Plastic degradation and upcycling by the gut microbiome of yellow mealworms

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Project goals: This project discovers and reconstructs the plastic degradation pathways distributed across the gut microbiome of yellow mealworms (larvae of *Tenebrio molitor*) to develop enhanced capabilities for biologically based polymer recycling.

Plastics, initially selected for their durability and environmental resiliency, pose a significant environmental challenge for modern economies. Polystyrene (PS), high- and low-density polyethylene (HDPE and LDPE), and polypropylene (PP) are produced at a rate of more than 228 million tonnes globally each year. However, none currently have robust infrastructures for mechanical or chemical recycling and ultimately become polluting waste streams. To address this need, we pursue biological strategies for plastics depolymerization. We focus on the microbiomes of insect larvae (colloquially called worms) as they degrade plastics more rapidly than microbial isolates and do not require clean plastics or pretreatment. In particular, the microbiome of yellow mealworms is unique in that its host does not appear to contribute to degradation of a wide range of plastics. While bacterial community members have been identified, the specific pathways responsible for biodegradation remain to be elucidated and the potential contributions of fungal members are unexamined. Additionally, emerging evidence suggests that nutrient supplementation enhances plastic metabolism up to 70% and gives rise to a gut community structure distinct from that without additional nutrients. However, it is unclear if nutrient supplementation induces microbes to participate in in the plastic degradation or if it supports an optimal community composition for function.

As a first step to address these gaps, we characterized the consumption rates of PS, LDPE, HDPE, and PP via *T. molitor* larvae in the presence and absence of co-fed oats as a nutritional supplement. The consumption rates of PS, LDPE, and HDPE were 20.4, 12, and 1.1, mg (100 larvae)$^{-1}$d^{-1}, respectively, in agreement with established studies. However, oat supplementation enhanced plastics consumption by 158.8, 60, and 232.1 %, respectively. These studies establish the use of oats as a potent supplement for enhancement of PS and LDPE consumption rates, up to double that obtained with established supplements, and validated HDPE consumption by *T. molitor*.

Antibiotic and antifungal selection studies supported the role of both fungal and bacterial populations in plastics consumption. Mealworms treated with penicillin/streptomycin to remove their bacterial population consumed PS 15% faster (60.6 mg (100 larvae)$^{-1}$d^{-1}) than untreated mealworms. Similarly, amphotericin B antifungal treatment selecting for bacteria enhanced PS consumption by 4.1% (55 mg (100 larvae)$^{-1}$d^{-1}). These results suggest that fungal communities in the mealworm gut microbiome are likely to play an important role in the plastic consumption and that the inter-kingdom relationship between bacteria and fungi may be antagonistic.

Worm-consumed plastics were chemically modified beyond simple mechanical degradation validating biological mechanisms for plastics depolymerization. Fourier transform infrared spectroscopy (FTIR) analysis of frass (excrement) from mealworms fed PS revealed incorporation of oxygen not found in untreated controls. Moreover, benzene ring cleavage was observed for treated PS samples. Similarly, FTIR spectra of the frass from mealworms fed LDPE revealed the incorporation of carbonyl and alcohol groups. Thermogravimetric analyses (TGA) of frass also confirmed the changes in physical properties, supporting the biodegradation of PS and LDPE via *T. molitor* microbiomes. Finally, gel permeation chromatography (GPC) of the frass of *T.molitor* larvae fed PS confirmed a decrease in polymer molecular weight and an increase in polydispersity. Taken together, these results demonstrate that the plastics being ingested by the larvae are being depolymerized.

Microbiome community analysis via 16s and ITS sequencing revealed a rich consortium of bacteria and fungi. The bacterial community was more diverse than the fungal community with observed taxa belonging to the bacterial phyla Firmicutes, Tenericutes, Proteobacteria, Actinobacteria, Spirochaetes, Bacteroidetes, and Fusobacteria, and fungal Ascomycota, Basidiomycota and Mucoromycota. As expected, mealworm diet led to unique community structures adapted to degradation of the fed plastic substrate. However, oats co-supplementation frequently selected for taxa that were not observed in plastics only or oats only controls suggesting currently unrecognized interactions. Despite these unique community structures, microcosms of communities in planktonic culture selected for with LDPE, HDPE, PS, and PP diet were all able to grow on LDPE as a sole-carbon source. Finally, our community analyses revealed obligate anaerobic genera such as *Selbadella* associated with PP degradation, suggesting potentially novel oxygen-independent pathways for plastics depolymerization.

In summary, our ongoing work has characterized plastic consumption rates in *T. molitor* microbiomes, revealing novel strategies to structure gut microbial populations for enhanced degradation. Plastics were noted to be metabolized and not only mechanically degraded by both bacterial and fungal communities that contribute to plastic degradation even independent of the host mealworm. Future studies will probe the contributions of individual taxa within these communities and generate systems-level insight into their metabolic pathways to develop consortia enriched in plastic degradation activity, and identify novel enzymes from community members.

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