Identification of Viable Bacteria in Environmental Waters using EMA-qPCR

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Our research provides a simple and efficient method to identify viable pathogens in the environment. As a tool it is important because it provides an accurate description of the microbial population from any given source.

Advances in molecular techniques have improved pathogen detection sensitivity and specificity as well as reduced the time to result. However, issues related to cell viability and/or infectivity continue to prevent adoption of DNA Microarrays, Polymerase Chain Reaction (PCR), and Quantitative Polymerase Chain Reaction (qPCR) methodologies by public health, water, and wastewater agencies for routine monitoring. A microarray that simultaneously identifies a number of indicator organisms and pathogens is an ideal solution for public health agencies. In the process of examining this approach it became evident that the issue of cell viability is of utmost importance. Our goal is to develop a simplistic assay that couples ethidium monoazide bromide (EMA) treatment for viability with qPCR to allow accurate quantification of viable target populations. These targets can be comprised of water quality indicator bacteria and/or pathogens. The method can be applied to source water, irrigation and food processing waters, as well as recreational waters and point and nonpoint discharges that enter sensitive water bodies. The problem with many molecular-based identification strategies is the persistence of DNA after cell death, thus bacterial culture techniques remain the accepted standard in determining cell viability by the United States Food and Drug Administration (USFDA) and the Environmental Protection Agency (USEPA). However, EMA treatment allows amplification of target DNA from culturable, and viable but non-culturable cells, but prevents PCR amplification of DNA from non-viable or injured sources, thus avoiding the issue of DNA persistence. In the future, our approach can easily be adapted for microarray analyses where real-time monitoring can be achieved. Given current standards, optimization is most cost effective using qPCR methodologies. Such a method would improve pathogen monitoring in environmental waters and potentially increase plant operation efficiencies by rapidly providing information on bacterial populations important in treatment processes. To date, we focused on wastewater effluents as these sample types have proven to be the most difficult matrices for molecular analysis.

We adopted a method based on treating our samples with EMA prior to DNA extraction in order to prevent PCR amplification of nonviable cells. This approach is based on qPCR using dual labeled probes that allows for the identification and enumeration of genetic markers specific for a target pathogen from wastewater effluents. Optimization of this assay was performed by evaluating treatment with EMA concentrations of 0, 1, 2, 3, 5, 7.5 μg/ml on wastewater effluents from primary, secondary activated, and secondary trickling filter treatment processes. Our qPCR genetic targets were *E. coli* specific and identify the H7 flagellar antigen gene associated with E. coli O157:H7, *fli*C, and the beta-glucuronidase gene, *uid*A. Throughout the study, it became evident that qPCR targets were being amplified in heat-killed samples, therefore we used environmental scanning electron microscopy (ESEM) to determine if EMA was able to penetrate the cell membranes.

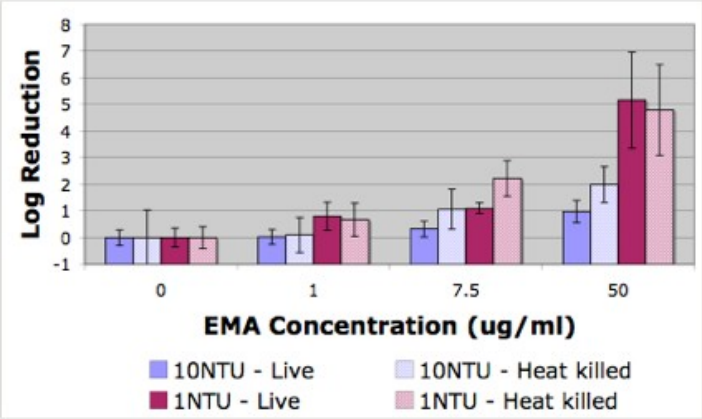

Figure 1.

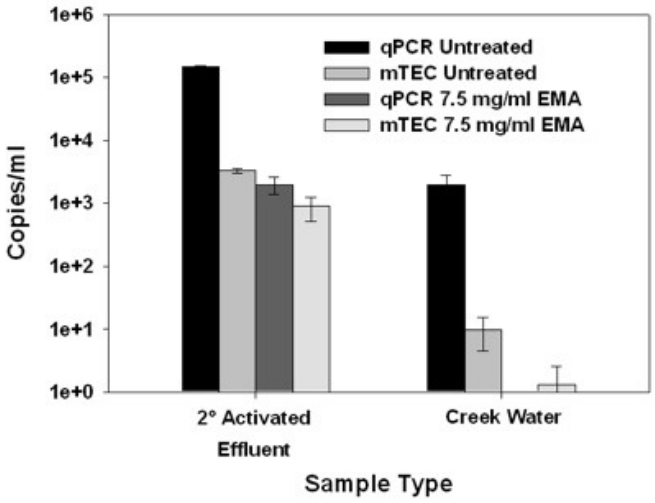

Figure 2.

Results indicated a culture age effect when treated with EMA to differentiate live and heat-killed bacteria. EMA treatment of pure cultures produced over a 4 log reduction between live and heat-killed samples treated with > 2μg/ml EMA. Additionally, the 5h and 40h cultures were most sensitive to EMA treatment. In environmental samples, EMA treatment proved to be improved after turbidity was reduced (Figure 1). Samples diluted to 1NTU were completely inhibited when treated with 50μg/ml EMA. However, treatment with 7.5μg/ml EMA produced a 1 log reduction between live and heat-killed *E. coli* from primary clarifier effluent diluted to 1NTU. However, creek water samples were completely inhibited by this concentration (Figure 2). In both sample types, it is clear that DNA from non-viable, injured, or viable but non-culturable cells were being amplified in the absence of EMA treatment. ESEM analysis allowed us to visualize the bacteria to determine if EMA concentration affected cells differently based on growth phase. Cultures at 5h, 18h, and 40h were representative of early log phase, late log phase, and early stationary phase growth, respectively. ESEM images of live bacteria treated with 7.5μg/ml were unable to produce a qualitative difference due to the limited resolution we were able to obtain.

This research is important to water quality because recreational waters such as rivers, lakes, and beaches continue to be impacted by high bacterial counts. Public health agencies can take advantage of the rapid and reliable analysis of recreational waters for microbial contamination because this method reduces error in molecular methods by eliminating non-viable sources from the analysis. Wastewater treatment utilities can benefit by optimizing plant operation parameters directly based on actual bacteria populations rather than bacterial indicators. Our research provides a simple and efficient method to identify viable pathogens in the environment. As a tool it is important because it provides an accurate description of the microbial population from any given source.

Professional Presentations

Gedalanga, P.B. and B.H. Olson. Comparison of *Escherichia coli* Populations in a Lake Reservoir Enumerated using EMA-qPCR with direct DNA Analysis for Viable but Non-Culturable Bacteria and Membrane Filtration on mTEC Media. 108th American Society for Microbiology General Meeting. Boston, MA, June 1 – 5, 2008.

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