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Gut Plastisphere Microbiota-Mediated Polystyrene Depolymerization in the Plastivore Insect *Tenebrio molitor*

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Abstract

Polystyrene (PS) is extensively employed in various industrial applications. Its recalcitrant to depolymerization and biodegradation leads to detrimental environmental persistence, necessitating finding sustainable bioresources such as microbiota and biocatalysts that could degrade plastics. Here, we investigated the role of gut plastisphere microbiome of plastivore *Tenebrio molitor* (yellow mealworm) in the degradation of PS. Metagenomic sequencing using Long-Read Nanopore (LRN) MinION platform was employed to profile the gut microbiome of the *T. molitor* larvae fed with PS in comparison with a control diet. The depolymerization of PS in the gut was characterized by FTIR and ^{1}H NMR analyses. The LRN sequencing of full-length metagenomic 16S rDNA delineated the species-level identification of *Tenebrionibacter* (*T. intestinalis*), *Enterococcus* (*E. faecalis*, *E. canis*, *E. thailandicus*, *E. hermanniensis*), *Spiroplasma* (*S. lampyridicola*, *S. gladiatoris*) and *Clostridium* (*C. cellulovorans*) genera in the gut microbiota of *T. molitor*. PS allotrophagy by *T. molitor* was associated with the elevated levels of *T. intestinalis* (1–1.2-fold), *E. faecalis* (1.2–1.5-fold), *E. canis* (1.1–3.3-fold) and *C. cellulovorans* (7.2-fold) in the gut microbiota. Various *Enterococcus* spp. were only associated with PS allotrophagy, indicating their role in plastic degradation in the gut plastisphere. Furthermore, FTIR and ^{1}H NMR analyses revealed the presence of new functional groups (–OH, C=O) in the frass of PS-fed mealworm, signifying PS depolymerization after ingestion by the mealworms. Taken together, we identified the link between the gut plastisphere microbiota of the plastivore *T. molitor* larvae and PS depolymerization, highlighting the gut plastisphere microbiota as a unique niche for identifying microbes and enzymes that could help develop innovative plastic biodegradation solutions.

Keywords: Gut microbiota, *Tenebrio molitor*, Plastic degradation, FTIR, ^{1}H-NMR, Nanopore sequencing

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