Heterologous production of curcuminoids in E. coli through an artificial biosynthetic pathway

Joana L. Rodrigues

MIT Portugal Bioengineering Systems, Department of Biological Engineering, School of Engineering, University of Minho, Braga, Portugal
joana.joanalucia@deb.uminho.pt

Background: Master in Biological Engineering/ University of Minho/ Portugal
Starting year: 2010
Supervisors: Lígia L. Rodrigues[1]; Kristala L. J. Prather[2]; Leon Kluskens[1]
[1]Department of Biological Engineering, University of Minho
[2]Department of Chemical Engineering, Massachusetts Institute of Technology

ABSTRACT

Curcuminoids are natural pigments from plants that have been reported as potential cancer-fighting drugs. Nevertheless, they have a poor bioavailability. Cellular uptake is low, and they are quickly metabolized once inside the cell, requiring repetitive oral doses to achieve sufficient concentration inside the cell for therapeutic activity. The aim of this work is to engineer an artificial biosynthetic pathway for the production of curcuminoids by *Escherichia coli*. Starting from the substrate tyrosine, the curcumin pathway involves several enzymatic steps: conversion of tyrosine to *p*-coumaric acid; conversion of *p*-coumaric acid to caffeic acid; production of caffeoyl-CoA from caffeic acid; production of feruloyl-CoA from caffeoyl-CoA; and finally the production of curcumin from feruloyl-CoA and possibly other curcuminoids, due to enzyme promiscuity. The enzymes involved in the two first enzymatic steps are tyrosine ammonia lyase from *Rhodotorula glutinis*, P450 CYP199A2 from *Rhodopseudomonas palustris*, and the redox partners *pdr* from *Pseudomonas putida* and *pux* from *R. palustris*. Coumaric acid and caffeic acid were successfully produced. A coumaroyl-CoA ligase from *Arabidopsis thaliana* is being explored for the conversion of the different carboxylic acids into their corresponding CoA esters. Different combinations of this enzyme and caffeoyl-CoA 3-methyl transferase may lead to the production of different curcuminoids. For the last step of the pathway two approaches are being studied: the use of diketide-CoA synthase and curcuminoid synthase from Curcuma longa, and curcumin synthase from Oryza sativa that itself catalyzes both steps. Curcumin and bisdemethoxycurcumin were produced using both approaches and their production was confirmed by HPLC analysis, as well as by the yellow color of the culture supernatant. Successful construction of the curcuminoids biosynthetic pathway would mark a significant step forward in the *in situ* production of these poorly soluble, anti-carcinogenic compounds.

Acknowledgments: The author thanks the Master student Rafael Araújo for the support given in the laboratory.