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DEVELOPING A NOVEL MICROBIAL HOST AND SYNTHETIC BIOLOGY TOOLS FOR VALORIZING WASTE POLYETHYLENE TEREPHTHALATE AND LIGNIN-DERIVED COMPOUNDS

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Polyethylene terephthalate (PET) represents 8% (by weight) 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 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 microbial strains capable of growing on both TPA and EG generated from PET as sole carbon sources. To overcome this critical challenge, we have performed strain screening to discover a *Rhodococcus* strain (named RPET) that can grow well on the alkaline hydrolysis products of PET as the sole carbon source without any purification step. Notably, this strain was able to tolerate and grow on a mixture of TPA and EG at extremely high concentrations (up to 0.3M each, total 0.6M) and high osmolarity resulting from alkaline hydrolysis and pH neutralization. The resultant pH neutralized media supported RPET's growth (up to 0.4 g dry cell weight per g PET) without any purification and sterilization step except for their dilution to make up to 0.6M of monomer concentrations.

Adipic acid, a monomer for nylon production, is currently produced from petroleum derivatives, requiring an alternative process for its sustainable "green" production. Muconate can be converted into various chemicals, including adipic acid. Using non-model organisms, multiple labs have demonstrated muconate production from lignin-derived aromatic compounds, with glucose used as a growth substrate. *Rhodococcus opacus* is well suited for valorizing lignin [1-8], but developing this promising chassis had been challenging due to limited genetic engineering tools. To address this issue, we have developed various synthetic biology tools [9-12], including a gene repression system based on CRISPR interference (CRISPRi) and a knockout method. Notably, many synthetic biology tools, developed for *Rhodococcus opacus* [12], were functional in different related species and strains such as RPET.

In this presentation, we will discuss our CRISPRi tool's utility for waste valorization. We have developed and optimized the CRISPRi system, which uses a T7 RNA polymerase system to express a small guide RNA, demonstrating the maximum repression efficiency up to 85% [13]. We also provide a cloning strategy that enables constructing multiple CRISPRi plasmids without any PCR step, facilitating this GC-rich organism's engineering. Using the optimized CRISPRi system, we confirmed the annotated roles of four metabolic pathway genes related to the consumption of benzoate, vanillate, catechol, and acetate. Additionally, we showed our tool's utility by demonstrating the inducible accumulation of muconate. While the maximum muconate yield obtained using our tool was 30% of the yield obtained using gene knockout, our tool showed its inducibility and partial repressibility. Finally, we will discuss our effort for PET conversion into carotenoids and muconate as two demonstration products [14]. This work represents the promise for valorizing waste PET and lignin-derived compounds using *Rhodococcus*.

[1] Chatterjee, A., DeLorenzo, D. M., Carr, R., Moon, T. S., Bioconversion of renewable feedstocks by Rhodococcus opacus. Current opinion in biotechnology 2020, 64, 10-16. [2] Davis, K., Moon, T. S., Tailoring microbes to upgrade lignin. Current opinion in chemical biology 2020, 59, 23-29. [3] Henson, W. R., Campbell, T., DeLorenzo, D. M., Gao, Y., et al., Multi-omic elucidation of aromatic catabolism in adaptively evolved Rhodococcus opacus. Metabolic engineering 2018, 49, 69-83. [4] Yoneda, A., Henson, W. R., Goldner, N. K., Park, K., et al., Comparative transcriptomics elucidates adaptive phenol tolerance and utilization in lipid-accumulating Rhodococcus opacus PD630. Nucleic Acids Res. 2016, 44, 2240–2254. [5] Henson, W. R., Hsu, F.-F., Dantas, G., Moon, T. S., Foston, M., Lipid metabolism of phenol-tolerant Rhodococcus opacus strains for lignin bioconversion. Biotechnology for biofuels 2018, 11, 339. [6] Roell, G. W., Carr, R. R., Campbell, T., Shang, Z., et al., A concerted systems biology analysis of phenol metabolism in Rhodococcus opacus PD630. Metabolic engineering 2019, 55, 120-130. [7] Hollinshead, W. D., Henson, W. R., Abernathy, M., Moon, T. S., Tang, Y. J., Rapid metabolic analysis of Rhodococcus opacus PD630 via parallel C-metabolite fingerprinting. Biotechnology and bioengineering 2016, 113, 91-100. [8] Anthony, W. E., Carr, R. R., DeLorenzo, D. M., Campbell, T. P., et al., Development of Rhodococcus opacus as a chassis for lignin valorization and bioproduction of high-value compounds. Biotechnology for biofuels 2019, 12, 192. [9] DeLorenzo, D. M., Henson, W. R., Moon, T. S., Development of Chemical and Metabolite Sensors for Rhodococcus opacus PD630. ACS Synthetic Biology 2017, 6, 1973–1978. [10] DeLorenzo, D. M., Moon, T. S., Selection of stable reference genes for RT-qPCR in Rhodococcus opacus PD630. Scientific reports 2018, 8, 6019. [11] DeLorenzo, D. M., Moon, T. S., Construction of Genetic Logic Gates Based on the T7 RNA Polymerase Expression System in Rhodococcus opacus PD630. ACS Synthetic Biology 2019, 8, 1921-1930. [12] DeLorenzo, D. M., Rottinghaus, A. G., Henson, W. R., Moon, T. S., Molecular Toolkit for Gene Expression Control and Genome Modification in Rhodococcus opacus PD630. ACS Synthetic Biology 2018, 7, 727-738. [13] DeLorenzo, D.M., Diao, J., Carr, R., Hu, Y., Moon, T.S., An Improved CRISPR Interference Tool to Engineer Rhodococcus opacus. ACS Synthetic Biology. doi.org/10.1021/acssynbio.0c00591. [14] Moon, T.S. et al. U.S. Provisional Patent ApplicatioN.