Title: New Approaches to the Assessment of Microbes in Groundwater: Application to Bioremediation and Detection of Pathogens

Project I.D. : DNR Project #155

Investigator: Mary Lynne Perille Collins, Dept. of Biological Sciences, UW-Milwaukee

Period of Contract: 7/1/00 - 6/30/02

Background/Need: Groundwater monitoring, Bioremediation, Detection of pathogens

Objectives: 1) Development of molecular methods for the detection of a variety of bacteria in groundwater, including relevant pathogens

2) Development of molecular methods for the assessment of the population of methanotrophic bacteria in groundwater

Methods: Objective 1: The direct polymerase chain reaction (DPCR) was applied to the detection and quantification of a range of bacteria in groundwater.

Objective 2: The use of DPCR in conjunction with single-stranded conformational polymorphism (SSCP) was evaluated for analysis of methanotrophs in groundwater.

Results and Discussion: DPCR detection of bacteria in environmental water samples was optimized and a general strategy developed. Methanotrophic bacteria were detected and quantified in groundwater and other environmental samples. Phototrophic bacteria and the water-borne pathogens *Escherichia coli* O157:H7 and *Helicobacter pylori* were also detected.

SSCP was used to "fingerprint" the populations of methanotrophic bacteria in environmental samples. Changes in the fingerprint during the course of a monitored bioremediation were observed.

Conclusions and Implications: Because traditional culture-based methods of bacterial detection are of limited use for environmental applications, molecular methods for the detection and quantification of bacteria are essential. DPCR is an improved molecular approach that should facilitate assessment of the presence of specific bacteria. This is relevant to monitoring of bioremediation and of particular importance for pathogens, which may be may not be detected by culture-based methods.

To accurately predict the process of natural attenuation and to maximize the potential for bioremediation, it is necessary to understand the microbial processes at work. Ideally, it would be useful to know the composition of the bacterial population at a site and to be able to correlate particular microbes with reductions in the target chemical and with amendments of the site. This would lead to improved methods for monitoring and improved strategies for *in situ* bioremediation in which treatments of the site could be designed to stimulate of activity of those bacteria most effective in the degradation.

This research project developed an SSCP method for the analysis of populations of methanotrophic bactera. This should provide a useful means to assess the role of these organisms in degradative processes in the environment. It would be valuable to extend both DPCR quantification and SSCP population analysis to other bacterial groups that play a role in bioremediation. This would provide a more complete picture of the microbial processes at work and could serve as the basis for improved remediation strategies and monitoring recommendations.

Research products:

Research papers

Fode-Vaughan, K. A., C. F. Wimpee, C. C. Remsen and M. L. P. Collins. 2001. Detection of bacteria in environmental samples by Direct PCR without DNA extraction. BioTechniques 31: 598-607.

K.A. Fode-Vaughan, J. A. Benson, J. S. Maki,and M. L. P. Collins. 2002. Detection of Pathogens in Environmental Samples by Direct PCR. (submitted)

Papers Presented at Scientific Meetings

Collins, M. L. P. 2000. Detection of bacteria in environmental samples by direct PCR. Invited presentation at the Midwest Molecular Microbial Ecology Conference. DeKalb IL, July 2000.

K. A. Fode-Vaughan, C. F. Wimpee, J. Maki, M. L. P. Collins. 2001. Detection of bacteria in environmental samples by Direct PCR without DNA extraction. General Meeting of the American Society for Microbiology, Orlando FL, May 2001

K. A. Fode-Vaughan. 2001. Analysis of bacterial populations in natural samples using direct PCR. presentation to the Milwaukee Microbiology Society, Milwaukee WI, May 2001.

Thesis

K. A. Fode-Vaughan. 2001. Development of methods for the detection, quantification, and community analysis of methanotrophic bacteria in environmental samples. M.S. thesis. University of Wisconsin-Milwaukee.

Invention Disclosures

Method to detect *Escherichia coli* O157:H7 in environmental samples and food by Direct PCR, filed in the Graduate School, University of Wisconsin-Milwaukee

Detection of *Helicobacter pylori* by Direct PCR, filed in the Graduate School, University of Wisconsin-Milwaukee

Keywords: PCR, Direct PCR, methanotrophic bacteria, bioremediation, TCE, *Helicobacter pylori*, *E. coli* O157:H7, Shiga toxin

Funding: Wisconsin Department of Natural Resources

Final Report: A final report containing more detailed information on this project is available for loan from Wisconsin's Water Library, University of Wisconsin - Madison, 1975 Willow Drive, Madison, Wisconsin 53706 (608) 262-3069.