Robust methods for detecting inorganic arsenic with biosensor bacteria using luminescence measurement and fish imaging

Anne Rantala, Daniel Paul and Matti Karp
Department of Chemistry and Bioengineering, Tampere University of Technology, P.O.Box 541, 33101, Tampere, FINLAND

Introduction

Inorganic arsenic determination is based on bioluminescence produced by the bioreporter cells in response to arsenite exposure. A bioluminescence-producing arsenic-inducible bacterium *Escherichia coli* XL-1 (parsRluxCDABE) (Hakkila, 2004) was used in this study as the reporter organism. These sensor cells express luciferase upon exposure to arsenite, the activity of which was detected by measurement of cellular bioluminescence. Arsenate (V) is spontaneously reduced by the cells to arsenite (III) and hence can also indirectly cause luciferase synthesis. The data obtained by the two methods, instrumental luminescence measurement and visualization with CCD camera were used to show the performance on the biosensor cells.

Method

Luminescence measurement

Freeze-dried *E. coli* XL-1 (parsRluxCDABE) were rehydrated and used as fresh cells in arsenic analysis to produce a standard curve. Assay mixtures were prepared directly in the 96-well microtiter plate containing equal volumes of each As standard and reconstituted biosensor cell suspension.

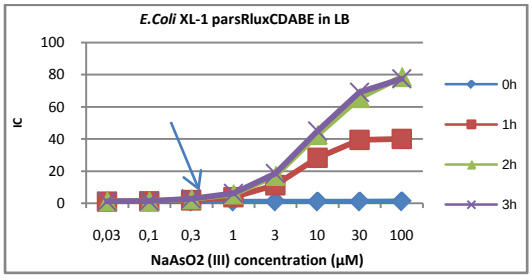

Fig.1. Induction coefficiencies from the *E. coli* XL-1 (parsRluxCDABE) cells in LB is plotted as a function of the arsenite concentration in the assay, ranging from 0 to 100 *µM* As(III).The detection limit is shown with the arrow.

Assays were incubated with shaking (300 rpm) at 37 °C for 180 min. Luminescence was measured once an hour (Chameleon multilabel Detection Platform luminometer, Hidex Oy, Turku, Finland). Induction coefficients were calculated using the formula IC = *Li*/*L*b, where IC is the induction coefficient, *Li* is the luminescence value of the sample, and *L*b is the luminescence value of a blank.

Visualization

An agar diffusion assay (ADA) was performed for the comparison of arsenic detection on plate. Logarithmically grown cells (*E. coli* XL-1 parsRluxCDABE) were added to soft agar supplemented with appropriate antibiotic, mixed gently and poured on top of the LA agar plates containing fish filet (Baltic herring, *Clupea harengus membras*) samples soaked in 5 ml of 30 µM arsenic (Fig. 2a) and 5 ml of milliQ water (Fig. 2b) for 24 hours. Biophotonic imaging station (IVIS Xenogen, Caliper life sciences) was used to visualize the arsenic detection. Exposure time at each measurement point was 30 sec. The more red intensity the higher is the concentration around the filet.

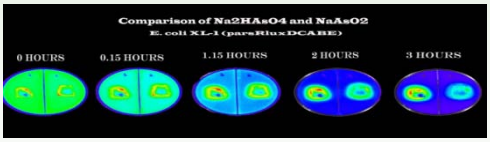

Fig.2a. Comparison of arsenic using fish sample immersed in 5 ml of 30 µM Na_2HAsO_4 (left spots) and $NaAsO_2$ (right spots) for 24 hours and exposed to *E. coli* XL-1(parsRluxCDABE) for up to 3 hours

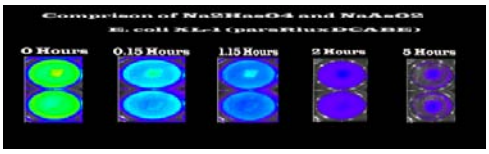

Fig.2b. Fish sample immersed in 5 ml of milli-Q water for 24 hours and exposed to *E. coli* XL-1(parsRluxCDABE) for up to 3 hours.

Conclusion

The intensity of the bioluminescence is proportional to the arsenite concentration in the luminescence assay measurements; detection limit being 0.3 µM. Theoretical explanation was confirmed with the visualization method: the higher is the arsenic concentration, the higher the luminescence is seen in biophotonic imaging. Response intensities by the sensor cells on inorganic arsenic demonstrate the correlation of the two methods used in this study (data not shown). Also the use of reagent-like freeze-dried bacteria in the luminescence measurement make the sensors available as robust detectors, which can simply be reconstituted and used, thus enabling rapid and simple analysis of inorganic arsenic.

TAMPERE UNIVERSITY OF TECHNOLOGY

Reference: Hakkila K., Green T., Leskinen P., Ivask A., Marks R., Virta M. (2004) Detection of bioavailable heavy metals in EILATox-Oregon samples using whole-cell luminescent bacterial sensors in suspension or immobilized onto fibre-optic tips. J.Appl Toxicol. 24(5):333–42.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n° 211326: CONffIDENCE project (www.conffidence.eu).