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Mechanistic insights into polystyrene and polyethylene depolymerization by *Galleria Mellonella* and screening of involved gut bacteria

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Abstract
The synthetic plastic pollution, driven by the accumulation of recalcitrant polystyrene (PS) and polyethylene (PE), represents an urgent ecological challenge due to its environmental persistence and high molecular weight. Conventional waste treatments often suffer from high energy demands and toxic emissions, making green biodegradation strategies highly sought after. This study provides critical mechanistic insights into the concurrent depolymerization of PS and PE foam configurations by the larvae of *Galleria mellonella* over a 25-day experimental trial. The larvae exhibited robust polymer consumption, achieving a maximum weight reduction of 68.0% for thermo black sheets, followed by 61.0% for thermocol and 58.0% for thermo white sheets. Fourier Transform Infrared Spectroscopy (FT-IR) of the larval gut fluid confirmed rigorous *in vivo* oxidative depolymerization. This was evidenced by the distinct splitting of aliphatic carbon-hydrogen backbones, a depletion of resonance-stabilized aromatic benzene ring signatures, and the formation of oxidized intermediate carbonyl (C=O) functional groups. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) verified the presence of salivary proteins, corroborating an insect-mediated initial surface modification phase. Concurrently, a highly metabolic plastic-degrading bacterial strain was isolated from the gut microbiota. Identified via 16S rRNA gene sequencing as *Klebsiella aerogenes* WA1 (GenBank: PX855136.1), the isolate displayed broad phenotypic versatility and carbohydrate utilization capabilities optimal for surviving polymer-contaminated ecosystems. Ultimately, these findings elucidate a powerful host-microbiome synergistic framework that can be leveraged to develop sustainable, bio-inspired engineering solutions to mitigate synthetic plastic waste globally.

Keywords: Polystyrene, Polyethylene, *Galleria mellonella, Klebsiella aerogenes WA1*

Introduction
Plastic pollution has emerged as one of the most critical environmental challenges due to the extensive production, and improper disposal of synthetic polymers (Kibria *et al*., 2023) [9]. Polystyrene (PS) and polyethylene (PE) is one of the most extensively manufactured synthetic polymers worldwide and is widely utilized in packaging materials, disposable food containers, insulation products, and electronic applications due to its lightweight structure, durability, and low production cost (Hassan *et al*., 2022) [8]. The extensive consumption and improper disposal of PS have resulted in severe environmental contamination, particularly in terrestrial and aquatic ecosystems (Kumar *et al*., 2021; Osabuohien, 2017) [11, 17]. However, the same physicochemical properties that make PS commercially valuable, including its hydrophobicity, high molecular weight, aromatic backbone, and strong carbon–carbon bonds, also render it highly resistant to natural degradation (Mohanan *et al*., 2020) [14]. As a result, massive quantities of PS accumulate in terrestrial and aquatic ecosystems, where they gradually fragment into microplastics and nanoplastics, posing serious ecological and human health risks through bioaccumulation and food chain transfer (Benson *et al*., 2022; Siddiqui *et al*., 2023) [3, 19]. Conventional plastic waste management methods such as landfilling, incineration, and chemical recycling are associated with major drawbacks including toxic emissions, secondary pollution, high energy consumption, and limited economic feasibility (Fayshal, 2024) [6]. Consequently, there is increasing global interest in developing sustainable and environmentally friendly biodegradation strategies for synthetic plastics (Fayshal, 2024) [6].

Recent studies have demonstrated that several insects possess an unexpected capacity to consume and degrade synthetic polymers, opening a new frontier in plastic biodegradation research. Among these insects, the larvae of the greater wax moth, *Galleria mellonella*, commonly known as waxworms, have attracted considerable scientific attention because of their remarkable ability to degrade PE and PS (Lou *et al*., 2020 [12]; Ma *et al*., 2025). Waxworms naturally feed on beeswax within honeycombs, a substrate chemically similar to synthetic polyolefins due to the presence of long-chain hydrocarbons (Kong *et al*., 2019) [10]. This evolutionary adaptation is believed to contribute to their ability to attack plastic polymers. Previous investigations reported significant weight loss of PE and PS films after exposure to waxworms, accompanied by the formation of oxidized functional groups, reductions in molecular weight, and generation of degradation intermediates such as long-chain fatty acids and oxidized hydrocarbons (Hasan *et al*., 2026; Kong *et al*., 2019) [7, 10]. Furthermore, studies revealed that the gut microbiota of waxworms, including bacterial genera such as *Bacillus*, *Serratia*, *Pseudomonas*, *Enterobacter*, and *Lysinibacillus*, play critical roles in polymer depolymerization and metabolism (Ma *et al*., 2025). Suppression of gut

microorganisms through antibiotic treatment significantly reduces plastic degradation efficiency, confirming the importance of host–microbe synergistic interactions during biodegradation (Wang *et al*., 2024) [22].

In addition to microbial involvement, recent evidence suggests that biological secretions and enzymes produced by waxworms may directly participate in polymer oxidation and chain scission (Ali *et al*., 2025; Nwagu *et al*., 2025.; Sanluis-Verdes *et al*., 2022) [2, 16, 18]. Studies on waxworm saliva identified oxidizing enzymes capable of initiating rapid depolymerization of PE under ambient conditions without requiring harsh pretreatment processes such as UV irradiation or thermal oxidation (Ali *et al*., 2025; Nwagu *et al*., 2025) [2, 16]. These findings challenge the traditional paradigm that abiotic oxidation is the essential first step in plastic biodegradation and indicate that insect-derived enzymes may represent a novel and efficient mechanism for polymer degradation. Despite these advances, the precise biochemical pathways, metabolic intermediates, structural modifications, and microbial dynamics involved in PS degradation by *Galleria mellonella* remain insufficiently understood (Kong *et al*., 2019; Lou *et al*., 2020 [10, 12]; Ma *et al*., 2025). Most available studies primarily focus on PE degradation, while detailed investigations on PS biodegradation mechanisms and biological characterization are still limited (Lou *et al*., 2020; Ma *et al*., 2025; Nwagu *et al*., 2025; Sanluis-Verdes *et al*., 2022; Wang *et al*., 2024) [2, 12, 16, 18, 22].

Therefore, the present study focuses on PS biodegradation by *Galleria mellonella* (Waxworm) mechanisms and biological characterization to investigate the degradation efficiency of waxworms toward PS and to elucidate the associated biological and physicochemical mechanisms. The study aims to evaluate structural and chemical changes in PS during biodegradation, identify potential degradation intermediates, characterize the role of gut-associated microorganisms, and understand the synergistic interactions between the host insect and its microbiota. Understanding these mechanisms may contribute to the development of sustainable biotechnological approaches for plastic waste management and provide valuable insights into environmentally compatible solutions for mitigating PS pollution.

Materials and Method

Sample Collection

Different types of PS foam (Thermocol, Thermo white sheet, Thermo black sheet - density, 0.9 g/cm^3) was purchased from Zhenchuang Plastic Co. (Jiangsu, China).

Wax worm- Galleria Mellonica

Third- to fifth-instar larvae of the greater wax moth (Galleria mellonella), with a mean body length of 15 – 25 mm, were procured from Micro worms India Pvt Ltd, Karnataka, India. Commercial supplier, ensuring a healthy and genetically diverse population. The worms were acclimated to laboratory conditions 28^0 C dark condition the experiment to ensure optimal performance (Lou *et al*., 2020) [12].

Experimental Set Up

Prior to testing, the larvae were fed food containing mixed nutrients, including PS. The larvae were starved for 36 h before the experiment to prevent any effect of previously eaten food. In this study were three identical trials, were denoted as established reproducibility and accuracy results. The strong prepared to were Carefully provide a Consistent environment for the worms prior to introducing the Substrate the initial weight of the worms was precisely recorded using a digital balance. Similarly, the initial weight of the Substrate was also documented. System a contained Thermocol (PS), System b thermo white sheet (PE foam), System c thermo black seat (PE foam) and control only nutrition. The containers were placed in an incubator maintained at 27.5 ± 0.5 °C and with humidity of 75 ± 5%. The survival rate of the larvae and the weight loss of the plastic were measured every 5 days for 21 days. Dead larvae were removed immediately to prevent any possible disease (Ali *et al*., 2023; Lou *et al*., 2020) [1, 12].

Fourier Transform Infrared Spectroscopy (Ft-Ir)

FT-IR was performed to investigate changes in the chemical structure and functional groups of the polymer following biodegradation. Prior to analysis, the frass samples were dried in a hot-air oven for 12 h to remove residual moisture. FTIR spectra were recorded using a Spectrum One FTIR spectrometer (PerkinElmer, Shelton, CT, USA) over a wavenumber range of 4000–500 cm^{-1} with a spectral resolution of 4 cm^{-1}. Each spectrum was obtained from a minimum of five scans to ensure analytical reliability (Ma *et al*., 2025).

Protein Isolation from Wax Worms Saliva

Larvae of 150–300 mg were used for saliva collection. Briefly, a glass capillary connected to a mouth pipet was placed at the buccal opening and the liquid was collected. Five to ten microliters of saliva were collected from each worm. The proteins in the saliva measured via SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis). Saliva for PE application was immediately used. Occasionally, frozen saliva-only can be utilized. To prepare resolving gel (10-15% acrylamide, Tris-HCl pH 8.8) and stacking gel (pH 6.8) with SDS and polymerization reagents (TEMED, APS). To mix protein sample with SDS loading buffer, boil at 95-100°C for 5-10 minutes. Load samples into wells, add protein marker, and assemble electrophoresis apparatus. Run gel at 100-150 V, proteins separate by size. Stain gel with Coomassie Brilliant Blue or Silver Stain, de-stain with methanol and acetic acid (Sanluis-Verdes *et al*., 2022; Spínola-Amilibia *et al*., 2023) [18, 20].

Anatomy of Gut and Enrichment Culture of Gut Microbiota

Gut microbiota enrichment was conducted based on the method (Ma *et al*., 2025) [2]. 30 wax moth larvae (*Galleria mellonella*) were allocated into dietary treatment. Larvae were surface-sterilized by sequential immersion in 75 % (v/v) ethanol for 1 min, followed by sterile 0.85 % (w/v) NaCl for 1 min to eliminate epibiotic contaminants. After blotting residual liquid with sterile filter paper, dissections were performed under aseptic conditions in a Class II biosafety cabinet using autoclaved instruments. Intact guts were homogenized in 1 mL sterile 0.85 % (w/v) NaCl using a mortar pre-chilled to 4C to preserve bacterial viability. For enrichment cultures, 0.5 mL of gut homogenate was inoculated into 250 mL Erlenmeyer flasks containing 100 mL of LB medium supplemented with 1 g sterile PS powder. All flasks underwent aerobic incubation at 35C, a temperature suitable for Galleria gut microbiota activity with continuous orbital shaking at 160 rpm for 72 h (Ma *et al*., 2025) [2].

Isolation and Screening of Gut Bacteria from Gut Microbiota

To screen for gut bacteria with PS degrading capability, sterilized PS powder was incorporated as the sole carbon source into a minimalsalts medium (MSM) modified from (Das and Kumar, 2015) (0.2 g/L $MgSO_4 \cdot 7H_2O$, 1.0 g/L NH_4NO_3, 0.01 g/L $CaCl_2$, 0.5 g/L KH_2PO_4, and 1.5 g/L Na_2HPO_4 at pH 7.2). Subsequently, 5 mL of an enriched gut microbiota culture derived from Galleria mellonella larvae was inoculated into the medium. Cultures were incubated aerobically at 35C with agitation at 160 rpm for 10 days. To acclimatize the microbial consortium to PE, 5 % (v/v) of the inoculum from the preceding culture was transferred every 10 days into fresh MSM supplemented with PS as the sole carbon source. This sequential sub-culturing was performed for a total of two enrichment cycles.

To isolate single colonies, 1 mL of enriched culture was serially diluted from 10^{-1} to 10^{-6} in sterile 0.85 % (w/v) NaCl solution. 200 µL of each dilution were spread-plated onto PE-incorporated MSM agar plates (1.5 % w/v agar) and incubated aerobically. Isolates were further screened for enhanced PE-degrading potential. 15 mL of sterilized molten Luria-Bertani (LB) medium supplemented with 1.5 % (w/v) agar was dispensed into 90 mm diameter Petri dishes and allowed to solidify at room temperature. 200 µL of cell suspensions of pure isolates at varying dilutions were spread uniformly onto the surface of the solidified LB agar. Sterile PE film was then aseptically overlaid onto the inoculated agar surface. Control plates consisted of LB agar overlaid with PE film but without bacterial inoculation. To evaluate the intrinsic growth capacity of isolates on the medium in the absence of PE, cell suspensions were also plated directly onto LB agar without PE film overlay. Pure isolates exhibiting potential for PE degradation were cryopreserved in 20 % (v/v) glycerol at − 80C for long-term storage. Working cultures were maintained on MSM slants at 4C for routine laboratory use (Ali *et al*., 2025; Ma *et al*., 2025; Sanluis-Verdes *et al*., 2022; Wang *et al*., 2024) [2, 18, 22].

Identification of bacteria

Genomic DNA was extracted from bacterial cultures for 16S rRNA gene amplification via polymerase chain reaction (PCR). A 50 µL reaction mixture containing 2 µL of each universal primer (27 F and 1492 R; 10 µmol/L) (Lane, 1991), 2 µL DNA template, 25 µL Taq DNA Master Mix, and 21 µL ddH_2O was subjected to thermal cycling under the following conditions: initial denaturation at 94C for 5 min; 30 cycles of denaturation (94C, 50 s), annealing (55C, 48 s), and extension (72C, 1 min); with final extension at 72C for 10 min. PCR products were verified by agarose gel electrophoresis prior to commercial sequencing (Sangon Biotech, Shanghai, China). Resultant sequences were analyzed using the NCBI BLAST web interface (http://blast.ncbi.nlm.nih.gov; (Boratyn *et al*., 2013), with phylogenetic reconstruction performed in MEGA 6.0 using maximum likelihood methodology and 1000 bootstrap replicates (Duraimurugan *et al*., 2025) [5].

Result and Discussion

Physical Conformation of Consumption PS

After 30 days of feeding, the PE film from the experimental group displayed extensive damage, including multiple distinct perforations and fissures across its surface (Fig. 1). These findings confirm that wax moth larvae are capable of consuming and breaking down polyethylene. The weight of all polymer samples decreased progressively during the 25-day exposure to *Galleria mellonella* larvae, indicating continuous degradation was presented in Fig.2. Among the tested materials, the thermo black sheet exhibited the highest weight loss (68.0%), followed by thermocol (61.0%) and the thermo white sheet (58.0%). The greater degradation of the thermo black sheet suggests that differences in polymer composition, additives, and surface characteristics influence the susceptibility of the material to larval consumption and subsequent biodegradation. The observed reduction in polymer weight is consistent with previous reports demonstrating the plastic-degrading capability of *Galleria mellonella*. Ma *et al*. (2025) [13] reported significant weight loss of agricultural PE films following larval feeding and highlighted the synergistic role of gut-associated microorganisms in polymer degradation (Ma *et al*., 2025). Similarly, Ali *et al*. (2023) [1, 2] showed that bacteria isolated from the gut of plastic-feeding waxworms further degraded PE fragments generated during larval feeding (Ali *et al*., 2025) [2]. The progressive weight loss observed in the present study suggests a comparable mechanism, in which polymer fragmentation by *Galleria mellonella* facilitates microbial degradation within the gut. Overall, the substantial weight reductions (58–68%) demonstrate the potential of *Galleria mellonella* as an effective biological system for the degradation of polystyrene-based plastic waste.

Wax Moth Survival and Weight Loss

The variation in wax weight during the three independent experimental trials is presented in Fig. 3 (a–c). In general, the amount of wax decreased progressively throughout the 25-day experimental period, indicating continuous wax consumption by *Galleria mellonella* larvae during exposure to the different PS materials. The control groups showed only a slight reduction in wax weight, whereas the polymer-treated groups exhibited comparatively greater wax consumption, suggesting increased nutritional demand associated with polymer ingestion and biodegradation. In the first trial, the thermocol treatment exhibited a continuous decline in wax weight from 510 to 410 mg, while the thermo white sheet decreased from 490 to 470 mg. The thermo black sheet showed only minor variation during the experiment. In the second trial, the greatest reduction was observed in the thermocol treatment, where the wax weight decreased from 360 to 250 mg, followed by the thermo white sheet (410 to 270 mg) and the thermo black sheet (490 to 370 mg). Similarly, in the third trial, the thermo white sheet showed the highest wax consumption, with the weight decreasing from 480 to 280 mg, whereas the thermocol and thermo black sheet treatments declined from 550 to 460 mg

and 530 to 360 mg, respectively. Despite slight variations among the three experimental trials, the overall trend consistently demonstrated continuous wax utilization by the larvae during the degradation process.

The progressive reduction in wax weight indicates that *Galleria mellonella* larvae relied on wax as an important energy source while simultaneously consuming polystyrene. Since PS alone provides limited nutritional value, wax likely supplied the essential carbon and energy required to sustain larval growth, metabolism, and gut microbial activity during the biodegradation process. Consequently, continuous wax consumption may have enhanced larval feeding activity and supported the depolymerization of the polymer. These observations are consistent with the pioneering work of Bombelli *et al.* (2017) [17], who first demonstrated that *Galleria mellonella* larvae rapidly degraded PE while maintaining normal feeding activity (Moro *et al.*, 2017). They proposed that the degradation process involved both larval enzymes and associated gut microorganisms. Similarly, Ali *et al.* (2025) [2] reported that waxworms consumed PE together with their natural diet, suggesting that dietary nutrients support the metabolic processes required for plastic biodegradation (Ali *et al.*, 2025). Furthermore, Ma *et al.* (2025) [2] observed continuous feeding of *Galleria mellonella* larvae during PE degradation and identified gut bacterial strains capable of further degrading polymer fragments generated after larval ingestion (Ma *et al.*, 2025). Likewise, Ali *et al.* (2023) [1] demonstrated that efficient polyethylene-degrading bacteria isolated from waxworm guts significantly enhanced polymer degradation through enzymatic oxidation and depolymerization (Ali *et al.*, 2025) [2].

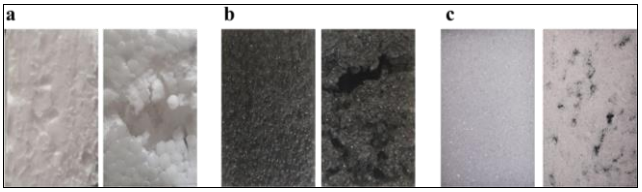


Fig. 1: Polystyrene and polyethylene physical consumption after treatment

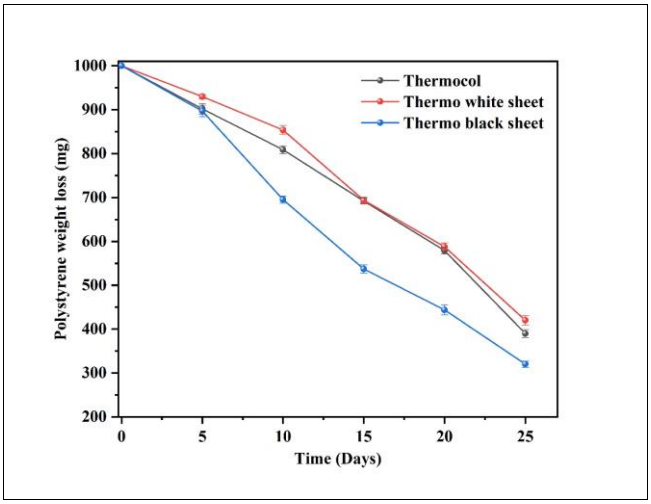
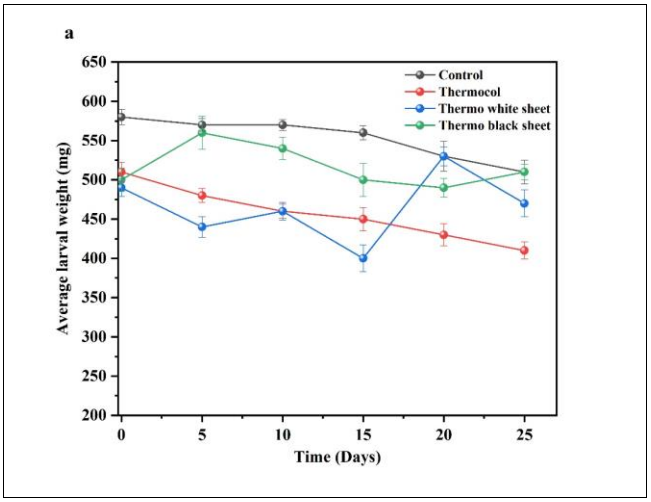
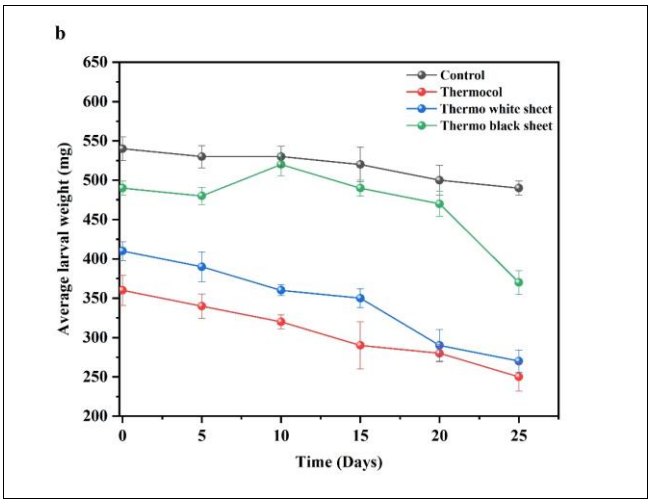


Fig 2: Average polystyrene and polyethylene weight by *Galleria mellonella* utilization





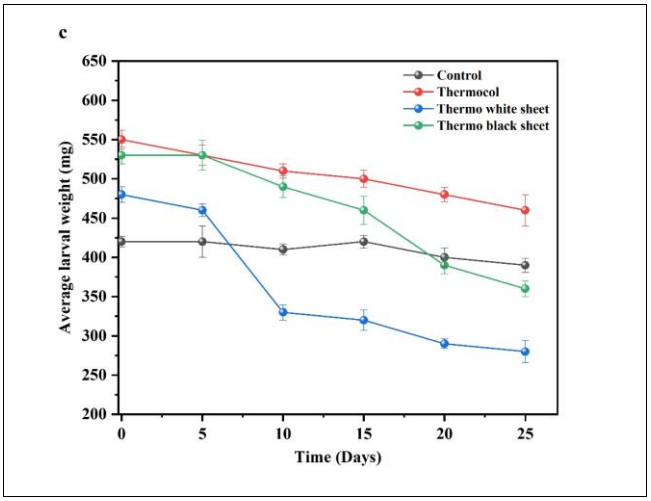


Fig 3: Average larval (*Galleria mellonella*) weight during Polystyrene utilization

Fourier Transform Infrared (FT-IR)

The FT-IR spectra of the polymeric samples are illustrated in Fig. 4, showcasing the distinctive transmission profiles recorded across the wavenumber range of 4000 to 500 cm^{-1}. Fig. 4a, corresponds to the pristine PS matrix, exhibiting its hallmark aromatic signatures. The low-intensity peaks emerging slightly above 3000 cm^{-1} are assigned to the sp^2 hybridized aromatic C-H stretching vibrations of the phenyl ring. The structural integrity of the PS is further verified by the sharp, dominant absorption band centered near 695 cm^{-1},

accompanied by a distinct peak near 750 cm^{-1}, which collectively signify the strong out-of-plane C-H bending and aromatic C=C ring deformation modes of a mono-substituted benzene ring 17. In contrast, Fig. 4b displays the typical spectral footprint of pure PE, characterized by a highly clean aliphatic profile. The spectrum is dominated by two exceptionally sharp and intense doublet peaks at 2916 cm^{-1} and 2848 cm^{-1}, which represent the asymmetric and symmetric stretching vibrations of the backbone methylene ($-CH_2$) groups, respectively. Additionally, the well-resolved peak at approximately 1465 cm^{-1} is attributed to the $-CH_2$-scissoring deformation, while the sharp peak near 718 cm^{-1} is assigned to the long-chain methylene rocking vibration, confirming a highly linear, unbranched PE structure (Ma *et al*., 2025).

Fig. 4c represents the blended or composite material, clearly revealing the simultaneous integration of both PS and PE chemical domains. The high-intensity aliphatic doublet at 2916 cm^{-1} and 2848 cm^{-1} remains highly prominent, indicating that the methylene backbone from the PE phase forms a major structural component (Spínola-Amilibia *et al*., 2023) [20]. Concurrently, the appearance of a broadened, strong absorption envelope in the fingerprint region between 1000 cm^{-1} and 1100 cm^{-1}, alongside the structural perturbations and peaks below 800 cm^{-1}, confirms the successful incorporation of the aromatic benzene ring features from the PS phase. Furthermore, a broad, shallow absorption band is visible in the 3300 to 3500 cm^{-1} regions of Fig. 4c, which can be attributed to localized surface oxidation, trapped atmospheric moisture, or complex interfacial hydroxyl (-OH) interactions developing between the two distinct polymer phases (Chen *et al*., 2022) [4]. Ultimately, the systematic coexistence of both the sharp aliphatic stretching doublets from the PE phase and the specific aromatic fingerprint bands from the PS phase within Fig. 4c provides definitive spectroscopic evidence of a successful chemical integration or physical blending between the two polymers.

Fig. 5 presents a comparative view of the gut fluid profiles, distinguishing between larvae maintained under a standard control (Fig. 5a) and those actively fed a plastic of PS and PE (Fig. 5b). Fig. 5a illustrates the intrinsic biological matrix baseline of the *G. mellonella* gut fluid without plastic ingestion. This profile is fundamentally characterized by a dominant, broad, and intense hydroxyl (-OH) stretching band centered around 3400 cm^{-1}, reflecting the highly aqueous environment of the digestive tract alongside endogenous mucus and proteins (Chen *et al*., 2022) [4]. In the fingerprint region, Fig. 5a exhibits a sharp peak at approximately 1640 cm^{-1}, corresponding to the Amide I (C=O stretching) vibration, and a secondary peak near 1400 cm^{-1} representing the Amide II (N-H bending and C-N stretching) configurations of biological enzymes and proteins (Chen *et al*., 2022 [4]; Ma *et al*., 2025). Concurrently, weak aliphatic bands can be observed near 2920 cm^{-1} and 2850 cm^{-1}, which stem naturally from endogenous fatty acids, lipids, or residual dietary structural waxes in the larval gut. Fig. 5b reveals profound chemical alterations and spectral transformations following the ingestion and digestion of the PS/PE plastic mixture, confirming aggressive *in vivo* polymer depolymerization. While the sharp, highly defined aliphatic backbone stretching peaks of pristine PE and the rigid aromatic signatures of pristine PS are completely dominant in raw polymer blends, their profile changes dramatically inside the ingested gut fluid. In Fig. 5b, the aliphatic $-CH_2$-asymmetric and symmetric stretching bands near 2924 cm^{-1} and 2853 cm^{-1} undergo distinct splitting, sharpening, and an alteration in relative intensity (Ali *et al*., 2025; Chen *et al*., 2022; Ma *et al*., 2025) [2, 4]. This direct modification of the carbon-hydrogen aliphatic backbone signals significant chain-scission of the PE long-chain structure, breaking high-molecular-weight polymers down into low-molecular-weight oligomers and intermediate fatty acids.

Simultaneously, the fingerprint region of Fig. 5b shows the evolution of multiple new, well-defined intermediate absorption bands between 1000 cm^{-1} and 1500 cm^{-1} that are absent or significantly weaker in the control gut fluid (Fig. 5a). The notable increase and broadening of the absorption complex around 1645 cm^{-1} to 1715 cm^{-1} strongly suggests the generation of carbonyl (C=O) functional groups, such as ketones, aldehydes, or carboxylic acids (Moro *et al*., 2017). This provides undeniable proof of an oxidative depolymerization pathway. This oxidation breaks down the highly stable carbon-carbon bonds of both the PE aliphatic chain and the PS aromatic system. Furthermore, the sharp depletion of the pristine PS aromatic ring signatures (above 3000 cm^{-1} and below 750 cm^{-1}) when compared to raw plastic baselines confirms that the *G. mellonella* gut environment, driven by specialized screening of involved gut bacteria, successfully targets and ruptures the resonance-stabilized benzene rings of polystyrene (Ali *et al*., 2025; Ma *et al*., 2025) [2]. The accumulation of oxidized intermediate metabolites alongside broken aliphatic segments embedded within the biological matrix of Fig. 5b confirms that plastic consumption is not merely a physical mastication process, but a true biochemical depolymerization mechanism catalyzed by the larval digestive enzymes and screening gut microbiomes.

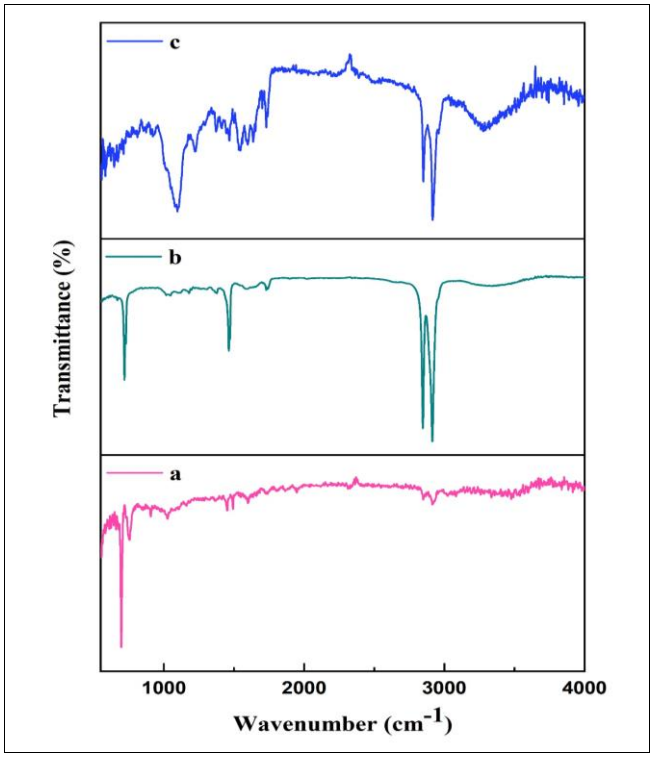


Fig 4: Fourier Transform Infrared of Polystyrene and polyethylene. a. Thermocol, b. Thermo White Sheet, c. Thermo Black Sheet.

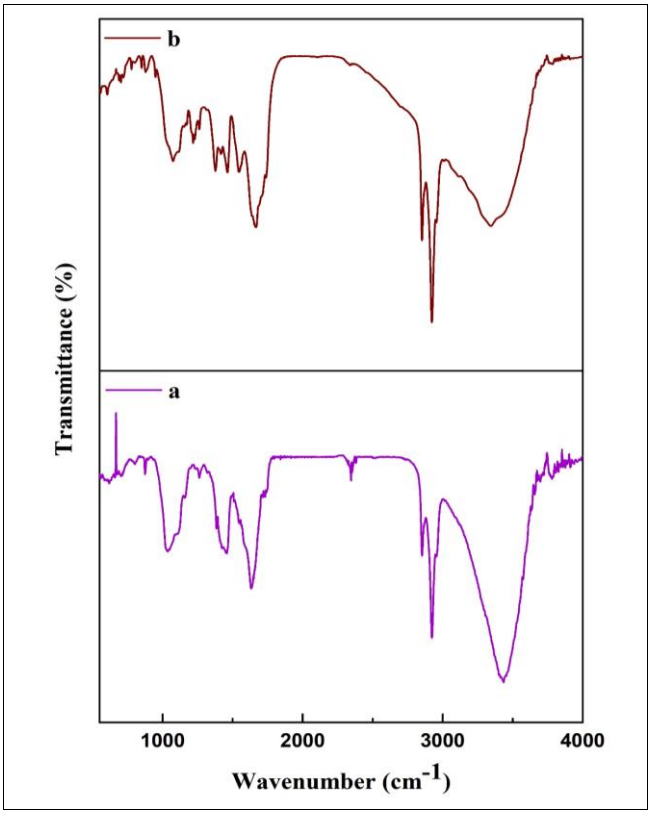


Fig 5: Fourier Transform Infrared of *G. mellonella* gut fluid profile. a. control *G. mellonella*, b. PS and PE treated *G. mellonella.*

Protein Isolation

Protein extracts obtained from the saliva of *Galleria mellonella* larvae were analyzed by SDS-PAGE to examine the presence of extracellular proteins potentially associated with PS depolymerization (Fig. 6). The electrophoretic profile revealed detectable protein staining in all saliva samples, confirming the successful extraction of salivary proteins. However, the gel exhibited broad protein staining with limited resolution of individual protein bands, suggesting the presence of complex protein mixtures and proteins of relatively low abundance. Previous studies have suggested that *Galleria mellonella* secretes oxidative and hydrolytic enzymes capable of initiating the modification of synthetic polymers. The proposed that enzymes present in larval secretions contribute to the initial oxidation of PE prior to microbial degradation. Likewise, the salivary secretions from waxworms accelerate PE oxidation, supporting the hypothesis that insect-derived enzymes participate in the early stages of polymer breakdown (Vital Vilchis & Karunakaran, 2024) [21]. More recently, studies investigating waxworm-associated biodegradation have emphasized that polymer depolymerization is mediated through the combined action of larval enzymes and gut microorganisms rather than by microbial activity alone. The detection of salivary proteins in the present study supports the proposed host–microbiome degradation mechanism, in which salivary enzymes may facilitate the initial surface modification and depolymerization of polystyrene, thereby increasing its accessibility to gut-associated microorganisms for subsequent biodegradation. Although SDS-PAGE provides preliminary evidence for the presence of salivary proteins, identification of the active enzymes responsible for PS depolymerization will require detailed proteomic and enzymatic analyses.

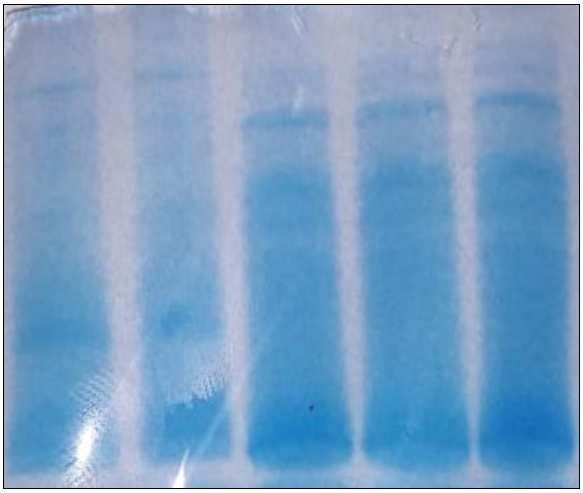
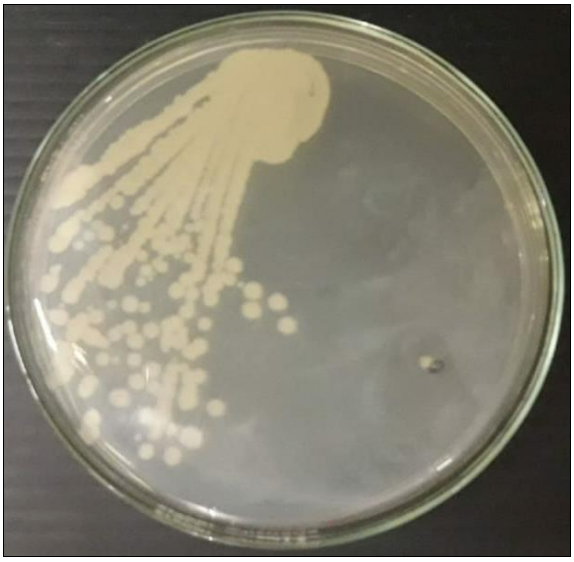
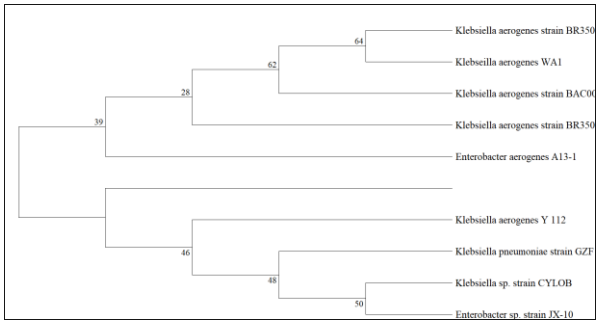


Fig 6: SDS-PAGE analysis of proteins isolated from wax worm saliva.



a



b

Fig 7: Colonies from subculture using LB agar. a. *Klebsiella aerogenes* WA1 pure strain, b. Phylogenetic tree relationship of *Klebsiella aerogenes* WA1

Isolation and Identification of Bacteria

The biochemical characteristics of the isolate (Table. 1) were consistent with those of WA1 (Fig. 7a). The isolate formed large (2–4 mm), white colonies, was negative, rod shaped, and motile bacteria. It exhibited positive reactions for Voges–Proskauer, citrate utilization, catalase, glucose, lactose, adonitol, arabinose, sorbitol, lysine utilization, ornithine utilization. Negative reactions were observed for indole, methyl red, oxidase, phenylalanine deaminase, and H_2S production. The ability to utilize multiple carbohydrates

and citrate, together with positive nitrate reduction and urease activity, demonstrates broad metabolic versatility and physiological adaptability. These traits are advantageous for survival in polymer contaminated environments, where diverse carbon sources and intermediate degradation products are present. The 16S rRNA sequence exhibited a 99.05 % similarity to *Klebsiella aerogenes* WA1 and has been deposited in the NCBI GenBank, acquiring the accession number PX855136.1 (Fig. 7b). Combined with 16S rRNA gene sequencing, the biochemical characterization confirmed the isolate as *K. aerogenes* WA1 and suggests its potential involvement in the biodegradation of polystyrene-derived compounds (Duraimurugan *et al*., 2025) [5].

Table 1: Biochemical test analysis for potential bacterium strain of *Klebsiella aerogenes* WA1.

S. No	Biochemical test	Result
1.	Colony morphology (size)	2 to 4 mm
2.	Colour	White
3.	Growth temperature range (°C)	25 – 42°C
4.	Gram staining	Negative (rod shape)
5.	Motility	Positive
6.	Indole Production	Negative
7.	Methyl Red Test	Negative
8.	Voges-Proskauer Test	Positive
9.	Citrate Utilization	Positive
10.	Oxidase test	Negative
11.	Catalase	Positive
12.	H_2S production	Negative
13.	Glucose	Positive
14.	Lactose	Positive
15.	Phenylalanine deaminase	Negative
16.	Adonitol	Positive
17.	Arabinose	Positive
18.	Sorbitol	Positive
19.	Citrate utilization	Positive
20.	Lysine utilization	Positive
21.	Ornithine utilization	Positive
22.	Urease Test	Positive
23.	Nitrate Reduction	Positive

Conclusion

This study successfully demonstrated that *Galleria mellonella* larvae serve as an efficient biological system for the depolymerization of highly resilient polystyrene and polyethylene materials. Over a 25-day period, the larvae achieved substantial polymer mass reductions up to 68.0%, directly influenced by composition and surface characteristics. Spectroscopic analysis via FT-IR confirmed that plastic utilization transcends mere physical mastication, driving a true biochemical transformation that breaks down stable carbon backbones and aromatic rings into intermediate carbonyl metabolites. SDS-PAGE of salivary secretions highlighted the role of insect-derived enzymes in early-stage polymer oxidation. Furthermore, the isolation of *Klebsiella aerogenes* WA1 from the gut microbiota uncovered a versatile bacterial partner capable of utilizing diverse degradation intermediates. Together, this synergistic host-microbiome relationship provides an essential biochemical baseline for scaling up sustainable, eco-friendly biotechnological configurations to manage global synthetic plastic waste.

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