Synthetic biology approach for the valorisation of caffeic acid in *S. cerevisiae*

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Residues from coffee consumption are currently disposed mainly by either composting or anaerobic digestion. To further valorise the components of spent coffee grounds (SCGs), we aim to develop a novel and synthetic microbiological route able to convert one the main characteristic compounds, caffeic acid, into valuable molecules to be used as scaffold for pharmaceutical synthesis. We genetically modified *Saccharomyces cerevisiae* by an approach of synthetic biology CRISPR-mediated: the first step of the metabolic pathway involves the amination of caffeic acid, by the enzyme phenylalanine/tyrosine ammonia lyase (PAL/TAL), with L-3,4-dihydroxyphenylalanine (L-DOPA) as product and precursor to different natural compounds of medicinal use. To investigate on the preferred reaction direction of different ammonia lyase enzymes, the combination of pHs, buffers and ammonia sources and titers needed to be tested. We therefore developed a biosensor able to detect the presence of intracellular L-DOPA. The genome of *S. cerevisiae* was modified to express the DOD gene, from two different plants, coding for 4,5-DOPA-extradiol-dioxygenases: this enzyme oxidases L-DOPA to 4,5 seco-DOPA, which spontaneously cycles into betalamic acid. This compound spontaneously reacts with primary amines obtaining betaxanthins, whose yellow colour is easily detected. After assessing the functionality of the biosensor, we focused our attention on exploring different combinations, using yeast cell both in a whole-cell reaction condition or in a growing state. Additionally, several putative fungal transporters were expressed in the yeast-based biosensor to promote internalization of caffeic acid from a formulated media. Taken together, this work explores the valorisation of an untapped building block from a residual biomass, by the use of unconventional reaction routes and a biosensor.