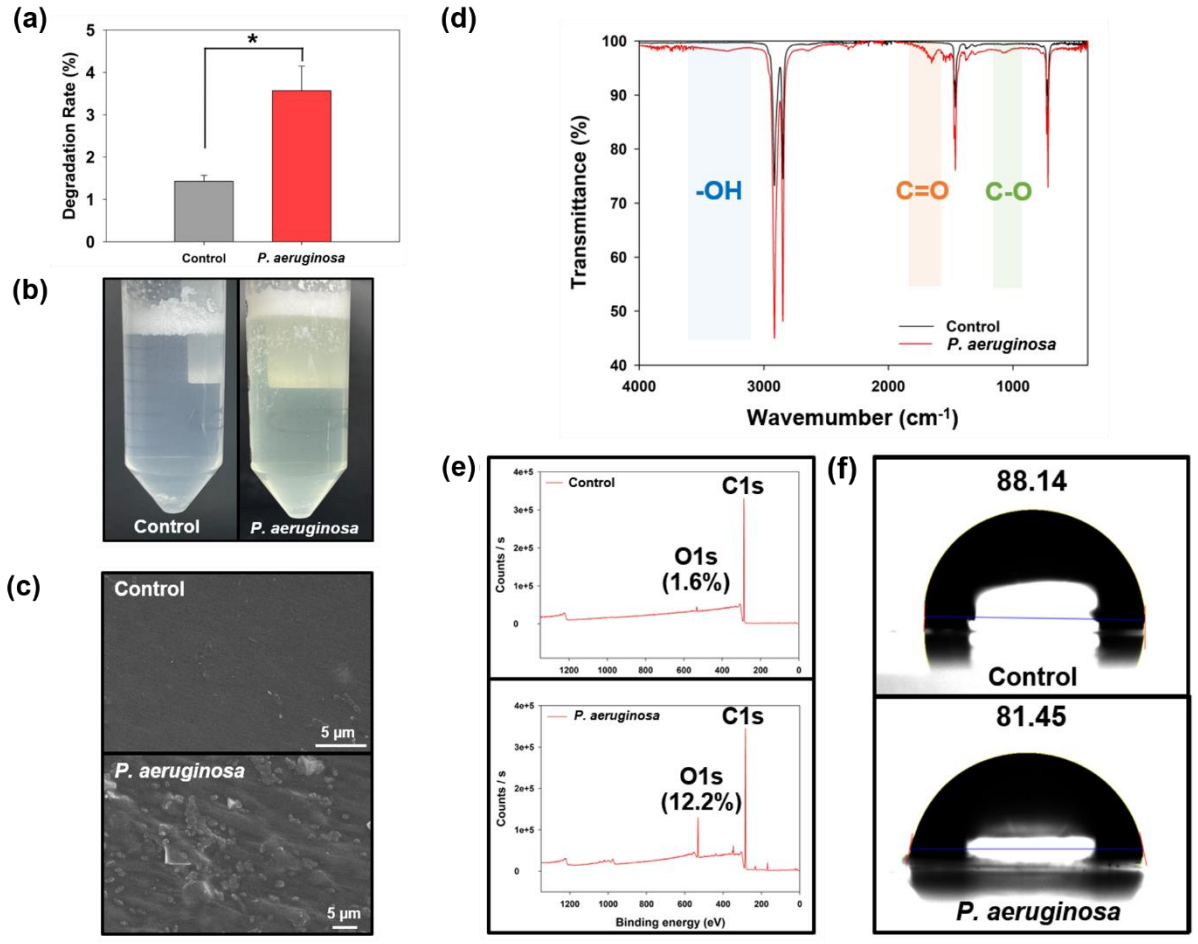

Figure 1. Polyethylene-degrading bacteria, *P. aeruginosa.* (a) Polyethylene degradation rate over 7 days by *P. aeruginosa*. (b) *P. aeruginosa* proliferation using polyethylene as the carbon source. (c) SEM image showing surface erosion of polyethylene by *P. aeruginosa*. (d) Analysis of chemical groups on polyethylene surfaces using FT-IR. (e) Elemental analysis of polyethylene surface using XPS. (f) Contact angle measurement on polyethylene surface.

Table 1. Colony-forming unit of *P. aeruginosa* cultured with low-density polyethylene as the carbon source

Time (days)	**D0**	**D1**	**D2**	**D3**
CFU/mL	4.93×10^5	3.77×10^6	1.83×10^7	3.65×10^7
Time (days)	**D4**	**D5**	**D6**	**D7**
CFU/mL	5.27×10^7	5.03×10^7	7.17×10^7	6.67×10^7

The degradation of LDPE by *P. aeruginosa* was chemically validated through FT-IR measurements. Culturing *P. aeruginosa* resulted in the detection of peaks corresponding to -OH (3200–3600 cm^{-1}), C=O (1700 cm^{-1}), and C-O (1050 cm^{-1}) in the FT-IR spectra (Figure 1d) [18]. This finding was further corroborated by XPS analysis, which indicated an increase in oxygen content after *P. aeruginosa* cultivation (Figure 1e). Contact angle analysis revealed a decrease in the contact angle (81.45°) compared with that of the control (88.14°), signifying an increase in hydrophilicity. During plastic biodegradation, plastic is oxidized, enhancing microbial biofilm formation and enzyme reactions through increased plastic hydrophilicity (Figure 1f) [4, 19]. Collectively, these analyses confirmed the utilization and proliferation of *P. aeruginosa* using LDPE as the carbon source.

3.2. Transcriptomic Analysis of *P. aeruginosa*

Transcriptome analysis of *P. aeruginosa* cultured with LDPE as the sole carbon source revealed the upregulation of 1170 genes and downregulation of 1023 genes (Table S3). Principal component analysis distinctly clustered samples from the control and PE groups, indicating transcriptional changes associated with polyethylene metabolism (Figure 2a). To

elucidate the functions of these genes, upregulated and downregulated genes were categorized by related pathway using the KEGG database and sorted based on the number of hits (Figure 2b, 2c). ABC transporters may be involved in the transport of intermediate molecules generated during polyethylene degradation [20]. Additionally, enzymes responsible for degrading hydrophobic ring structures such as caprolactam and xylene contribute to polyethylene oxidation [21].

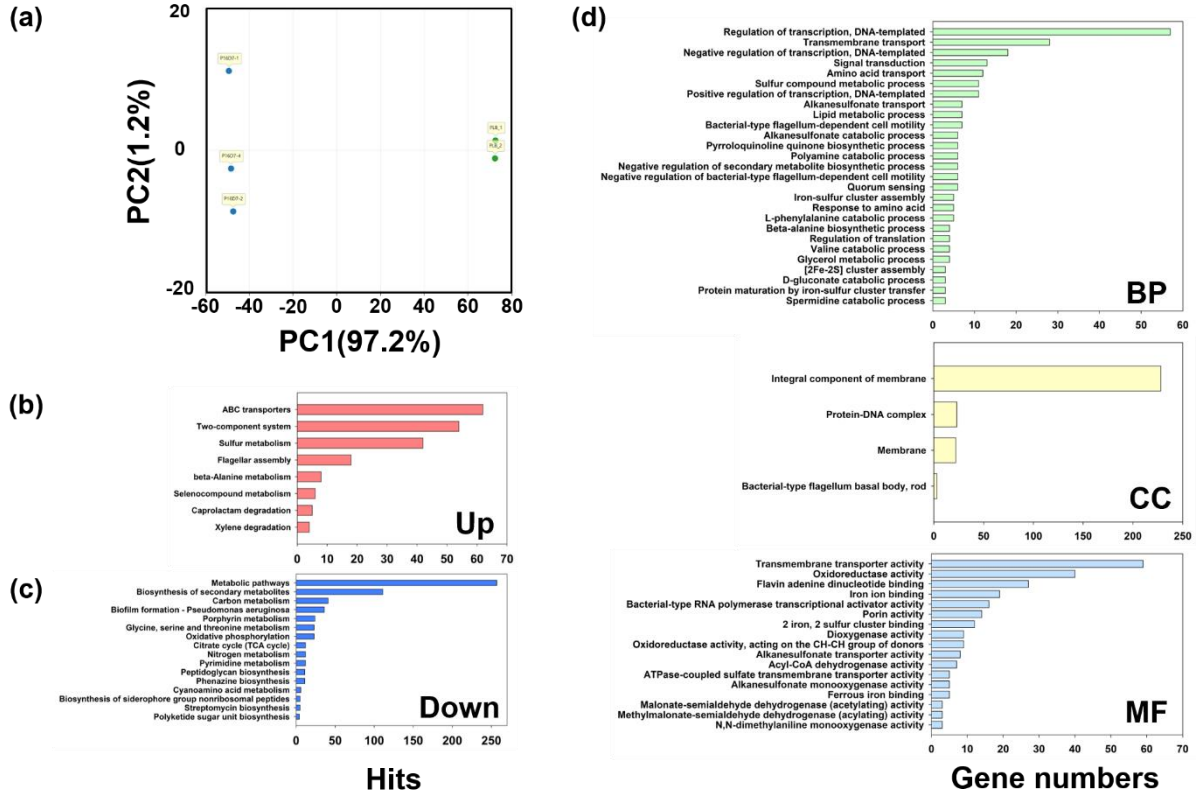

Figure 2. Transcriptome analysis of *P. aeruginosa* cultured with polyethylene as the carbon source. (a) Changes in gene expression patterns of *P. aeruginosa* observed through PCA plot. (b) Pathways associated with upregulated genes analyzed using the KEGG database. (c) Pathways associated with downregulated genes analyzed using the KEGG database. (d) Biological processes, cellular components, and molecular functions associated with upregulated genes analyzed using the GO database.

Subsequently, genes were categorized based on their associated biological processes using the GO database (Figure 2d and S1). Transmembrane transport may be involved in the transport of polyethylene metabolic intermediates. The lipid metabolic process was found to participate in the intracellular metabolism of depolymerized polyethylene intermediates [22]. Owing to the structural similarity between polyethylene and lipids, polyethylene enters the TCA cycle through lipid metabolic pathways within cells [13]. Additionally, an amino acid catabolic process was identified, and it is noteworthy that the relevant amino acids, L-phenylalanine and valine, contained hydrophobic residues. These results suggested the potential degradation of hydrophobic polyethylene intermediates through hydrophobic amino acid metabolism. Analysis of genes based on their cellular component, which represents their expression location, revealed upregulation in the membrane and downregulation in the cytoplasm. This result suggests that enhanced expression of transport proteins is required for the transportation of polyethylene intermediates.

Genes were categorized based on molecular function, revealing an increase in transmembrane transporter activity, in line with KEGG and biological process analyses. Moreover, oxidoreductase activity increased, specifically involving genes acting on the CH-CH group. Polyethylene is a polymer composed solely of carbon and hydrogen and is directly influenced by oxidoreductases acting on the C-H groups, playing a crucial role in plastic degradation [23]. Oxidoreductases are the enzymes involved in oxidation/reduction processes, such as oxygenases, oxidases, and dehydrogenases. Among these, the most crucial enzyme in the initial stages of plastic degradation is oxygenase, which incorporates oxygen into plastics [24]. The upregulated genes include monooxygenases and dioxygenases, both of which require electron donors. Flavin adenine dinucleotide (FAD) binding signifies the activation of oxygenases that receive electrons from flavoproteins and FADH2 during plastic oxidation (Tables S4 and S5) [25]. Additionally, oxygenases commonly feature iron-sulfur clusters in their

active sites or rely on iron-sulfur proteins as electron donors [26]. The upregulation of iron-sulfur clusters and ferrous iron binding signifies the activation of these oxygenases.

3.3. Proteomic Analysis of *P. aeruginosa*

Proteomic analysis of *P. aeruginosa* cultivated with LDPE as the sole carbon source revealed upregulation and downregulation of 888 and 870 proteins, respectively (Table S6). Principal component analysis demonstrated distinct clustering of samples from the control and PE groups (Figure 3a). The overall protein analysis results closely resembled those of the transcriptome analysis. KEGG database analysis highlighted the involvement of fatty acid degradation and lipoic acid metabolism in the metabolism of polyethylene intermediate molecules (Figure 3b, 3c) [13, 27]. Additionally, an increase in the degradation of hydrophobic amino acids (valine, leucine, isoleucine, alanine, and tryptophan) was observed. These results suggested the potential degradation of hydrophobic polyethylene intermediates through hydrophobic amino acid metabolism.

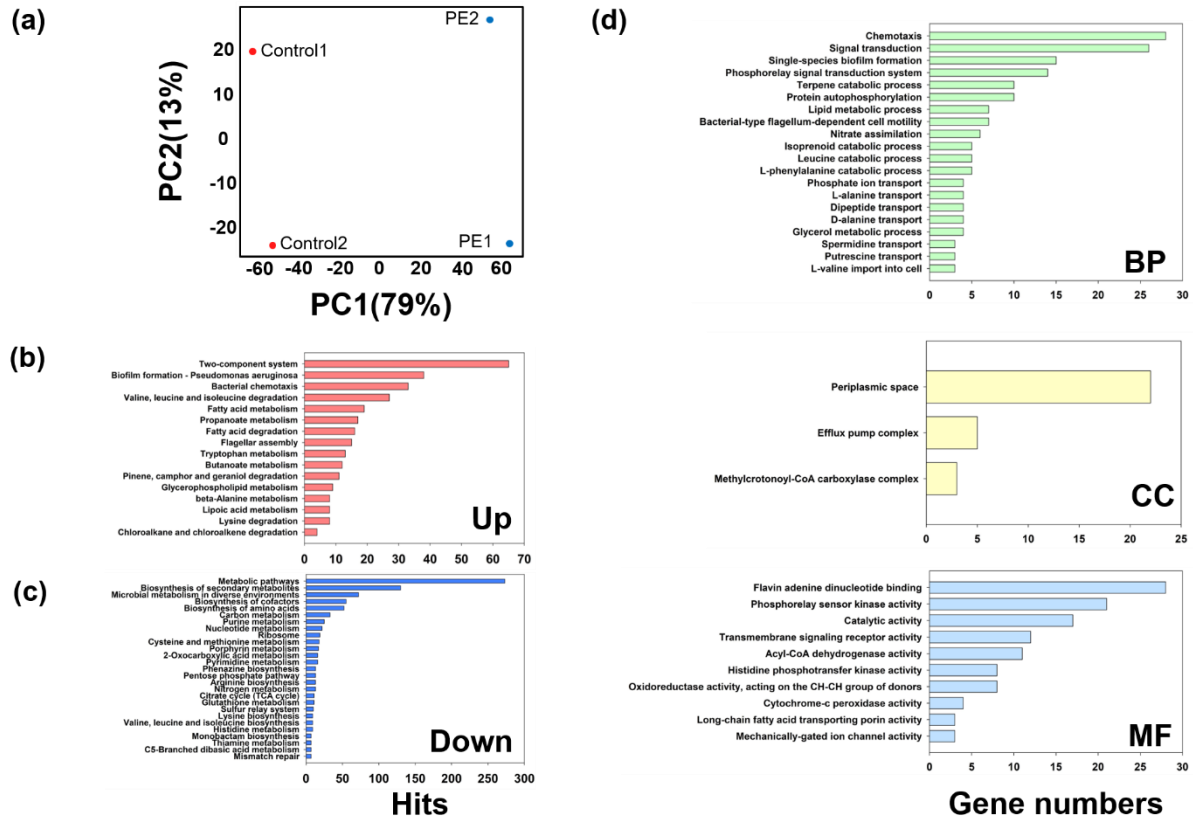

Figure 3. Proteome analysis of *P. aeruginosa* cultured with polyethylene as the carbon source. (a) Changes in protein expression patterns of *P. aeruginosa* observed through PCA plot. (b) Pathways associated with upregulated proteins analyzed using the KEGG database. (c) Pathways associated with downregulated proteins analyzed using the KEGG database. (d) Biological processes, cellular components, and molecular functions associated with upregulated proteins analyzed using the GO database.

By utilizing the GO database to categorize proteins according to biological processes, lipid metabolic and hydrophobic amino acid catabolic processes (Leucine and L-phenylalanine) were increased, corroborating the findings from the KEGG analysis (Figure 3d, S2). Additionally, nitrate assimilation was upregulated. As polyethylene comprises only carbon and hydrogen and lacks nitrogen, microorganisms can convert polyethylene metabolic intermediates into amino acids via nitrogen fixation [28]. Increased hydrophobic amino acid

transport (L-alanine, D-alanine, and L-valine) further supports this possibility. Analysis of proteins based on cellular components revealed upregulation of proteins in the periplasmic space, with downregulation in the cytosol and cytoplasm. Long-chain fatty acid-transporting porin activity increased, suggesting its involvement in the intracellular transport of polyethylene degradation intermediates [29]. Furthermore, the upregulation of oxidoreductase activity acting on the CH-CH group of donors indicates the expression of enzymes directly engaged in polyethylene degradation. Flavin adenine dinucleotide binding suggested the potential involvement of enzymes receiving electrons from $FADH_2$ during polyethylene degradation [30].

3.4. Putative Plastic Oxygenase: Phenylalanine Monooxygenase and Depolymerase: Isocitrate Lyase

Hydrophobic amino acid and lipid metabolism emerged as commonly identified metabolic pathways in both the transcriptome and proteome analyses (Table 2). Although lipid metabolism is considered a key pathway for plastic degradation, our analysis suggested the potential degradation of hydrophobic intermediates via the hydrophobic amino acid degradation pathway [14]. Examination of gene and protein functions revealed consistent upregulation of transporter proteins and oxidoreductase activities in the CH-CH group. Transporter proteins, such as long-chain fatty acid-transporting porins, facilitate the transport of hydrophobic intermediate molecules into cells, whereas oxidoreductases play a crucial role in plastic degradation [13, 22, 31].

Table 2. Functional analysis of the genes and proteins upregulated during polyethylene degradation

Function		KEGG	GO
Transmembrane transport	Transcriptome	ABC transporter, Two-component system	Integral component of membrane, Transmembrane transporter activity, Porin activity
	Proteome	Two-component system	Long-chain fatty acid transporting, porin activity
Lipid metabolism	Transcriptome	Lipid metabolic process	Acyl-CoA dehydrogenase activity
	Proteome	Fatty acid degradation, Lipoic acid metabolism	Lipid metabolic process, Acyl-CoA dehydrogenase activity
Amino acid metabolism	Transcriptome	Beta-Alanine metabolism	Polyamide catabolic process, amino acid catabolic process (L-phenylalanine, Valine)
	Proteome	Amino acid degradation (Valine, Leucine, Isoleucine, Lysine)	Amino acid metabolism (L-phenylalanine, Leucine, L-alanine, D-alanine, L-valine), Nitrate assimilation
Oxidoreductase activity	Transcriptome	Sulfur metabolism	Iron-sulfur cluster assembly, Oxidoreductase activity (acting on the CH-CH group of donors), Dioxygenase activity, Monooxygenase activity
	Proteome	-	Oxidoreductase activity (acting on the CH-CH group of donors), Cytochrome-c peroxidase activity

Among the 247 genes that were upregulated simultaneously in both the transcriptome and proteome, the majority were associated with transporter functions, acyl-CoA dehydrogenase (involved in lipid metabolism), and largely uncharacterized hypothetical proteins (Table S9). Genes related to oxygenases that are capable of initiating the initial oxidation of polyolefin plastics are composed of only carbon and hydrogen, including cytochrome P450, alkane monooxygenase, and phenylalanine monooxygenase [10, 11, 22]. Notably, the mRNA expression level of phenylalanine monooxygenase increased by 3.16-fold in microbial samples degrading PE, and the enzyme expression, analyzed through proteomic analysis, increased by 5.84-fold. However, the role of phenylalanine monooxygenase in plastic degradation remains unclear.

Phenylalanine monooxygenase acts on the phenyl residue of L-phenylalanine, a hydrophobic amino acid, forming a hydroxyl group and oxidizing L-phenylalanine into L-tyrosine [32]. Unlike cytochrome P450 and alkane monooxygenase, which are membrane proteins, phenylalanine monooxygenase is a cytoplasmic enzyme [33]. This characteristic renders phenylalanine monooxygenase suitable for plastic degradation applications. Phenylalanine monooxygenase is expressed in soluble form, which enables its mass expression and purification. Therefore, this enzyme is well-suited for investigating functions and improving performance. Additionally, the ease of large-scale production of phenylalanine monooxygenase makes it highly suitable for plastic degradation.

After oxidition by oxidizing enzymes, polyethylene must be depolymerized into low molecular weight hydrocarbons to be fully degraded by microorganisms. Unlike polyester plastics, which are depolymerized through ester bonds, polyethylene is composed of C-C bonds. Previous studies have suggested that esterases, such as lipases, which are involved in the degradation of polyester plastics, may also play a role in the depolymerization of polyethylene [9]. However, experimental evidence for polyethylene depolymerization is limited. In our study, we observed a 5.18-fold increase in the expression of ICL, an enzyme that cleaves the C-C bonds in isocitrate to produce glyoxylate and succinate [34]. ICL shows great potential for involvement in polyethylene depolymerization because of its ability to cleave C-C bonds, which distinguishes it from previously proposed esterase candidates.

3.5. Enzymatic LDPE Degradation via Phenylalanine Monooxygenase and Isocitrate Lyase

3.5.1. Phenylalanine monooxygenase and Isocitrate lyase purification

The proteins produced from recombinant *phhA* and *aceA* in *E. coli* BL21(DE3) were purified using affinity chromatography and confirmed by SDS-PAGE (Figure S3). A protein of

approximately 30 kDa was observed, which is consistent with the known size of phenylalanine monooxygenase, indicating successful protein expression. The protein derived from *aceA* was approximately 59 kDa, matching the expected size of ICL. Moreover, no other proteins were visible in the bands corresponding to each protein, confirming the successful purification.

3.5.2. Degradation step1: polyethylene oxidation by phenylalanine monooxygenase

Polyethylene, a polymer composed of carbon and hydrogen, undergoes oxidation as the initial step in its biodegradation process. Phenylalanine monooxygenase can introduce a hydroxyl (-OH) group into C-H bonds. FT-IR analysis was conducted to examine the chemical functional groups on the surface of LDPE. When treated with PAH, C-O groups at 1050 cm^{-1} and hydroxyl groups (-OH) at 3000–3600 cm^{-1} were identified (Figure 4a) [35]. Subsequently, the surface carbon binding energies of LDPE treated with phenylalanine monooxygenase were examined using XPS (Table S10). In the control group, only C-C bonds were detected at 284.5 eV, whereas in the PAH-treated group, C-O bonds were identified at 285.6 eV, constituting 10.6% of the total bonds (Figure 4d, 4f) [4, 36, 37]. This finding was supported by the oxygen elemental analysis, with minimal oxygen detected in the control group (Figure 4e). Although polyethylene is composed solely of carbon and hydrogen, XPS, which is highly sensitive, can detect trace amounts of oxygen in a the surrounding environment of the device. In the enzyme-treated group, significant increase in C-O bonds at 532.2, C=O bonds at 531.1, and COO- at 533.4 eV were observed, indicating the oxidation ability of phenylalanine monooxygenase on polyethylene (Figure 4g) [38]. The C-O bond observed in the C1s and O1s scans was attributed to the formation of C-OH by PAH. This result was in line with the XPS findings, confirming the introduction of hydroxyl groups by phenylalanine monooxygenase and the presence of C-O bonds in LDPE. The thermal stability of LDPE was assessed using TGA, revealing thermal decomposition at 479.3 °C in the control group (Figure 5a). Plastics exhibit distinct thermal decomposition temperatures, and alterations in these temperatures signify plastic degradation.

In the PAH-treated group, the decomposition temperature decreased, with maximum decomposition rates observed at 424.28 and 461.93 °C, indicating LDPE oxidation and formation of intermediate molecules [39].

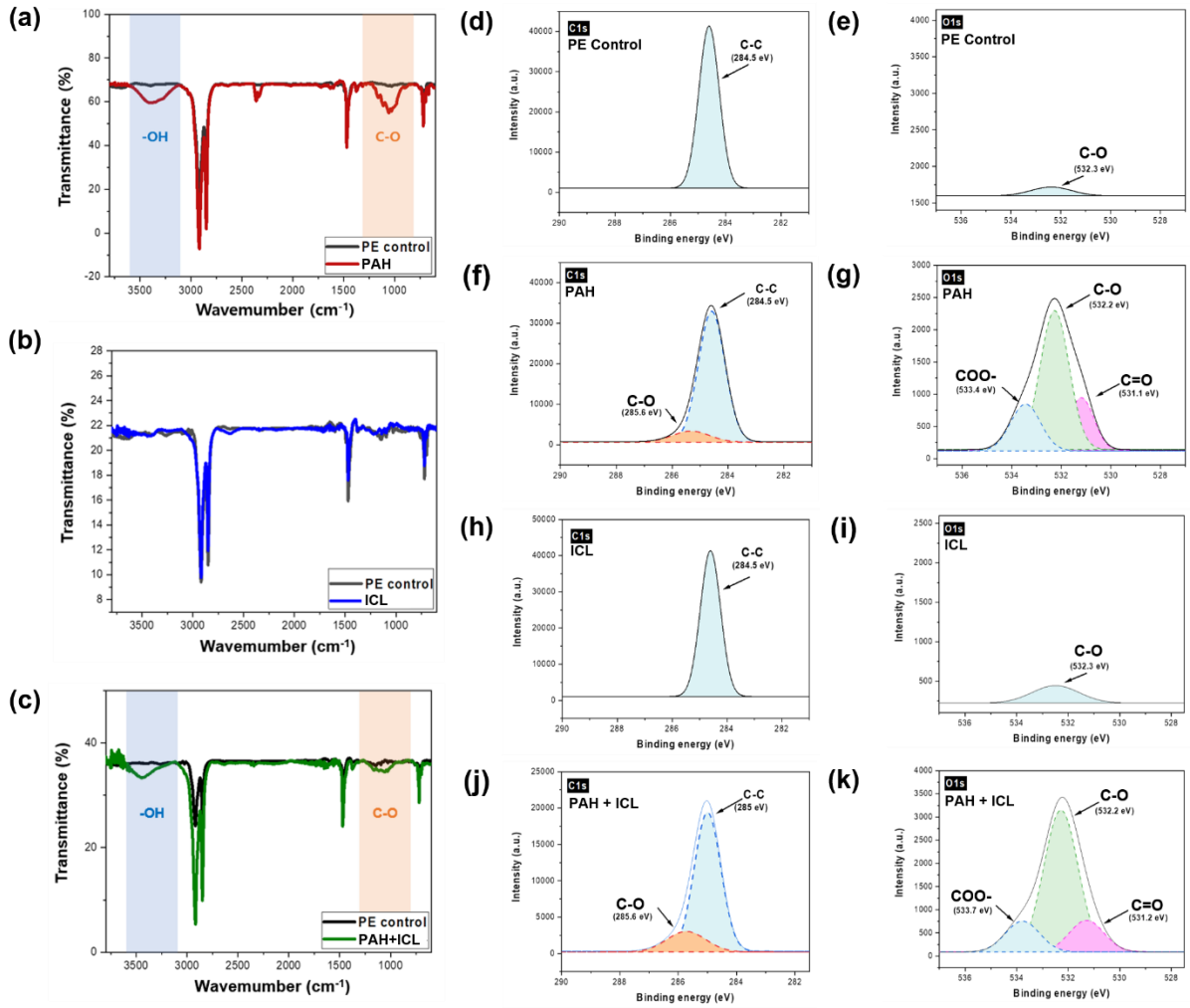

Figure 4. FT-IR and XPS analyses of polyethylene surface treated with phenylalanine monooxygenase and isocitrate lyase. Analysis of chemical functional groups using FT-IR for (a) PAH-treated, (b) ICL-treated, and (c) PAH+ICL-treated samples. Analysis of binding energy using XPS for (d) C1s and (e) O1s binding energy of the polyethylene control sample. (f) C1s and (g) O1s binding energy of the PAH-treated sample. (h) C1s and (i) O1s binding energy of the ICL-treated sample. (j) C1s and (k) O1s binding energy of the PAH+ICL-treated.

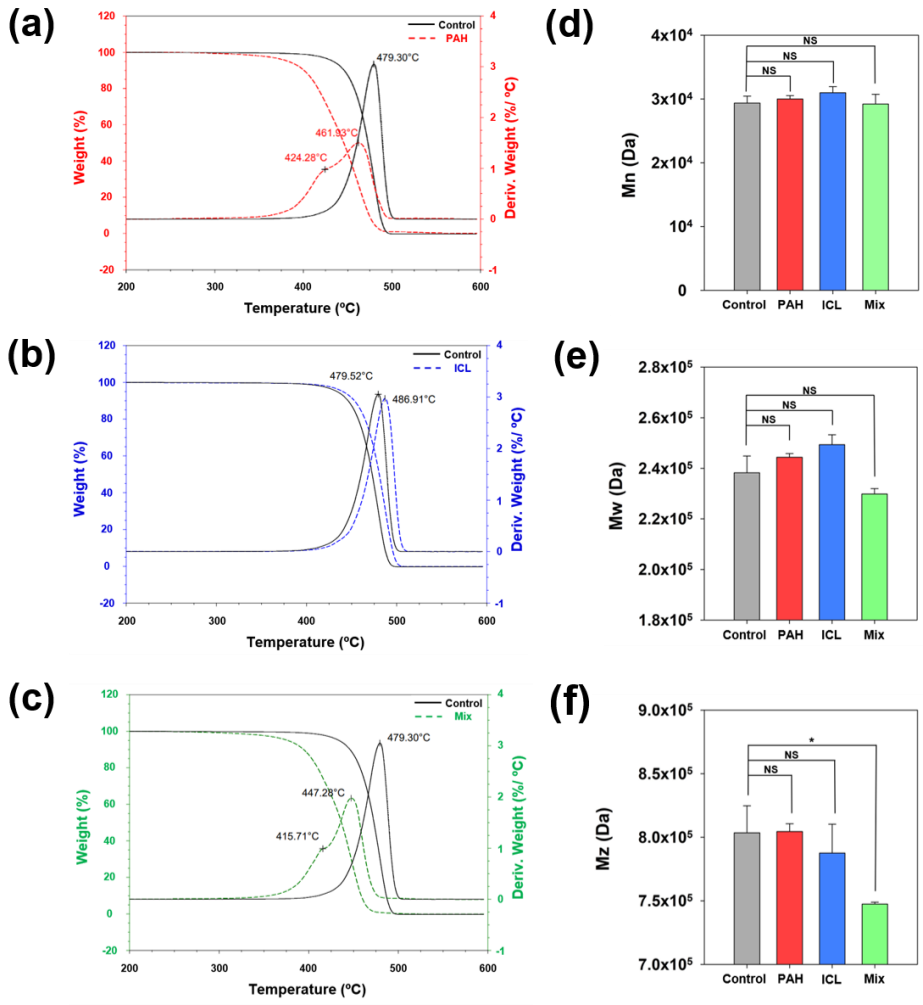

Figure 5. Analysis of polyethylene degradation by PAH and ICL. Thermal stability analysis using TGA for (d) PAH-treated, (e) ICL-treated, and (f) PAH+ICL-treated samples. (g) Number-average molecular weight (Mn) of control, PAH, ICL, and PAH+ICL (Mix) samples. (h) Weight-average molecular weight (Mw) of control, PAH, ICL, and PAH+ICL (Mix) samples. (i) Z-average molecular weight (Mz) of control, PAH, ICL, and PAH+ICL (Mix) samples. The asterisk (*) indicates a statistically significant difference (p=0.065) analyzed using Student's t-test.

3.5.3. Degradation step2: polyethylene depolymerization by isocitrate lyase

When ICL was applied to the LDPE film, FT-IR analysis showed results identical to those of the control group, indicating that no oxidation occurred (Figure 4b). Additionally, XPS analysis revealed no significant differences between the control and isocitrate lyase-treated

groups, confirming that ICL lacks the ability to oxidize plastic (Figure 4h, 4i). TGA analysis indicated a slight increase of approximately 7 °C in the decomposition temperature but with minimal impact compared with the PAH-treated group (Figure 5b). When both PAH and ICL were applied simultaneously, FT-IR, XPS, and TGA results were nearly identical to those obtained by treating LDPE with PAH alone, suggesting that PAH was responsible for the oxidation of polyethylene (Figure 4c, 4j, 4k, 5c). The molecular weight of enzyme-treated LDPE was analyzed to assess the role of ICL as a depolymerase (Table 3) [18]. There was no significant difference in the Mn among the control, PAH, ICL, and PAH+ICL experimental groups (Figure 5e). While the Mw did not decrease when PAH and ICL were applied individually, a decrease of approximately 3.5% from 238,251 to 229,864 Da was observed when both enzymes were applied simultaneously (Figure 5f). Similarly, while the Z-average molecular weight (Mz) did not decrease when PAH and ICL were applied individually, a decrease of approximately 7.0% from 803,487 to 747,396 Da was observed when both enzymes were applied simultaneously (Figure 5g). The statistical significance between the control and PAH+ICL groups was assessed using Student's t-test, resulting in a p-value of 0.065. Biological plastic degradation reactions often proceed via limited-extent depolymerization reactions. In cases of broad depolymerization, where enzymes react uniformly with all molecular weights of the material, changes are observed in the Mn, Mw, and Mz values. However, to a limited extent, depolymerization and effective enzyme reactions with low molecular weight substances lead to significant variations in Mn, whereas effective reactions with high molecular weight substances result in significant variations in Mz [40]. Depolymerization mediated by PAH and ICL resulted in changes in Mz, indicating effective enzyme reactions with high molecular weight substances in limited extent depolymerization. A limited extent of depolymerization was also observed in the GPC curve. LDPE treated with PAH showed no significant difference from the control, indicating that PAH did not contribute to depolymerization. However, when

treated with ICL, the curve shifted toward the higher molecular weight region, suggesting that a limited extent of depolymerization primarily occurred in the lower molecular weight range. However, no statistically significant difference was observed in molecular weight due to low efficiency. In samples simultaneously treated with both PAH and ICL, a reduction in the number of molecules was observed in the high molecular weight region. (Figure S4). Further research on precise enzymatic reaction mechanisms is necessary to understand the underlying causes of such phenomena.

Table 3. Changes in molecular weight of low-density polyethylene films treated with phenylalanine monooxygenase and isocitrate lyase (mean±standard deviation, n=2). Mn, number-average molecular weight Mw, weight-average molecular weight; Mz, z-average molecular weight.

Sample	Mn (Da)	Mn Change (%)	Mw (Da)	Mw Change (%)	Mz (Da)	Mz Change (%)
Control	29373 ± 1086	-	238251 ± 6635	-	803487 ± 21274	-
PAH	29997 ± 534	+2.1%	244363 ± 1458	+2.6%	804535 ± 6147	+0.1%
ICL	30982 ± 967	+5.5%	249371 ± 3902	+4.7%	787503 ± 22776	-2.0%
PAH + ICL	29216 ± 1534	-0.5%	229864 ± 2126	-3.5%	747396 ± 1508	-7.0%

This result suggests that although ICL cannot effectively depolymerize polyethylene itself, it exhibits depolymerization activity in PAH-oxidized polyethylene [36, 41]. Since isocitrate is more oxidized molecule compared to polyethylene, primary oxidation may be necessary for ICL activity. Furthermore, while most previous studies focused on identifying a single enzyme capable of degrading plastic, the results of this study indicate at least a two-step process of oxidation and depolymerization during polyethylene degradation, highlighting the need for sequential reactions involving multiple enzymes [10]. Moreover, the results demonstrated that ICL, which is capable of breaking the C-C bonds of oxidized polyethylene, can catalyze the depolymerization of polyethylene.

4. Conclusion

We conducted transcriptome and proteome analyses of the plastic-degrading microbe *P. aeruginosa* to identify genes and enzymes with increased expression levels. Using the KEGG and GO databases, we analyzed the associated metabolic pathways and confirmed the potential involvement of lipid and hydrophobic amino acid metabolism in polyethylene degradation. Subsequently, genes showing elevated expression levels in both transcriptome and proteome analyses were selected and oxygenases involved in the initial oxidation of plastics were identified. Remarkably, phenylalanine monooxygenase, which was previously unrecognized in plastic degradation, was identified along with alkane monooxygenase and cytochrome P450 monooxygenase, both of which are known for their involvement in the initial oxidation of plastics. Furthermore, among the upregulated enzymes, ICL, capable of breaking the C-C bond in polyethylene, was identified as a potential depolymerase.

The oxidative capability of enzyme was confirmed by the formation of C-O, C=O, and -OH functional groups on the LDPE film after treatment with phenylalanine monooxygenase. ICL did not affect LDPE oxidation when used alone; however, it exhibited depolymerization activity in oxidized LDPE when co-treated with phenylalanine monooxygenase. This study delineated the degradation of polyethylene into two stages, oxidation and depolymerization, and demonstrated degradation by purifying the enzymes involved at each stage. In particular, lyases capable of breaking the C-C bond in polyethylene were identified and proposed as depolymerization enzymes.

This study had limitations in qualitatively demonstrating the oxidative and depolymerization abilities of plastic. Hence, further research, such as enzyme kinetic studies, is required to develop quantitative measurement methods for degradation rates. Additionally, subsequent studies are necessary to analyze the structures of phenylalanine monooxygenase and isocitrate

lyase discovered in this study and to engineer them for enhanced degradation performance, such as directed evolution. Furthermore, exploring the potential industrial applications of enzymes through mass production and purification can significantly contribute to addressing plastic pollution.

Author Contributions

§ Hong Rae Kim and Ye Eun Lee contributed equally to this paper.

Hong Rae Kim: Conceptualization, Investigation, Formal analysis, Visualization, Writing-Original Draft; Ye Eun Lee: Investigation, Formal analysis, Visualization; Dong-Eun Suh: Investigation, Funding acquisition; Donggeon Choi: Investigation, Writing-Review & Editing, Sukkyoo Lee: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Project administration, Funding acquisition, Revision of draft

Acknowledgement

This study was supported by the Tech Incubator Program for Startups funded by the Ministry of Small and Medium Enterprises and Startups, Republic of Korea (No. S3136229). This work was also supported by the DGIST R&D Program of the Ministry of Science and ICT, Korea (21-BRP-04 and 22-BRP-04) and the DGIST start-up fund.

Data availability

RNA sequencing data are deposited in the NCBI database under the ID codes PRJNA1100243, SRR28677042, and SRR28677047. LC-MS/MS data are deposited in the PRIDE database under the accession code PXD051434.

References

(1) Geyer, R.; Jambeck, J. R.; Law, K. L. Production, use, and fate of all plastics ever made. *Science advances* **2017**, *3* (7), e1700782.

(2) MacLeod, M.; Arp, H. P. H.; Tekman, M. B.; Jahnke, A. The global threat from plastic pollution. *Science* **2021**, *373* (6550), 61-65. DOI: 10.1126/science.abg5433 From NLM Medline.

(3) Ali, S. S.; Elsamahy, T.; Al-Tohamy, R.; Zhu, D.; Mahmoud, Y. A.; Koutra, E.; Metwally, M. A.; Kornaros, M.; Sun, J. Plastic wastes biodegradation: Mechanisms, challenges and future prospects. *Sci Total Environ* **2021**, *780*, 146590. DOI: 10.1016/j.scitotenv.2021.146590 From NLM Medline.

(4) Kim, H. R.; Lee, H. M.; Yu, H. C.; Jeon, E.; Lee, S.; Li, J.; Kim, D. H. Biodegradation of Polystyrene by Pseudomonas sp. Isolated from the Gut of Superworms (Larvae of Zophobas atratus). *Environ Sci Technol* **2020**, *54* (11), 6987-6996. DOI: 10.1021/acs.est.0c01495 From NLM Medline.

(5) Yang, J.; Yang, Y.; Wu, W. M.; Zhao, J.; Jiang, L. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ Sci Technol* **2014**, *48* (23), 13776-13784. DOI: 10.1021/es504038a From NLM Medline.

(6) Ahmed, T.; Shahid, M.; Azeem, F.; Rasul, I.; Shah, A. A.; Noman, M.; Hameed, A.; Manzoor, N.; Manzoor, I.; Muhammad, S. Biodegradation of plastics: current scenario and future prospects for environmental safety. *Environ. Sci. Pollut. Res. Int.* **2018**, *25* (8), 7287-7298. DOI: 10.1007/s11356-018-1234-9 From NLM Medline.

(7) Singh, R.; Mehrotra, T.; Bisaria, K.; Sinha, S. Environmental hazards and biodegradation of plastic waste: Challenges and future prospects. *Bioremediation for Environmental Sustainability* **2021**, 193-214.

(8) Ghatge, S.; Yang, Y.; Ahn, J.-H.; Hur, H.-G. Biodegradation of polyethylene: a brief review. *Applied Biological Chemistry* **2020**, *63*, 1-14.

(9) Yeom, S. J.; Le, T. K.; Yun, C. H. P450-driven plastic-degrading synthetic bacteria. *Trends Biotechnol* **2022**, *40* (2), 166-179. DOI: 10.1016/j.tibtech.2021.06.003 From NLM Medline.

(10) Yun, S.-D.; Lee, C. O.; Kim, H.-W.; An, S. J.; Kim, S.; Seo, M.-J.; Park, C.; Yun, C.-H.; Chi, W. S.; Yeom, S.-J. Exploring a new biocatalyst from Bacillus thuringiensis JNU01 for polyethylene biodegradation. *Environmental Science & Technology Letters* **2023**, *10* (6), 485-492. Jeon, H. J.; Kim, M. N. Functional analysis of alkane hydroxylase system derived from Pseudomonas aeruginosa E7 for low molecular weight polyethylene biodegradation. *International Biodeterioration & Biodegradation* **2015**, *103*, 141-146.

(11) Yoon, M. G.; Jeon, H. J.; Kim, M. N. Biodegradation of polyethylene by a soil bacterium and AlkB cloned recombinant cell. *J Bioremed Biodegrad* **2012**, *3* (4), 1-8.

(12) Tournier, V.; Duquesne, S.; Guillamot, F.; Cramail, H.; Taton, D.; Marty, A.; Andre, I. Enzymes' Power for Plastics Degradation. *Chem Rev* **2023**. DOI: 10.1021/acs.chemrev.2c00644 From NLM Publisher.

(13) Zadjelovic, V.; Erni-Cassola, G.; Obrador-Viel, T.; Lester, D.; Eley, Y.; Gibson, M. I.; Dorador, C.; Golyshin, P. N.; Black, S.; Wellington, E. M. H.; et al. A mechanistic understanding of polyethylene biodegradation by the marine bacterium Alcanivorax. *J Hazard Mater* **2022**, *436*, 129278. DOI: 10.1016/j.jhazmat.2022.129278 From NLM Medline.

(14) Gravouil, K.; Ferru-Clement, R.; Colas, S.; Helye, R.; Kadri, L.; Bourdeau, L.; Moumen, B.; Mercier, A.; Ferreira, T. Transcriptomics and Lipidomics of the Environmental Strain Rhodococcus ruber Point out Consumption Pathways and Potential Metabolic Bottlenecks for Polyethylene Degradation. *Environ Sci Technol* **2017**, *51* (9), 5172-5181. DOI: 10.1021/acs.est.7b00846 From NLM Medline.
(15) Langmead, B.; Trapnell, C.; Pop, M.; Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **2009**, *10* (3), R25. DOI: 10.1186/gb-2009-10-3-r25 From NLM Medline. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; Genome Project Data Processing, S. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **2009**, *25* (16), 2078-2079. DOI: 10.1093/bioinformatics/btp352 From NLM Medline.
(16) Anders, S.; Pyl, P. T.; Huber, W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* **2015**, *31* (2), 166-169. DOI: 10.1093/bioinformatics/btu638 From NLM Medline.
(17) Lee, H. M.; Kim, H. R.; Jeon, E.; Yu, H. C.; Lee, S.; Li, J.; Kim, D.-H. Evaluation of the biodegradation efficiency of four various types of plastics by Pseudomonas aeruginosa isolated from the gut extract of superworms. *Microorganisms* **2020**, *8* (9), 1341. Tamnou, E. B. M.; Arfao, A. T.; Nougang, M. E.; Metsopkeng, C. S.; Ewoti, O. V. N.; Moungang, L. M.; Nana, P. A.; Takang-Etta, L.-R. A.; Perrière, F.; Sime-Ngando, T. Biodegradation of polyethylene by the bacterium Pseudomonas aeruginosa in acidic aquatic microcosm and effect of the environmental temperature. *Environmental Challenges* **2021**, *3*, 100056. Kale, S. K.; Deshmukh, A. G.; Dudhare, M. S.; Patil, V. B. Microbial degradation of plastic: a review. *J. Biochem. Technol.* **2015**, *6* (2), 952-961.
(18) Wu, W. M.; Criddle, C. S. Characterization of biodegradation of plastics in insect larvae. *Methods Enzymol* **2021**, *648*, 95-120. DOI: 10.1016/bs.mie.2020.12.029 From NLM Medline.
(19) Yang, Y.; Yang, J.; Wu, W. M.; Zhao, J.; Song, Y.; Gao, L.; Yang, R.; Jiang, L. Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 2. Role of Gut Microorganisms. *Environ Sci Technol* **2015**, *49* (20), 12087-12093. DOI: 10.1021/acs.est.5b02663 From NLM Medline.
(20) Borst, P.; Zelcer, N.; van Helvoort, A. ABC transporters in lipid transport. *Biochim Biophys Acta* **2000**, *1486* (1), 128-144. DOI: 10.1016/s1388-1981(00)00053-6 From NLM Medline.
(21) Kadri, T.; Rouissi, T.; Kaur Brar, S.; Cledon, M.; Sarma, S.; Verma, M. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. *J Environ Sci (China)* **2017**, *51*, 52-74. DOI: 10.1016/j.jes.2016.08.023 From NLM Medline. Zhang, Z.; Hou, Z.; Yang, C.; Ma, C.; Tao, F.; Xu, P. Degradation of n-alkanes and polycyclic aromatic hydrocarbons in petroleum by a newly isolated Pseudomonas aeruginosa DQ8. *Bioresour Technol* **2011**, *102* (5), 4111-4116. DOI: 10.1016/j.biortech.2010.12.064 From NLM Medline.
(22) Kong, H. G.; Kim, H. H.; Chung, J. H.; Jun, J.; Lee, S.; Kim, H. M.; Jeon, S.; Park, S. G.; Bhak, J.; Ryu, C. M. The Galleria mellonella Hologenome Supports Microbiota-Independent Metabolism of Long-Chain Hydrocarbon Beeswax. *Cell Rep* **2019**, *26* (9), 2451-2464 e2455. DOI: 10.1016/j.celrep.2019.02.018 From NLM Medline.
(23) Inderthal, H.; Tai, S. L.; Harrison, S. T. Non-hydrolyzable plastics–an interdisciplinary look at plastic bio-oxidation. *Trends Biotechnol.* **2021**, *39* (1), 12-23.

(24) Liang, Y.; Wei, J.; Qiu, X.; Jiao, N. Homogeneous Oxygenase Catalysis. *Chem Rev* **2018**, *118* (10), 4912-4945. DOI: 10.1021/acs.chemrev.7b00193 From NLM Medline. Abbasian, F.; Lockington, R.; Mallavarapu, M.; Naidu, R. A Comprehensive Review of Aliphatic Hydrocarbon Biodegradation by Bacteria. *Appl Biochem Biotechnol* **2015**, *176* (3), 670-699. DOI: 10.1007/s12010-015-1603-5 From NLM Medline.

(25) Morikawa, M. Dioxygen activation responsible for oxidation of aliphatic and aromatic hydrocarbon compounds: current state and variants. *Appl Microbiol Biotechnol* **2010**, *87* (5), 1595-1603. DOI: 10.1007/s00253-010-2715-z From NLM Medline.

(26) Britt, R. D.; Rao, G.; Tao, L. Bioassembly of complex iron-sulfur enzymes: hydrogenases and nitrogenases. *Nat Rev Chem* **2020**, *4* (10), 542-549. From NLM PubMed-not-MEDLINE.

(27) El-Sherif, D. M.; Eloffy, M. G.; Elmesery, A.; Abouzid, M.; Gad, M.; El-Seedi, H. R.; Brinkmann, M.; Wang, K.; Al Naggar, Y. Environmental risk, toxicity, and biodegradation of polyethylene: a review. *Environ. Sci. Pollut. Res. Int.* **2022**, *29* (54), 81166-81182. DOI: 10.1007/s11356-022-23382-1 From NLM Medline.

(28) Yang, Y.; Hu, L.; Li, X.; Wang, J.; Jin, G. Nitrogen Fixation and Diazotrophic Community in Plastic-Eating Mealworms Tenebrio molitor L. *Microb Ecol* **2023**, *85* (1), 264-276. DOI: 10.1007/s00248-021-01930-5 From NLM Medline. Ding, M. Q.; Yang, S. S.; Ding, J.; Zhang, Z. R.; Zhao, Y. L.; Dai, W.; Sun, H. J.; Zhao, L.; Xing, D.; Ren, N.; et al. Gut Microbiome Associating with Carbon and Nitrogen Metabolism during Biodegradation of Polyethene in Tenebrio larvae with Crop Residues as Co-Diets. *Environ Sci Technol* **2023**, *57* (8), 3031-3041. DOI: 10.1021/acs.est.2c05009 From NLM Medline.

(29) LeMoine, C. M.; Grove, H. C.; Smith, C. M.; Cassone, B. J. A very hungry caterpillar: polyethylene metabolism and lipid homeostasis in larvae of the greater wax moth (Galleria mellonella). *Environmental science & technology* **2020**, *54* (22), 14706-14715. Mishra, R.; Chavda, P.; Kumar, R.; Pandit, R.; Joshi, M.; Kumar, M.; Joshi, C. Exploring genetic landscape of low-density polyethylene degradation for sustainable troubleshooting of plastic pollution at landfills. *Science of The Total Environment* **2024**, *912*, 168882.

(30) Tischler, D.; Kumpf, A.; Eggerichs, D.; Heine, T. Styrene monooxygenases, indole monooxygenases and related flavoproteins applied in bioremediation and biocatalysis. *Enzymes* **2020**, *47*, 399-425. DOI: 10.1016/bs.enz.2020.05.011 From NLM Medline. Kaur, T.; Lakhawat, S. S.; Kumar, V.; Sharma, V.; Neeraj, R. R. K.; Sharma, P. K. Polyaromatic Hydrocarbon Specific Ring Hydroxylating Dioxygenases: Diversity, Structure, Function, and Protein Engineering. *Curr Protein Pept Sci* **2023**, *24* (1), 7-21. DOI: 10.2174/1389203724666221108114537 From NLM Medline.

(31) Tao, X.; Ouyang, H.; Zhou, A.; Wang, D.; Matlock, H.; Morgan, J. S.; Ren, A. T.; Mu, D.; Pan, C.; Zhu, X.; et al. Polyethylene Degradation by a Rhodococcous Strain Isolated from Naturally Weathered Plastic Waste Enrichment. *Environ Sci Technol* **2023**, *57* (37), 13901-13911. DOI: 10.1021/acs.est.3c03778 From NLM Medline.

(32) Joh, T. H.; Hwang, O.; Abate, C. Phenylalanine hydroxylase, tyrosine hydroxylase, and tryptophan hydroxylase. *Neurotransmitter Enzymes* **1986**, 1-32.

(33) Barnaba, C.; Gentry, K.; Sumangala, N.; Ramamoorthy, A. The catalytic function of cytochrome P450 is entwined with its membrane-bound nature. *F1000Research* **2017**, *6*. Austin, R. N.; Born, D.;

694 Lawton, T. J.; Hamilton, G. E. Protocols for purifying and characterizing integral membrane AlkB
695 enzymes. *Hydrocarbon and Lipid Microbiology Protocols: Biochemical Methods* **2016**, 133-147.
696 (34) Kretzschmar, U.; Khodaverdi, V.; Jeoung, J. H.; Gorisch, H. Function and transcriptional regulation
697 of the isocitrate lyase in Pseudomonas aeruginosa. *Arch Microbiol* **2008**, *190* (2), 151-158. DOI:
698 10.1007/s00203-008-0381-7 From NLM Medline.
699 (35) Jiang, S.; Su, T.; Zhao, J.; Wang, Z. Biodegradation of polystyrene by Tenebrio molitor, Galleria
700 mellonella, and Zophobas atratus larvae and comparison of their degradation effects. *Polymers* **2021**,
701 *13* (20), 3539.
702 (36) Nyamjav, I.; Jang, Y.; Park, N.; Lee, Y. E.; Lee, S. Physicochemical and structural evidence that
703 Bacillus cereus isolated from the gut of waxworms (Galleria mellonella larvae) biodegrades
704 polypropylene efficiently in vitro. *Journal of Polymers and the Environment* **2023**, *31* (10), 4274-
705 4287.
706 (37) Ji, S. H.; Yoo, S.; Park, S.; Lee, M. J. Biodegradation of low-density polyethylene by plasma-
707 activated Bacillus strain. *Chemosphere* **2024**, *349*, 140763. DOI: 10.1016/j.chemosphere.2023.140763
708 From NLM Medline.
709 (38) Cheng, Y.; Chen, J.; Bao, M.; Li, Y. Surface modification ability of Paracoccus sp. indicating its
710 potential for polyethylene terephthalate degradation. *International Biodeterioration &*
711 *Biodegradation* **2022**, *173*, 105454.
712 (39) Peng, B. Y.; Su, Y.; Chen, Z.; Chen, J.; Zhou, X.; Benbow, M. E.; Criddle, C. S.; Wu, W. M.; Zhang, Y.
713 Biodegradation of Polystyrene by Dark (Tenebrio obscurus) and Yellow (Tenebrio molitor)
714 Mealworms (Coleoptera: Tenebrionidae). *Environ Sci Technol* **2019**, *53* (9), 5256-5265. DOI:
715 10.1021/acs.est.8b06963 From NLM Medline.
716 (40) Yang, L.; Gao, J.; Liu, Y.; Zhuang, G.; Peng, X.; Wu, W. M.; Zhuang, X. Biodegradation of expanded
717 polystyrene and low-density polyethylene foams in larvae of Tenebrio molitor Linnaeus (Coleoptera:
718 Tenebrionidae): Broad versus limited extent depolymerization and microbe-dependence versus
719 independence. *Chemosphere* **2021**, *262*, 127818. DOI: 10.1016/j.chemosphere.2020.127818 From
720 NLM Medline. Peng, B.-Y.; Li, Y.; Fan, R.; Chen, Z.; Chen, J.; Brandon, A. M.; Criddle, C. S.; Zhang, Y.;
721 Wu, W.-M. Biodegradation of low-density polyethylene and polystyrene in superworms, larvae of
722 Zophobas atratus (Coleoptera: Tenebrionidae): Broad and limited extent depolymerization.
723 *Environmental Pollution* **2020**, *266*, 115206.
724 (41) Brandon, A. M.; Gao, S. H.; Tian, R.; Ning, D.; Yang, S. S.; Zhou, J.; Wu, W. M.; Criddle, C. S.
725 Biodegradation of Polyethylene and Plastic Mixtures in Mealworms (Larvae of Tenebrio molitor) and
726 Effects on the Gut Microbiome. *Environ Sci Technol* **2018**, *52* (11), 6526-6533. DOI:
727 10.1021/acs.est.8b02301 From NLM Medline.

728