SYNBIOBACTHER – Engineering “therapeutic” bacteria

Joana L. Rodrigues, Rui M. Pereira, Leon D. Kluskens, Lígia R. Rodrigues

IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057, Braga, Portugal

Breast Cancer

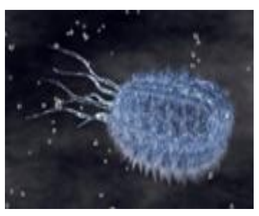
Problem:

- Over 1.2 million persons will be diagnosed / yr
- Ultrasound is not always successful: sometimes it just heats the tumour without destroying it

Solution:

- **Synthetic Biology** strategies to engineer a model bacterium to trigger the release of a therapeutic agent (curcumin) concurrent with ultrasound.

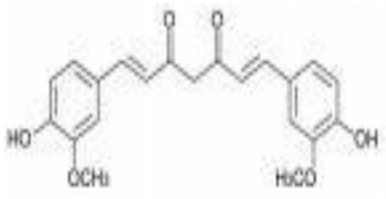
E. coli



Engineered curcumin pathway triggered by heat

Breast cancer

Combined treatment: “therapeutic” bacteria ultrasounds



In situ production of curcumin

Curcumin

- **Potential:** anti-proliferative capability, anticarcinogenic and chemopreventive effect
- **Problem:** Poor bioavailability (cellular uptake is slow and quickly metabolized once inside cells)
- **Solution:** *in situ* production

Figure 1: Construction of a new therapeutic bacterium, in which the curcumin biosynthetic pathway (therapeutic agent) will be inserted whose expression will be triggered *in situ* by the heat emitted by ultrasound treatment.

Experimental steps & Results

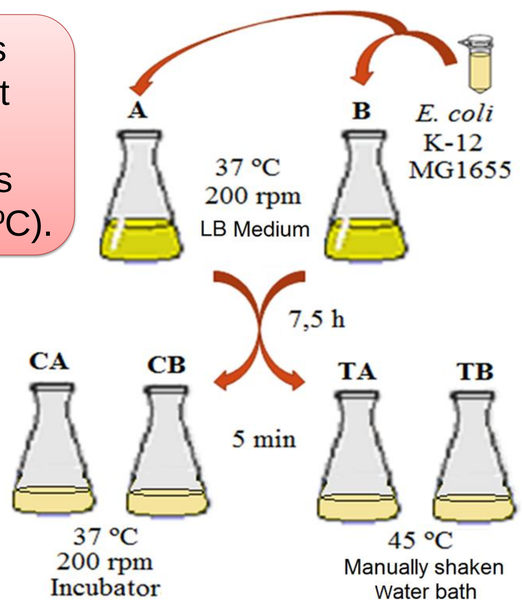
Task 1 Selection of the best heat shock promoters in *E. coli* (microarrays) and validation (qRT-PCR)

Task 2 Promoters validation by the construction of a stress probe with a reporter gene (GFP)

Task 3 Design and construction of the biosynthetic pathway for the production of curcuminoids in *E. coli*

Task 4 *In vitro* validation of the engineered bacterium

Experiments conducted at different temperatures (37 ºC and 45 ºC).



High expression and induction ratio of *ibpA*, *dnaK* and *fxsA* genes

Microarrays (promoters selection) ↔ Real-time PCR (promoters validation)

RNA extraction

Table 1 - Induction ratios of the heat shock genes

Technique	Flask experiment	Induction Ratios		
		dnaK	*ibpA*	*fxsA*
qRT-PCR	A	58.49	3541.14	12.13
	B	48.50	48.84	5.17
Microarrays	A	9.47	61.35	12.68
	B	6.23	49.9	24.72

Discussion & Conclusions

The genes identified with a higher induction ratio and expression by microarrays were in accordance with the ones previously reported in the literature. The *ibpA, fxsA* and *dnaK* promoters were identified as the strongest. The preliminary results obtained using real time PCR also corroborated the ones previously obtained using microarrays. Nevertheless, the samples analyzed by microarrays and real-time PCR were not exactly the same since several independent experiments were conducted to generate enough amount of RNA to conduct the analysis. Currently, the promoters validation through the construction of a stress probe using green fluorescent protein as a reporter gene (task 2) is in progress. In the future, these promoters will be used to trigger the biosynthetic pathway for the production of curcuminoids that will be designed and introduced in *E. coli*.

Acknowledgements

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