Research Brief 191:
Portable Biosensing Systems

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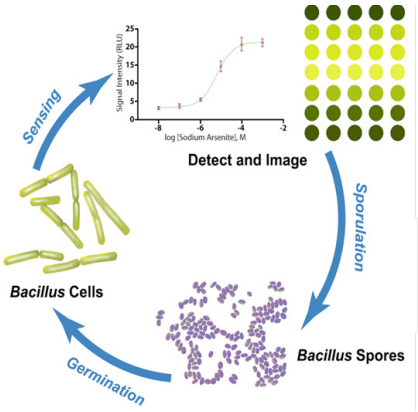
Superfund Research Program

http://www.niehs.nih.gov/srp/

Background

Whole-cell sensing systems are used widely for environmental, pharmacological, and clinical chemistry bioassays. These systems use genetically engineered, living cells that contain biospecific recognition elements for the detection of analytes of interest. After binding the target analyte, a regulatory protein activates gene transcription. This results in expression of a reporter protein and generation of a detectable signal that is directly related to the concentration of analyte. SRP-funded researchers have developed and validated whole-cell sensing systems to detect catechols, arsenic, and dioxin-like halogenated aromatic hydrocarbons (See Research Briefs 79, 113, and 150, respectively).



Whole-cell sensing systems have multiple advantages over standard laboratory methods:

- They are sensitive, selective, and rapid..
- They do not require highly trained personnel or costly analytical equipment, so analytical costs are significantly reduced.
- Because the process requires that the sensing cells uptake the contaminant of interest, whole-cell sensing systems measure the bioavailability of the compound.
- The systems are easily adapted for miniaturization and high-throughput analyses.

The major limitation to the use of whole-cell sensing systems for field analysis is the lack of effective preservation methods for long-term storage and transport. Freeze- and vacuum-drying, continuous culture, and encapsulation in organic or inorganic polymers have been tested. In addition to challenges with cell viability, these approaches introduce the potential for interference of the sample matrix components with the sensing system response.

Advances

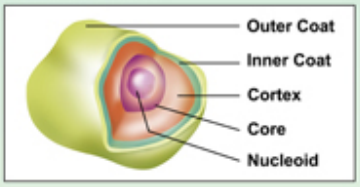
Building on her work at the University of Kentucky Superfund Research Program, Dr. Sylvia Daunert has developed a new method for the long-term preservation, storage, and transport of whole-cell sensing systems. Her research team uses spore-forming bacteria as host microorganisms for the development of whole cell sensing systems. Spores of these bacterial cells are generated as robust preservation, storage, and transport vehicles of the sensing cells and then converted to fully active vegetative sensing cells when needed.

Dr. Daunert's lab group developed spore-based sensing systems using *Bacillus subtilis* and *Bacillus megaterium* for detection of arsenic and zinc, respectively. They conducted a series of tests to assess the applicability of the system for analysis of sample matrices such as blood serum and freshwater, and demonstrated that the spore-based sensing systems could:

- Detect arsenic concentrations as low as 1×10^{-7} M in freshwater and serum samples..
- Detect zinc concentrations as low as 1×10^{-6} M in freshwater and serum samples.
- Be stored for up to 24 months at room temperature.
- Be stored for up to 12 months in extreme temperature and humidity/drought conditions.

The complete assays, including spore germination and analyte detection can be performed in 2.5 hours or less. This method not only provides a way to stabilize the biosensing systems with minimum storage requirements but also opens up the possibility of application of such sensors for field analysis. The spores can be immobilized on paper strip platforms or at the tip of an optical fiber, with the sensing cells emitting light in response to a target compound.

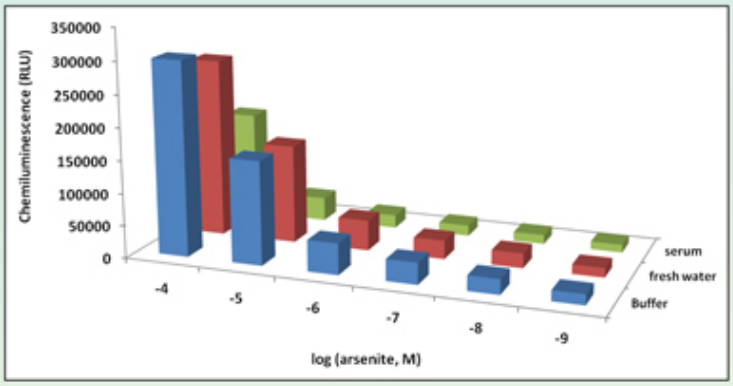
Spores are dry, naturally hardy, dormant cells that can survive most environmental challenges (extreme temperatures, desiccation, and exposure to noxious chemicals) for long periods of time. The most important function of spores is to lock the bacterial DNA into a stable crystalline state within a multilayer shell, which excludes any toxic molecule that may be present, thus preserving the microorganism's genetic material.



Spores are capable of regaining their full metabolic activities in favorable environmental conditions. Spores can sense even small amounts of nutrients, such as sugars and amino acids, and respond to them by germinating to viable, growing, and metabolically active cells.

Significance

This new method of preservation, storage, and transport of whole-cell biosensing systems will facilitate their application for broad use in on-site applications. Dr. Daunert demonstrated that the assays can be performed efficiently in environmental and biological sample matrixes, without loss of analytical performance of the sensing systems.



Detection of arsenite by the spore-based whole-cell sensing system in buffer, freshwater and serum. The detection limits for arsenite are within the physiological levels in serum and the EPA standard of 10 parts per billion (ppb) for drinking water.

These spore-based sensing systems are well-suited for integration into miniaturized portable platforms, creating analytical tools with that could be developed into a rapid, high-throughput, field portable.

The resistance of spores to extreme conditions such as dry and wet heat, freezing temperatures, and desiccation suggests that these whole-cell sensing systems could be of great use in harsh environments. This would be of particular importance in developing countries, which often experience difficult environmental conditions and have poor storage and transportation facilities.

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To learn more about this research, please refer to the following sources:

Amol Date, Patrizia Pasini, and Sylvia Daunert. 2010. Integration of spore-based genetically engineered whole-cell sensing systems into portable centrifugal microfluidic platform. *Analytical and Bioanalytical Chemistry* 398:349-356. doi:10.1007/s00216-010-3930-2
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Amol Date, Patrizia Pasini, Abhishek Sangal, and Sylvia Daunert. 2010. Packaging Sensing Cells in Spores for Long-Term Preservation of Sensors: A Tool for Biomedical and Environmental Analysis. *Analytical Chemistry* 82:6098-6103. doi:10.1021/ac1007865
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