Toxics MultiBiosensor

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INTRODUCTION

The aim of our study was to design a multiple biosensor for toxic compounds present in water, taking advantage that microorganisms are cheap, self-regenerable and it is possible to get a quick response. To achieve the challenge of having a bacteria able to detect many compounds, we started with separate biosensors.
The first design was an *E. coli* that could detect trihalomethanes and, specifically, chloroform.
The second approach was to detect contamination by excess of nutrients, quantifying the presence of nitrite and phosphate.

PROJECT 1: Trihalomethanes

Why trihalomethanes?

Trihalomethanes (THM), and chloroform in particular, are toxic recalcitrant compounds. An important source of THM is the chlorination of drinking water or swimming pool water.

Fig. 1. Chloroform molecule

CHLOROFORM: Biobricks design

Sayavedra et al[1] isolated 2 genes (*mbla* and *clpB*) of *Nitrosomonas europaea* that are selectively induced by chloroform. In order to obtain an *E. coli* strain behaving as *Nitrosomonas*, our idea was to co-express the *N. europaea amo* (ammonia monooxygenase) encoding gene with either *mbla* or *clpB*:

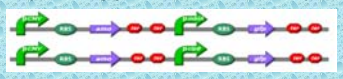

Fig. 2. Chloroform detection biobricks for *E. coli*

In this way, depending on the amount of chloroform in the medium, we should be able to detect more or less intensity of green fluorescence in *E. coli* cells.

[1] Sayavedra-Soto et al. *Construction of recombinant Nitrosomonas europaea expressing green fluorescent protein in response to co-oxidation of chloroform.* Appl Microbiol Biotechnol (2009)

Materials and Methods

As *N. europaea* has a very slow growth rate and *amo* isolation by PCR failed, we therefore decided to transform *E. coli* with the plasmids containing the *gfp* gene under the *mbla*- and *clpB* promoters provided by Prof. Sayavedra (that is, without *amo*).

The standard GFP expression experiment performed is described in *Fig. 3.* CF concentration, induction time, growth phase and OD were modified in an attempt to find the best conditions.

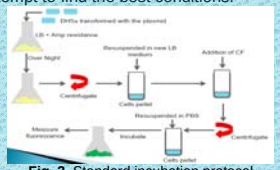

Fig. 3. Standard incubation protocol

Results

Although basal fluorescence was observed, no chloroform-induced fluorescence was detected.

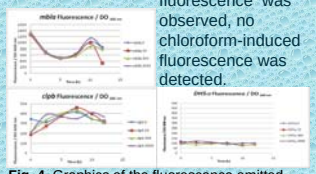

Fig. 4. Graphics of the fluorescence emitted

Problems	Why?	Solution/ Testing	Result
Activation of GFP → MilliQ® or distilled water?	Distilled water has little ammount of CF	Use MilliQ® water	No difference observed
Possible variation in the CF concentration	High volatility of CF	Gas chromatography	No significant loses in 24 h of incubation
Saturation of the GFP fluorescence	Inclusion bodies	- Incubation in PBS - Addition of chloramphenicol - ↓ incubation T - confocal microscopy	No inclusion bodies

Fig. 5. Confocal microscopy image of *E. coli* with GFP

Kinetic modeling

The modeled system is divided into the three phases where the chloroform appears: air phase, bulk liquid and inside the cell.
All reactions have been assumed as mass action.
Model Statements:
- GFP response is high around pH 6.
- GFP response increases with promoter concentration

Behavior as a biosensor:
The model is sensitive enough to detect initial low concentration of chloroform (around 1 ppb), and for values up to 20 ppb leads to a saturated response.

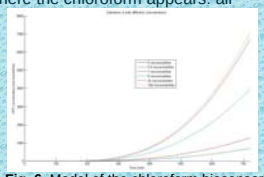

Fig. 6. Model of the chloroform biosensor

Conclusions

- GFP basal expression in the cells with *mbla/clpB* + GFP plasmids
- Addition of CF did not change this GFP basal expression

↓

- Both promoters are recognized by an *E. coli* transcription factor
- Although *E. coli* has its own *clpB*, its regulatory machinery can not recognize *Nitrosomonas'* one

Future work

- Introduce the *amo* gene in the expression cassette
- Find transcriptional regulators allowing induction of *mbla* and *clpB* promoters by phosgene in *E. coli*

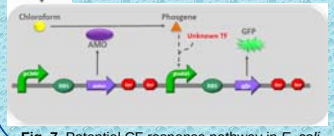

Fig. 7. Potential CF response pathway in *E. coli*

PROJECT 2: Nitrite and Phosphate

Why nitrite and phosphate?

Both are important contaminants of water. Phosphate excess in water bodies provokes eutrophication (algal blooming, excessive aquatic macrophyte growth and oxygen depletion), resulting in a decrease of water quality.
Nitrite is highly toxic for people, specially for babies, and for aquatic live. Moreover, nitrite can develop nitrosamines, very carcinogenic compounds.

NITRITE: Biobricks design

nir promoter from *N. europaea* that is still functional and constitutive in *E. coli*

NSR: nitrite sensitive repressor from *N. europaea*, in absence of nitrite binds to the promoter and stops transcription

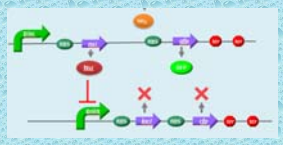

Fig. 8. Biosensor in absence of nitrite

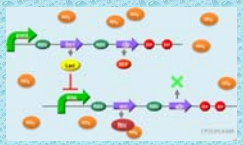

Fig. 9. Biosensor in presence of nitrite

PHOSPHATE: Biobricks design

phoA promoter from *E. coli* that is activated in absence of phosphate

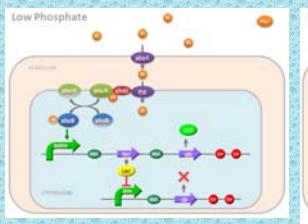

Fig. 10. Biosensor in absence of phosphate

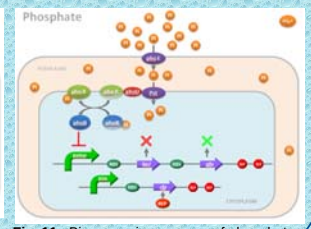

Fig. 11. Biosensor in presence of phosphate

Materials and methods

- PCR isolation and cloning of required elements from *N. europaea* and *E. coli* DH5α.
- Selection of required existing biobricks
- "Cut and paste" to make the new constructs = biobricks we want

New regulatory system to be used for researchers!!!

NsrR (nitrite sensitive repressor)

+ p*nirK*

p*nir* is constitutive in *E. coli*

↓

It is repressed if NSR is produced!

↓

With the addition of NO_2^- to the medium, NSR is released and the synthesis begins.

Kinetic modeling

A mathematical model of a theoretical phosphate biosensor was developed to study the production of GFP and RFP, regulated by inorganic phosphate. The model includes:
- External phosphate transport and across the cell membrane
- Detection of the phosphate concentration at the cell surface
- Signal transduction cascade
- Gene regulatory network (including activation and repression of *phoA* promoter)

20 ordinary differential equations and 1 algebraic expression were integrated using an algorithm suitable for stiff equations in Matlab

Accomplishments

- Cloning of all genetic elements
- Submitting BioBrick Bba_K248001 (*nsrR*)

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