

Use of Microarrays and qPCR/RT-qPCR for Characterization of Viral Populations within Water Supplies Affected by Agricultural Activities

Ronald L. Crawford, PhD

University of Idaho

Grant Number: 2008-35102-04635

Justification:

Viruses are oftentimes the most abundant biological entity in natural waters. In addition to causing disease, they play a dominant role in microbial communities by serving as a major genetic reservoir that promotes evolution via mutation of members of biological communities. Gastroenteritis, infectious hepatitis, meningitis, respiratory diseases, and eye infections are among the common diseases caused by water-borne. Pathogenic viruses transmitted by drinking water cause ~6.5 million illnesses per year in the United States. Four classes of viruses have been identified as prime contaminant candidates to monitor; however, the lack of technologies for virus detection is still a hurdle in drinking water monitoring efforts. Unfortunately, viral analysis is beyond the capabilities of most water quality assurance laboratories because detection techniques that do exist are expensive, time- and labor-intensive, and require highly skilled and trained personnel. Better viral monitoring techniques are needed to address these shortcomings. This is especially true for environments such as watersheds impacted by animal wastes generated by the agricultural industry.

Objectives:

The objectives of the research are to develop and validate a protocol for use of custom-designed microarrays in combination with qPCR and RT-qPCR to characterize viral contaminants in agriculturally impacted waters, specifically those affected by dairy practices.

Progress to date:

This project is new, but initial experiments are underway. Initial wastewater samples have been collected from the University of Idaho (UI) dairy wastewater lagoon (Moscow, ID) and processed for viral and bacterial nucleic acids for probing of two viral microarrays. One array is the Combimatrix Pan Vira array (probes for ~1000 known human viruses). A second array was designed by us specifically for this project and will include 10 replicates each of probes for >500 bacteriophages (partial phage gene sequences) and their hosts (conserved 16S rRNA gene regions). Initial hybridization and PCR-based experiments are underway to identify viruses and/or phages that are signatures of water contamination by dairy wastewaters.

Impacts:

This research will allow us and others to implement effective monitoring methods in support of efforts to understand the sources, fate, and transport of microbial pathogens in surface water and groundwater, in wastewater-based irrigation systems, and in rural agricultural watersheds nationwide. The protocol we will develop will require only a minimum number of high-through-put analyses and can be readily adopted for use in centralized public health laboratories.