Design and construction of a new biosynthetic pathway for the production of curcuminoids in *Escherichia coli*

J. L. Rodrigues

1MIT Portugal Program, Department of Biological Engineering, School of Engineering, University of Minho - Campus de Gualtar 4710-057 Braga – Portugal, joana.joanalucia@deb.uminho.pt

**Supervisors:** Project Coordinator/Supervisor in Portugal: Lígia Rodrigues, Universidade do Minho  
**Project Coordinator at MIT:** Kristala Prather, Massachusetts Institute of Technology  
**Co-Supervisor:** Leon Kluskens, Universidade do Minho

Curcuminoids are produced by plants and due to their potential as novel cancer-fighting drugs they have recently attracted increased attention. 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 goal of this PhD project is to engineer a synthetic pathway for curcuminoid in a model bacterium and trigger its release concurrent with ultrasound treatment. The proposed tasks involve several design and engineering steps to program *Escherichia coli* to execute the new synthetic pathway triggered by a temperature increase. The heat shock response machinery of *E. coli* will be used as a sensor in the design of the model bacterium. Afterwards, the gene sequences of the enzymes that catalyze each reaction in the curcuminoid pathway will be synthesized and introduced in the *E. coli* genome applying several cloning strategies.

Data from several well documented experiments on *E. coli* in relevant conditions that have been published were analyzed to select the most expressed heat shock genes in *E. coli* with the strongest heat shock promoters. The *ibpA, dnaK* and *fxsA* gene promoters were chosen based on their induction rates and expression and were validated by RT-qPCR and subsequently through the construction of a stress probe using an adequate reporter gene.

The new synthetic pathway for curcuminoids production that has been designed starts from tyrosine as a substrate and involves five enzymatic steps. Recently the first two enzymes, tyrosine ammonia lyase from *Rhodotorula glutinis* and 4-coumarate 3-hydroxylase from *Saccharothrix espanaensis* were cloned in a duet plasmid. Currently, the production of the two intermediate metabolites and the functionality of the constructs are being confirmed by HPLC and gel protein analysis.