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Heterologous production of plant natural polyphenolic compounds in *Escherichia coli*

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Plants secondary metabolites are important for their survival and are usually considered as high-value chemicals. However, they are generally present in low amounts and accumulate during long growth periods. Their extraction is often problematic since their purification from mixtures containing compounds with similar structures is difficult originating low yields also subject to environmental and regional factors. In addition, chemical synthesis is normally too complex and environmentally unfriendly. Therefore, the biosynthesis of high-value chemicals in engineered organisms has emerged as a competitive alternative compared to chemistry-based methods.

Curcuminoids and coumarins are polyphenolic compounds produced in plants in low amounts that exhibit interesting pharmacological properties. In this study, we report the construction of an artificial pathway using codon-optimized enzymes for the production of these compounds in *Escherichia coli*. Both types of polyphenolic compounds can be produced from tyrosine or hydroxycinnamic acids as precursors. To produce curcumin, the most studied curcuminoid for therapeutic purposes, 4-coumaroyl-CoA ligase (4CL) from *Arabidopsis thaliana*, curcuminoid synthase from *Oryza sativa* or diketide-CoA synthase and curcumin synthase from *Curcuma longa* were used. Using this pathway 354 mg/L of curcumin was produced, which corresponds to the highest concentration obtained so far using a heterologous host. Curcumin was also produced for the first time using tyrosine as precursor and caffeic acid as an intermediate. Other curcuminoids, such as bisdemethoxycurcumin and demethoxycurcumin were also produced using as precursors tyrosine or hydroxycinnamic acids (*p*-coumaric acid or a mixture of *p*-coumaric and ferulic acids, respectively). Based in this pathway, a similar pathway was constructed to produce coumarins. The enzymes 4CL

and *p*-coumaroyl-CoA 2'-hydroxylase from *Ipomoea batatas* were used to produce the coumarins umbelliferone, scopoletin and esculetin from *p*-coumaric acid, ferulic acid and caffeic acid, respectively. Approximately 20-50 mg/L of each coumarin was produced. The optimization of coumarins production from tyrosine is being conducted.