Review

Latest Trends on Insect Larvae, Their Enzymes and Their Gut Microbiota to Degrade PlasticLatest Trends on Insect Larvae, Their Enzymes and Their Gut Microbiota to Degrade Plastic

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Review

Latest Trends on Insect Larvae, Their Enzymes and Their Gut Microbiota to Degrade Plastic

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Simple Summary: Plastic pollution represents a great environmental problem around the world. Less than 10% of the plastic ever made is being recycled and the rest is either incinerated, accumulated in landfills or thrown into the natural world where it becomes a severe health threat for animals and humans. Thus, novel, efficient and environmentally-friendly solutions are urgently needed. In this regard, the most novel scientific breakthrough was around 2014 when scientists discovered the incredible ability of some insect larvae to feed on plastic. This review covers all the larvae with this ability reported since then, especially the waxworm, mealworms and superworms, as well as the first adult insect "palstivores": the termites. It also reports on their gut microorganisms and enzymes that contribute to plastic uptake.

Abstract: Plastic pollution is one of the biggest current global threats to the environment given that petroleum-based plastic is recalcitrant and can stay in the environment for decades, even centuries, depending on the specific plastic type. Since less than 10% of all the plastic ever made is recycled and other solutions (such as incineration or landfill storage) are pollutant methods, new environmentally friendly solutions are needed. In this regard, the latest biotechnological discovery on this topic is the capability of insect larvae to use plastic polymers as carbon feedstock. This present review describes the most relevant information on insect larvae capable of degrading plastic, mainly from *Galleria mellonella* (Fabricius, 1798), *Tenebrio molitor* (Linnaeus, 1758) and *Zophobas atratus* (Fabricius, 1776) but also adds new information about other less studied "plastivore" insects such as the termites. The review covers from the very first work describing plastic degradation by larvae in 2014 all the way till the very latest research available (till June 2024) focusing on the identification of a wide variety of plastic-degrading microorganisms isolated from larvae's gut to date and on the understanding of the potential molecular mechanisms present for the degradation to take place. It also describes the latest discoveries which include the identification of novel enzymes from the waxworm's saliva.

Keywords: plastic degradation; plastivore larvae; waxworms; mealworms; superworms

1. Introduction

Plastic pollution represents one of the major global challenges of this era and yet, it has been reported that only the 9% out of the 9 billion tons of plastic ever produced had been recycled [1]. As plastic pollution accumulates in the environment at alarming rates new and more effective solutions to address this problem are needed. A novel biotechnological approach is to degrade this plastic into its original monomers using microorganisms and their enzymes so that these monomers can be potentially later upcycled into new high-value products [2]. An even newer biotechnological trend is the use of insect larvae for the same purpose [3].

Even though, observation of common insect pests such as *Rhyzopertha dominica* (commonly referred to as the lesser grain borer) and *Tenebroides mauritanicus* (Cadelle beetle) penetrating packaging materials has been observed since the 1950's, the main concern at the time was to protect packaged food from these invaders [4,5].

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The first scientific report suggesting the revolutionizing idea of using insects to fight plastic pollution did not come until 2014 using the larvae *Plodia interpunctella* [6,7]. This report was followed by the first report of full PS mineralization into CO_2 by *T. molitor's* larvae [8] and the first observation of the waxworm (*Galleria mellonella's* larvae) degrading PE in 2017 [9]. The same year, this discovery hit the news and was published in National Geographic to reach the general public where Dr. Federica Bertocchini was acknowledged as the discoverer [10]. She and her research group in Spain , later in 2022, identified 4 novel waxworm saliva enzymes responsible for this degradation and named them as Demetra, Cibeles, Ceres and Cora which are the first plastic-degrading enzymes ever isolated from an invertebrate organism [11,12]. Other relevant events also include the introduction of the term "plastivore" to describe insect larvae or any other organism capable of using plastic as carbon feedstock [13] and the report of plastic-degrading yeasts from adult termites gut [14]. All these important events are illustrated in **Figure 1** in chronological order.

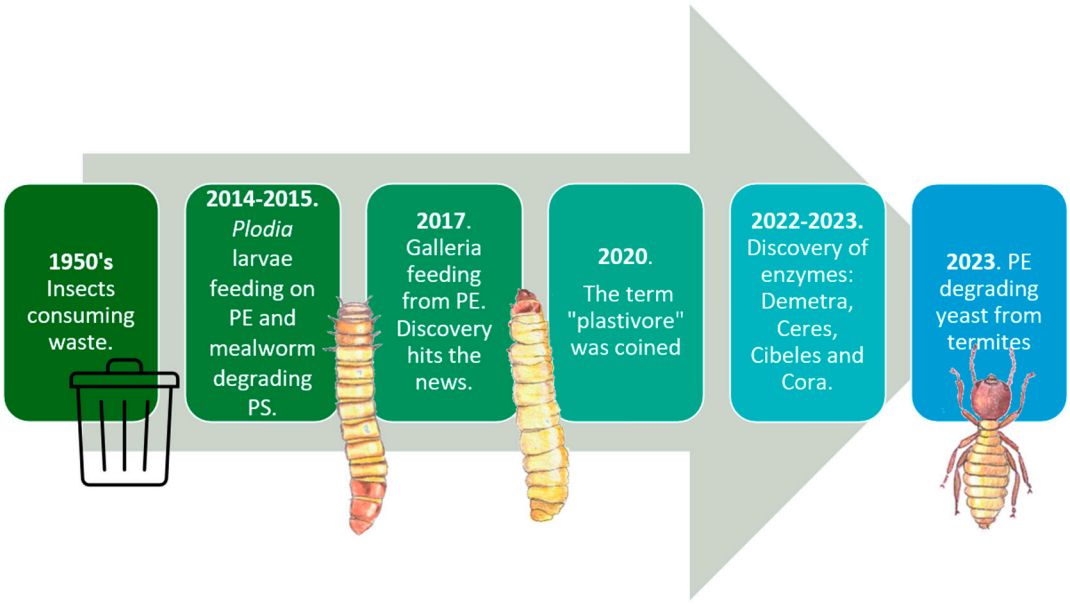

Figure 1. Main historic events related to insects degrading petroleum-based plastic.

The number of scientific papers related to plastic-eating larvae is growing every year and yet, the number of papers to date are still scarce. Till June 2024 only 366 papers resulted from the keyword search "insect larvae to degrade plastic" from PubMed and only a couple of them are literature reviews. The first literature review published ever that summarizes insect degradation of plastics was in the year 2021 [15] and the latest is from in 2024 [16] and there are very few reviews in between [3,17]. This review expands the knowledge on plastivore larvae even more and includes the latest research information to date (Till June 2024). Specifically, it is the most comprehensive and thorough literature review about the waxworm (*Galleria's mellonella* larvae) published to date, but it also reviews mealworms (*Tenebrio molitor*) and superworms (*Zophobas atratus*), focusing on the identification of plastic-degrading microorganisms that have been identified in these larvae's gut and on the understanding of the potential molecular mechanisms present on the larvae for the degradation to take place. It also describes the latest discoveries which includes the identification of novel enzymes from the waxworm's saliva and the first potential adult plastivores: the termites.

2. Plastic Pollution

Around 8300 million metric tons of virgin plastics had been produced by 2017 from non-renewable petrochemical feedstocks and only a small proportion has been recycled or incinerated. It is estimated that if current trends continue, approximately 1200 metric tons of plastic waste will accumulate in landfills or in the natural environment by 2050. Whilst this analysis includes

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thermoplastics, thermosets, polyurethanes (PURs), elastomers, coatings, and sealants, it mainly focuses on the most abundant resins and fibres: high-density polyethylene (HDPE), low-density and linear low-density PE (LPE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), poly-ethylene terephthalate (PET), and PUR resins; and polyester, poly-amide, and acrylic (PP&A) fibres [18].

Molecular structure of these common plastic resins (81% plastics) along with their density, crystallinity, life span in the environment [19,20], common uses and demand distribution by resin type in the year 2018 in Europe [21] are reported in **Figure 2.** The rest 19% of resins that are not presented in **Figure 2** include PTFE for cable coatings in communications, PMMA for touch screens, PC for roofs and eye glasses, PBT as optical fibre , ABS for keyboards, LEGO toys and others [21]. Half-life of PU is still unknown [22].

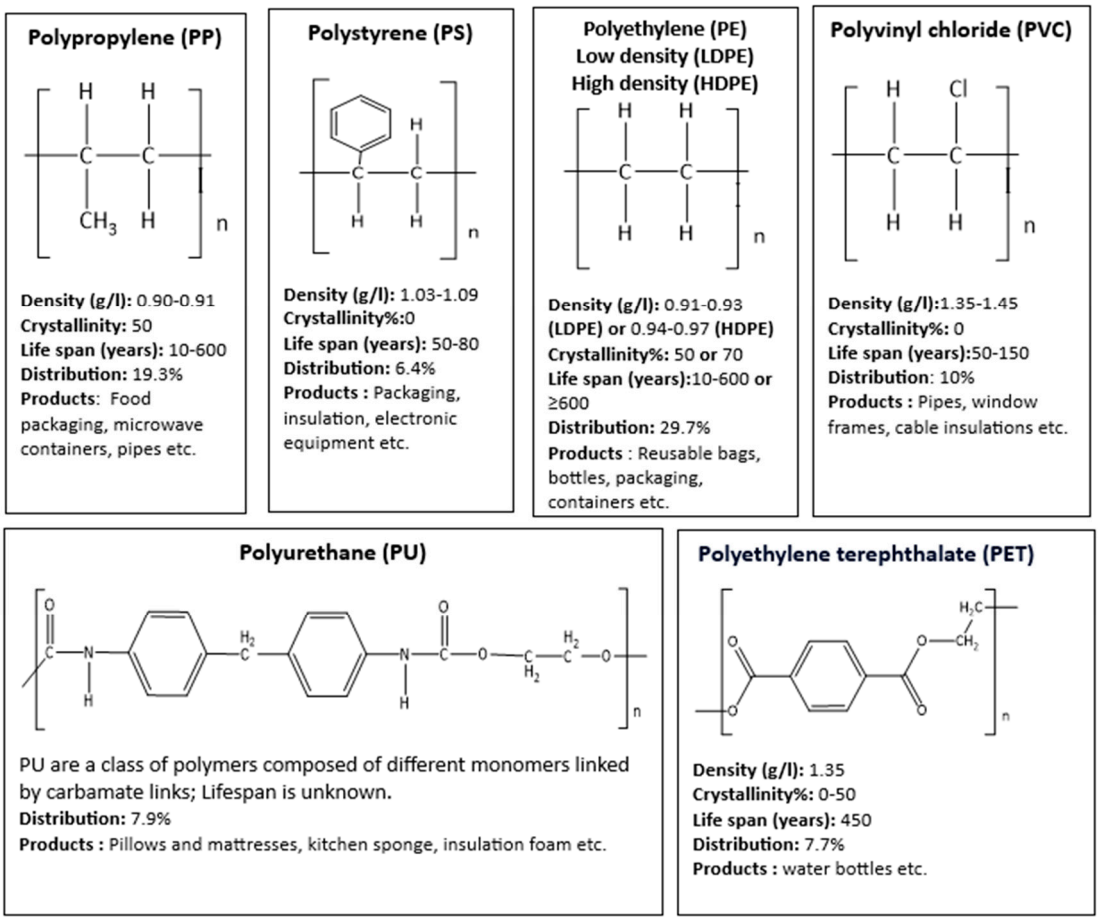

Figure 2. Structure, density, crystallinity, life span and common uses if the most abundant plastic resins [19,20]. Half-life of PU is still unknown [22].

The ubiquitous distribution of plastic contamination in both terrestrial and marine environments has identified the phenomenon as a key geological indicator of the Anthropocene [23] which is an epoch of time defined by the domination of humanity over surface geological processes [24]. Plastic pollution is a serious issue that affects animal and human life. In the sea, for example, it has been reported that over 260 of marine species including mammals, seabirds, turtles and invertebrates become entangled or ingest plastic waste which impair their movement, feeding and reproductive capabilities or cause internal lacerations and ulcers ultimately resulting in death [25].

One of the major problems of plastic pollution is that incomplete degradation of plastics in the environment leads to accumulation of microplastics (particles of less than 5 mm) rather than complete mineralization of the material [26,27]. In humans, microplastics enter the human body through inhalation, ingestion and dermal contact and although more studies related to the human health hazard related to microplastics are needed, some potential hazards include metabolic

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disorder, inflammation, oxidative stress and multisystem adverse effects (respiratory and digestive) [28,29] as well as potential male and female fertility issues [30]. It can also induce DNA damage and oxidative stress which in turn leads to carcinogenesis [31]. Unfortunately, this threat is now imminent as there has been microplastics found in the marine environment, soil, in drinking water and even in commonly consumed food like fish, vegetables, sugar, honey and salt [32]. Studies also show that these microplastics are indeed present in human blood [33], stool, lungs, placenta, internal organs [34] and in the reproductive system [35].

Some solutions for this problem include recycling, incineration or disposal of plastics in designated landfills. Unfortunately, even though, plastic recycling has existed for decades, scientists estimate that only the 9% is recycled globally, some 12% is incinerated and a greater 79% is either in landfills or in the environment [36]. These numbers are surprising because, in principle, most plastics are recyclable, however there are many factors that represent a barrier towards recycling. For example, contamination of the items in the form of labels, food or mixing of the recycling bins with other products may inhibit recycling entirely. Some plastic items are a complex bend of chemical additives which are harmful for human health which makes recycling dangerous for workers and other items are so unique that they cannot be recycled together [36].

Incineration is a method that could permanently degrade and eliminate plastic waste, nevertheless, the residual ashes from municipal incinerators are still a source of microplastics [37] moreover, the process releases toxic volatile organic compounds to the air. These compounds include chlorinated and aromatics such as benzene and chloroform [38]. Consequently, more environmentally friendly solutions are needed.

3. Degradation of Plastics-A General Perspective

Degradation of plastics can take place because of abiotic and biotic factors present in the environment as shown in **Figure 3.** When the biotic degradation results in fragments or microplastics, the process is considered bio-disintegration of the polymer, whereas if the polymer is entirely assimilated and mineralized inside the cell, it is considered biodegradation [26]. Alternatively, a polymer is considered as being degraded by abiotic factors when there is any change that may cause depolymerization, change in physical properties, alteration of chemical composition, mass loss or complete mineralization into carbon dioxide and water [39,40].

Overall mass loss is the parameter commonly used to study plastics degradation rates [40]. These rates are hard to estimate because of the wide variety of factors affecting the process in different environments but some plastic life-span estimations are presented in **Figure 3.**

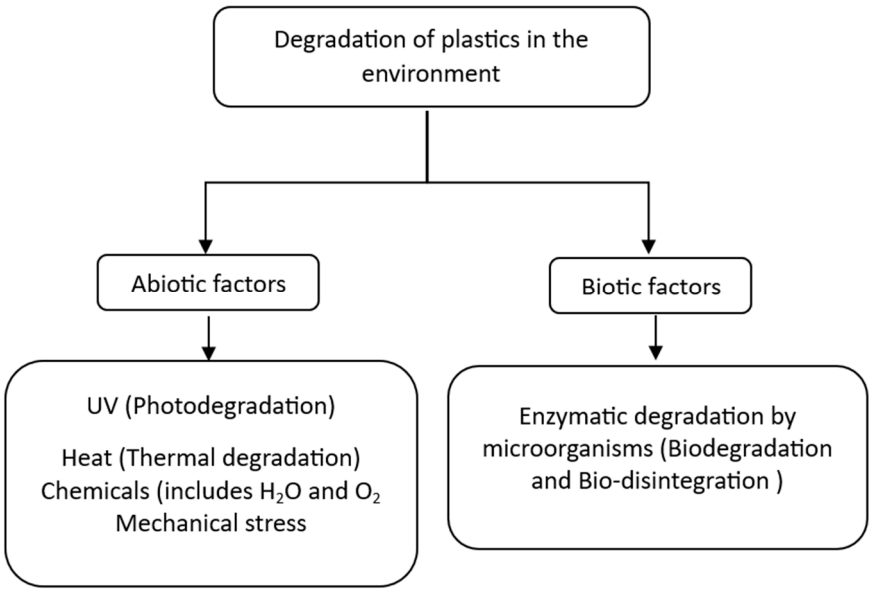

Figure 3. Types of plastic degradation factors in the environment. [26].

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From all the mechanisms described above, one of the most important mechanisms is photodegradation [26]. In photodegradation, high energy ultraviolet (UV) irradiation UV-B (290–315 nm) and medium energy UV-A (315–400 nm) initiate radical-mediated plastic degradation [26,41,42].

4. Microbial Degradation of Plastics

One of the most relevant events with regards of microbial degradation of plastics was the discovery of the new bacteria species, *Ideonella sakaiensis* in 2016 outside a bottle-recycling facility in Japan [43]. This bacterium breaks down PET using two novel enzymes. The first one was labelled as PETase (NCBI accession number A0A0K8P6T7.1) and converts PET to Bis(2-Hydroxyethyl) terephthalate (BHET), TPA and mono(2-hydroxyethyl) terephthalic acid (MHET) which in turn is converted to more terephthalic acid (TPA) and ethylene glycol (EG) monomers by the MHETase enzyme [44]. (NCBI accession number A0A0K8P8E7.1) as shown in **Figure 4**. After this discovery, dozens of other new PETases have also been identified from several other bacteria. Example: *Vibrio gazogenes, Oleispira antarctica, Polyangium brachysporum* [45], *Marinobacter sp*.[46], *Ketobacter sp*. and *Thermobifida* [47].

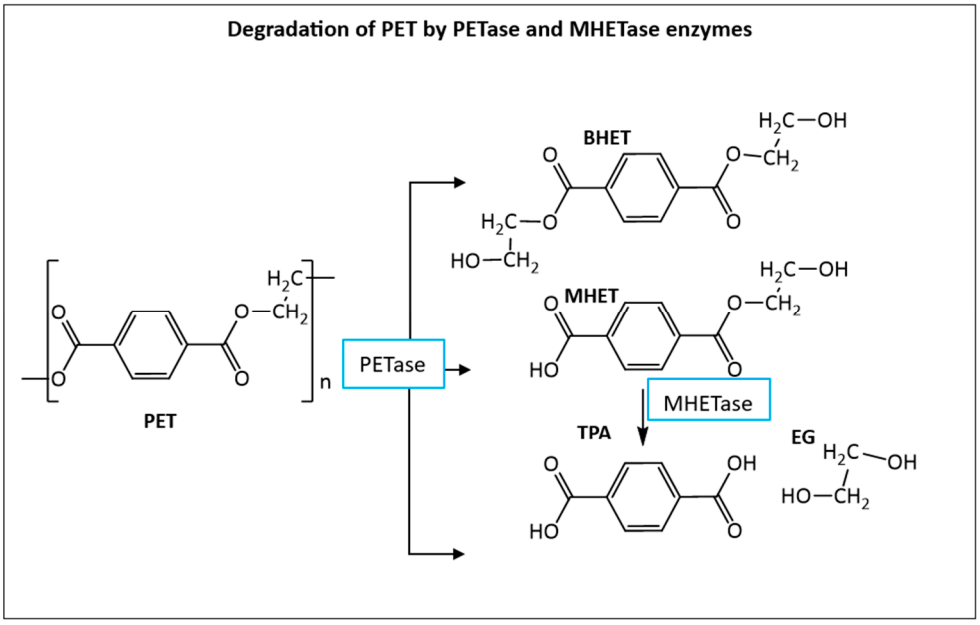


Figure 4. PETase enzymes degrades PET into Bis(2-Hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalic acid (MHET) and terephthalic acid (TPA). MHETase enzyme further degrades MHET into more TPA and ethylene glycol (EG).

The final PET degradation products, terephthalic acid (TPA) and ethylene glycol (EG), can be both either further metabolized by the cells through the Krebs cycle for biomass accumulation or they can be converted into high value products [48]. TPA can be converted, for example, into vanillic acid, muconic acid, catechol, pyrogallol, gallic acid and adipic acid [48] while ethylene glycol can be separated and used mainly to produce polyester fibres and for antifreeze products [49,50]. BHET can also be used in the industry for making resins, coatings, foams and tissue scaffolds [51] or can be further hydrolysed, inside the cell, into more MHET and TPA by esterase enzymes [52].

The number of research publications reporting plastic-degrading microorganisms keeps increasing every day and by 2020, there had been reported approximately 436 different species [53]. These species include bacteria from the classes *Actinobacteria*, *Firmicutes*, *Cyanobacteria*, *Proteobacteria* y *Bacteroidetes* [53] while plastic-degrading fungi are found in eleven classes in the fungal phyla Ascomycota (*Dothideomycetes, Eurotiomycetes, Leotiomycetes, Saccharomycetes*, and *Sordariomycetes*), Basidiomycota (*Agaricomycetes, Microbotryomycetes, Tremellomycetes, Tritirachiomycetes*, and *Ustilaginomy-cetes*), and Mucoromycota (*Mucoromycetes*) [54].

 doi:10.20944/preprints202411.1632.v1

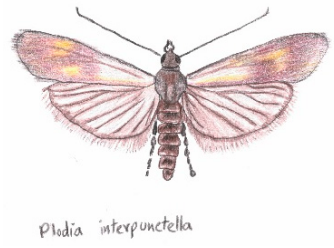
To name a few, bacteria such as *Cupriavidus necator* H16 [55] , *Pseudomonas putida* LS46, and *Pseudomonas putida* IRN22 have also been discovered to degrade polyethylene [56] while *Pseudomonas putida* CA-3 can be fed with styrene to accumulate intracellular polyhydroxyalkanoates [57] and a unique *Raoultella* sp. DY2415 strain from petroleum-contaminated soil can degrade PE and PS film [58] while the fungus *Aspergillus fumigatus*, and *Phanerochaete chrysosporium* degrade a wide range of plastics [53]. The countries that have isolated the most strains are Japan (14.1%) and India (13.8%) [53].

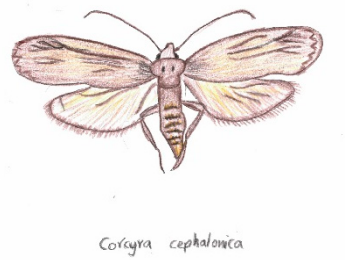
In an effort to compile all this new information, in 2022 the database PlaticDB was created (https://plasticdb.org/). To this date the database contains 753 organisms and 219 proteins that include cutinases, esterases, PETases etc. [59]. Cutinases, specifically, are hydrolases that degrade the cutin which is a component of higher plants cuticle and they have been extensively studied to degrade plastics (PET, PE, PU, Poly (butylene succinate(PBS) and Poly (ε-caprolactone) (PCL)) [60]. They are usually isolated from thermophilic actinomycetes such as *Thermobifida fusca (KEGG:* Tfu_0882)*[61].*

However, despite the number of microorganisms and enzymes available, most of them has low activity levels and are not thermostable [43,62]. As a result, efforts have been made to engineer these proteins to increase activity and thermostability. Examples of these enhanced proteins are ThermoPETase, HotPETase, [63,64] , DuraPETase[65] and the novel FAST-PETase (FAST-PETase: functional, active, stable and tolerant PETase) [66] from *Ideonella sakaiensis.* Moreover, for a more environmentally friendly approach, the native *I. sakaiensis* PETase has also been successfully expressed in the chloroplast of the microalgae *Chlamydomonas reinhardtii [67].*

5. Insect Plastic Degradation Order: Lepidoptera (Butterflies and Moths)

Lepidoptera is an order of winged insects and it is the 2nd largest order there is with approximately 180,000 described species [68]. Aside from the wings, the more representative features are the presence of scales and the proboscis (tubular sucking organ) [69]. Larvae of the following insects from this order have been reported to have plastic-degrading capabilities: A. grisella, P. interpunctella, C. cephalonica, S. frugiperda. (Refer to **Figure 5**)





Achroia Grisella (Lesser wax moth) HDPE and LDPE *Citrobacter freundii* and *Bacillus* sp.	***Plodia interpunctella*** (Indian meal moth) PE *Meyerozyma guilliermondii* and *Serratia marcescens Bacillus YP1*	***Corcyra cephalonica*** (Rice moth) LDPE no microorganism identified yet.

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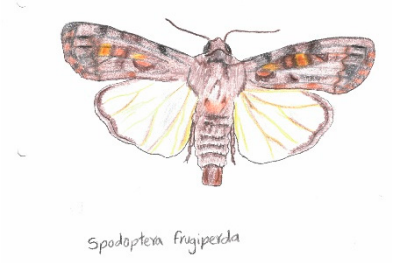

Spodoptera frugiperda
(Fall armyworm)
PVC
Klebsiella sp. EMBL-1

Figure 5. Insects from the order lepidoptera whose larvae has been reported to have plastic-degrading capabilities. The larvae's common name is reported as well as the plastic degraded and the microorganism associated. Note: insect figures do not represent real scale. *Achroia Grisella* [70,71], *Plodia interpunctella* [7,72] *[6], Corcyra cephalonica* [73], *Spodoptera frugiperda* [74].

The Waxworm Galleria Mellonella (Fabricius, 1798) [Lepidoptera: Pyralidae] to Degrade Plastic

Commonly referred as the greater wax moth, *Galleria mellonella* is a natural honeycomb pest that has contributed to the decline of bee populations at global scale due to the larvae's capability to feed on wax [75]. In science, these larvae's importance has gradually increased as a model organism for biomedical studies [76]. They are specially used as an infectious-disease model due to the presence of an immune system that is similar to that of vertebrates [77] (See **Figure 6**).

Figure 6. Galleria mellonella. The female wax moth average body length is 15–20 mm. The male is considerably smaller and darker [75].

To date (June 2024) the PubMed entry "*Galleria mellonella* to degrade plastic" gives 43 entries from which only 28 are related to the larvae's capacity to degrade plastic and are summarised here.

Degradation of plastics using the larvae from *Galleria mellonella* (commonly referred as waxworm) is a fairly novel research topic: The first experiment reporting the capability of this insect to degrade polyethylene (PE) was presented in 2017 when Bombelli, Howe and Bertocchini [9] left worms in a polyethylene bag and observed that they were eating it. The plastic degradation capability of *Galleria mellonella* has also been found true for other petroleum-based plastics such as expanded polystyrene and polypropylene [78] and for the bio-plastic polylactic acid (PLA).[79] *Galleria mellonella* is naturally capable of decomposing long-chain hydrocarbons from beeswax without the help of intestinal microorganism using specific carboxylesterases, lipases and fatty-acid metabolism related enzymes. It has been hypothesized that a similar metabolic approach is used to degrade plastic by the waxworm. [80] However, plastic is not nutritious enough as studies show that most larvae (≥50%), growing on an exclusive PE diet lose weight and die in between 3 and 15 days, indicating that supplementary diet is necessary [81-83] or the use of older larvae (last developmental stage 25-30 mm) [84] for this type of plastic bioremediation to take place. Pre-treating low density polyethylene under solar radiation for 15 days before feeding the larvae with the material is also being suggested as another technique to increase plastic degradation rate and larvae survival [85].

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Polyethylene degradation starts with the expression of salivary enzymes after exposing the larvae to the material [86]. Sanluis-Verdes, Colomer-Vidal, Rodríguez-Ventura, Bello-Villarino, Spinola-Amilibia, Ruiz-López, Illanes-Vicioso, Castroviejo, Cigliano, Montoya, Falabella, Pesquera, González-Legarreta, Arias-Palomo, Solà, Torroba, Arias and Bertocchini [11] discovered and published the first report of 2 novel enzymes isolated from the wax worm saliva with the capability of oxidizing and depolymerizing polyethylene (PE) after only few hours of exposure to the material at room temperature and neutral pH. These enzymes, named as Demetra (NCBI accession number: XP_026756396.1) and Ceres (NCBI accession number: XP_026756459.1) are classified as arylphorin and hexamerin respectively. Gas Chromatography-Mass Spectrometry (GC-MS) was used to confirm the presence of degradation products such as small oxidized aliphatic chains in PE treated with saliva. This discovery opened the way to new ground-breaking solutions for plastic waste management. A closely related protein (81% sequence identity with Demetra) is also described in the study and named as Cibeles. (NCBI accession number XP_026756460.1) Cibeles forms a heterocomplex with Demetra but has not proven to degrade PE on its own [11]. A fourth PE-degrading protein was later identified and named Cora (NCBI accession number XP_026749149.2) late in 2023.[12] The protein 3D structure of all these proteins have been elucidated [12].

Microplastic and plastic depolymerization products are then swallowed and further processed in the gut, where the microbiome plays a key role in plastic degradation [17]: *Desulfovibrio vulgaris*, *Enterobacter* sp. D1 [78,87], *Acinetobacte*r [13], the fungus *Aspergillus flavus* PEDX3 [88] and the fungus *Cladosporium halotolerans* [89] are examples of microorganisms isolated from the waxworm's gut with reported capability of degrading PE on experiments *in vitro*. For the case of A. *flavus,* for example, microplastics of HDPE were degraded into microplastics with lower molecular weight when exposed to the fungi in liquid culture for 30 days. Chemical changes on the microplastic particles such as the appearance of hydroxyl, carbonyl and ether groups also validate the degradation. Two laccase-like multicopper oxidases enzymes are believed to be responsible for this degradation .[88] Highly similar results were observed when the fungus *Cladosporium halotolerans* was cultivated in a HDPE microparticle suspension [89].

Likewise, the bacteria *Lysinibacillus fusiformis*, *Bacillus aryabhattai*, and *Microbacterium oxydans*, isolated from the whole worm's body extract are able to degrade and grow using low-density polyethylene LDPE as carbon source [56].

To add up , other microorganisms from the worm have been studied and proven to be capable of acting over other plastics different from polyethylene (PE). Example: *Bacillus cereus* can degrade polypropylene (PP) *in vitro* [90] while mastication of expanded polystyrene (EPS) and polypropylene (PP) increase the abundance of *Enterococcus sp.* in the gut [78]. Also, the genus *Bacillus*, *Serratia* and the bacteria identified as *Massilia* sp. FS1903 have been associated with polystyrene (PS) degradation [83,91] .Some enzymes, pathways and gut microorganisms mentioned in th is section to degrade plastic by waxworms are summarized in **Figure 7** and **8.**

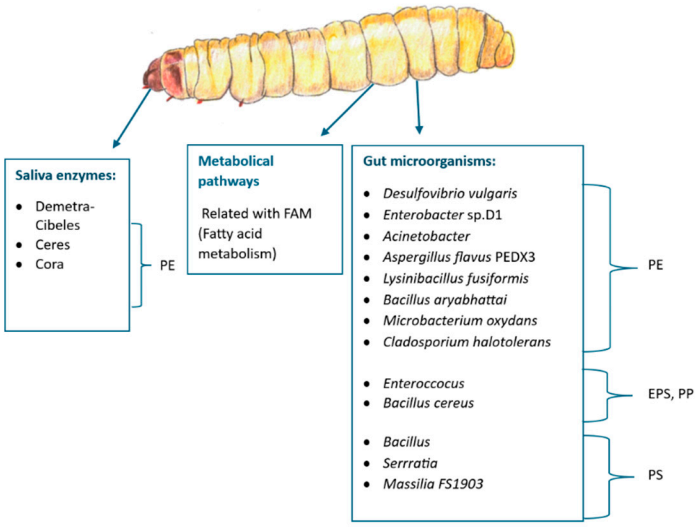

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Figure 7. The greater wax worm uses different biological tools to degrade plastic: saliva enzymes, metabolic pathways and gut microorganisms.

Despite of all the above studies, plastic degradation in the gut cannot be solely attributed to microbiota presence. Gut RNA sequencing and biochemical approaches showed that polyethylene-fed larvae show enhanced fatty acid metabolism (FAM) [92,93]. Additionally, early this year 2024, an improved version of the whole genome of *Galleria mellonella* was published (GenBank: JAPDED000000000.1) [94]. In this study various new putative probable PE-degrading enzymes found *are highlighted.*

As for the case of polystyrene metabolism a list of possible styrene-degrading enzymes present in the wax worm have been published and two potential metabolic pathways have been proposed [95,96]: the styrene oxide–phenylacetaldehyde [97] pathway that has also expressed in the presence of beeswax [98] (Refer to **Figure 8A**) and the 4-methylphenol–4- hydroxybenzaldehyde–4-hydroxybenzoate pathway (**Figure 8B**) which takes place prior to the β-oxidation pathway (main FAM process) in the larvae's intestines [96].

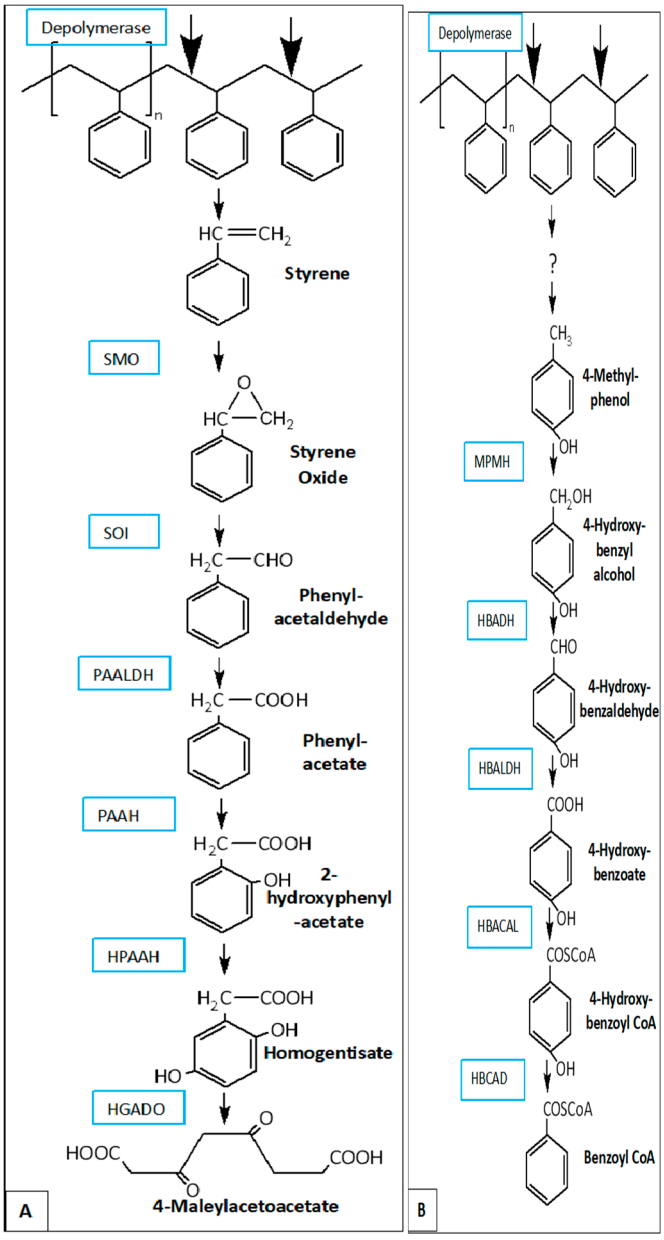

Figure 8. Proposed metabolic pathways for PS degradation in G. mellonella. [96] **SMO**: styrene monooxygenase; **SOI:** styrene oxide isomerase; **PAALDH**: phenacetaldehyde dehydrogenase;

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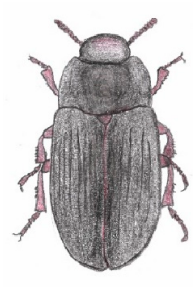
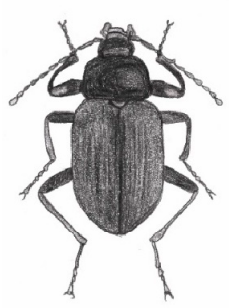
PAAH: phenylacetate hydroxylase; **HPAAH**: 2-hydroxyphenylacetate hydroxylase; **HGADO**: homogentisate 1,2-dioxygenase; **MPMH:** 4-methylphenol methyl hydroxylase; **HBADH**: 4-hydroxybenzyl alcohol dehydrogenase; **HBALDH**: 4-hydroxybenzaldehyde dehydrogenase; **HBACAL**: 4-hydroxybenzoic acid-CoA ligase; **HBCAD**: 4-hydroxybenzoyl-CoA reductase[96].

6. Insect Plastic Degradation Order: Coleoptera (Beetles and Weevils) [99]

The order Coleoptera represents the largest group of insects with 40% of known insect species. In this group, generally, the wings develop internally but some have no wings [99]. Plastivore Coleoptera larvae include the larvae from the beetles *Alphitobius diaperinus*, *Plesiophthalmus davidis*, *Tribolium castaneum* and *Uloma*. The type of plastic and microbiome associated with these insects are shown in **Figure 9.**

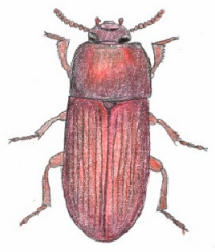


Alphitobius diaperinus
(Lesser mealworm)
PS
Aspergillus, Trichoderma, Penicillium, Pseudomonas and *Kocuria*.



Plesiophthalmus davidis
PS
Serratia

Tribolium castaneum
(Red flour beetle)
PS
Acinetobacter sp.



Uloma sp. (0)
(PS)
no microorganism identified yet

Figure 9. Insects from the order coleoptera whose larvae has been reported to have plastic-degrading capabilities. The larvae's common name is reported as well as the plastic degraded and the microorganism associated. Note: insect figures do not represent real scale. *Alphitobius diaperinus* [100], *Plesiophthalmus davidis [101], Tribolium castaneum* 1 [102], *Uloma sp. (0)* (PS)[103].

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Commonly referred to as mealworm, *Tenebrio molitor's* larvae is commonly used as protein source for domestic animals (monogastric animal feed) [104] , for fish [105] and can also be grown for human consumption [106,107]. In science, it has been studied as a model for cellular and humoral immunity against pathogenic infections [108]. A representation of an adult beetle is shown in **Figure 10.**

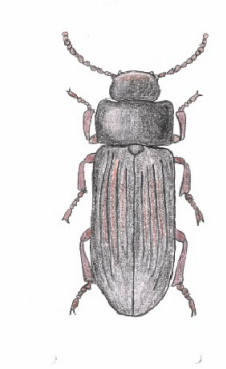

Figure 10. *Tenebrio molitor*. Adults are commonly 13 to 18 millimetres long. *T. molitor* is dark brown or black.[109].

Starting from 2015, to date (June 2024) the entry in PubMed "*Tenebrio* to degrade plastic" gives 82 entries from which 67 are related to the larvae's capacity to degrade plastic. Most of those papers were used for this review.

The mealworms, at the moment, are growing a reputation as polystyrene (PS) plastic eaters [8,83,93,110-116]. This larvae is capable of converting ≈47% of the ingested Styrofoam (a common PS product) into CO_2 and the residue (≈49%) is excreted as fecula with a limited fraction incorporated into biomass [8]. Although *Galleria mellonella,* can degrade PS, [117] the mealworms lose their capacity to degrade PS plastic when the bacteria gut is inhibited [115,118] which suggest a strong dependency on microbiota to degrade PS. Even so, it has also been demonstrated that the mealworm secretes emulsifying factors that increase plastic bioavailability in the gut [113] as well as a wide range of oxidases, cytochrome P450, monooxygenase, superoxidase, and dehydrogenase and other enzymes related to the fatty acid metabolism [119,120].

Mealworms of approximately 3-4 instars (20–25 mm in length) fed only with PS are able to survive and complete their entire life cycle and emerge into adult beetles [8,116,120]. This could partially be explained by the fact that gut microbiome, specially from the genus *Klebsiella*, is capable of nitrogen fixation, thus the worm is supplied with this element as well [121]. Still, it is recommended to supplement with corn flour (*T. obscurus*) or wheat bran (*T. molitor*), sucrose and hydrate with H_2O [122] to increase the PS degradation rate and enable breeding of a second generation with favourable capabilities for PS degradation as well [114,116,123,124]. It has been hypothesized that the mechanism used to degrade PS is similar to the mechanism described for *Galleria mellonella* in **Figure 8A** in which PS is degraded into styrene first [93] and after several intermediate steps the benzene ring is destroyed [117] and assimilated through the β-oxidation pathway [96].

Additionally, *T. molitor* larvae can also biodegrade polyethylene (PE) through the mechanism presented in **Figure 11** [112,125,126] . They can also degrade PP, PVC [127-129], Nylon 11 Polymers[130,131], Polyethylene terephthalate (PET) [119] , melamine formaldehyde (MF)[132] and the biopolymer PLA but the mechanism for this polymers remains unknown [133]. *T. molitor* can even chew and ingest polyurethane (PU) but the digestion/degradation of this plastic has not been demonstrated [134-136]. During the COVID-19 pandemic, polypropylene (PP) face mask production and contamination increased considerably and it was observed that *T. molitor* can consume face masks.[137] The capability of biodegradation can be affected by the molecular weight, branching and crystallinity of the material [138].

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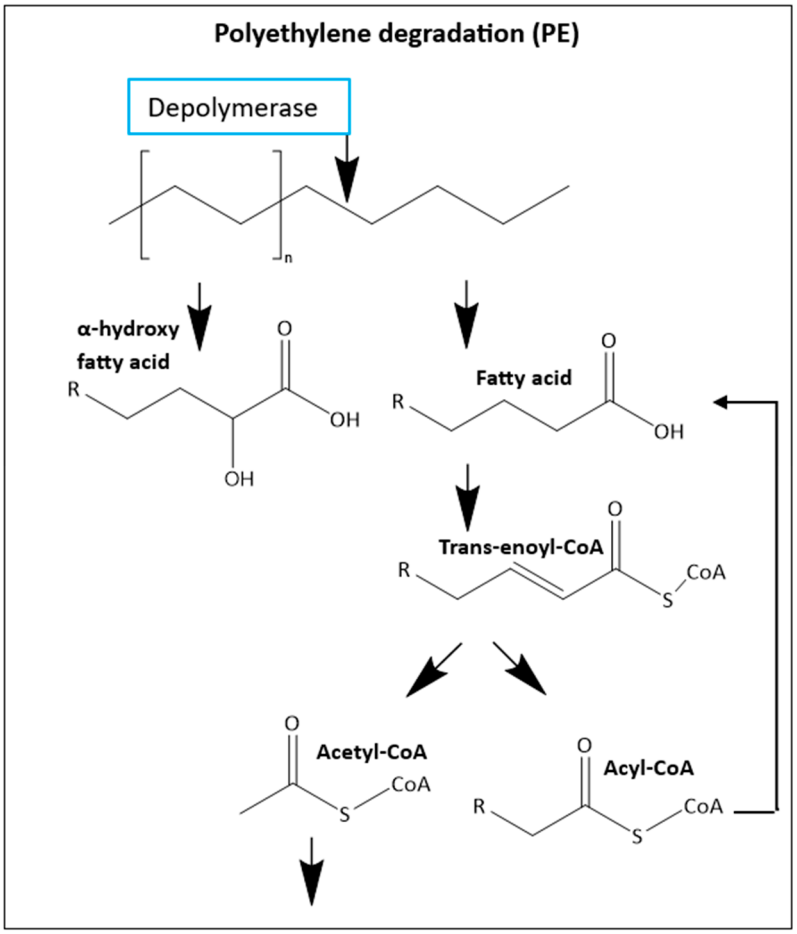

Figure 11. Proposed mechanism of PE degradation in *Tenebrio molitor* larvae (mealworms) presented by Zhong, Nong, Xie, Hui and Chu. [93].

The mealworm's gut bacteria *Exiguobacterium* sp. strain YT2 degrades polystyrene (PS) [115]. *Citrobacter* sp. and *Kosakonia* sp. were also found in the gut and strongly relate with PE and PS consumption [112]as well as the bacteria *Priestia megaterium* S1 [139]. Other strains related to PS degradation include Erwinia olea, Lactococcus lactis, Lactococcus garviae [122] *Serratia marcescens, Pseudomonas aeruginosa, Acinetobacter septicus, Agrobacterium tumefaciens, Klebsiella grimontii, Pseudomonas multiresinivorans, Pseudomonas nitroreducens, Pseudomonas plecoglossicida,* and *Yokenella regensburgei* [110,111,113,140]. Bacteria from the families *Enterobacteriaceae,* such as *Enterobacter hormaechei LG3 [141])*, the families *Spiroplasmataceae, Enterococcaceae,* [114,117] *Staphylococcus* and *Rhodococcus* [120] also play a role in PS degradation. It is important to note that a study, where mealworms from 3 different regions in China were compared, showed that larvae from different regions have different metabolism [142] which suggests that the gut microbiota can change depending on the environment of the larvae, diet and even depending on the PS molecular weight provided [143], yet PS consumption is ubiquitous to this species. [144]

Additionally, the family *Enterobacteriaceae* has also been linked with polyurethane (PU) degradation along with the family *Hafnia* [145] while the genus's *Spiroplasma, Dysgonomonas* and *Hafnia-Obesumbacterium* are associated with PET degradation [119]. Gut bacteria from *Tenebrio molitor* is even capable of degrading vulcanized poly(cis-1,4-isoprene) rubber (vPR) (strain Acinetobacter sp. BIT-H3) [146]. It is also capable of degrading PVC and PP but the genre of these microorganisms has not been elucidated [127,128] . *Tenebrio molitor's* microbiome related to plastic-degradation is illustrated in **Figure 12.**

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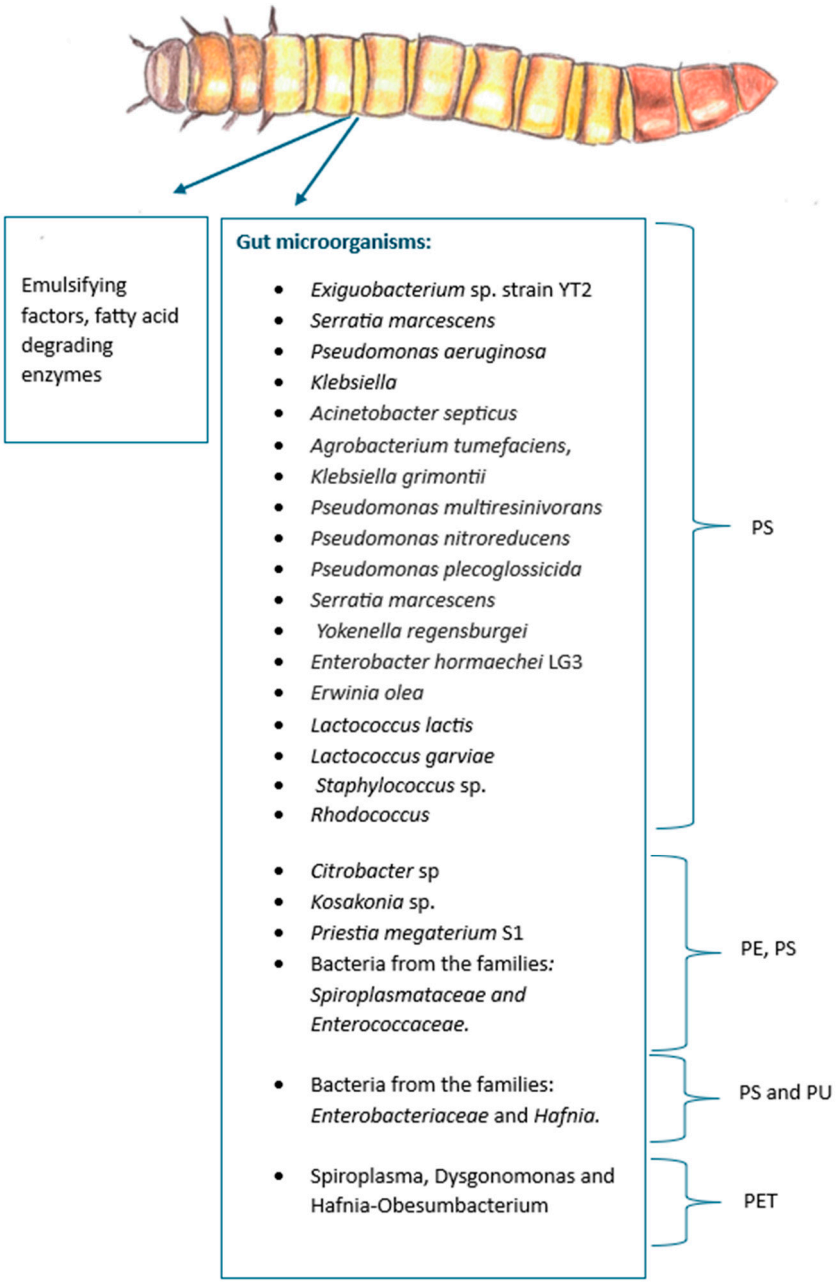

Figure 12. *Tenebrio molitor* uses a wide variety of gut microorganisms to degrade plastics.

Interestingly enough, Although less studied, a comparison between the yellow mealworms (T. molitor) and the dark ones (T. obscurus) show that the latter degrade PS at higher rates [114]. T. obscurus larvae also degrades LDPE using gut bacteria mainly from the genus Spiroplasma and Enterococcus [147,148].

The Superworm Zophobas Atratus (Fabricius, 1776) [Coleoptera: Tenebrionidae] to Degrade Plastic

Zophobas morio (Fabricius, 1776) is a dark beetle [Coleoptera: Tenebrionidae] that is currently considered as the same species as *Zophobas atratus*. It was also previously identified as *Tenebrio morio* and/or *Helops morio* and commonly referred as the giant mealworm beetle which has been a matter of confusion and controversy [149]. (**Figure 13**) *Zophobas* larvae is commonly refer as the superworm [149]. This larvae is highly nutritious and it is promising as fish, poultry and pig feed [149].

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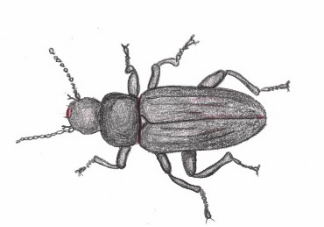

Figure 13. Adults are dark and 38- to 57mm long.

To date (June 2024) the entry in PubMed "*Zophobas atratus* to degrade plastic" gives 17 entries from which all 17 are related to the larvae's capacity to degrade plastic. All 17 studies were used for this review.

Zophobas is also a main polystyrene (PS) consumer. A comparison in a 30 days long experiment, between the larvae of *Tenebrio molitor* (yellow mealworm), *Galleria mellonella* (greater wax moth) and *Zophobas atratus* (superworm) showed that the latter has the strongest polystyrene consumption capacity and the highest survival rate of the three [117] being able to consume 4 times more PS than the yellow mealworm per day [150]. Superworms also outdid the yellow mealworms by 11 folds on PU consumption in another study [145]. But, similarly to *Tenebrio*, this capability is lost when the gut microbiota is supressed using antibiotics [150].

Moreover, new research indicates that the superworm's microbiota is also capable of degrading PE, PP, PVC and PET[151-154] and even polyurethane (PU) [155] , melamine formaldehyde (MF)[132], ethylene vinyl acetate (EVA)[156] and polybutylene succinate (PBS)[157].

PE and PS degradability is being attributed to *Pseudomonas aeruginosa* [152,158] and *Enterococcus* (also associated with PU degradation)[155], *Citrobacter* with PE and PVC [153], *Brevibacterium* [159] and *Dysgonomonas* and *Sphingobacterium* with PS and *Mangrovibacter* with PU degradation [155] (Summarized in **Figure 14**).

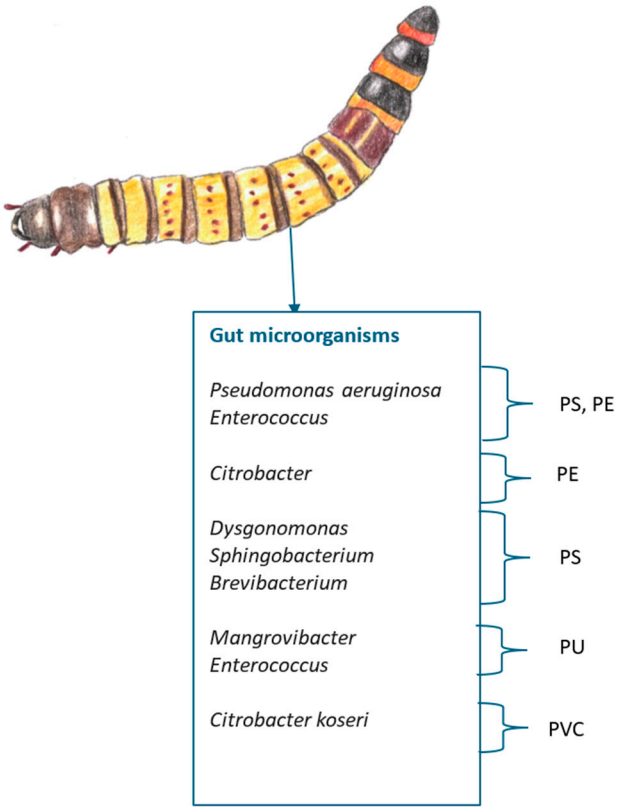

Figure 14. *Zophobas* can digest a wide variety of plastics with the aid of gut microbes.

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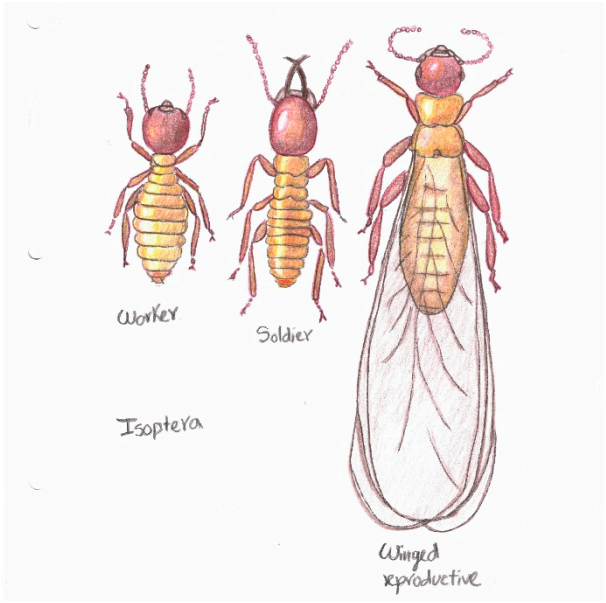
Little is known about the mechanisms these specific larvae use to degrade plastic but PS degradation seems to be partly achieved by the synergistic effect of the generation of reactive oxygen species (ROS) inside the gut and the production of oxidases and other enzymes by the microbiome [160].

PS was achieved by the synergistic effect of ROS and complex functional microbes and enzymes as well as other enhancing factors in the gut of larvae.

both compounds [80].

Insect plastic degradation. Order: Blattodea (cockroaches and termites)[161]

Termites have a physical appearance that is similar to ants as observed in **Figure 15** . They used to be classified in their own order named Isoptera. However, new studies show that they are actually closely related to cockroaches and should be classified in the same order Blattodea [161]. The most characteristic feature of these insects is that they feed on wood, which is composed mainly of polymers of cellulose, hemicellulose and lignin [162,163]. Lignocellulose and plastic polymers have similar physicochemical features, for example, their carbon chains have similar chemical bonds and hydrophobicity properties which has led to believe that termites could also be plastivore organisms [164]. The relationship between lignocellulose and plastic consumption is further supporter by the fact that, as mentioned before, some enzymes like cutinases which are involved in degrading plants are very well documented plastic degraders [60,61].



Isoptera
LDPE
The yeast consortium *Sterigmatomyces* halophilus, *Meyerozyma guilliermondii*, and *Meyerozyma caribbica*.

Figure 15. Termites live in colonies formed by worker, soldier and winged reproductive termites which are represented in this figure. a. [14].

In fact, higher adult termites (*Nasutitermes nigriceps*) have been observed and reported degrading wood-HDPE plastic composites (WPCs) in one study from Yucatan, Mexico [165]. In a later study, a group of previously isolated yeast symbionts from the guts of the adult *Coptotermes formosanus* (termite) showed low density PE degrading capabilities [14,166]. Termites are the first

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example of adult insects degrading plastic as all the previously reported degradation has been reported on larvae.

Other wood-eating insects examples include the pest *Chrysobothris* sp. which is a beetle that attacks cedar trees, [167] the emerald ash borer (*Agrilus planipennis*) which attacks ash trees, [168] the red bay ambrosia beetle (*Xyleborus glabratus*) that attacks laurel trees, [169] and the Asian long horned beetle (*Anoplophora glabripennis*) that attack maple trees. [170] in between others woodboring beetles [171].

Other Organisms from the Class Insecta to Degrade Plastic

The list of plastivores in this review includes all the insects mentioned in previews reviews [3] [16,172] from the orders Lepidoptera and Coleoptera and expands to the order Blattodea that could be further explored in the future for plastic degradation. It was confirmed in 2023 that 3 novel yeasts isolated from termite's gut (Blattodea) can metabolise polyethylene (PE) into alkanes, aldehydes, ethanol, and fatty acids [14].

Interestingly, similar to *Galleria mellonella*, the larvae from *Achroia grisella* and from *Uloma* feed on the long-chain hydrocarbon beeswax and can degrade the plastics PE and PS (pre-print study) [173]. The larvae from *Plodia interpunctella* is another larvae that eats both beeswax and PE [6]. The positive relationship between capability for eating beeswax and capability for eating plastic might not be a coincidental one but rather a case of cause and consequence since, as mentioned before in this document, it has been suggested that similar metabolic approaches are used to degrade both compounds [80].

The number of plastivore insects is growing every day and in the latest 2024 review [174] the authors suggested a large variety of insects with potential plastic-degrading capabilities which include insects from the order Diptera (Example: Black soldier fly), Blattodea (several type of cockroaches) and Orthoptera (Ex. The cricket *Gryllodes sigillatus*) as well as other families within the already studied order Coleoptera (Example: the lesser grain borer or *Rhyzopertha dominica* ,the rice weevil or *Sitophilus oryzae*, and cigar beetle or *Lasioderma serricorne*) and Lepidoptera (The larvae of *Hofmannophila pseudospretella* or the Brown House moth). However, scientific studies are needed to confirm whether there is actual degradation by these species and which microorganisms/enzymes could be responsible. Another recent review estimated that over 23 species of insects (including the 12 insects described in this review) have been observed consuming plastics [175] and the list could expand even further in the future. If a positive relationship between wax degradation and plastic degradation is confirmed then other wax-eating larvae could be explored. An example of this type of larvae would be the American wax worm *Vitula edmandsii* which is a honeybee comb pest [176]. Similarly, other woodboring beetles or any other xylophagous (wood diet) larvae or insect could be a good candidate for research.

Analysis of Plastic Degradation After Exposure to Insect Larvae

Several techniques and protocols are available for characterization of degraded polymer after exposure to larvae. The simplest method is to measure plastic weight loss over time. [70] Afterwards, the plastic polymer can be closely inspected for changes in the surface morphology using scanning electron microscopy (SEM)[101]. To check for chemical modifications one of several options is the analysis using X-Ray photoelectron spectroscopy (XPS)[101].

Once the plastic has been digested, frass can be collected and analysed using different techniques. For example, Brandon, Gao, Tian, Ning, Yang, Zhou, Wu and Criddle [112] measured the molecular weight of the polymer using gel permeation chromatography (HT-GPC) followed by characterization of the degradation products through magnetic resonance (1H NMR) and Fourier Transform Infrared Spectroscopy (FTIR). Thermal gravimetric analysis (TGA) can also be used to analyse thermal changes from plastic to frass.

In order to isolate plastic-degrading microorganisms, faecal matter collected has to be diluted and plated in Tryptic soy agar (TSA) and in a defined media as described in literature [131] to obtain isolated colonies. To confirm the capability of these colonies to degrade plastic several tests can be

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performed such as the clear zone assay in agar plate and turbidity measurements in liquid culture. [139] Gas Chromatography-Mass Spectometry (GC-MS), which is an analytical technique used to identify and quantify compounds, can also be used to confirm the presence of PE degradation products in liquid culture such as small oxidized aliphatic chains. [11]

Challenges and Future Perspectives

Even though plastivore insects are a new exciting avenue for bioremediation of plastic pollution, several challenges need to be overcome before the technology can be industrialized.

One of these challenges is to provide optimal, standardized conditions for larval rearing at the industrial scale to ensure reproducible results in terms of larvae quality (weight, survival rate lipid content etc.) and plastic degradation rate. Environmental conditions such as light exposure, temperature and ventilation greatly affect the development of the larvae. In the case of *Galleria mellonella*, for example, constant exposure to light significantly reduces its size and delays metamorphosis, so they need to be grown in darkness at a temperature of 28–32°C. Providing ventilation is also highly important not only to provide oxygen but to prevent infections too [177]. Therefore, for the use of live larvae for plastic degradation, we consider essential to have a contained and controlled area (a dark greenhouse for example) with controlled conditions. The other indispensable benefit of the use of an enclosed area is the responsible containment of insects that are recognized as pests.

Another problematic source of variability at industrial scale is the chemical composition and properties of the waste plastic used as feedstock. Evidence shows that the presence of other contaminants present in plastic pollution such as plastic additives, in between other factors, may affect larvae's digestion [178]. This challenge could be overcome by processing the plastic waste prior to feeding the larvae as it is normally processed for plastic recycling: 1) Sorting and categorizing: In this step several types of plastics need to be separated from each other, 2) Washing: Impurities that can be toxic for the larvae are removed , 3) Shredding: Plastic is broken down into much smaller pieces and 4) Testing: At this point, the plastic pieces are tested for their quality and density [179]. Additionally, other novel steps shall be explored, for example, (as mentioned previously) pre-treating PE under solar radiation for 15 days before feeding the larvae increases plastic degradation rate and larvae survival [85].

There are also concerns raised about the economic feasibility of the technology due to the high cost of breeding the larvae and the lack of sufficient research to obtain high-value end products. It has been calculated that it would cost more than €300 to degrade 1 ton of low LDPE plastic using ≥ 4 tons of waxworms or mealworms in approximately 38 days [180] while recycling 1 ton of LDPE costs less than €250 in less time [181]. In this regard, due to their high fat content, the waxworm, the yellow mealworm and even PE-plastivore larvae from *Corcyra cephalonica* (up to 60%, 38% and 43.3% respectively) could potentially be used for biodiesel production [182,183]. Another solution suggested is the extraction of chitin from adult plastic-fed *Tenebrio molitor's* exoskeleton [184] which can then be processed to use as biomedical materials, food additives, cosmetic ingredients, agricultural materials, analytical reagents and others [185]. Larvae could also, potentially, be used as animal feed as some studies show that there are no microplastic nor nano plastic residues present in the frass as a result of plastic consumption by larvae [186]. However more studies are needed to corroborate safety.

On the other hand, it is important to note that also this same study [180] calculated that the process to degrade 1 ton of plastic using larvae would also release ≥ 4 tons of CO_2 to the atmosphere which is a much larger number than the ≈2.9 tons of CO_2 that would be produced during plastic incineration [187]. This information is worrying and lead us to wonder if this technology can be used as a sustainable process. In future the process will need a more comprehensive Life Cycle Assessment [188] and the possible co- implementation of a CO_2 capture system.

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Lastly, scale up standardization and the production of high-value end products could be achieved using other , more advanced, biotechnological approaches such as the recombinant expression of insect-derived novel enzymes or the use of gut-isolated microorganisms to degrade plastics in cell culture. Insect cells specifically, are already successfully used as factories for biomanufacturing of several proteins, vaccines and vectors for gene therapy [189] while the industrial cultivation of microorganisms to obtain biotechnological products has been practiced for thousands of years, starting from the production of wine, beer and bread [190]. Cell culture plastic degradation would allow the recovery of plastic monomers that can be converted into high-value components. For example, as mentioned above, TPA monomers from PET degradation can be transformed into vanillin [191] which is considered the second most important flavouring agent, after saffron, and has a wide variety of applications in the food and beverage industry but also in the pharmaceutical industry and for production of home-use products such as perfumes and deodorants [192].

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