

Title: Engineering novel microbes for upcycling waste plastic and solving climate crisis

Authors: Jinjin Diao, Yifeng Hu, and **Tae Seok Moon*** (tsmoon@wustl.edu)

Institutions: Washington University in St. Louis

Website URL: <https://sites.wustl.edu/moonlab/>

Project Goals: The goal is to develop a consolidated biological process to upcycle waste polyethylene terephthalate.

Abstract Text:

The use of petroleum-based products has negative impacts on our planet, leading to climate crisis. Polyethylene terephthalate (PET) represents 8% of global solid waste. PET chemical recycling has been an option to solve this global problem, but it suffers from its relatively high process cost and the extremely low price of virgin PET. One solution to address this issue is to upcycle waste PET rather than recycle it to generate the same PET typically with low quality. PET upcycling can be achieved by depolymerizing PET into terephthalic acid (TPA) and ethylene glycol (EG) and biologically converting these monomers into value-added products. However, there are only a handful of reports demonstrating microbes capable of growing on both TPA and EG generated from PET as sole carbon sources. To overcome this limitation, we have performed strain screening to discover a *Rhodococcus* strain RPET that can grow well on the alkaline hydrolysis products of PET as the sole carbon source without any purification step. Notably, this strain can grow on a mixture of TPA and EG at extremely high concentrations (up to 0.6M) and high osmolarity resulting from alkaline hydrolysis and pH neutralization. The resultant media supported RPET's growth without any purification and sterilization step except for their dilution to make up to 0.6M of monomer concentrations. In addition, many synthetic biology tools, developed for a related species *Rhodococcus opacus* (1,2), were functional in RPET, facilitating its engineering. We will discuss our effort to develop this novel chassis for waste PET valorization with PET conversion into carotenoids and muconate as two demonstration products (3).

1. DeLorenzo, Rottinghaus, Henson, Moon. 2018. ACS Synthetic Biology 7:727-38
2. DeLorenzo, Diao, Carr, Hu, Moon. 2021. An improved CRISPR interference tool to engineer *Rhodococcus opacus*. ACS Synthetic Biology 10:786-98
3. Moon et al. 2021. U.S. Provisional Patent Application

Key words: climate crisis; polyethylene terephthalate; plastic upcycling; synthetic biology; metabolic engineering

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