Increasing wax ester production in *Acinetobacter baylyi* for bioconversion of plastic waste

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Abstract: The current models of plastic production and consumption are tied to huge environmental costs, particularly for single-use plastics. As a result, there is a growing interest in the use of new bio-derived and sustainable polymers. *Acinetobacter baylyi* ADP1 is the best-known microorganism for the production of wax esters (WE). WE are industrially valuable lipid compounds that can be used as precursors for sustainable polymers with polyethylene-like properties[1]. Taking advantage of *A. baylyi's* versatile metabolism, we aim to revalorize monomers from the oxidative depolymerization of plastics[2] and convert them into chemically recyclable bioplastic based on WE. However, to exploit bacterial WE industrially, we need to increase their natural production rates. The fatty acid biosynthesis pathway provides the precursors for WE synthesis. In this study, we engineered *A. baylyi* by deleting the genes *aceA* (encoding for isocitrate lyase) and a putative *fadE* (encoding for acyl-CoA dehydrogenase) to enhance the pool of fatty acids (FA). The effects of these mutations were evaluated using a luminescent biosensor[3] which enables real-time monitoring of WE production. It appears that *aceA* deletion increases WE formation without affecting growth on aromatic substrates derived from the oxidative depolymerization of plastics, although an increased lag phase was observed for adipic acid. This finding is particularly relevant since the glyoxylate shunt is essential for cells to grow on non-glycolytic carbon sources. However, *A. baylyi* catabolizes these compounds through the β-ketoadipate pathway, resulting in the production of acetyl-CoA and succinyl-CoA, which can replenish essential intermediates of the tricarboxylic acids cycle. Future transcriptomic analyses will help elucidate the FA and WE synthesis pathways in order to identify new targets for increasing WE production.

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