Exploring bacterial and yeast strains as hosts for terephthalic acid upcycling into value-added organic acids

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Abstract.

Plastic pollution has become a major concern because of their persistence in the environment, compounded by microplastic release and bioaccumulation. Polyethylene terephthalate (PET) is the most extensively manufactured plastic globally, constituting >15% of all solid waste. Advancements on PET enzymatic hydrolysis enabled the possibility of recycling at an industrial scale, while other mitigations strategies for plastic contamination await future developments. This work focuses on the upcycling of one of the PET-derived monomers, terephthalic acid (TPA), by adopting and comparing engineered bacterial and yeast strains, with a focus on the production of protocatechuic acid (PCA), an industrially relevant compound. TPA assimilation has not been previously documented in *Saccharomyces cerevisiae*, and two bacterial gene clusters (*tph* operon) have been engineered into this strain with the EASY-MISE toolkit through the assembly of novel polycistronic plasmids. Moreover, as these enzymes depend on an iron-sulphur cluster, the strains were further engineered to circumvent iron regulation and increase its availability. In parallel, we tested a *Pseudomonas putida* strain carrying the *tph* operon previously engineered to produce PCA. The PCA production profiles by the bacterial and yeast strains was compared in bioreactor cultures by implementing a biosensor originally designed for bacteria. Overall, the results suggest that the bacterial *chassis* is a promising starting point for efficient TPA conversion into PCA and process scale-up.