Innovative utilization of Escherichia coli for PET valorization: a pathway to amino acids production

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One of the major concerns in today's world is the microplastic pollution caused by unsustainable usage and disposal. Microplastics (mainly polyethylene terephthalate, PET) with their potential risks to organisms, are dramatically impacting the marine environment and having extreme repercussions for human beings. In the modern era, the exponential growth of knowledge and increased awareness presents a significant opportunity within the field of applied science. In this scenario, the ProPla project (Proteins from Plastic) proposes the recovery of PET microplastics from wastewater and their conversion into valuable amino acids, such as L-Ala, using a combination of protein engineering and systems biology approaches.
A novel biosynthetic pathway (made by 9 enzymes also containing the LCC PET-degrading enzyme) has been developed for the bioconversion of PET into pyruvate, and has been divided in two major modules, then integrated into the *E. coli* K12MG1655 strain using CRISPR/Cas9 technology. With the first module we exploit optimized enzymes to convert PET into protocatechuic acid (PCA). The subsequent step focuses on the transformation of PCA into pyruvate, a key intermediate for amino acids production. Through our bottom-up approach to gene integration, we have obtained preliminary results in achieving stable integration of the enzymatic pathway required for the bioconversion of PCA into pyruvate. Using Flux Balance Analysis and related constraint-based techniques, we will determine the optimal combinations of metabolic fluxes leading to the maximum production of L-Ala (previously published in: Cazzaniga, P. et al. (2014) Metabolites 4,1034). Upon establishing a stable cell factory, a large amount of pyruvate will be generated and will be then effectively converted into valuable amino acids utilizing either a single or multiple additional enzymatic steps.
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