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A SYNTHETIC BIOLOGY APPROACH TO ENGINEER ''THERAPEUTIC'' BACTERIA

S.O.D. Duarte[1]; J. Paulo[1,2]: D. Machado[3,4]; I. Rocha[3]; F.J.M. Mergulhão[2]; L.R. Rodrigues[3]; G.A. Monteiro[1,5]

[1]*Institute of Biotechnology and Bioengineering, Centre for Chemical and Biological Engineering, Instituto Superior Técnico, Lisbon, Portugal*

[2]*Department of Chemical Engineering, Faculty of Engineering, Universidade do Porto, Oporto, Portugal*

[3]*Institute of Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal*

[4]*Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark*

[5]*Department of Bioengineering, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisbon, Portugal*

The high incidence and mortality of solid tumors like breast cancer makes the development of novel therapeutic agents a high priority. Curcumin, a natural substance from the rhizome of *Curcuma longa*, has captured the attention of the scientific community. Pre-clinical trials and extensive research has demonstrated its ability to prevent cancer. Indeed, curcumin has been shown to target critical genes involved in angiogenesis, apoptosis, cell cycle and metastasis, and consequently to inhibit cell growth. Currently, the clinical use of curcumin is mainly limited by its poor bioavailability which implies repetitive oral doses in order to achieve the therapeutic concentrations inside the cell. The idea of the present work is to design a strategy that could link the common technique used to treat solid tumors (ultrasound) with the therapeutic effects of curcumin. The plan is to use the temperature increase (consequence of ultrasound treatment) to trigger the *in situ* expression of curcumin by engineered bacteria. *Escherichia coli* was chosen as the model organism in which the genes involved in the curcumin pathway will be cloned.

Those genes (4-coumarate: CoA ligase, diketide-CoA synthase and curcumin synthase) were successfully cloned under the control of a temperature sensitive promoter (dnaK). The proof-of-concept that the dnaK promoter can be induced by a temperature increase, leading to the expression of the 3 necessary genes, is currently being tested, using several biochemical assays.

Moreover, several knockouts (KO) of specific genes from the *E. coli* K-12 MG1655 genome were performed in order to maximize the production of curcumin. The deletion strategy, as well as the definition of the non-essential genes to be KO, was determined *in silico*. This strategy included one single KO (*gnd* gene) and the multiple KO of five non-essential genes for aerobic growth (*fumA*, *fumB*, *fumC*, *ccmA* and *argO*) and *serA* gene for anaerobic growth. After optimizing the genes expression under the control of the temperature inducible promoter, the several KO will be transformed with this construction to confirm the improvement of curcumin production.

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