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

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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.

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.

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.

References

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