Development of a novel biosensor for the detection of arsenic in drinking water

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Abstract: We sought to develop a whole-cell biosensor for the detection of arsenic in drinking water, a major problem in Bangladesh and West Bengal. In contrast to previously described systems, our biosensor would give a pH change as output, allowing simple detection with a pH electrode or pH indicator solution. We designed and modelled a system based on the arsenate-responsive promoter of the *Escherichia coli* arsenic detoxification system, using urease to increase pH in the absence of arsenate, and β-galactosidase (LacZ) to decrease pH in the presence of arsenate. The pH-reducing β-galactosidase part of the system was constructed and tested, and was found to give a clear response to arsenate concentrations as low as 5 ppb arsenic, well below the World Health Organisation (WHO) recommended limit of 10 ppb.

1 Initial project ideas

In our initial discussions, we considered a variety of projects, including a biosensor for detection of water contamination, and a hybrid biological-electrical device such as a variable resistor. Ultimately we decided to combine these two ideas to develop a whole cell biosensor that responds to arsenic by producing a measurable pH change which can be easily detected with a pH electrode.

2 Usefulness of a biosensor for arsenic in drinking water

Arsenic contamination in drinking water is a serious problem in many parts of the world, and is particularly associated with Bangladesh and West Bengal, where many tube wells were inadvertently drilled through arsenic bearing sediments, resulting in drinking water contaminated with arsenate (AsO_4^{3-}) and arsenite (AsO_3^{3-}) anions [1–3]. Consumption of water with elevated arsenic levels over a prolonged period leads to arsenicosis, resulting in skin lesions and various cancers. Many millions of people worldwide are at risk. The current WHO recommended limit for drinking water is 10 ppb arsenic; in many countries a more relaxed limit of 50 ppb is still in operation. Current field tests for arsenic detection are not altogether satisfactory, requiring toxic chemicals and having a significant false negative rate.

A simple, cheap and sensitive field assay for arsenic levels would therefore be extremely useful. A whole cell microbial biosensor, with an arsenic-responsive promoter linked to a suitable reporter gene, might be one way of achieving this [4, 5]. Arsenic biosensors have been previously reported [for example, 6, 7], but have mainly relied on luminescent or fluorescent reporter genes, which require expensive equipment and trained technicians, and are not really suitable for field use. Other biosensors have used the LacZ/Xgal reporter system, but this is difficult to quantify, and Xgal (5-bromo-4-chloro-3-indoyl-ß-D-galactoside) is expensive and requires refrigeration. By contrast, a sensor giving a pH response would allow a simple quantitative measurement using a cheap pH electrode or solid state device (ISFET, ion-sensitive field effect transistor), or even just a pH indicator solution giving a colour change.

3 Design of the system

We devised a system based on the plasmid-encoded arsenic resistance operon of *Escherichia coli*. This is controlled by two repressor proteins, ArsR (responding to low concentrations of arsenate or arsenite) and ArsD (responding to higher concentrations)[8, 9]. Each is negatively autoregulated. To induce an increase in pH, we chose to use urease, which breaks down urea, $(NH_2)_2CO$, to release ammonium ions. This is used in diagnostic microbiology to distinguish urease-positive bacteria such as *Proteus*, since the pH can rise above 9. To induce a decrease in pH, we chose to use *lacZ*. This encodes β-galactosidase, which catalyses the essential first step in the fermentation of lactose to acetic and lactic acids (mixed acid fermentation) in *E. coli* and related organisms. This reaction is also used in diagnostic microbiology, since the pH can fall below 4.5.

In our design, the activity of the biosensor is initiated by exposure to lactose. Urease is expressed from a hybrid promoter repressed by both lambda cI repressor and LacI repressor. In the presence of lactose, but absence of arsenate, urease is induced and the pH rises. When low amounts of arsenate are present, an ArsR-repressed promoter is induced, leading to expression of lambda cI repressor, switching off urease production. Thus the pH remains neutral. If higher amounts of arsenate are present, *lacZ* expression is induced through an ArsD-responsive

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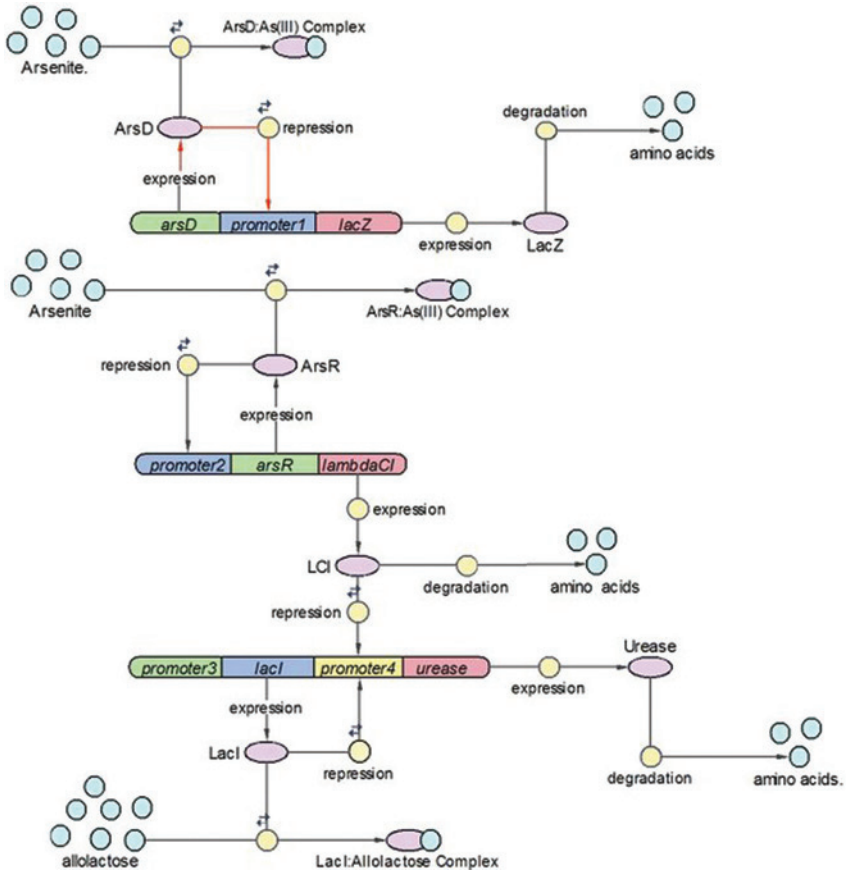

Fig. 1 *Schematic diagram of the complete biosensor system*

promoter, leading to a fall in pH. By using multiple promoters in this way, a high sensitivity and high dynamic range (the range of arsenate concentrations over which arsenate concentration can be estimated from the response) are achieved. A schematic diagram of the system is shown in Fig. 1.

4 Modelling

This system was modelled using an ordinary differential equation (ODE)-based model, with parameters estimated based on the literature. The model showed good induction of urease and repression of *lacZ* in the absence of arsenate, and repression of urease and induction of *lacZ* at high arsenate levels. Sensitivity analysis was also conducted in order to determine which parameters had the greatest effect on the urease and *lacZ* responses. For example, it was found that the parameter having the greatest effect on the steady-state level of *lacZ* expression in the presence of arsenate was the degradation rate of ArsD.

5 Testing the concept

To demonstrate that a detectable pH change could be achieved in the laboratory, we constructed a BioBrick bearing the *E. coli* chrososomal *ars* promoter and negatively autoregulated *arsR* gene (BBa_J33201). The chromosomal *ars* operon is similar to the plasmid-encoded one we had originally envisaged using, but is controlled solely by ArsR and has no equivalent of the second repressor, ArsD [10, 11]. We also made a BioBrick (BBa_J33202) of the *lacZ'* gene encoding the N-terminus of *lacZ*, which complements the *lacZ*ΔM15 mutation found on the chromosome of laboratory strains of *E. coli* such as JM109 and XL1Blue. These BioBricks were joined to generate BBa_J33203. Unfortunately, we were not able to obtain template DNA for the plasmid encoded *arsR* and *arsD* genes we had intended to use within the time frame of the competition. We therefore also cloned the *ars* promoter and *arsR* gene from *Bacillus subtilis* [12], to test whether this might have a sufficiently different affinity for arsenate to be useful in this context. This was joined to *lacZ'* to generate BBa_J33206. In experiments using JM109/pSB1A2-BBa_J33203, concentrations of arsenate as low as 5 ppb gave a significant decrease in pH at incubation times above 5 h, persisting to over 20 h, in a non-optimised medium based on Luria-Bertani medium with 2% w/v lactose (Fig. 2). The response was easily detected with a pH electrode and could also be visually assessed using the pH indicator methyl red, which has a pKa around 4.8–5.0. The equivalent system using BBa_J33206 unfortunately did not show a response to arsenate, with even arsenate-free controls giving a rapid drop in pH, suggesting

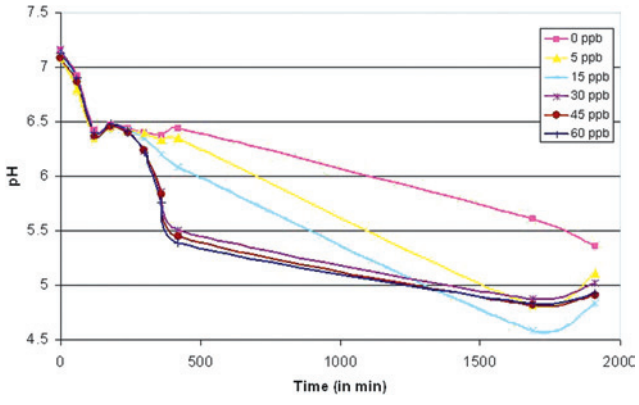

Fig. 2 *pH response of E. coli JM109/pSB1A2-BBa_J33203 to varying arsenate concentrations*

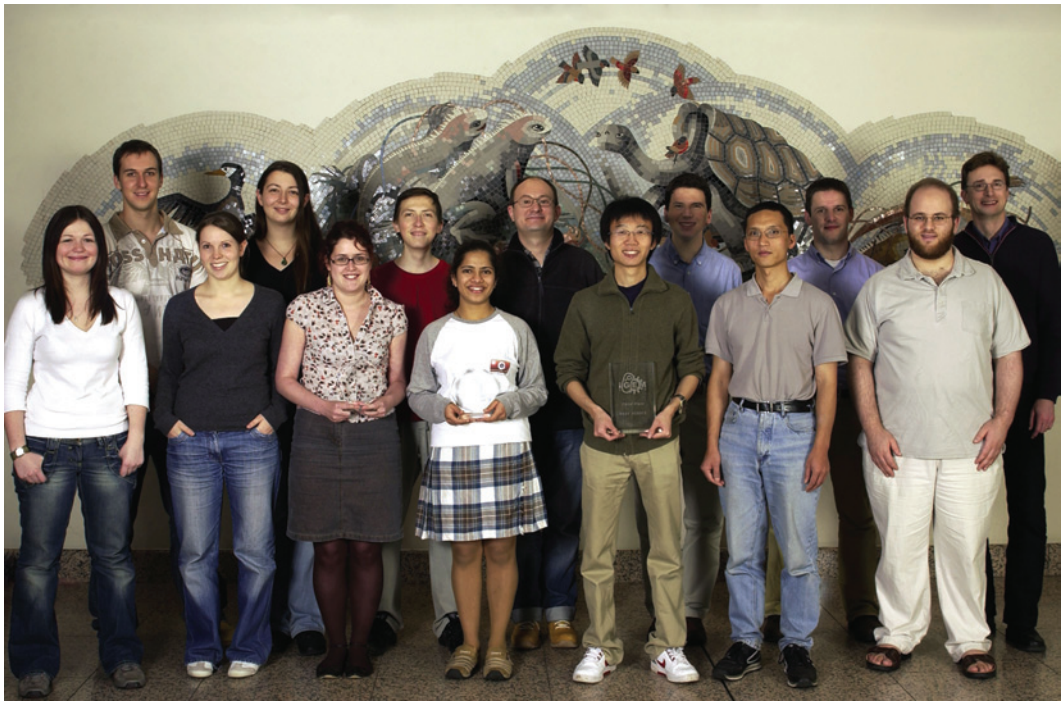

Fig. 3 *The University of Edinburgh iGEM2006 team from left to right: Jennifer Wilson; Kim de Mora;Bryony Davidson; Jelena Aleksic; Judith Nicholson; Sergii Ivakhno; Sreemati LalgudiSeshasayee; Dr Laszlo Kozma-Bognar; Yizhi Cai; Dr Chris French; Dr Hongwu Ma; Dr Alistair Elfick; Farid Bizzari; Prof. Andrew Millar*

high background activity due to incomplete repression of this promoter in *E. coli*.

6 Generation of other parts

For our system, we also needed a urease part to increase pH in the absence of arsenate. The most obvious choice would have been the urease gene cluster present in some strains of *E. coli*; however, this consists of around 5 kb of DNA with seven genes (*ureDABCEFG*, where *ureABC* are the genes encoding the urease subunits, and the other genes encode accessory factors required for proper insertion of the nickel cofactor) and contains six forbidden restriction sites which would have to be mutated out individually before the gene cluster could be converted to a BioBrick. After searching the literature, we found that the *Bacillus subtilis* urease gene cluster consists of only three genes, *ureABC*, which can nevertheless be assembled into a functioning urease in *E. coli* without the requirement for the usual accessory proteins [13]. Unfortunately, this gene cluster also contains two forbidden restriction sites, EcoRI and SpeI.

To check that this urease would be suitable for our purposes, the *ureABC* region was cloned in pGemT-easy (Promega) and pBluescript SK+ (Stratagene). In both constructs, activity was demonstrated in *E. coli*, with pH rising to 9 after incubation in LB with 0.2% w/v urea. Having decided that this urease would be suitable, we used site-directed mutagenesis to remove the two forbidden restriction sites. This was successfully achieved, but the mutant gene cluster gave no detectable urease activity. Sequencing revealed a possible frameshift mutation in *ureC* as well as two non-silent single nucleotide changes as compared to the published sequence. Thus we were unable to generate a urease BioBrick during the time available.

The final part required for our system was the hybrid promoter repressed by both lambda cI and LacI. This was generated by fusing the P_{RM}-P_R region of bacteriophage lambda, including cI binding sites OR1, OR2 and OR3, to the 3′ end of the lac promoter region including the LacI binding site. The N-terminal region of *lacZ* was also included, so that *lacZ'* expression could be used to test regulation of the promoter. This BioBrick was designated BBa_J33205. Unfortunately, we did not have time to build the constructs necessary to test the regulation of this part.

Fig. 4 *The University of Edinburgh iGEM 2006 team logo*

7 Conclusions

Even though we were not able to build our complete design in the time available, we have demonstrated that a simpler version, *E. coli* JM109/pSB1A2-BBa_J33203, gives a good pH response to arsenate concentrations as low as 5 ppb arsenic, with a dynamic range in the region of 0–20 ppb, in a non-optimised system. This can be detected with a pH electrode or a pH indicator (methyl red) which

changes from yellow to red when the pH falls below about 5. Recalling that the WHO limit is 10 ppb, this device is suitable for further development, and could potentially be the basis for a cheap and useful sensor to help prevent the ongoing tragedy of chronic arsenic poisoning. Also, we have submitted the functioning arsenic-responsive promoter to the Registry (BBa_J33201), so we hope that others may be inspired to develop even better arsenic biosensors in the future.

8 Acknowledgments

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