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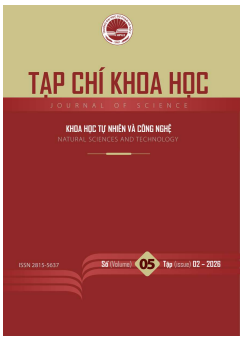

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Polyethylene degradation ability of superworms (*Zophobas morio*) and isolation of polyethylene-degrading microorganisms from superworm gut and landfill soil

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Abstract

Plastic pollution, particularly polyethylene (PE), represents a major global environmental challenge. This study aimed to evaluate the ability of superworms (*Zophobas morio*) to consume polyethylene and to isolate bacterial strains with potential PE-degrading capacity from landfill soil and the superworm gut. After 28 days of feeding on PE as the sole carbon source, an average PE mass reduction of 11 ± 2.25 mg/week (n = 3) was observed. Microbial isolation resulted in 11 bacterial strains, of which three strains (D2, D4, and RS1) grew on selective media containing PE as the sole carbon source. These findings provide preliminary evidence of the role of superworms and associated microorganisms in polyethylene consumption. However, further studies using physicochemical, enzymatic, and molecular approaches are required to confirm actual biodegradation mechanisms.

Keywords: *Zophobas morio, polyethylene biodegradation, plastic-degrading bacteria, landfill soil, gut microbiota*

1. Introduction

Plastic waste has become a serious global environmental issue due to its increasing accumulation in natural ecosystems [1], [2]. Plastic pollution is considered one of the most urgent environmental challenges today because of its far-reaching impacts on biodiversity, ecosystem function, and human health [3]. Since the mid-20th century, global plastic production has grown exponentially reaching more than 400 million tons annually. A substantial proportion of this plastic is ultimately released into the environment as waste after use [4].

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The potential health risks posed by microplastics have become a major concern. Evidence of microplastics being present in drinking water, food, and even within human tissues, including genetic mutations, impaired brain function, respiratory issues, and detection in the placenta, has raised alarms about their bioavailability and toxicity [5], [6], [7]. Experimental and epidemiological studies suggest that prolonged exposure to microplastics may induce oxidative stress, inflammatory responses, and endocrine disruption [8], [9]. In addition, microplastics can adsorb and transport toxic chemicals, and this property increases their potential risks to human health [10]. These particles can enter the food chain through aquatic environments, sea salt, and drinking water, and they pose potential threats to food safety and public health [5].

Among various types of plastics, polyethylene (PE) is one of the most widely produced materials and accounts for a large proportion of global plastic production. PE is commonly used in plastic bags, food packaging, bottles, and other household products. Due to its high resistance to degradation, PE persists in the environment for long periods causing serious ecological impacts and posing potential risks to living organisms [11], [12], [13], [14].

In light of this situation, the search for biological degradation solutions for PE has garnered growing interest from scientists worldwide. In recent years, numerous studies have highlighted the remarkable biodegradation potential of certain insect species, particularly waxworms and grain-eating larvae, in consuming and reducing the mass of PE. Notably, species such as the mealworm (*Tenebrio molitor*) and the superworm *Zophobas morio* (also known as *Z. atratus)* have been shown to ingest and metabolize PE through the action of gut microbiota [15], [16], [17]. Symbiotic microorganisms in their digestive systems, including various bacterial and fungal strains, are capable of oxidizing, depolymerizing, and converting complex organic polymers into simpler forms. This emerging research direction shows potential for environmentally friendly and sustainable plastic waste management.

In Vietnam, although plastic pollution is becoming increasingly serious, research on PE biodegradation using microorganisms or insect digestive ecosystems remains limited. Most studies have focused primarily on morphological observations or preliminary assessments of plastic consumption ability, without delving deeper into the isolation, selection, and biological characterization of microbial strains capable of degrading PE. Therefore, conducting in-depth research in this field is both scientifically valuable and practically significant for environmental protection.

In this context, the present study is designed as an exploratory investigation to (i) evaluate the polyethylene consumption ability of *Zophobas morio* and (ii) isolate bacterial strains with potential polyethylene-degrading capacity from landfill soil and the superworm gut. This study aims to provide preliminary data for future mechanistic and applied research on plastic biodegradation in Vietnam.

2. Experimental methods

2.1. Evaluation of polyethylene nylon degradation by superworms

Superworms (*Zophobas morio*) were reared under controlled laboratory conditions (25°C, relative humidity 60–70%). Healthy larvae were selected, and unhealthy individuals were excluded prior to the experiment.

The experimental design included two groups:

- Control: Superworms fed with cornmeal

- Treatment: Superworms fed with PE as the sole carbon source

Each experimental unit contained approximately 16 g of larvae (~80–100 individuals) and 150 mg of PE (3 pieces × 50 mg). The selected sample size and PE dosage were based on previous studies to ensure measurable changes in mass.

Measurements were conducted at 7-day intervals over 28 days. Each treatment was performed in triplicate (n = 3) [18].

PE consumption was evaluated using the gravimetric method, a commonly used preliminary indicator in biodegradation studies.

2.2. Soil and gut sample collection

After 28 days of rearing under the two experimental conditions, digestive system samples were collected from the superworms in both groups for microbial analysis.

Soil samples were collected according to Vietnamese National Standard TCVN 7538-6:2010 (ISO 10382-6:2019) - *Soil Quality - Sampling - Guidance on collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*.

The soil was collected from the landfill site in Xuan Hoa Ward, Phuc Yen City, Vinh Phuc Province. Samples were taken directly from the landfill, at a depth of 10 - 15 cm below the surface. Multiple sampling points were selected randomly, but at the same depth. The soil was placed in zip-lock bags with clearly labeled information (sampling location and date), then transported to the laboratory for bacterial isolation. Samples were stored at 4°C until further processing.

2.3. Enrichment method

Enrichment was performed using liquid carbon-free basal medium (LCFBM) containing PE as the sole carbon source. This method was selected to promote the growth of microorganisms capable of utilizing polyethylene under carbon-limited conditions [19].

1g of soil sample or 1 g of superworm gut sample was diluted with 9 mL of distilled water. Then, 5 mL of the diluted sample was transferred into 95 mL of LCFBM and incubated with shaking at 180 rpm at 30ºC. After two weeks of shaking incubation, the culture was transferred into fresh LCFBM. This subculturing process was repeated two more times [20].

Table 1. Composition of LCFBM

Composition	**Concentration**
KH_2PO_4	0.7 g/L
K_2HPO_4	0.7 g/L
$MgSO_4$.$7H_2O$	0.7 g/L
NH_4NO_3	1 g/L
NaCl	0.005 g/L
$FeSO_4.7H_2O$	0.002 g/L
$ZnSO_4.7H_2O$	0.002 g/L
$MnSO_4.H_2O$	0.001 g/L
Distilled water	1000ml
PE	1 g/L

2.4. Bacterial Isolation Method

After six weeks of enrichment, the shaking culture from the soil sample was serially diluted to appropriate concentrations. First, 10 mL of the culture was transferred into a flask and mixed with 90 mL of distilled water, and shaken well to obtain a 10^{-1} dilution. Five penicillin vials were prepared and numbered from 2 to 6, each containing 9 mL of sterile distilled water. Using a micropipette, 1 mL of the 10^{-1} dilution was added to vial number 2 and mixed well to obtain a 10^{-2} dilution. This process was repeated sequentially to reach a 10^{-6} dilution in the final vial [21]. The same dilution procedure was applied to the shaking culture from the superworm gut sample.

After dilution, 100 µL of each diluted sample was spread onto MPA agar plates. The samples were spread evenly across the surface of the agar using a sterile spreader. Each Petri dish was labeled with the isolation date, sample type, and dilution concentration, then wrapped in paper. The plates were incubated at 30ºC for 24 to 48 hours, after which individual bacterial colonies were observed.

Table 2. Composition of MPA medium

Composition	Concentration
Meat extract	5 g/L
Pepton	10 g/L
NaCl	5 g/L
Distilled water	1000ml
pH	7

Note: Add 2% agar when preparing solid media.

2.5. Selection of plastic-degrading strains

Isolated bacterial colonies were purified and transferred to CFBAM medium, which contains polyethylene as the sole carbon source. After incubation at 30°C for 7 days, strains that showed growth were considered to have potential plastic-degrading ability.

The CFBAM medium, a solid agar medium, is used to isolate PE-degrading bacteria [19].

Table 3. Composition of CFBAM

Composition	Concentration
KH_2PO_4	0.7 g/L
K_2HPO_4	0.7 g/L
$MgSO_4.7H_2O$	0.7 g/L
NH_4NO_3	1 g/L
NaCl	0.005 g/L
$FeSO_4.7H_2O$	0.002 g/L
$ZnSO_4.7H_2O$	0.002 g/L
$MnSO_4.H_2O$	0.001 g/L
Distilled water	1000ml
PE	1 g/L
Agar	15 g/L

2.6. Statistical analysis

All experiments were conducted in triplicate (n = 3). Data are presented as mean ± standard deviation. Analysis of variance (ANOVA) was performed using Statistica version 10.0 and means were compared using Duncan's multiple range test at a significance level of $p \leqslant 0.05$.

3. Results and discussion

3.1. Polyethylene consumption by superworms

After 28 days of rearing in an environment supplemented with PE nylon bags, the weight of the superworms and the mass of the PE nylon were measured. The changes in superworm weight and PE mass are shown in Figure 1 and Table 4.

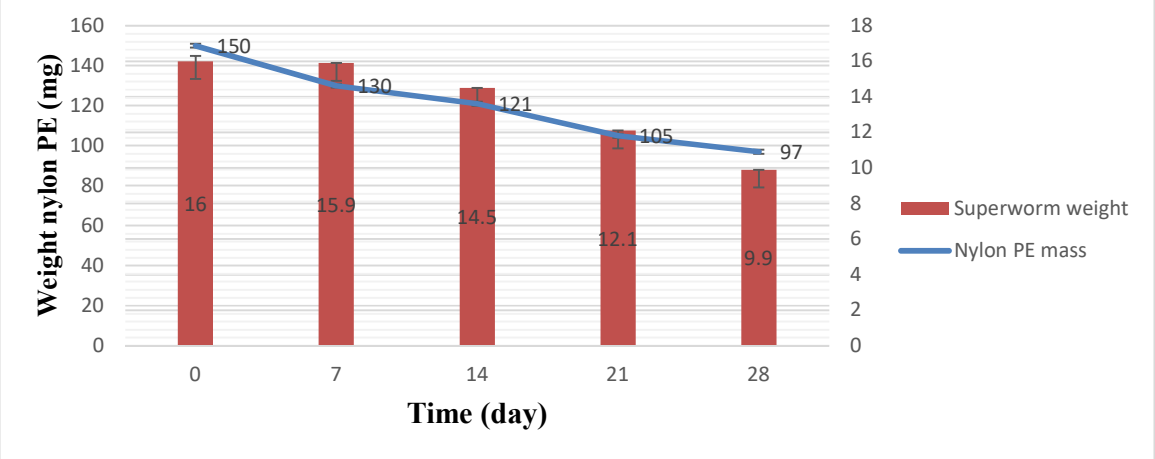


Figure 1. Correlation chart between superworm weight and PE mass

Table 4. Changes in superworm body weight under experimental and control conditions

Time (days)	Experimental (PE diet) (g)	Control (cornmeal diet) (g)
0	16.0 ± 0.13	16.0 ± 0.18
7	15.9 ± 0.11	16.4 ± 0.22
14	14.5 ± 0.24	17.1 ± 0.11
21	12.1 ± 0.12	17.8 ± 0.38
28	9.9 ± 0.35	18.6 ± 0.12

The results indicate that both the body weight of the superworm *Zophobas morio* and the mass of the PE gradually decreased over the experimental period. On average, according to formula 2, the amount of PE consumed by the superworms reached 11 ± 2.25 mg per week, with the highest consumption recorded in weeks 2 and 3. These results indicate that *Zophobas morio* is capable of utilizing PE as an alternative carbon source, although the metabolic efficiency remains low. After 28 days of cultivation, the PE mass decreased by an average of 11 ± 2.25 mg/week, while the superworm weight also gradually declined throughout the study period, suggesting that part of the energy derived from PE consumption was used for metabolism and basic metabolic functions.

These findings are consistent with recent international publications regarding the ability of *Zophobas morio* and other insects with similar mechanisms to consume and degrade PE. Specifically:

According to Luo *et al. (*2021), the PE consumption rate of *Zophobas atratus* averaged 0.3 mg/larva/day under conditions without additional nutrient supplementation. This result shows that the species can use PE as an alternative carbon source, though the energy extraction efficiency from the

synthetic polymer is low, leading to a reduction in body biomass during the experiment [16].

Additionally, Peng *et al.* (2020) reported that *Zophobas atratus* larvae could consume both low-density polyethylene (LDPE) and polystyrene (PS), with an average LDPE consumption rate of 52.9 ± 3.1 mg/100 larvae/day over a 28-day experiment. However, gel permeation chromatography (GPC) analysis revealed that LDPE depolymerization occurred only to a limited extent, with most high molecular weight polymers excreted as microplastics, indicating a low level of biodegradation efficiency [18].

Notably, Luo *et al.* (2021) also observed changes in the activities of gut enzymes (lipase, protease, and amylase) along with shifts in the gut microbial community structure when larvae fed on PE, suggesting a role of symbiotic microorganisms in polymer oxidation and chain cleavage [16]. This aligns with Peng *et al.* (2020), who concluded that bacteria such as *Citrobacter* sp. and enzymes like arylesterase are directly involved in the degradation of PE and PS in the gut of *Zophobas atratus* [18].

Synthesizing these findings suggests that *Zophobas atratus* can consume and reduce the mass of PE, but the biodegradation process mainly occurs at the initial depolymerization stage, supported by gut microorganisms. This opens potential applications for this species in plastic waste management research but also highlights the need to enhance biodegradation efficiency through microbiome optimization or enzyme supplementation.

Thus, the results of the current study not only align with international research trends but also contribute additional scientific data from Vietnam regarding the biological potential of *Zophobas morio* in PE degradation. The observed reduction in PE mass across the feeding stages, together with physiological changes in the worms and endogenous enzyme activity, clearly demonstrate that *Zophobas morio* possesses both mechanical (chewing and fragmenting plastic) and biological (enzymatic and microbial) degradation capabilities during the PE breakdown process, opening promising avenues for the biological treatment of plastic waste in the future.

However, research on the PE consumption capacity of *Zophobas morio* remains limited. Therefore, further in-depth studies on the biodegradation potential of PE by *Zophobas morio* are necessary, especially given the increasing concern over pollution from PE plastic bags and films. Moreover, analyzing the diversity of the gut bacterial community is essential to identify bacterial groups involved in the biodegradation process of PE.

a) b)

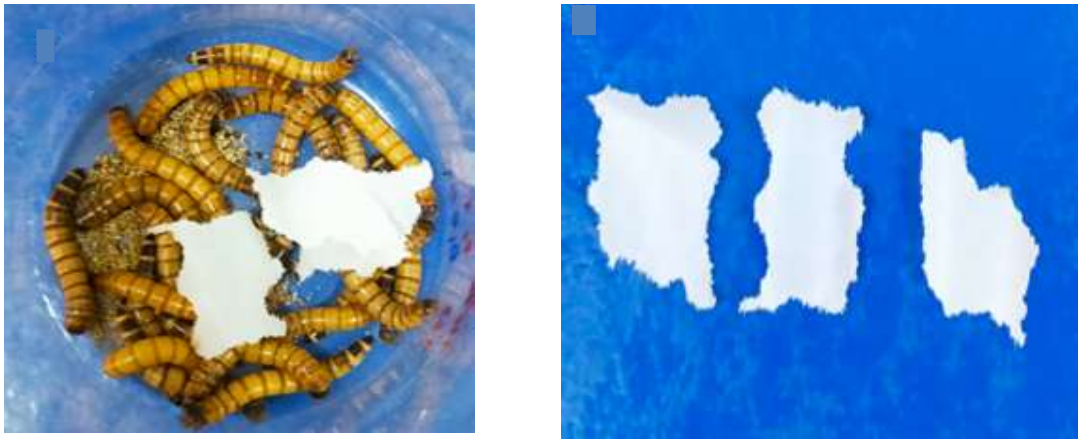


Figure 2. Polyethylene degradation ability of superworms after 28 days in formula 2
(a: Superworms in PE environment after 28 days
b: PE after 28 days)

3.2. Isolation of bacteria from long-term landfill soil samples and superworm gut samples

From soil samples collected at a long-term landfill site, eight bacterial colonies with different morphological characteristics were isolated, corresponding to eight distinct bacterial strains. These strains were designated as D1, D2, D3, D4, D5, D6, D7 and D8.

Similarly, from the gut samples of *Zophobas morio* superworms, the isolation process yielded three bacterial colonies with distinct morphological features, representing three potential bacterial strains, labeled as RS1, RS2, and RS3.

These results indicate that both sample sources, the landfill soil and the superworm gut, harbor diverse microorganisms that may play important roles in the biodegradation of PE. This provides a solid foundation for subsequent selection and evaluation of the PE degradation activity of each strain in further experiments.

Figure 3. Results of microbial isolation on MPA agar medium

A total of 8 strains were isolated from landfill soil, while 3 strains were obtained from the superworm gut. The higher diversity observed in soil samples suggests a richer microbial community compared to the gut environment.

However, no quantitative comparison of microbial abundance was performed, which represents a limitation of this study.

3.3. Selection of bacterial strains capable of degrading PE

Bacterial strains that were purified on MPA agar medium were further transferred to the selective CFBAM medium to evaluate their ability to grow under conditions where PE serves as the sole carbon source.

After the incubation period, among the 8 initially isolated and purified bacterial strains, only 3 strains, D2, D4, and RS1, demonstrated clear growth on CFBAM medium. These results indicate that these strains have the potential to utilize or degrade PE by adapting and thriving in an environment lacking conventional organic nutrients.

The morphological characteristics of the three selected bacterial strains from the long-term landfill soil samples and the digestive system of *Zophobas morio* are detailed in Table 5 below. These features

are important for preliminary identification and taxonomic classification of bacterial strains capable of degrading nylon PE bags in subsequent research steps.

Based on the results presented in Table 5, it can be observed that:

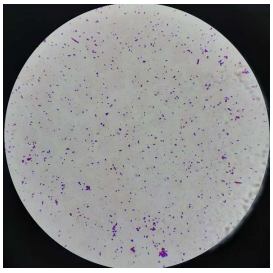
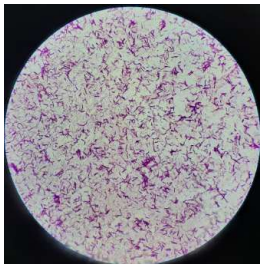
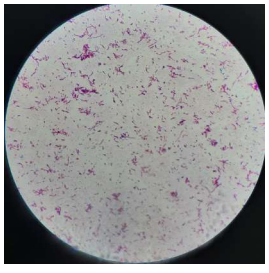
Strain D2 forms white colonies with a wrinkled surface and irregular edges, and its cell morphology was identified as Gram-positive cocci.

Strain D4 produces transparent white, round colonies with a slightly wrinkled surface and thin edges; the cells were identified as Gram-negative rods.

Strain RS1 forms opaque white, round colonies with a convex and smooth surface and thick edges; the cells exhibit Gram-negative rod morphology.

The morphological characteristics of the colonies and the Gram staining results indicate clear biological differences among the three bacterial strains, suggesting they belong to different bacterial groups.

Table 5. Morphological characteristics of 3 bacterial strains capable of degrading PE

Characteristic	**Strain code**		
	D2	**D4**	**RS1**
Figure			
Color	White	Translucent white	Opaque white
Colony characteristics	Wrinkled surface, irregular edges	Round, slightly wrinkled surface, thin edge	Round, convex and smooth surface, thick edge
Cell morphology	Cocci (spherical bacteria)	Bacillus	Bacillus
Gram staining	(+) 	(-) 	(-) 

Note: (−) negative; (+) positive

4. Conclusion

This study provides preliminary evidence that *Zophobas morio* can consume polyethylene under laboratory conditions, with an average reduction of 11 ± 2.25 mg/week. Additionally, three bacterial strains (D2, D4, RS1) capable of growing on polyethylene-containing media were isolated from landfill soil and superworm gut samples.

However, further studies incorporating molecular identification, enzymatic analysis, and physicochemical characterization are required to confirm true polyethylene biodegradation mechanisms.

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