Transforming plastic waste: harnessing *E. coli* for sustainable PET recycling and valorisation

Alex Pessina [1], Isabella Cecilia Rizzo[1], Caren Battaglia[3], Davide Miani[3], Chiara Damiani [1,2], Luca Brambilla [1,2], Gianluca Molla [3], Elena Rosini [3], Loredano Pollegioni [3], Marco Vanoni [1,2]

Corresponding author: *alex.pessina@unimib.it*

[1] *Department of Biotechnology and Biosciences, University of Milano-Bicocca*

[2] *SYSBIO – Centre of Systems Biology, Italy*

[3] *"The Protein Factory 2.0" Lab, Department of Biotechnology and Life Sciences, University of Insubria*

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Abstract:

A significant issue facing the world today is the pollution from microplastics resulting from unsustainable consumption and disposal practices. Microplastics, primarily composed of polyethylene terephthalate (PET), pose potential risks to living organisms, severely affecting marine ecosystems and posing serious consequences for human health. In our current era, the rapid expansion of knowledge and rising awareness offer a substantial opportunity in applied sciences. In this context, the ProPla project (Proteins from Plastic) aims to extract PET microplastics from wastewater and transform them into valuable products by utilizing protein engineering and systems biology techniques. A novel biosynthetic pathway (made by 9 enzymes also containing the LCC PET-degrading enzyme) has been developed for the bioconversion of PET into substrates for bacteria central carbon metabolism, then integrated into the *E. coli* K12MG1655 strain using CRISPR/Cas9 technology. Using our bottom-up approach to gene integration, we systematically verified the functionality of the entire metabolic pathway by confirming the formation of intermediate substrates and end products at each step. We assessed the complete pathway conversion through both in vitro and cell-based bioconversion experiments as proof of concept. Additionally, several in silico analyses have been conducted to enhance the efficiency and optimization of the process. These included Flux Balance Analysis and related constraint-based modelling techniques, aimed at identifying the optimal combinations of metabolic fluxes that lead to determine the most suitable growth conditions and the required gene knockouts to increase the products yield. Upon establishing a stable cell factory, a large amount of primary carbon sources will be generated and will be then effectively converted into valuable products utilizing either a single or multiple additional enzymatic steps.

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