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Decay of low-density polyethylene by bacteria extracted from earthworm's guts: A potential for soil restoration

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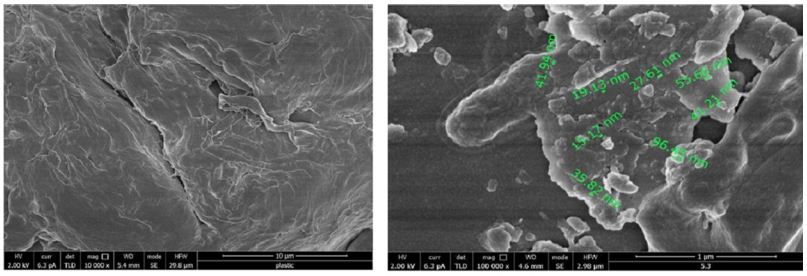
HIGHLIGHTS

- 60% of microplastics content is decayed with isolated bacteria from *L. terrestris* gut.
- Microplastics size is reduced by the bacteria and nanoplastics are produced.
- Different volatiles were emitted in the treatments with bacteria and microplastics.

GRAPHICAL ABSTRACT



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ABSTRACT

Low-density polyethylene (LDPE) is the most abundant source of microplastic pollution worldwide. A recent study found that LDPE decay was increased and the size of the plastic was decreased after passing through the gut of the earthworm *Lumbricus terrestris* (Oligochaeta). Here, we investigated the involvement of earthworm gut bacteria in the microplastic decay. The bacteria isolated from the earthworm's gut were Gram-positive, belonging to phylum Actinobacteria and Firmicutes. These bacteria were used in a short-term microcosm experiment performed with gamma-sterilized soil with or without LDPE microplastics (MP). We observed that the LDPE-MP particle size was significantly reduced in the presence of bacteria. In addition, the volatile profiles of the treatments were compared and clear differences were detected. Several volatile compounds such as octadecane, eicosane, docosane and tricosane were measured only in the treatments containing both bacteria and LDPE-MP, indicating that these long-chain alkanes are byproducts of bacterial LDPE-MP decay.

1. Introduction

Plastic pollution is recognized as a serious global problem. Whereas (micro)plastic pollution in aquatic systems is widely recognized, research concerning plastic pollution in the terrestrial ecosystem began just a few years ago (Huerta Lwanga et al., 2016; Huerta Lwanga et al.,

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2017; Maas et al., 2017; Rillig et al., 2017). Long-term assessments of plastic pollution in soils treated with plastic mulch or with urban sludge containing microplastics are non-existant (Rillig, 2012; Nizzetto and Langaas, 2016). However, there is strong evidence that agricultural soils treated with sludge or plastic mulch are suffering from severe plastic pollution thus monitoring and developing restoration techniques are urgently needed (Steinmetz et al., 2016; Zheng et al., 2017).

Low-density polyethylene (LDPE) is the most common petroleum-based plastic used for mulch in agricultural fields. It is a linear hydrocarbon polymer consisting of long chains of ethylene monomers $(C_2H_4)n$. Recently, Huerta Lwanga et al. (2016) revealed that the earthworm *Lumbricus terrestris* exposed to microplastic LDPE (<150 μm) could uptake LDPE and decrease its particle size (to <50 μm in the earthworm cast) thus facilitating the decay of LDPE as it passes through the gut. We hypothesized that the gut microbiome of *Lumbricus terrestris* contributed to this decay. Although, it is known that microbes isolated from the gut of the larvae of *Plodia interpunctella* (mealmoth) can partially biodegrade PE (Yang et al., 2014) nothing is known so far about the ability of the microbes from earthworm to degrade LDPE.

Therefore, the aim of the present study was to isolate and identify bacteria from the gut of *Lumbricus terrestris* and to test their effect on LDPE microplastic (LDPE-MP) decay in the soil microcosm.

2. Material and methods

2.1. Bacteria extraction from earthworm gut

Earthworms (*Lumbricus terrestris* species) were exposed to microplastic treatments for 60 days. The treatments consisted of surface microplastics mixed with *Populus nigra* litter w/w as described in Huerta Lwanga et al. (2016).

Six worms were collected from the 7% w/w microplastic treatment. Each worm was rinsed thoroughly with Ethanol and dried carefully with paper tissue. Earthworms were then frozen at −18 °C. Under sterilize conditions, the earthworms were defrosted and opened from the ventral side of the body. The opening was made carefully with the help of sterile chirurgical scissors from the prostomial ring (first ring of the body) to the last ring of the body. The incision was intentionally shallow in order to cut only the epithelium of the worm and keep the gut intact. The epithelium was carefully pushed to one side with the help of sterile entomological nails. Each earthworm was treated separately and the process of gut extraction was done on one worm at the time. The gut of each earthworm was extracted carefully by cutting the internal under tissue, which keeps the epithelium attached to the gut. Once the whole gut was extracted, it was immediately deposited inside a sterile container (one container per gut), which was sealed and stored at −18 °C until the bacterial extraction procedure could be carried out. The bacterial extraction was performed under sterile conditions by adding 3 ml of sterile phosphate-buffer to the container with the earthworm gut and mixing at 20 °C for 45 min. Aliquots of 100 μL of the mixture were used for making series of dilutions. From each dilution, 150 μL was spread in triplicate over 1/10 strength Tryptic Soy Broth agar (TSBA) (Garbeva and de Boer, 2009). The plates were then incubated for seven days at 20 °C.

2.2. Bacterial sequencing and identification

PCR amplification of the 16S rRNA genes from the bacterial isolates was performed with isolated DNA using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol. The PCR mix contained 0.6 μM each of primers pA and 1492r (Edwards et al., 1989), 1 μL template and 1× GoTaq G2 Green Master Mix (Promega) containing GoTaq G2 DNA polymerase, reaction buffer (pH 8.5), 3 mM MgCl2 and 400 μM of each dNTP. The thermal protocol was as follows: initial denaturation at 95 °C for 2 min and 34 subsequent cycles at 95 °C for 30 s, at 55 °C for 60 s, and 72 °C for 45 s. The final elongation was at 72 °C for 10 min. The 16S rRNA PCR product of each bacterial isolate was purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and sent for sequencing to Macrogen (Amsterdam).

Sequences were trimmed and aligned by using DNAbaser Sequence Assembler v4.36 and NCBI nucleotide database and BLASTn algorithm (Altschul et al., 1990).

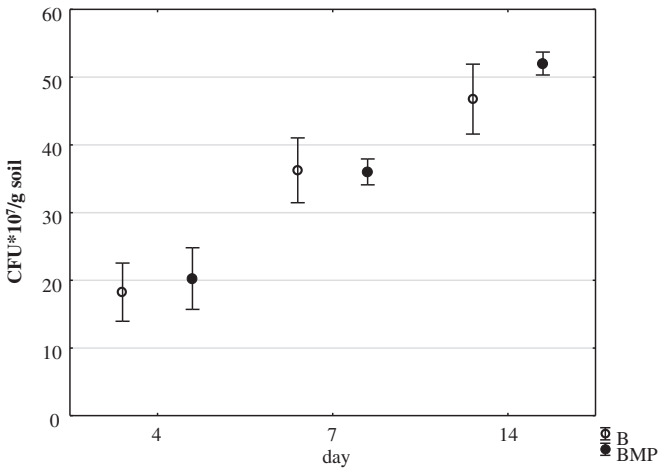

Fig. 1. Bacteria growth (CFU per gram soil) during the experiment.

2.3. Exposure of bacteria to microplastics and bacterium enumeration

Sandy soil with a low carbon-content and a low amount of mineral nitrogen (0.2 mg/kg nitrite and nitrate) and phosphate (1.1 mg/kg) was collected from an old river dune site near the village of Bergharen (51°10′N, 05°40′E) in the Netherlands. The soil was dried, sieved (ø 2 mm), and gamma-sterilized by Synergy Health Ede B.V. (Netherlands, Schulz-Bohm et al., 2017).

150 μm of low density Polyethylene (LDPE) particles were mixed with 20 g of gamma sterile soil (sandy soil with pH of 5.5 and 0.33% organic matter) at a concentration of 1% (w/w).

The isolated bacteria were inoculated into this soil mixture at a concentration of 10^5 CFU per g soil for each strain. A control treatment was also established (mixture without bacteria). Four replicas per treatment

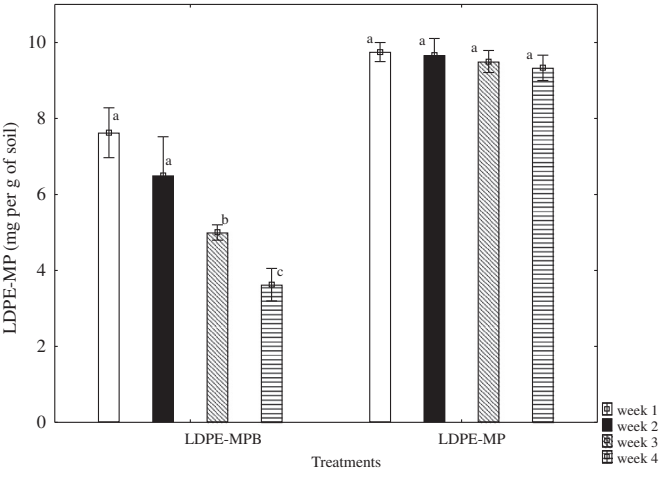

Fig. 2. LDPE-MP decay during 4 weeks with (LDPE-MPB) and without bacteria (LDPE-MP).