Bacterial Biosensors to Measure Arsenic in Potable Water

Worldwide, arsenic is one of the most important inorganic pollutants in drinking water. Particularly alarming is the situation in Bangladesh where more than one million people are already suffering from arsenic poisoning. In order to test each one of the roughly 9 million private drinking water wells, an inexpensive, reliable and sensitive field method is needed. For this reason, an EAWAG team has developed a new biosensor for arsenic. The paper strip test uses genetically modified bacteria that produce blue coloration even at low arsenic concentrations. EAWAG has applied for a patent for this sensor.

Inorganic arsenic is a common contaminant of potable water throughout the world [1–3]. Being usually of geochemical origin, arsenate and arsenite can occur in ground waters in concentrations up to 1 or 2 mg per liter. The safety limit for arsenic in drinking water in most countries is 10 µg or 50 µg per liter. Chronic exposure to arsenic, even at low concentrations around 50 µg per liter, leads to an increased risk for arsenosis and arsenic-mediated cancers. Therefore, it is important that arsenic-containing waters are not used as a drinking water source. Rather unfortunately, current regions in the world with the highest exposure to arsenic in potable water are those with the lowest scientific infrastructure, such as Bangladesh and Vietnam [1, 2]. Furthermore, the drinking water supply in both countries is organized very locally, with individual households each having their own tube well. Since it has now become clear that the accurate prediction of the contamination level of potable water in individual tube wells is very difficult because of strong local and seasonal variations in arsenite concentrations, accurate analyses of the water quality do form an important strategy in arsenic mitigation, as long as no effective treatment methods for arsenic removal are available.

Arsenic Measurements

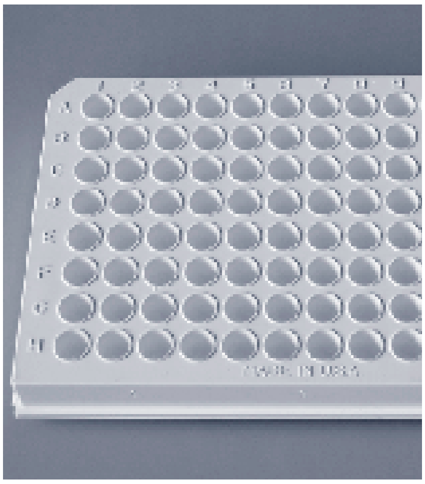
Traditionally, arsenic is measured with colorimetric tests, like the mercuric bromide stain method. However, this method, which is the basis of several commercial field test kits, has been shown to be insufficiently accurate at the level around 70 µg arsenic per liter and below and, moreover, gives rise to arsine gas and heavy metal (Zn, Hg, Sn) contamination. In contrast, arsenic measurement by atomic absorption spectrophotometry or atomic fluorescence spectroscopy is very accurate and reliable, but requires substantial financial investment. Therefore, an easy, accurate and inexpensive arsenic test system using genetically modified bacteria as biosensors has been developed at EAWAG. How was this made possible?

Exploit the Bacterial Defense Mechanisms Against Arsenic

Arsenic is not only toxic for humans and animals; arsenic toxicity is even found in simple organisms like bacteria. They have a few relatively effective biochemical mechanisms to deal with arsenic ions which have entered the cell (Fig. 1). Two well known bacterial proteins deal with the most common forms of arsenic: arsenite and arsenate. One protein is a pump which is integrated into the bacterial cell wall and removes any arsenite from the interior of the cell to the outside, where it can do no harm. The

Photographs: J.R. van der Meer, EAWAG

Bacterial cells of the light producing biosensor must always be kept on ice.



For the assay, the cells are incubated with different water samples in a 96-well plate.

The intesity of light emission as a measure of arsenic pollution is analysed by a luminometer.

other protein is called "arsenate reductase" and reduces arsenate to arsenite.

A further cellular protein is needed in order to elicit the defense reponse whenever arsenic is present in the cell's interior. This protein, called ArsR, is an arsenite sensing protein. It has two binding capacities: In the absence of arsenite, it binds to a specific element on the DNA and thereby prevents the arsenic defense genes from becoming transcribed by RNA polymerase (Fig. 1). Repression, however, is not complete and small amounts of ArsR, the arsenate reductase and the arsenite pump are always present. When arsenite enters the cell, ArsR changes its habits; it will immediately bind to the arsenic compound and lose affinity for the DNA binding site with the result that the protein "falls off" the DNA. As a consequence, ArsR no longer represses the defense mechanism so that the arsenic pump, and the arsenate reductase are produced by the cell in larger amounts.

For the development of the arsenic biosensor, we took advantage of the biochemical capacities of ArsR. However, our interest was not to have the defense mechanism produced when the cell experiences arsenite, but some other protein or enzyme which can easily be measured. This is where gene technology comes into play. By genetic engineering, the bacterial cells can be altered in a way that they produce a light response, or a fluorescent signal, or a colorimetric reaction when they come into contact with arsenite [4]. Therefore, we linked together the gene coding for the ArsR protein, the DNA binding site for ArsR, and a reporter gene coding for a light producing enzyme, the luciferase (Fig. 1). After transfecting *Escherichia coli* bacteria with this DNA construction, the biosensor in principle was ready [5].

An Accurate Light Producing Liquid Biosensor

How to use the new bacterial biosensor? In their most simple form, the biosensor cells are precultured in a liquid medium until a cell density of about 2×10^8 cells per ml is reached. The bacteria are harvested and resuspended in a salt solution with glycerol, in which the cells can be aliquoted and frozen at –80 °C. Such aliquots stay viable for periods longer than 5 years. For an assay, the cells are thawed, diluted with fresh culture medium (to revive them) and mixed with the aqueous sample to be measured. At the same time, a standard calibration assay is performed with known arsenite concentrations between 0 and 0.5 µM (0 and 40 µg arsenic per liter). The volume of the assay mixture can be as low as 200 µl and many assays can be carried out simultaneously in 96-well plates. For the assay, the cells are incubated at 30 °C for a time period of at least 30 minutes. During this time, the bacterial luciferase is produced. After incubation, one drop of substrate for the bacterial luciferase (n-decanal) is added, mixed

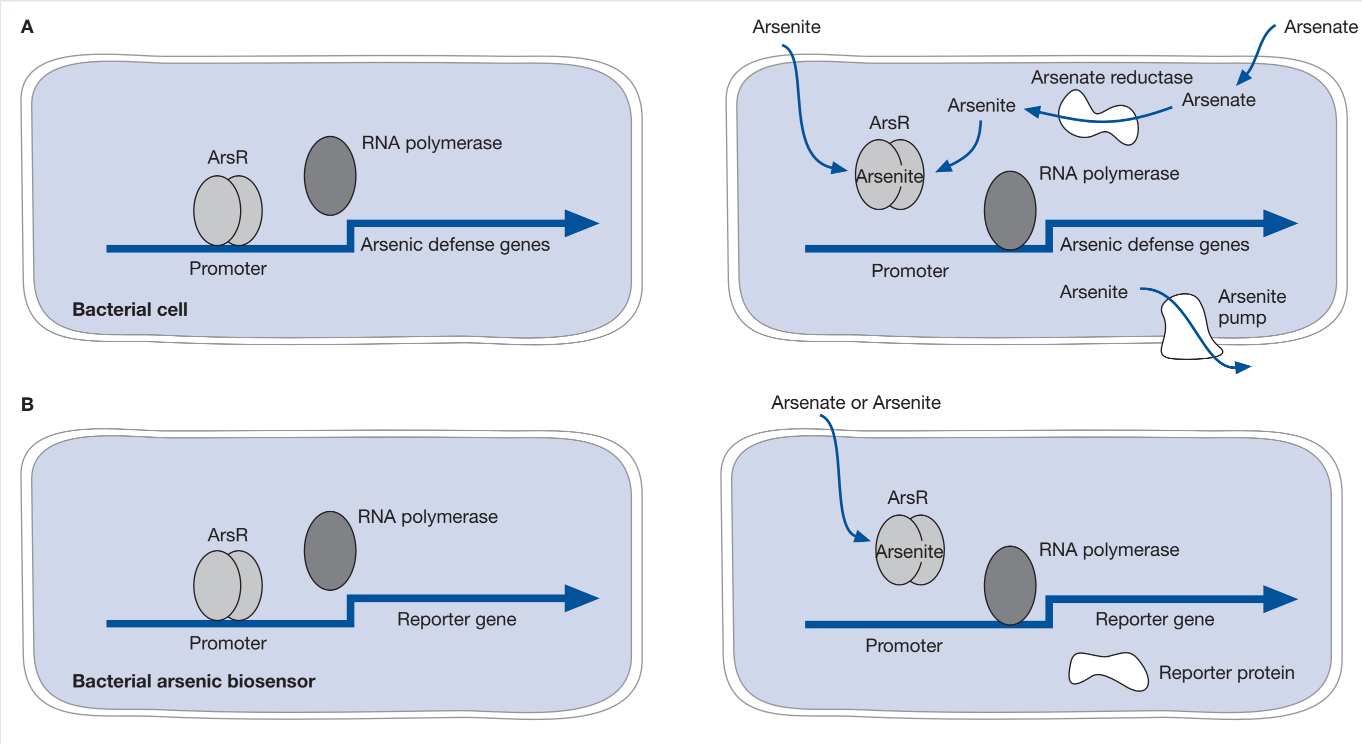


Fig. 1: Principle of the arsenic biosensors. A) The ArsR regulatory protein binds to a specific DNA element and thus inhibits the expression of the genes coding for the arsenic defense mechanism. When arsenite enters the cell, it binds to ArsR. ArsR dissociates from the DNA and RNA polymerase can access. The arsenic defense genes (the ArsR protein itself, the arsenite pump and the arsenite reductase) are expressed in high amounts. B) In the biosensor cells, the ArsR control DNA element is coupled to a reporter gene. Expression of the reporter gene is inhibited by ArsR, but when arsenite enters the cell, it again dissociates and the reporter gene product is synthesized. These products are either luciferase, green fluorescent protein or β-galactosidase.

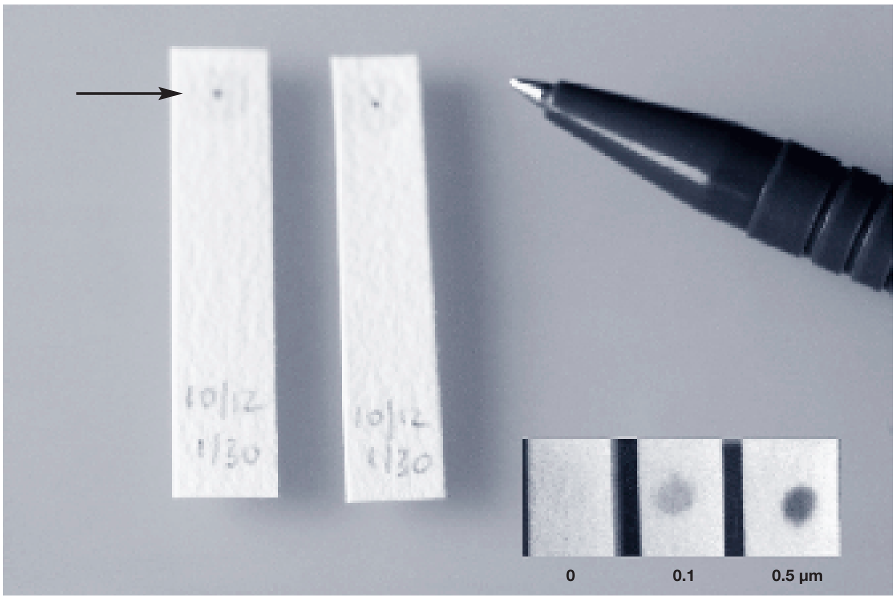


Fig. 3: The arsenic paper strip test: paper strips (4 x 0.5 cm) with spots of dried bacterial cells (arrow). After incubation with arsenite containing water samples, the cells produce β-galactosidase. The activity of this enzyme can be visualized by conversion of a substrate into a blue molecule (inset). Color intensity depends on the concentration of arsenite.

and the light emission measured in a luminometer. The calibration curve with these biosensor cells is usually linear within the range of 0 to 0.5 µM arsenite (Fig. 2). At high concentrations or for unknown samples, different dilutions have to be made in order to perform accurate measurements.

A Simple Paper Strip Biosensor

For two main reasons, the use of the above described biosensor is not easy enough and remains restricted to the laboratory: a rather expensive luminometer has to be installed and the handling of liquid bacterial cultures is too critical in the field. We therefore tried to develop another biosensor system where the genetically modified bacterial cells are immobilized on small paper strips [5]. Instead of the luciferase reporter gene, this second system contains a gene for the enzyme β-galactosidase that produces a color reaction in the presence of arsenic. These biosensor cells are also grown in culture broth, but mixed after harvesting with a solution containing various sugars, amino acids and gelatine. Small amounts of this mixture are pipetted on paper strips (Fig. 3) and carefully dried at controlled temperature and partial vacuum. The cells on the paper strips remain active for about one month storage at temperatures between −20 and 30 °C. For an assay, a paper strip is placed in a vial with 1 ml of aqueous sample, incubated for 30 minutes at 30 °C and taken out. A drop of substrate for the β-galactosidase enzyme is added to the paper and – depending on the amount of β-galactosidase – converted into a blue product. The color intensity is a qualitative measure for the amount of arsenite which the cells have been exposed to. When compared to a standard solution with 10 µg or 50 µg arsenite per liter, one can judge if the arsenic level is above or below the drinking water limit (Fig. 3). Thus, the paper strip biosensor is less accurate and has a shorter storage life than the liquid biosensor but seems to be more appropriate for utilization in the field.

Unsolved Questions

So far laboratory practice. Many important questions and problems remain before we can think of using the biosensors routinely outside the laboratory. For example, how can the quality of the biosensor cells (i.e., their immediate activation potential) be guaranteed? How good are biosensor measurements when compared to chemical analyses? Does the chemical composition of the aqueous sample influence the biosensor response? Would local authorities in developing countries be sufficiently skilled to carry out the biosensor test reliably? What happens with the genetically modified biosensor bacteria after the test? Solutions to these questions may only be achieved by proceeding step-by-step. EAWAG has applied for a patent for the arsenic biosensor at the European patent office. At the moment, potential industrial partners are being traced who might be interested in licensing the arsenic biosensor technology and funding additional developments. Further improvement and testing of the biosensors will hopefully result in a realistic comparison of the biosensor test with chemical methods and its usefulness.

Jan Roelof van der Meer, portrait see page 8.

Coauthor: Judith Stocker

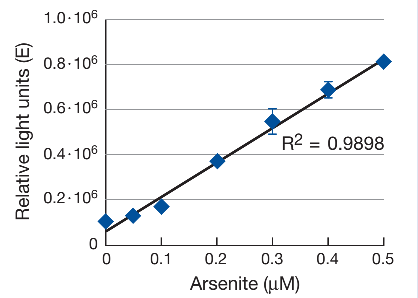


Fig. 2: Example of a calibration curve with the luciferase biosensor. Increasing amounts of arsenite (in a range of 0.05 to 0.5 µM) result in a linear increase in light production by the sensor cells.

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