

Synthetic biology approaches to engineer new pathways for the production of plant secondary metabolites

Joana L. Rodrigues¹, Rafael G. Araújo¹, Kristala L. J. Prather², Leon D. Kluskens and Lúcia R. Rodrigues¹

1 Centre of Biological Engineering, University of Minho, Portugal

2 Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, USA

joana.joanalucia@deb.uminho.pt

Secondary metabolites from plants are important sources of high-value chemicals, many of them being pharmacologically active. These metabolites are commonly isolated through inefficient extractions from natural biological sources and are often difficult to synthesize chemically. Therefore, their production using engineered organisms has lately attracted an increased attention. Curcuminoids, an example of such metabolites, are produced in *Curcuma longa* and exhibit anti-cancer and anti-inflammatory activities. Herein we report the construction of an artificial biosynthetic pathway for the curcuminoids production in *Escherichia coli*. Different 4-coumaroyl-CoA ligases (4CL) and polyketide synthases (diketide-CoA synthase (DCS), curcumin synthase (CURS) and curcuminoid synthase) were tested. The highest curcumin production (70 mg/L) was obtained by feeding ferulic acid and with the *Arabidopsis thaliana* 4CL1 and *C. longa* DCS and CURS enzymes. Other curcuminoids (bisdemethoxy- and demethoxycurcumin) were also produced by feeding coumaric acid or a mixture of coumaric and ferulic acids, respectively. Curcuminoids, including curcumin, were also produced from tyrosine through the caffeic acid pathway. To produce caffeic acid, tyrosine ammonia lyase and 4-coumarate 3-hydroxylase were used. Caffeoyl-CoA O-methyltransferase was used to convert caffeoyl-CoA to feruloyl-CoA. This pathway represents an improvement of the curcuminoids heterologous production. The construction of this pathway in another model organism is being considered, as well as the introduction of alternative enzymes.