Microbial Community Microarrays to Assess Mercury Contamination

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Problem Statement

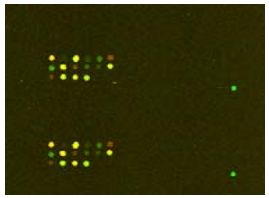
A variety of pathogens, toxins, and pollutants currently pose significant regulatory, legal, and health concerns for any business, farm, residence, municipality, or recreational water park that must meet appropriate water quality and safety standards. Currently, the detection of these agents requires many different types of equipment and tests that are often labor-intensive, time-consuming, and expensive. Mercury (Hg) is one example of an important chemical pollutant because it is known to be a serious hazard posing acute and serious health risks to living organisms (US EPA 2001, US EPA 2005a).

Mercury enters water supplies through natural deposit erosion, factory and refinery discharges, landfill/cropland runoffs, and coal power plant emissions (US EPA 2005a, b). It can be converted into a highly toxic compound, methyl mercury, by certain anaerobic bacteria (Osborne, *et al.* 1997, Nascimento and Chartone-Souza 2003). In 1992, the US EPA required all water suppliers to test for mercury annually, to monitor quarterly if the ion was found, and to install appropriate removal systems as long as the problem persisted (US EPA 2005a). Since 2002, the number of water sources finding mercury has increased substantially in the United States, and in 2005, the EPA responded by initiating restrictions on mercury emissions from power plants. Since mercury can accumulate in living tissues, higher concentrations tend to be associated with higher trophic levels of the natural food chain. Consequently, ongoing, low-level consumption of mercury-laden food poses a chronic health risk to humans.

In addition, mercury remains in the environment long after the pollution source is discovered and removed (US EPA 2005b). Thus, the costs of ongoing testing, ongoing filtration of water and remediation, and closure of contaminated sediment or water sources far exceed the initial costs of removing the pollution source, emphasizing the desirability of methods designed for early and rapid detection. Currently, the assays approved by EPA for mercury, such as cold vapor atomic absorption spectrometry (US EPA 2003), require specialized equipment and present such technical difficulties that most municipalities outsource the required testing. Further, there are assays that utilize several mercury metabolizing genes found in the environment, but these tests are too difficult and expensive to use routinely.

Technology Description

Prototype Microbial Community Microarray (WaterChip™)



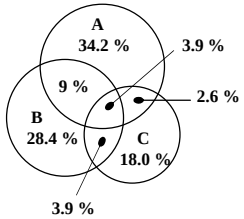
Efforts to develop faster, more economical testing alternatives include a variety of DNA diagnostic technologies. It is well-established that "DNA fingerprinting" methods, such as the microarray and amplification by PCR, are useful for the direct detection of pathogenic microbes (e.g. Warsen, *et al.* 2004). However, microarrays may need to be capable of simultaneously detecting bioindicator microbes of chemical pollutants before commercial applications for sediment and water quality testing become commercially viable.

We are developing a freshwater microbial community microarray, the WaterChip™, to detect the presence of specific chemical and biological contaminants in environmental samples. The WaterChip™ prototype is a glass slide onto which DNA-containing solutions are spotted, dried, and bound. Each spot contains a unique single-stranded DNA probe sequence that corresponds to a specific microbial species. Further, each spot is printed at a specific location on the array grid. An array includes these probes ("DNA fingerprints") for up to thousands of microbes, including eukaryotes and prokaryotes, known pathogenic and nonpathogenic species, and unknown and non-culturable microbes that may commonly exist in a given freshwater environment (Rappé and Giovannoni 2003).

The WaterChip™ technology performs two functions: it sorts out and detects bioindicator DNA fingerprints derived from environmental samples, and the resulting pattern provides an indication of the presence of specific chemical and biological contaminants. To demonstrate the utility of this approach, Phase I work will focus on the recovery and application of DNA fingerprints for the detection of mercury (Hg) present in freshwater sediments.

Expected Results

Prokaryotic and eukaryotic microbes vary in abundance among geographically dispersed environments, consistent with the working hypothesis that local environmental conditions dictate abundance. From previous work, we expect to find that a relatively small number of sequences are predominately abundant in each project sample and that many of these sequences represent microbial species that exist across a range of watersheds. In the Venn diagram below, three samples of diverse origin are compared, illustrating the proportions of sequences (%) shared among them. In this case, only 3.9% of the recovered sequences were shared by all three samples. However, a comparison of mercury-contaminated samples is expected to reveal a much higher overall similarity.



Based on these expectations, the following specific outcomes are anticipated:

1. Mercury-tolerant microbes will be relatively abundant in the mercury-contaminated samples.
2. From the subset of sequences shared among mercury-contaminated samples, a group of reliable microbial rDNA bioindicators for the presence of mercury in freshwater sediment sources will be identified and validated.
3. These bioindicators will be associated with levels of mercury contamination, potentially distinguishing contamination levels that fall above and below the US EPA's maximum allowable contaminant level (0.002 mg/L).
4. The WaterChip™ will be enhanced by adding the capability to detect bioindicators of mercury contamination.

Potential Environmental Benefits

We anticipate several significant outcomes, which have bearing on commercial feasibility and environmental benefits: (1) the WaterChip™ will implement the same DNA technology already applied in the direct detection of important pathogens for the detection of microbes associated with chemical conditions; (2) the WaterChip™ will have the diagnostic sensitivity and specificity to detect community microbial bioindicators associated with the presence of an important pollutant – mercury; (3) the presence of mercury can be tested in widely dispersed water sources will be possible due to the association with specific microbial community patterns; (4) several novel and specific bioindicators (and potential microbial bioremediators) for mercury will be included as part of a commercial testing program; and (5) mercury levels in these samples will also be measured using approved EPA testing methodologies, providing a basis for using the WaterChip™ in the context of current EPA regulatory guidelines. For the potential customer, the ability to monitor for mercury contamination at virtually no additional cost, *as part of an ongoing routine monitoring plan*, will help to ensure that a mercury pollution event does not occur. Thus, long-term testing and remediation processes can be avoided.

Perhaps most importantly, the completion of Phase I project goals will demonstrate that the WaterChip™ can be used as an indirect detection assay for chemical contamination, in general. In Phase II, the mercury component of the WaterChipTM technology will provide a model for expanding testing capabilities to other environmental toxins and pollutants. Ultimately, we anticipate that this process will produce a tool that has the capacity to perform a rapid, broad spectrum analysis of numerous environmental characteristics in a single test. Such an "all-in one" diagnostic platform would create an opportunity to perform comprehensive analyses that are also economical, thus facilitating efforts to better protect the environment, as well as the public health.

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