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Low-density polyethylene sheet biodegradation by *Tenebrio molitor* and *Zophobas morio* larvae and metagenome studies on their gut bacteria

BERNADETTA OCTAVIA♥, ANNA RAKHMAWATI, SUHARTINI, LUTHFIA DWI RACHMANI, TITAN DWIKAMA PUTRA

Environmental Science and Applied Microbiology Research Group, Department of Biology Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta. Jl. Colombo No. 1, Karang Malang, Sleman 55281, Yogyakarta, Indonesia.
Tel./Fax.: +62-274-586168, ♥email: b_octavia@uny.ac.id

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Abstract. *Octavia B, Rakhmawati A, Suhartini, Rachmani LD, Putra TD. 2023. Low-density polyethylene sheet biodegradation by* Tenebrio molitor *and* Zophobas morio *larvae and metagenome studies on their gut bacteria. Biodiversitas 24: 878-886.* Low-density polyethylene (LDPE) is one of the types of plastics that are massively produced and used today. Strong and very stable are the characteristics of this type of plastic. However, its chemical and physical qualities such as hydrophobicity and high molecular weight are believed to make LDPE plastic resistant to degradation. Some microorganisms such as bacteria, fungi, and algae are known to be feasible to be used as plastic biodegradation agents although some of them still show low biodegradation ability. Recently, it was reported that *Tenebrio molitor* and *Zophobas morio* could degrade plastic. These larvae can eat several types of plastic, and one of them is LDPE. The ability to degrade LDPE plastics with their gut bacteria contributes synergistically to solving problems related to plastic waste in the future. Therefore, the diversity of the larvae gut bacteria needs to be explored more to find out the bacteria that may be involved in the biodegradation of LDPE plastic sheets. In this study, *T. molitor* and *Z. morio* were treated with LDPE sheet diet for 30 days. By the end of the treatment, the sheets were analyzed to see the waste reduction index and the surface using SEM-EDX. Then, the gut bacteria were extracted and analyzed through metagenome analysis. This study found that *T. molitor* and *Z. morio* could degrade LDPE sheet with weight reduction indexes of 44.6% and 16.76% respectively. Metagenomic analysis found that *T. molitor* has 441 OTUs with Corynebacteria as the dominating genus. Meanwhile, *Z. morio* has 511 OTUs with Citrobacter as the dominating genus. Both larvae also have genera that known to have the ability to degrade LDPE sheets.

Keywords: Biodegradation, LDPE, metagenome, *Tenebrio molitor, Zophobas morio*

INTRODUCTION

Recently, plastic has been widely used in daily life. As it is strong, cheap, and easy to form, plastic is commonly used as the material of bags. In 2019, plastic production has reached 368 million tons around the world (Ekanayaka et al. 2022). Unfortunately, most of the plastic waste produced by humans ends up in landfills and oceans. It is concerning that Indonesia contributes as the second largest producer of plastic waste after China (Lestari and Trihadiningrum 2019). Plastics such as polyethylene (PE) are resistant to degradation and have the potential to pollute the environment. Even plastic waste that ends up accumulating in landfills will only produce other wastes such as leachate and form smaller plastic components known as microplastics which can pollute the aquatic environment (Fibriarti et al. 2021).

Plastic polluting the environment has several negative impacts on living organisms. Plastic is known to not only affect individual organisms but also damage the ecosystem in the marine environment (Syranidou et al. 2019). In terrestrial ecosystems, plastic pollution is responsible for changing soil properties such as moisture, density, structure, and nutrient content. Moreover, it may interfere with plant nutrient absorption and affect their growth (Dissanayake et al. 2022). Several studies show that microplastics and other xenobiotic components carried by plastics can move through the food chain and potentially accumulate at the highest trophic levels such as humans and other organisms. The accumulation of microplastics in humans and other organisms can cause serious health problems (De-la-Torre 2020; Walkinshaw et al. 2020; Cverenkárová et al. 2021).

Alternatives to synthetic plastics such as biodegradable plastics are considered unable to solve existing problems because even though they have almost the same durability as synthetic plastic, synthetic plastic is still more cost-efficient in terms of production. Therefore, synthetic plastic producers are reluctant to produce biodegradable plastics (Khandare et al. 2021). Synthetic plastics such as low-density polyethylene (LDPE) are one of the types of plastics that are massively produced and used today. It is estimated that the amount of LDPE plastic used as plastic bags globally has reached 0.5-1 trillion annually (Nielsen et al. 2019). Strong and very stable are the characteristics of this type of plastic. However, its chemical and physical characteristics such as hydrophobicity and high molecular weight are responsible for their resistance to degradation, thus making LDPE plastic one of the types of plastic that mostly pollutes the environment (Fibriarti et al. 2021; Hariadi et al. 2021).

In general, LDPE plastic degrades through incineration, landfilling, recycling, thermal and chemical oxidation processes. Unfortunately, those methods can produce other toxic compounds and are expensive, so they are not effective to solve the global plastic problem (Sanniyasi et al. 2021). Environmentally friendly remediation methods so far have been extensively researched and focusing on biodegradation using microorganisms. Some microorganisms such as bacteria, fungi, and algae are known to be potentially used as plastic biodegradation agents, although some of them still show low biodegradation ability (Brandon et al. 2018). Thus, an investigation to look for other biodegradation agents needs to be carried out considering that the global plastic problem has become a critical issue.

Recently, it was discovered that several larvae that can degrade plastic are potential to be a solution for the plastics problem in the future. These larvae are *Galleria mellonella*, *Tenebrio molitor*, *Zophobas morio*, *Tribolium casteneum*, and *Tenebrio obscurus* (Yang et al. 2015; Peng et al. 2019; Cassone et al. 2020; Wang et al. 2020; Yang et al. 2020). *Tenebrio molitor* and *Z. morio* larvae are known to be able to consume plastic foam made of polystyrene (PS) and LDPE (Brandon et al. 2018; Peng et al. 2022). Researchers believe that these insect species have the ability to degrade LDPE plastics with their gut bacteria which contributes synergistically. The larvae's gut bacteria are known and believed to have an essential role, for example as insecticide resistance, depolymerization, and biodegradation of xenobiotic components in the digestive tract of insects (Xia et al. 2018; Lee et al. 2020; Barrionuevo et al. 2022; Peng et al. 2022). However, studies on the ability of larvae to eat LDPE in the form of plastic sheets are still limited. Therefore, the ability of *T. molitor* and *Z. morio* to biodegrade plastics needs to be further investigated since plastic pollution of LDPE sheets such as plastic bags has become a concern lately. Additionally, gut bacteria diversity needs to be further explored to find out the types of bacteria that may be involved in the biodegradation process of LDPE sheets through metagenomic analysis.

MATERIALS AND METHODS

Sample collection and biodegradation by larvae

LDPE sheets were purchased from a local factory in Bogor, Indonesia. Used in larvae plastic diet treatment, the LDPE sheet was cut into small pieces (3 cm x 3 cm in size). The LDPE sheets were weighted and then sterilized using methods described by Yang et al. (2014). There was a slight modification by soaking the sheets in 75% ethanol and then washed twice using sterile saline water. Ninety days old *T. molitor* and sixty days old *Z. morio* were collected from local farmers in Sedayu, Yogyakarta. Each larva was then measured for its length and weighed to collect the same average size of the larvae. The *T. molitor* larvae were approximately 2.18 ± 0.21 cm in length and 27.3 ± 0.02 mg/larva in weight, meanwhile, *Z. morio* were approximately 3.94 ± 0.34 cm in length and 608.5 ± 0.12 mg/larva in weight. Those larvae were then placed in earthenware to keep their temperature cold. Both *T. molitor* (n= 100) and *Z. morio* (n= 100) were treated for 1 week with a rice mill diet. The larvae were starved for 3 days, then given LDPE sheets previously weighed as their diet. LDPE sheets were collected and examined using stereomicroscope (Nikon SMZ800N, Japan) and Scanning Electron Microscope-Energy Disperse X-ray (SEM-EDX) (JEOL JSM-6510, USA) to see the destruction caused by both larvae after 30 days of treatment. LDPE sheets were also weighted to compare the size before and after treatment. LDPE weight reduction index were determined using the following formula (Putra and Ma'rufah 2022):

$$D = (W-R)/W$$
$$\% = D \times 100\%$$

Where:
W : Initial weight of LDPE Sheet
R : Final weight of LDPE Sheet
D : LDPE degradation level
% : Weight reduction index

Sample extractions and metagenome analysis

In order to collect gut tissues from *T. molitor* and *Z. morio*, the larvae were sterilized. They were submerged for 1 minute in ethanol 75%, then washed twice with sterile saline water (Vojvodic et al. 2013; Cucini et al. 2022). Then, the larvae were dissected using sterile scissors and a scalpel. The gut was then pooled into 50 ml centrifuge tubes. The deoxyribonucleic acid (DNA) genome was extracted using the cetyltrimethylammonium bromide (CTAB)/sodium dodecyl sulfate (SDS) method. Purity of DNA was monitored using 1% agarose gels. The extracted DNA was used for the PCR mixture. Polymerase chain reaction (PCR) amplification used 16S rRNA primer with the barcode. PCR product was detected with electrophoresis in 2% agarose. The sequence data process was then merged using FLASH (V1.2.7). Paired-end reads were assigned to samples based on their unique barcodes and truncated by cutting off the barcode and primer sequences to create tags. Sequence tags with more than or equal to (≥) 97% similarity were assigned to the same operational taxonomic units (OTUs).

RESULTS AND DISCUSSION

LDPE biodegradation by larvae

Based on this current study, the evidence that the larvae could chew the LDPE sheet was shown in Figure 1. The LDPE sheet was examined using a stereomicroscope to see the structure of the sheet after treatment. The control sheet showed smooth edges. Meanwhile, both of the sheets that were placed with larvae showed changes around the edges. The *T. molitor* treatment has some wrinkly and uneven edges. This LDPE sheet shows more damage than that of *Z. morio* treatment. *Zophobas morio* treatment causes less harm than the *T. molitor* treatment probably because some of the *Z. morio* larvae were dead during the experiment.

LDPE sheets were then examined using SEM-EDX. SEM analysis was conducted to show the surface of the LDPE sheet between control, *T. molitor*, and *Z. morio* after treatment. An EDX analysis was employed to find out if there was a change in the constituent elements of the LDPE sheet. SEM analysis of the LDPE sheet from the *T. molitor* and *Z. morio* treatments showed that there was microdeterioration and a bumpy surface. It is in contrast to the control treatment which showed no damage on a smooth surface with some wavy structure from the plastic weave and no damage visible at 5000x magnification (Figure 2.A). SEM analysis results from the *T. molitor* treatment show some flaky and irregularly carved structures (Figure 2.B). This damage may be caused by the chewing and movement activity of the larvae in the *T. molitor* treatment. *Zophobas morio* treatment using SEM analysis showed that there was some bumpy and wrinkled structure with some microdeterioration (Figure 2.C). *Zophobas morio* treatment showed less damage because *T. molitor* larvae were chewing and moving a lot more than those of *Z. morio*. It is proof that larvae belonging to the family Tenebrionidae have been reported to have the ability to chew and penetrate plastic materials like PE (Peng et al. 2020a).

Based on the EDX analysis, the carbon element showed that there was a difference between the control and the treatment larvae (Figure 3d). The control had 75.93% mass of carbon, but the *T. molitor* and *Z. morio* treatment showed a carbon mass of 74.64% and 74.63% respectively. The decrease in this carbon mass might occur because of what the larvae consume. Meanwhile, the decreasing mass of carbon could be proof that the larvae could degrade the plastics. A unique phenomenon occurred where the oxygen element was only found in the two LDPE sheets used as larvae diet but not in the control one (Figure 3d). This indicates the possibility of an oxidation reaction taking place in the treated sheets leads the detection of oxygen elements. According to Kim et al. (2022), oxidation of plastics can occur because of abiotic treatments such as exposure to UV light (photo-oxidation) and high temperatures (thermal-oxidation), but in this study, the researchers did not pre-treat the LDPE sheet. Therefore, the hypothesis is that in the process of eating plastic, the two species of larvae produce an oxidase enzyme, which was secreted through their mouths to help digest the plastic. This has not been reported before, but a recent study by Sanluis-Verdes et al. (2022) revealed that the saliva of *G. mellonella* larvae contains enzymes that can oxidize PE plastic films. Oxidation in the plastic degradation process is beneficial because the presence of oxygen leads to the formation of carbonyl and hydroxyl groups in the LDPE chain. Due to the instability of these two functional groups, LDPE becomes brittle and easily degraded (Zhu et al. 2020; Fibriarti et al. 2021).

Recent studies have found that *T. molitor* larvae can depolymerize LDPE and PS foam in their digestive tract even when larvae are given the antibiotic gentamicin (Peng et al. 2019; Yang et al. 2021a; Yang et al. 2021b). This indicates that the LDPE depolymerization process in *T. molitor* is independent or less dependent on their gut microbiota. However, Peng et al. (2020b) investigated the biodegradation of LDPE and PS foam by *Z. atratus* larvae (taxonomically co-specific with *Z. morio*). It was found that these larvae could not depolymerize both types of plastic when they were given antibiotic to suppress their gut microbiota. This study shows that depolymerization in the digestive tract of *Z. morio* depends on their gut microbiota. Depolymerization that occurs in the larval digestive tract may occur in two ways: board depolymerization (BD) and limited extent depolymerization (LD). BD can reduce the number-average molecular weight (M_n) and weight-average molecular weight (M_w) in a polymer, while LD can increase M_n but reduce M_w in a polymer. The *T. molitor* larva has been reported to be able to perform BD and LD on PE. Meanwhile, *Z. morio* is able to perform BD on PS and can only perform LD on PE (Peng et al. 2020b; Yang et al. 2020).

Moreover, other larvae from Order Coleoptera such as *T. obscurus* and *G. mellonella* are also known to be able to depolymerize plastic polymers such as PS without relying on their gut microbiota (Peng et al. 2019; Zhu et al. 2021). We suspect that this independent ability of depolymerization exists because of the physical and enzymatic processes that occur in the digestive system of the larvae. These processes cause LDPE to be depolymerized and degraded. The findings of a study conducted by Zhong et al. (2022) provided new evidence that *T. molitor* larvae were able to digest LDPE and PS, and it was suspected that the fatty acid degradation pathway played an important role in the digestion process.

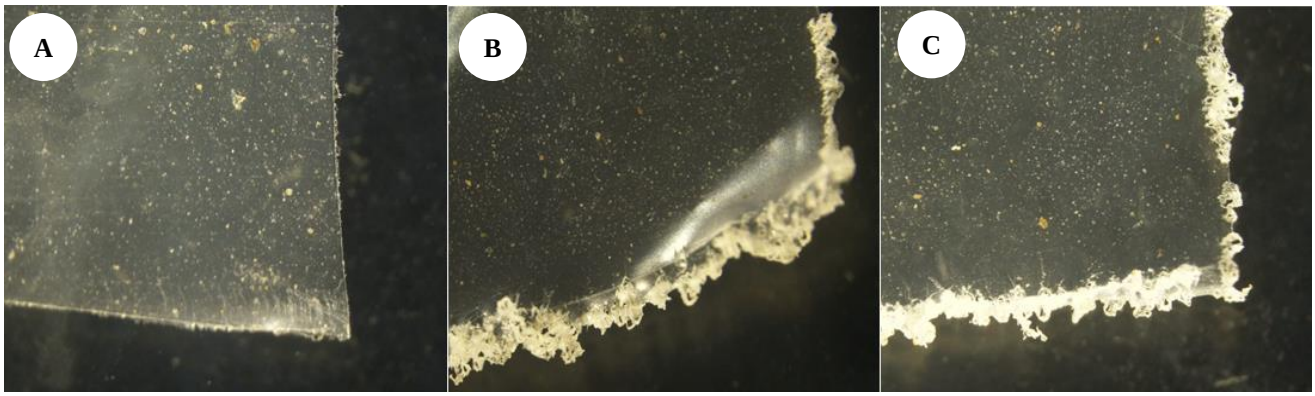


Figure 1. LDPE sheets condition after 30 days of treatment: A. Control, B. *Tenebrio molitor* treatment, C. *Zophobas morio* treatment

Biodegradation assay by larvae was carried out using 5 pieces of LDPE sheets which were 3 cm x 3 cm in size. They were fed to 100 *T. molitor* and *Z. morio* larvae. Measurement of the rate of degradation can be easily carried out by identifying the difference between the initial weight and the final weight of the LDPE sheet (weight reduction index). The results of LDPE sheet biodegradation using *T. molitor* larvae showed a lower weight reduction compared to previous studies using LDPE foam (Table 1). This is presumably due to the different shapes of LDPE. LDPE foam has a less compact structure than LDPE sheet, which makes it easier for the larvae to chew and consume it. In addition, the researchers believe that the process of plastic biodegradation by larvae can be carried out by feeding the larvae their natural food (Brandon et al. 2018; Bulak et al. 2021; Sanchez-Hernandez 2021; Pinchi et al. 2022). A study on *G. mellonella* larvae found that the addition of wheat bran did not significantly increase the value of PE and PS foam biodegradation in short-term incubation but increased the survival rate of these larvae. Therefore, in the future, plastic biodegradation can be carried out for a longer time so that a higher biodegradation value may be obtained (Lou et al. 2020). Wang and Tang (2022) reported that the addition of natural food as a co-diet with PS foam did not only increase biodegradability but also reduced the adverse effects on the development of *T. molitor* due to PS foam consumption.

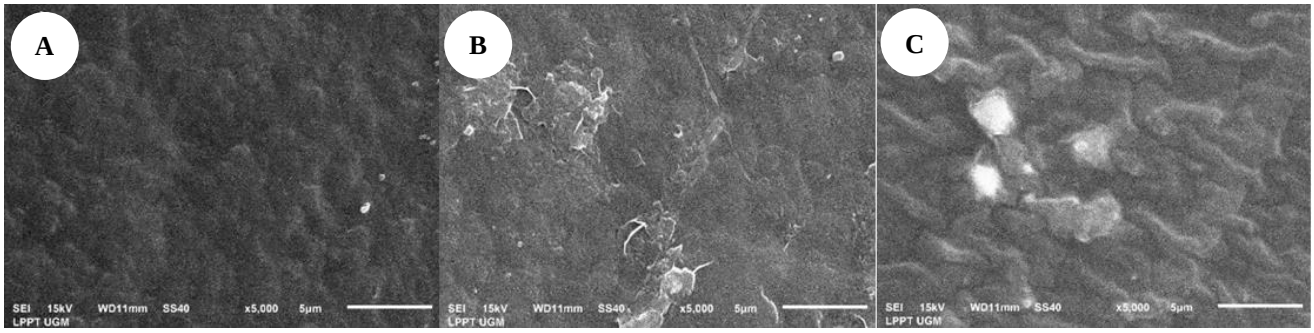
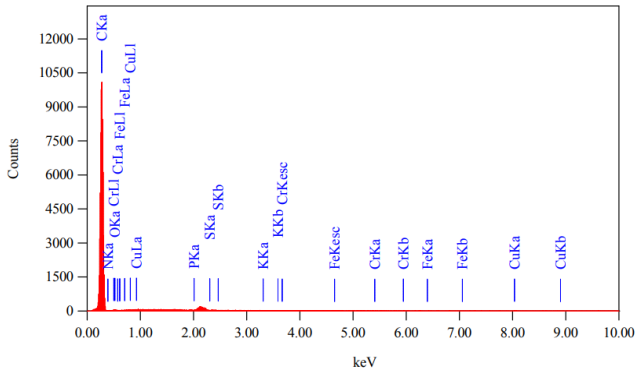
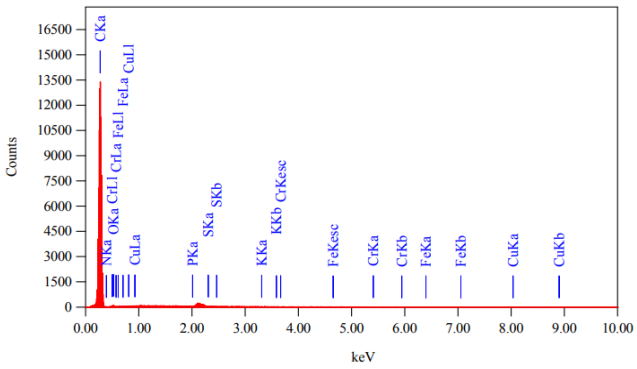


Figure 2. SEM LDPE sheets surface condition after 30 days of treatment: A. control, B. *Tenebrio molitor* treatment, C. *Z. morio* treatment



A



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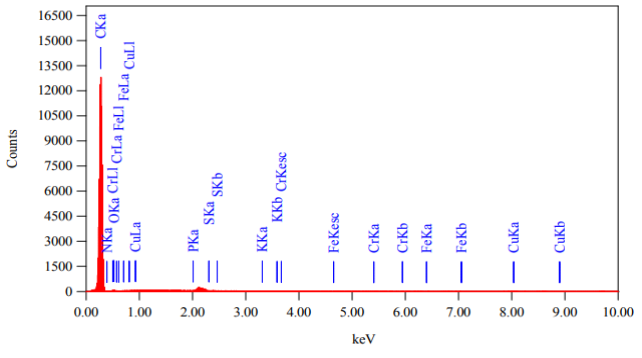

B

Element	Mass %			Atom %		
	Control	*T. molitor*	*Z. morio*	Control	*T. molitor*	*Z. morio*
C K	75.93	74.43	74.64	78.87	77.53	77.75
N K	23.62	24.13	24.19	21.04	21.55	21.60
O K	-	1.09	0.71	-	0.85	0.55
Fe K	0.02	0.04	0.03	0.01	0.01	0.01
Cu K	0.043	0.42	0.32	0.08	0.08	0.06
Total	**100**	**100**	**100**	**100**	**100**	**100**

D

Figure 3. EDX result of LDPE sheets after 30 days of treatment: A. control micrograph, B. *Tenebrio molitor* treatment micrograph, C. *Z. morio* treatment micrograph, D. mass and atom percentage table

Metagenome analysis

Alpha diversity was used to see species richness and equitability index in the microbial community. Species richness can determine the quantity of individual species per sample. Meanwhile, the equitability index shows the abundance of species and their evenness of distribution (Thukral 2017). Alpha diversity is measured based on their microbial community rarefaction curves and alpha diversity index. Rarefaction curves show that both curves have a similar kind of slope but are different in height (Figure 4). The number of species identified in the sequence can be seen from the increase in the curve. The steeper the curve, the greater the number of species that can be identified. The sloping curve indicates that fewer species have been identified because they are found from the initial sequence, so only rare species can be identified (Pangastuti et al. 2019). Both curves have reached the saturation phase, which indicates that most of the species were analyzed.

Zophobas morio sample curve has higher steepness which means that in this sample there are more identified species than the gut in *T. molitor* sample. A higher curve indicates that there are more species that can be sequenced. *Zophobas morio* has a higher detected species, which may be occurs due to the larvae eating less plastic than *T. molitor* during the experiment. This can be proven based on the weight reduction index obtained. Additionally, this can be caused by stress due to the consumption of LDPE, which makes the gut bacteria of *T. molitor* become lower than *Z. morio*. As the result, more species of gut bacteria from *Z. morio* survived and were detected compared to gut bacteria from *T. molitor*.

From the alpha diversity index table, some information such as species observed, Shannon, Simpson, Chao1, ACE, and coverage was obtained (Table 2). The total species that had been identified was 551 for *Z. morio* and 441 species for *T. molitor*. Shannon index and Simpson index were used to describe the species diversity of microbial communities (Monleón et al. 2019). It was perceived that the Shannon and Simpson indexes on *Z. morio* were higher than those on the *T. molitor* (Table 2). These values indicate that the *Z. morio* had greater species diversity in gut microbes than *T. molitor*. These great diversities are related to the number of species observed in which *Z. morio* had a higher value than *T. molitor*. Meanwhile, Chao1 and abundance-based coverage estimator were used to examine the species richness from their abundance in the sequence (Chao and Chiu 2016). As previously stated, Chao1 and ACE values from *Z. morio* samples were higher than those from *T. molitor*. These values show that the *Z. morio* had a greater species richness, which means it had a lot more species of gut microbes than the *T. molitor*. Both of their coverage was at 0.999, which means that most of the bacteria from the sample were present. Not all of the bacteria can be present because the amount of bacteria abundance is massive so it is possible to be not present at all.

Table 1. LDPE weight reduction compared to other studies

Larva	Source	Plastic type	Incubation time (days)	Weight reduction (%)	Reference
Tenberio molitor	Indonesia	LDPE sheet	30	44.6±0.02	*This study*
Zophobas morio	Indonesia	LDPE sheet	30	16.8±0.03	*This study*
Corcyra cephalonica	India	LDPE sheet	20	25	Kesti and Thimmappa (2019)
Tenberio molitor	USA	LDPE foam	32	51.8	Brandon et al. (2018)
Tenberio molitor	Poland	LDPE foam	58	69.71	Bulak et al. (2021)
Uloma sp.	India	LDPE foam	28	40.8	Kundungal et al. (2021)

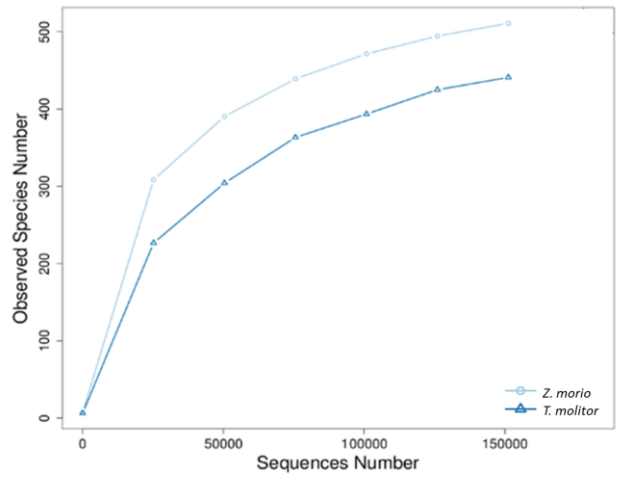

Figure 4. Rarefraction curves of the observed species

Based on metagenome analysis, 411 and 511 OTUs were found in the digestive tracts of *T. molitor* and *Z. morio* larvae respectively. These results indicated that the bacterial community in the digestive tracts of the two larvae was diverse. The larvae of *Z. morio* gut bacteria were dominated by the phyla Proteobacteria (53%) and Firmicutes (45%), while *T. molitor* contained Proteobacteria (8%), Firmicutes (57%), and Actinobacteriota (35%) (Figure 5a). This result is similar to the study conducted by Urbanek and Mirończuk (2020). It was found that *T. molitor* larvae gut bacteria treated with the PS diet were dominated by phyla proteobacteria, firmicutes, and actinobacteriota. Sun et al. (2022) also reported that the phyla Proteobacteria and Firmicutes dominated the gut bacteria community of the digestive tract of *Z. morio* with styrofoam diet.

Table 2. Alpha diversity sample indices

Sample	Observed species	Shannon	Simpson	Chao1	Ace	Goods coverage
Z. morio	511	4.139	0.895	606.136	565.186	0.999
T. molitor	441	4.024	0.866	482.786	506.800	0.999

Table 3. Plastic-degrading bacteria isolated from gut of several larvae species

Larva	Plastic type	Plastic-degrading gut bacteria	Reference
Tenberio molitor	PS	*Klebsiella oxytoca* ATCC 13182, *Klebsiella oxytoca* NBRC 102593, and *Klebsiella oxytoca* JCM 166	Machona et al. (2022)
		Citrobacter freundii, *Serratia marcescens*, and *Klebsiella aerogenes*	Brandon et al. (2021)
Zophobas morio	PS	*Bacillus megaterium*	Tan et al. (2021)
		Pseudomonas sp. DSM 50071	Kim et al. (2020)
		Pseudomonas sp. EDB1, *Bacillus* sp. EDA4 and *Brevibacterium* sp. EDX	Arunrattiyakorn et al. (2022)
Galleria mellonella	PE	*Enterobacter* sp. D1	Ren et al. (2019)
		Massilia sp. FS1903	Jiang et al. (2021)
Plodia interpunctella	PE	*Enterobacter asburiae* YT1 and *Bacillus* sp. YP1	Yang et al. (2014)
Spodoptera frugiperda	PVC	*Klebsiella* sp. EMBL-1 and *Klebsiella variicola*	Zhang et al. (2022)

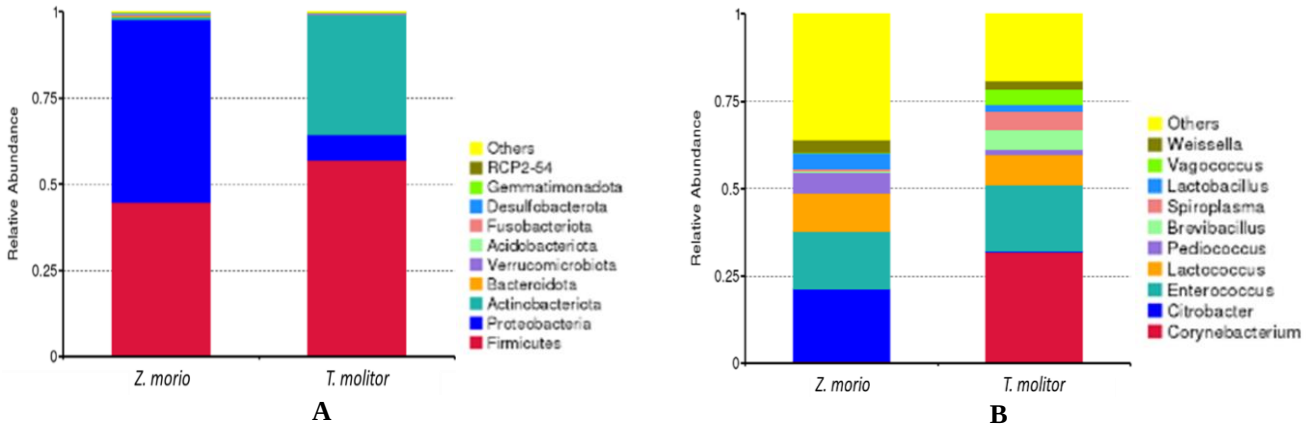

Figure 5. Top 10 Metagenome relative abundance. A. Phyla abundance of *Tenebrio molitor* and *Zophobas morio* gut bacteria, B. Genera abudance of *Tenebrio molitor* and *Zophobas morio* gut bacteria

Among the genera that dominated the two larvae, the ten most dominant genera were found. Those dominating in *T. molitor* were Corynebacterium, Enterococcus, Lactococcus, Pediococcus, Brevibacillus, Spiroplasma, Lactobacillus, Vagococcus, and Weissella. Meanwhile, Citrobater, Enterococcus, Lactococcus, Pediococcus, Brevibacillus, Spiroplasma, Lactobacillus, Vagococcus, and Wiessella were dominating *Z. morio* larva (Figure 5b). Corynebacterium which dominated *T. molitor* with 32% coverage was also reported to be one of the predominant genera on *T. molitor* with PS only diet (Lou et al. 2021). In addition, from the results of recent studies, Corynebacterium found in gut of *Z. morio* posses genes associated with PS degradation (Sun et al. 2022). This indicates the possibility that bacterial species in this genus have a role in the biodegradation of LDPE and PS in the digestive tract of *T. molitor*. Meanwhile, the dominant genus in *Z. morio* was Citrobacter with 21% coverage. Citrobacter is reported to be one of the bacteria that play a role in PS degradation which can be found in the gut microbiota of *Z. morio* (Jadaun et al. 2022). In addition, bacterial genera such as Corynebacterium and Citrobacter in both larvae were dominant because these bacteria are opportunistic pathogenic bacteria which in the presence of LDPE in digestive system cause dysbiosis due to the decrease of other gut microbiota. Other genera found in both gut bacteria that might contribute to LDPE biodegradation were Spiroplasma, Lactococcus, Enterococcus, Pediococcus, and Lactobacillus. Those genera were also reported by several studies to be associated with PE and PS foam diet treatment on *T. molitor* (Brandon et al. 2021; Lou et al. 2021; Tsochatzis et al. 2021).

Previous studies have revealed that bacteria isolated from the digestive tract of the larvae of several insect species have the ability to degrade various types of plastics

(Table 3). Their ability to degrade plastics is inseparable from the performance of enzymes. Enzymes that are associated with the degradation of plastic polymers are PEases, lipases, PETases, and MHETases (Bhardwaj et al. 2013; Yoshida et al. 2016; Li et al. 2019; Sanluis-Verdes et al. 2022). Yang et al. (2015) revealed that digestive enzymes produced by gut microbiota isolated from *T. molitor* larvae are believed to be the main enzymes that work in degrading PS plastic polymers. Furthermore, Mohanan et al. (2020) pointed out that the gut bacteria of *G. mellonella*, such as *Enterobacter* sp. have the enzymatic ability to cause oxidation and depolymerization reactions in PE films. Then, according to Kim et al. (2020), *Z. atratus* larvae gut bacteria such as *Pseudomonas* sp. secreted various enzymes which are reported to be associated with plastic degradation such as hydrolases, S-formylglutathione, and serine-hydrolase.

In conclusion, *T. molitor* and *Z. morio* larvae can degrade LDPE sheets without the addition of other food sources. Nevertheless, the weight reduction of LDPE sheets in this present study is still relatively low compared to other studies. Based on SEM analysis, LDPE suffered from microscopic damage. EDX analysis reveals that there is a possibility of an oxidation process occurring in LDPE, which we suspect is caused by the saliva that contains oxidase enzymes produced by the larvae. The enzymatic ability of the saliva of both larvae needs to be further explored because it can potentially become an alternative for enzymatic pre-treatment of plastic waste. In addition, metagenome analysis shows the domination of several gut bacteria genera. Those genera might be targeted to be isolated and developed as a ready-use inoculum used as a collaboration agent for the larvae to speed up biodegradation.

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