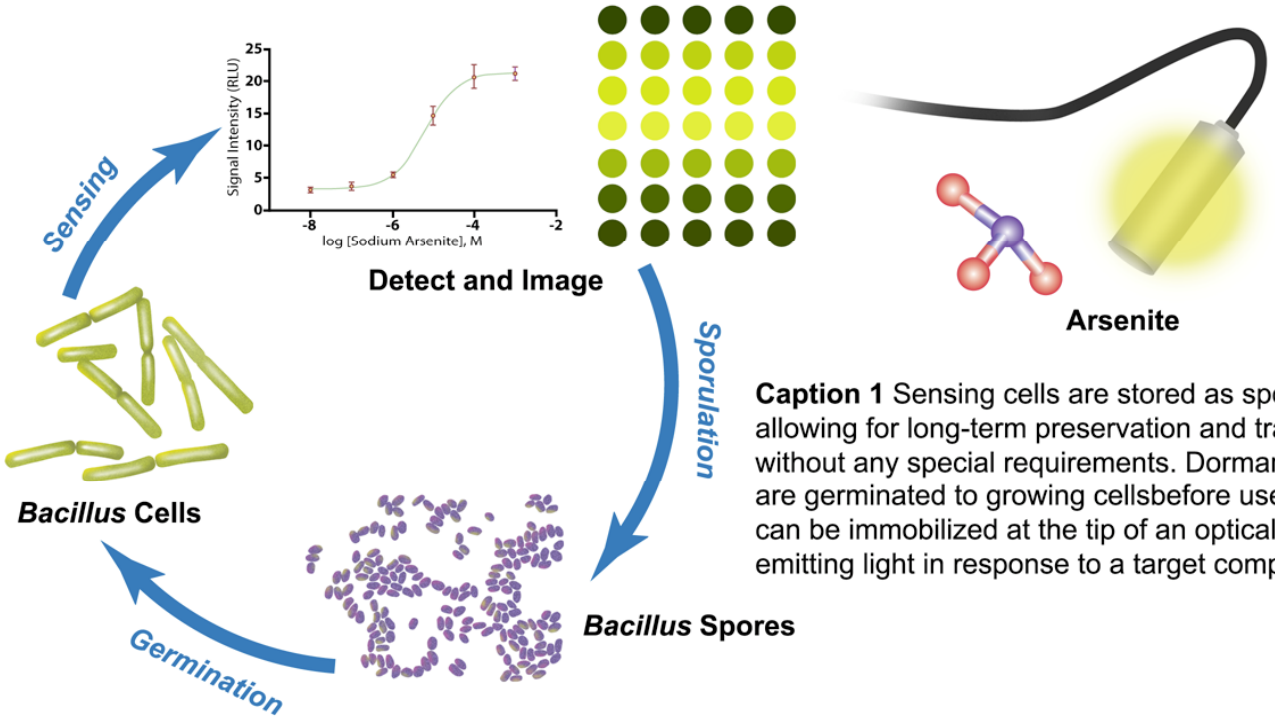
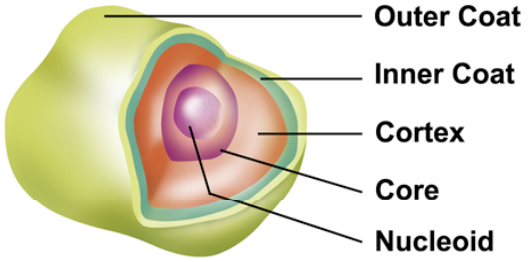
EXAMPLE **NSF Division of Chemistry Highlight** EXAMPLE

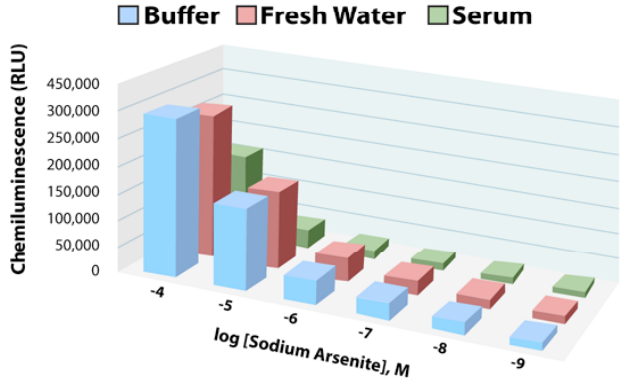
**Principal Investigator Name/Institution:** Sylvia Daunert University of Kentucky
**Award Number:** 0718844 (0416553)
**Highlight Title:** **Bacteria Comes to Life for Chemical Detection**




**Caption 1** Sensing cells are stored as spores, allowing for long-term preservation and transport without any special requirements. Dormant spores are germinated to growing cellsbefore use. The cells can be immobilized at the tip of an optical fiber, emitting light in response to a target compound.

**Caption 2** Illustration of a bacterial spore and its components. When subjected to stress, *Bacillus* forms metabolically inactive spores that lock the DNA into a dry, multilayer shell, thus preserving it for long periods of time, until conditions are suitable for re-germination.




**Caption 3** Detection of arsenite by the spore-based whole-cell sensing system in buffer, fresh water and serum. The detection limits for arsenite are within thephysiological levels and the EPA standards of 10 parts per billion or ppb.

| Storage time (months) | Room Temp. | Cold -20 °C | Wet Heat 28 °C/RH >80% | Dry Heat 37 °C | Dessication |
|---|---|---|---|---|---|
| 1 | $2.8 \times 10^{7}$ | $1.7 \times 10^{-7}$ | $3.2 \times 10^{-7}$ | $1.7 \times 10^{-7}$ | $3.2 \times 10^{-7}$ |
| 2 | $2.8 \times 10^{7}$ | $1.7 \times 10^{-7}$ | $1.7 \times 10^{-7}$ | $1.7 \times 10^{-7}$ | $3.2 \times 10^{-7}$ |
| 3 | $2.8 \times 10^{7}$ | $1.7 \times 10^{-7}$ | $3.2 \times 10^{-7}$ | $1.7 \times 10^{-7}$ | – |
| 4 | $2.8 \times 10^{7}$ | $1.7 \times 10^{-8}$ | $3.2 \times 10^{-7}$ | $3.2 \times 10^{-7}$ | $1.7 \times 10^{-8}$ |
| 5 | $2.8 \times 10^{7}$ | $5.0 \times 10^{-8}$ | $8.0 \times 10^{-8}$ | $1.0 \times 10^{-7}$ | $1.7 \times 10^{-8}$ |
| 6 | $2.8 \times 10^{7}$ | $8.0 \times 10^{-8}$ | $5.0 \times 10^{-8}$ | $1.7 \times 10^{-8}$ | $8.0 \times 10^{-9}$ |
| 24 | $2.8 \times 10^{7}$ | ---- | ----- | ----- | ----- |

**Caption 4** This table presents data on the effects of different storage conditions for a whole-cell biosensor for arsenic. Notably, the data points out the discovery that Professor Daunert's spore-based whole-cell sensing system is able to maintain its analytical performance for chemical detection upon storage at room temperature (RT) for up to 2 years.

**Credit:** Sylvia Daunert , University of Kentucky

**Project Outcome**

Professor Sylvia Daunert and her group at the University of Kentucky have recently demonstrated that their spore-based, whole-cell sensing systems based on living genetically engineered bacteria can survive and maintain analytical performance for detecting arsenic levels upon storage at room temperature for up to 24 months. Bacterial spores, or endospores, are dormant, non-reproductive structures produced by bacteria. They are able to withstand extreme environmental conditions for long periods of time and to resume full metabolic activity upon stimulation. Taking advantage of their unique properties, Daunert used the spore-forming bacteria *Bacillus subtilis* and *Bacillus megaterium* to develop two distinct sensing systems for both arsenic and zinc, respectively, as model analytes. Evaluation of biosensor analytical capability was performed to examine the detection limit, dynamic range, reproducibility, and selectivity, both before spore formation and after storage of the spores for a period of time. The arsenic or zinc sensing cells could be converted back to spores and "revived" (germinated) at a later time to generate viable and metabolically active cells. In the *B. subtilis* based sensing system, different species of arsenic are added to a solution of genetically modified *Bacillus* cells capable of sensing arsenic. Arsenic presence and concentration is determined when a chemical reaction occurs by means of special proteins incorporated in the bacterial cells; the special proteins “light-up” when the cells “sense” arsenic. Determination of the amount of arsenic detected in the sample is carried out by a sensitive optical detection system used for measuring very low light levels.

Overall research efforts with this NSF Chemistry grant resulted in demonstration of a new method for the long-term preservation, storage, and transport of whole-cell biosensing systems based on the formation of high-performing spores. This outcome represents an advance in extending the shelf life and transportability of luminescent sensing systems. This new methodology will allow for broad use in on-site applications for environmental and biomedical analysis.

**Does this Project Outcome Represent Potentially Transformative Research? If so, please provide explanation.**

This is the first time that spores have been employed as vehicles for harboring and storing biosensors. In that regard, we believe that our concept is unique and transformative as it represents a new means of thinking about packing and deploying sensors.

**Please summarize the Intellectual Merit (technical significance and background) of this project outcome in a few sentences. Explain why this outcome is notable and/or important.**

The work performed within this project successfully addressed one of the main limitations of whole-cell sensing systems, that is, their shelf-life and transportability. The developed spore-based method allows for long-term storage of sensing cells. The approach proved to be effective for storage in mild environments, e.g., at room temperature and holds promise for storage in harsh environments, as shown by the data obtained. Additionally, spore-based sensing systems are amenable to incorporation into portable devices for on-site sensing.

**Please summarize the major Broader Impacts of the project outcome in a few sentences. Explain why this outcome is notable and/or important. Broader Impacts include activities that advance discovery while teaching and training, seek to broaden participation of underrepresented groups, enhance infrastructure for research and education, disseminate information to enhance understanding of science and technology, and provide benefits to society.**

The availability of robust, portable whole-cell sensing systems will facilitate their use in environments and applications in which they could not be employed before. Specifically, in developing countries there is a need for rapid, simple and inexpensive analytical tools for monitoring human health and the environment. Whole-cell sensing systems meet the requirements; however, their extensive use in those countries has been hampered by the challenging environmental conditions, and the inadequate commercial distribution and storage facilities. This technology could be applied to evaluate human exposure to toxic compounds or the levels of a biomarker of disease, especially in remote areas of the Country. Thus, it could be employed in a variety of situations ranging from clinical analysis to environmental assessment.

**Publications Relevant to this Project Outcome**

February 2010 issue of Popular Science – *"Rebuilding America: 25 New Technologies to Rebuild Our Crumbling Infrastructure"*

Date, A; Pasini, P; Daunert, S, "Construction of spores for portable bacterial whole-cell biosensing systems", ANALYTICAL CHEMISTRY, p.9391, vol. 79, (2007). Published, 10.1021/ac701606

Date, A; Pasini, P; Daunert, S, "Fluorescent and bioluminescent cell-based sensors: strategies for their preservation", in "Whole Cell Sensing Systems", Advances in Biochemical Engineering/Biotechnology Series, S. Belkin and M.B. Gu, Eds., (2010). In press

**Website**

**http://www.chem.uky.edu/research/daunert/Daunert%20Research%20Group%20Website/Research%20Interests.html**

URL for project Website

**Send the completed Highlight to the Division of Chemistry at:**

**chemhighlights@nsf.gov**