Pyruvate production from protochatecuic acid, a degradation product of PET plastic

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Polyethylene terephthalate wastes are currently a heavily underused polymeric materials that represent an abundant source of bulk chemicals, useful for producing high value-added molecules. We focused on protocatechuic acid (PCA), an aromatic derivative originating from the depolymerization and biotransformation of raw materials. In details, PCA was used as the starting point for an enzymatic cascade bioconversion toward the production of pyruvate. The experimental approach is based on E. coli RARE, a strain with reduced catabolism toward aliphatic and aromatic aldehydes, transformed with up to 3 plasmids of the DUET series (pRSF-Duet 1, pET-Duet 1 and pCDF-Duet 1), to express a total of six proteins simultaneously: a dioxygenase (LigAB), a dehydrogenase (LigC), a hydrolase (LigI), a tautomerase (LigU), a hydratase (LigJ) and an aldolase (LigK). The main difficulties in establishing an efficient process arose from the hydration equilibria, involving the reactions catalyzed by LigI and LigJ enzymes and the tautomerization of 4-oxalmesaconate, which represents a kinetic bottleneck for the overall cascade (previously published in: Hogancamp TN et al. (2018) Biochemistry 19: 2837-2845). We proved that the additional LigU enzyme is necessary to establish a fast process: at the moment, up to 10 mM of PCA has been successfully consumed. The employed process involves a whole-cell set-up (expressing the LigAB and LigC enzymes), coupled with a subsequent conversion performed by purified LigI, LigU, LigJ and LigK enzymes. This mixed approach was chosen to avoid the funnelling of pyruvate, the final product, into the central metabolism. This bioconversion cascade represents a valuable starting platform to produce a range of high value-added amino acids, highlighting the great potential of underused waste materials. This work is part of the ProPla project (Fondazione Cariplo 2022).