GALLERIA MELLONELLA AND PE DEGRADATION

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INTRODUCTION

The accumulation of microplastic in the ecosystem is a significant concern worldwide. The presence of microplastic was reported in commercial seafood, drinking water, plankton, whales, and other marine organisms. Plastic fragments less than 5 mm are categorized as microplastics. Microplastics are classified into primary and secondary based on their size and formation. The primary microplastics are minute fragments made for commercial purposes, such as cosmetics, fishing nets, and fabrics. Secondary microplastics are fragments from the breakdown of oversized plastic products, such as water bottles, fishing nets, plastic bags, microwave containers, and other products. Several environmental factors, particularly sunlight and ocean waves, are responsible for the breakdown of these oversized plastics. Ever-increasing plastic usage and microplastic deposition lead to several hazardous ecological conditions such as toxic effects on flora and fauna, marine and soil contamination, releasing poisonous chemical constituents into the environment, etc. All these aroused an interest in the field of plastic or polymer degradation

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Fig 1: Plastic waste accumulation

The most prevalent synthetic polymers in the environment are polyamide (PA), polyethylene (PE), polystyrene (PS), polypropylene (PP), and acrylic (AC). PE, PP, and PS are the most abundant microplastics in the ecosystem, with which 92% is occupied by polyethylene (PE) and polypropylene (PP). The degradation of these materials is the current concern as they account for most plastic waste.

Thermal, photo-oxidative, mechanical, biological, and chemical methods are used in polymer degradation. Bacteria, fungi, and algae are also engaged in polymer degradation. Long-chain hydrocarbon metabolism is considered the significant stage in polymer degradation. Mechanisms regulating the degradation by microbes are still unknown, which drives the researchers to focus on the ability of insects in the metabolism of long-chain hydrocarbons. A wax moth species, *Galleria mellonella*, was reported for the potential results in the degradation of PEs. The habitats

and feeding behavior of *G. mellonella* larvae are highly distinct from those of other moth larvae, which consume honey, beeswax, and the pupae's skin while residing on honeycomb in beehives. Unique behavioral and chemical methods are needed to use these substances as an energy source. The biosynthesis of beeswax is carried out by bees and is made up of hydrocarbons, fatty acids, and wax esters. Interestingly, the structure of beeswax is similar to that of PE. Thus *G.mellonella* is considered to investigate the potentiality for metabolizing PE.

Galleria mellonella and Metabolism of Long-Chain Hydrocarbon Beeswax

Galleria mellonella, belongs to the Pyralidae moth family, having four stages in the life cycle - Egg, larval, pupae, and adult. Typically, the moth has four to six generations per year and lays its eggs in the early spring. They can be found where honeybees are cultivated. *G. mellonella* larvae parasitize honeybees, ingest pollen, cast skins of bee larvae, midribs of wax combs, trace amounts of propolis, and honey. PE is structurally similar to beeswax. The beeswax metabolism has a distinct degrading mechanism, making G. mellonella an attractive candidate for PE metabolism.

A study entitled “The *Galleria mellonella* Hologenome Supports Microbiota-Independent Metabolism of Long-Chain Hydrocarbon Beeswax” reported the degradation of beeswax in *G.mellonella* takes place even after the removal of intestinal microbiota by treating it with a mixture of antibiotics. For this, *G. mellonella*'s genome was investigated for the presence of genes encoding for the enzymes that have the potential to degrade long-chain hydrocarbons.

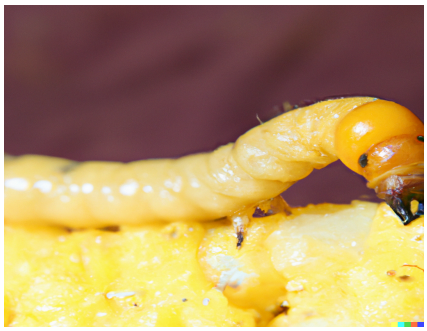

Fig 2: Larvae degrading beeswax

Also, the transcriptional study of the intestinal microbiota indicates that bacterial enzymes support the breakdown of short-chain fatty acids. Wax metabolism was investigated at the enzymatic and genetic levels in *G. mellonella,* which may aid in developing strategies for the bio-degradation of PE contaminants. The second instar larvae of G. mellonella were ingested with antibiotics to eliminate the intestinal flora in order to understand the role of the same in wax degradation. Groups of *G. mellonella* with and without intact intestinal microbiota were then compared for growth. Interestingly, constant body weight was expressed by the beeswax-fed group and a progressive increase in nutrition-rich diet groups even in the absence of intestinal flora. The body weight of both groups gradually declined after starvation. However, when given nutrition-rich food, the antibiotic-treated group's body weight dropped compared to the untreated group, demonstrating that the microbiota is involved in the breakdown of nutrition-rich food.

It was observed that *G. mellonella* could digest beeswax without the assistance of the gut flora. Therefore the body weight variations of *G. mellonella* were used to evaluate the decomposition of PE.

To reduce the impact of previously supplied food, all larvae were kept under starvation conditions for three days at the start of the experiment. As a result, after 15 days, the weight of the PE-treated G. mellonella remained constant. It gradually increased in the group fed a diet enriched in nutrients and progressively reduced in the starved group. To explore the role of the intestinal microbiota in the breakdown of PE, the gut microbiota was eliminated by feeding second-instar larvae (an antibiotic cocktail). Interestingly, the weight of the PE-fed group stayed constant irrespective of the intestinal microbiota. In contrast, the body weight of the group consuming nutrient-dense food steadily increased, and the weight of the group starving gradually declined.

13 insects were included in the orthologous gene comparison in order to identify the gene families involved in the breakdown of beeswax in G.mellonella, which is involved in the synthesis of long-chain fatty acids that are missing in other moths. *Galleria mellonella, Papilio xuthus, Bombyx mori, Amyelios transitella, Plutella xylostella*, and *Tetranych usurticae* were analyzed for comparison as an outgroup. Based on Gene Ontology (GO) and the KEGG pathway, *Galleria*

mellonella-specific genes mainly belong to peptidase-related gene families and sensory perception. Additionally, 333 and 340 gene families underwent significant contraction and expansion, respectively. Hydrolase activity, cytochrome P450 (CYP), and unsaturated fatty acid biosynthesis were overrepresented in *Galleria mellonella* among the expanded gene families. The pathways involved in fatty acid metabolism and biosynthesis, as well as peroxisome-proliferator-activated receptor (PPAR) signaling, were notably enriched and highly expressed, indicating the breakdown of long-chain hydrocarbons in *Galleria mellonella* and the usage of long-chain fatty acid derived from the wax.

LeMoine et. al(2020), hypothesized that it is important to note that *G.mellonella* is more than just a reservoir for microbes that break down PE; this process also has an immediate impact on its physiology. These experiments initially demonstrated that caterpillars deliberately and voraciously consume LDPE. The sequencing of RNA from the intestinal tissues provides mechanistic insights into the breakdown of LDPE. Particularly, PE-fed caterpillars maintain their usual digestive processes but exhibit a unique transcriptional profile associated with increased fatty acid metabolism. In Addition, the caterpillars fed LDPE alone kept significant lipid levels, which could be used as fuel later in their growth. The study found that the larvae's enzyme activity for alcohol dehydrogenase and lactate dehydrogenase is enhanced by the PE diet, which would likely improve their capacity to break down long-chain fatty alcohols, indicating *G. Mellonella* plays a significant role in the biodegradation of plastics, efficiently employing LDPE as the only source of nutrients.

Fig 3: Larvae degrading PE

100 caterpillar larvae of the *G. mellonella* species were fed with LDPE. Their daily intake was measured over 72 hours and confirmed that most fifth-instar caterpillars readily ingest LDPE, and most of the larvae consistently consumed LDPE. The researchers annotated each differentially expressed transcript according to its biological function to understand more about the changes in functional pathways. It is evident that the PE diet alters the expression of metabolic genes in the intestines. About 21% of the transcripts belonging to the most prevalent metabolic categories are related to amino acid metabolism (13%), carbohydrate metabolism (11%), and fatty acid metabolism (17%). It's interesting to note that the majority of transcripts engaged in fatty acid metabolism (73%) were elevated. The upregulated transcripts encode proteins that are considered to be involved in lipolysis, oxidation, and lipid synthesis. The fat body enzymes ADH and ALDH (contribute to β-oxidation) are found to be implicated in this pathway and subjected to further studies. Researchers noticed that ADH activity doubled in caterpillars fed PE while ALDH activity remained unaltered. Thus, the caterpillars have a stronger ability to digest alkanes, putative

byproducts of low-density polyethylene decomposition, even on a diet low in nutrients. This could eventually improve their ability to metabolize fatty acids.

The role of gut microbiota of *Galleria mellonella* in the biodegradation of PE

Barrionuevo et. al (2022), explains the alterations in gut microbiota due to the consumption of PE and PS by *G. Mellonella.* For this study, collected larvae were kept under nutrient conditions to ensure proper growth. To extract the digestive tubes, the larvae were dissected. The entire guts, including their contents, were used for the analysis. Analysis was done using three samples of *G mellonella* species, fed with polyester, PE, and beeswax. The Dice index approach was employed to gauge the co-occurrence of bacteria and fungus across the various plastic treatments. Using NMDS (Non-Metric Multidimensional Scaling), the distinctions between bacterial and fungal populations were made visible and the statistical significance between sample groups was evaluated using the PERMANOVA test. This study concluded that bacterial and fungal species were involved in plastic degradation and an integrated or symbiotic relationship was found. It was established through beta diversity (i.e., structure) comparisons of gut bacterial communities based on Operational taxonomic units (OTUs) and utilizing NMDS ordination that the bacterial communities in beeswax-fed and plastic-fed larvae had different compositions. Beta diversity analyses of the fungal microbiota's composition revealed negligible to no variations across treatments. *Proteobacteria, Pseudomonas, Lactobacillus, Fibrioacteria, P. citronellolis, Bifidobacterium, Butyricimonas, Alistipes, Fibrobacter*, and *Enhydrobacter* were the most prevalent bacteria found during the plastic digestion. *Ascomyota* was discovered to be the fungi in the highest concentration, followed by *Baridomycota* in an average concentration. The symbiotic interaction or integration of bacteria and fungi paves the way for joint metabolic processes.

Bryan J. Cassone et. al (2020), investigated the early stages of polyethylene breakdown and the function of the caterpillar gut microbiota. They manipulated and characterized the microbiome using various molecular and physiological methodologies. In the experiment, *G. mellonella* was grouped into honeycomb-fed, polyethylene-fed, and starved. A qPCR technique was used to quantify the bacterial count present in the intestinal DNA of *Galleria mellonella*. Using 16S amplicon sequencing, they identified the gut bacterial populations of *G. mellonella* linked to various feeding patterns. A total of 30 samples were taken. 3905 OTUs were found by QIIME analysis (Quantitative Insights Into Microbial Ecology). Bacteria were filtered down to the phylum level to identify the bacterial communities that are the most abundant in the caterpillar intestines. The majority (87.5%) of the organisms were proteobacteria, cyanobacteria (4.4%), and firmicutes (4.1%). Proteobacteria and Firmicutes were the only phyla consistently found in all samples. Researchers found 89 genera at the genus level, with the three most common including *Escherichia shigella* (28.5%), *Asaia* (20.3%), and *Acinetobacter* (13.1%). *Brevundimonas, Asaia, Aeromonas, Rhizobium, Pseudomonas, Vibrio, Shewanella, Ralstonia, Bacillus, Acinetobacter, Strenotrophomonas, and Escherichia-Shigella* were the 12 genera that were detected similarly in 90% of the samples. At the genus level, the comparisons of the relative bacterial abundance of the starved, honeycomb-fed, and polyethylene-fed groups were performed. The abundance of the genera *Asaia, Gluconacetobacter, Swaminathania, and Acetobacter decreased while Pseudocitrobacter, Escherichia coli, Serratia, Pantoea, Citrobacter, and Salmonella* showed a relative increase in plastic-fed caterpillars. Bacterial colonies isolated from the guts of the PE-fed group were cultured in a carbon-free media. Then they extracted the DNA and subjected it to 16s sequencing for identifying the microbes. According to analysis, the species belongs to the *Acinetobacter* genus. This genus, in particular, contains only aerobic species because the breakdown of PE requires oxidation of the stable carbon-carbon double bond. The researchers hypothesize that the genus is extensively involved in biodegradation as these bacteria can survive using PE as their only carbon source.

Impact of salivary glands proteome in *Galleria mellonella* on Polyethylene

Asal Peydaei et. al (2020), primarily focused on the role of the proteome in the salivary gland of the *Galleria mellonella* species on the breakdown of PE. In this study, the larvae of *Galleria mellonella* were introduced to low-density polyethylene for 12 hours. To obtain intact salivary glands, the last instar larvae—whose were first given a cold anesthetic. Larvae were cleaned entirely with 100% ethanol, followed by three washing stages. Using a glass tissue grinder,salivary glands and intestinal tissue were homogenized.

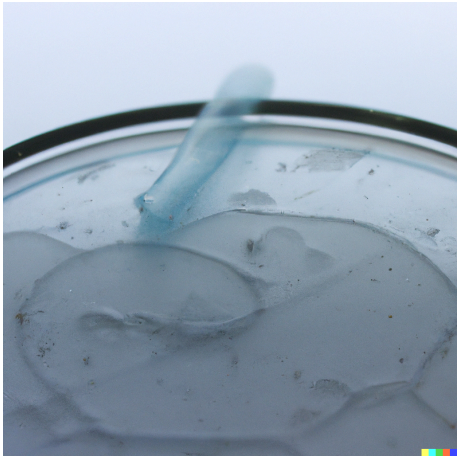

Fig 4: Salivary gland suspension

After incubation with PE, salivary gland suspension led to hole formation in LDPE. Large holes, deposits of debris, and fibers were visible in the larvae exposed to PE coupons. The KEGG database was used for basic functional annotation, and 341 proteins were found. 47 enzymes were involved in this degradation process, including oxidoreductases, transferases, and

hydrolases. Hole formation on the surface of FE coupons was observed after treating it with the salivary gland solution of *Galleria mellonella*. Infrared spectroscopy in transmission mode was used to identify the changes in physicochemical properties and new peaks were identified in treated samples. The result indicates a change in physicochemical properties.

After exposure to PE, there is a greater quantity of GST (glutathione S transferase) in the salivary glands, which indicates a metabolic change in the cells. Multiple inositol polyphosphate phosphatases 1 were upregulated in the salivary gland tissue, indicating the biochemical changes due to their exposure to PE. Species of proteins in salivary glands were found using a differential proteomic technique. It was discovered that carbohydrate metabolism, fatty acid metabolism, ribosome, and RNA transport were some of the biochemical processes related to the larval salivary glands. The alpha subunit of a trifunctional enzyme and apolipophorins were elevated in the PE-fed tissue sample. Finally, it was discovered that acetyl Co-A acyltransferase and trifunctional enzyme subunit beta are present in saliva. Additionally, a noticeable increase in the expression of fibrohexamerin was seen when PE was consumed.

Discussion

The most widely used synthetic polymer in the world is PE. Due to its resistance to natural degradation, it has accumulated in our landfills and the environment. It is crucial to develop efficient methods to accelerate the biodegradation process, and this has been the topic of extensive research in recent years. *G. mellonella* consumes beeswax from honeybee hives as part of its normal lifestyle. The significant structural similarity between PE and beeswax suggests that *G. mellonella* employs a similar degradation method. It was discovered that G. mellonella can produce long-chain fatty acids by metabolizing long-chain hydrocarbon wax without the aid of microbes. The researchers hypothesized that the great wax moth is expected to degrade natural and nutrient-rich lipids using a full complement of enzymes. Analyzing wax metabolism at the

enzymatic and genetic levels in *G. mellonella* may aid in developing strategies for the bio-degradation of PE contaminants. Reported studies mention that the salivary glands of G.mellonella play an important role in PE degradation. The researchers analyzed the surface changes of PE after the mastication, which resulted in the formation of holes and degradation intermediate, suggesting that *Galleria mellonella*'s salivary glands might aid in the breakdown of PE. FTIR and SEM analysis of the consumed PE coupons exhibited structural surface alterations. 481 proteins were found in the salivary glands after proteomic analysis, and 13 proteins showed differential expression between the PE-consumed larvae and those that didn't. There is still much to learn about the function of the intestinal flora of *Galleria mellonella* and the significance of host-microbiota associations in the breakdown of long-chain hydrocarbons. From various physiological, microbiological, and biochemical experiments, it is reported that the most frequently occurring bacteria discovered during the digestion of the PE included *Proteobacteria, Pseudomonas, Lactobacillus, Fibriobacteria, P. citronellolis, Bifidobacterium, Butyricimonas, Alistipes, Fibrobacter, Enhydrobacter*, and *Acinetobacter*. Compared to the other feeding regimes, PE-fed larvae had an abundance of *Acinetobacter sp*. that was almost 5000 times higher, which has been repeatedly associated with PE degradation. Thus it can be concluded that *G. mellonella* is a promising candidate for investigating its ability to degrade PE.

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