Ohio Lake Erie Protection Fund

Final Report

Grant Number: **SG 324-07**

Title: **Molecular Diagnostic Tool for the Potential Bioremediation of Lake Erie Sediments**

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Abstract:

Bacteria exhibit the ability to metabolize a wide array of contaminants and, as a result, reduce their toxicity to wildlife and humans. The general goal of this project was to design and evaluate, as a proof-of concept, a DNA microarray assay to evaluate bacterial detoxification mechanisms in contaminated sediments. The assay consists of 100 probes that measure activity of bacterial remediation genes in environmental samples. The utility of the assay on control bacterial strains has been demonstrated and experimental parameters are being optimized. Bacterial community DNA has been extracted from surface sediments associated with the Maumee River Area of Concern (AOC). Upon optimization of the assay, levels of bacterial remediation genes in polluted and relatively unpolluted sediments will be compared. This novel diagnostic tool will provide an indicator of the potential for microbial communities to remediate industrial organochlorines, heavy metals, and pesticides from contaminated sediments. The application of such indicators to contaminated sediments would be valuable in prioritizing contaminated sediments most in need of more traditional means of contaminant remediation.

Technical report:

The initiation of this project was postponed until September 2007 due to delays in the reception of funding. During the preliminary period of the project, 100 gene probes were designed at Bowling Green State University for the development of the proposed DNA microarray assay. These probes will allow us to assess the levels of bacterial genes involved in the detoxification of organochlorines, heavy metals, and pesticides within environmental samples (Figure 1). These probes correspond to target remediation genes present in the total DNA extracted from microbial communities indigenous to sediment samples. Subsequent to the binding of probes to targeted remediation genes, a measurable signal is emitted allowing us to quantify the levels of these genes in DNA extracted from the environment. The hypothesis underlying this project is that microbial communities from polluted sample sites will have greater levels of remediation genes when compared to unpolluted sites and, consequently, will provide a greater potential ability to remediate polluted sediments. As a result, the functional signal obtained from the assay could provide a diagnostic tool to assess the ability of bacteria to detoxify polluted sediments.

The synthesis of gene probes was performed by Integrated DNA Technologies, Inc. (see final accounting for pricing) as outlined in the original project timeline. The probes were subsequently sent to Duke University's Core DNA Microarray Center for attachment to glass slides (see final accounting for pricing) that provide a platform on which to bind probes to bacterial DNA extracted from environmental samples. A total of 70 glass slides were then printed, with each slide containing the 100 probes in triplicate to allow for greater experimental replication.

In order to experimentally evaluate the potential of the microarray assay to detect remediation genes, we first tested the microarray using DNA extracted from bacterial species known to possess target remediation genes. DNA was extracted from two bacterial species and both DNA samples were separately tagged with a fluorescent linker that allows quantification of DNA levels. Tagged DNA was then bound to gene probes using Invitrogen's Total Genomic Labeling System (see final accounting for pricing). The fluorescent labeling of DNA samples, as well as the binding of gene probes to DNA samples, was performed in our laboratory in the Department of Biological Sciences at Bowling Green State University. Labeled samples were

then taken to the University of Toledo's Health Sciences Campus' Core Genome Facility, where samples were scanned with a laser to detect the fluorescent signal emitted from positive probes, providing a quantifiable signal of target remediation genes

We are able to measure the levels of target remediation genes in separate bacterial DNA samples, as well as develop a composite image of gene levels in the pooled samples. To optimize DNA microarray conditions, various concentrations of DNA were tested (see Figure 2), as well as different binding conditions (such as the type of buffer used, binding time, and binding temperature). Increased concentrations of DNA in binding experiments has been demonstrated to provide a stronger signal-to-noise ratio, while binding time did not seem to be as significant of a factor. Importantly, these results demonstrate the ability of the designed DNA microarray assay to provide a measurable signal of genes involved in contaminant detoxification, one of the major goals proposed in the original project.

The optimization of experimental conditions is still being performed in our laboratory and will provide an additional critical step in the development of the proof-of-concept in regards to utilizing the DNA microarray assay as a diagnostic tool. For example, the ability to obtain identical results among replicates on the same glass slide will be critical to the reproducibility of the DNA microarray assay as an analytical tool. While various experimental parameters, such as DNA concentration and binding time, have been tested to optimize the ability of the DNA microarray to provide a functional signal of gene level, a greater signal-to-noise is desired to allow the detection of subtle differences in gene levels between samples.

Since the desired outcome of this project is to demonstrate a proof-of-concept of the DNA microarray as an analytical tool, we first need to develop and optimize the assay by assessing sediment samples from a system demonstrating a drastic gradient of environmental contamination (i.e., with well-documented high and low areas of contamination). In coordination with the Ohio EPA, surface sediment samples were collected from sites along the Little Scioto River in October 2007. The Little Scioto River is an EPA Superfund site that has a dramatic gradient of contaminants from a known point source. The contaminant concentrations at multiple sites along the Little Scioto River have been measured by the Ohio EPA and allow us to designate collected samples as contaminated or not contaminated. This initial testing will therefore serve as a control for the ability of the microarray assay to detect differential level of target remediation genes between polluted and unpolluted samples.

The assay can then be applied to Lake Erie Areas of Concern (AOCs) as described in the original grant application, which have generally been exposed to non-source point contamination and, therefore, may not have such dramatic gradients in contamination levels. Funds from the OLEPF Small Grant program were used to collect sediments from the Little Scioto River as well as from the Duck and Otter Creek Conservation Area, which is part of the Maumee River Area of Concern (see Figures 3 and 4). Sediments were transported to our laboratory at BGSU and stored at the appropriate temperature for DNA analysis of bacterial communities within samples. High-quality DNA (i.e., high concentrations and low contamination) was extracted from bacterial communities indigenous to both polluted and relatively nonpolluted sediment samples (see final accounting for pricing of DNA extraction kits).

Sediment samples were collected from Duck Creek in coordination with the Duck and Otter Creek Partnership, Inc. during August of 2008, as described in our interim report. Duck Creek is part of the Maumee River Area of Concern and the levels of various organic and heavy metal contaminants have been characterized throughout the creek. Distinct contaminant levels within proximate sediments allow us to compare microbial communities in relatively polluted and unpolluted sediments; in particular, distinct contaminant concentrations were measured near the origin of Duck Creek, Hecklinger pond. For example, concentrations of PAHs and PCBs in the eastern part of the pond were 801 mg/kg sediment and 0.340 mg/kg, respectively, while the western part of the pond had 1.1 mg/kg PAHs and undetectable amounts of PCBs (see Figure 4). A total of 8 sediment samples were collected from 2 sites (polluted and unpolluted) at Duck Creek.

High-quality DNA was obtained from bacterial communities dwelling within both polluted and relatively nonpolluted sediment samples collected from Duck Creek. Quality control tests included in the Invitrogen Total Genomic Labeling kits have been utilized to demonstrate the successful labeling of environmental DNA samples. Notably, this demonstrated that environmental DNA samples are free of impurities that would prevent binding of gene probes to environmental DNA samples, a common challenge in environmental DNA microarray experiments. Upon optimization of the DNA microarray, the potential utility of the assay to differentiate the levels of bacterial remediation genes in polluted and relatively unpolluted samples will be tested.

The purpose of this project was to develop a novel assay to assess the potential for natural remediation within contaminated sediments. This diagnostic tool may eventually be utilized in determining sites most in need of more traditional remediation methods, such as dredging. Such information could prove valuable to management organizations in deciding proper remediation strategies for ecosystem restoration. The Bouzat laboratory and the Duck and Otter Creek Partnership, Inc. continue contact and these results will ultimately be presented at one of the latter's meetings. The results of this study will be published in an appropriate academic journal, as well as presented at scientific meetings, such as those hosted by the International Association for Great Lakes Research and the American Society for Limnology and Oceanography. Finally, data from this project will be valuable in applying for external funds from agencies such as the Department of Energy and National Science Foundation. Since being awarded this grant from the Ohio Lake Erie Commission, the Bouzat laboratory has been awarded with a National Science Foundation Doctoral Dissertation Improvement Grant for a related but separate project that will examine microbial diversity and natural remediation in Duck Creek.

Activities completed that were proposed in the original timeline:

Bioinformatic survey of PAHs, PCBs and heavy-metal resistance genes	**Completed**
Development of DNA amplification primers for environmental detection of organic xenobiotics and metal resistance genes	**Completed**
Synthesis of DNA probes for xenobiotic metabolism genes	**Completed**
Printing of Microarray	**Completed**
Sampling of Lake Erie sediments (coordinated through collaborators)	**Completed**
Microarray Experiments	
Optimization	**Completed**
Environmental Samples	**Continue**
Data analyses and writing	**Continue**
Proposal preparation and submission	**Scheduled**

Products generated from this Project:

Presentation in which OLEPF was acknowledged:

Indicators of Natural Remediation in Contaminated Sediments within the Lake Erie Basin. Presentation by Matt Hoostal at University of Findlay, June 2008.

Publication in which OLEPF was acknowledged:

Hoostal, M.J., Bidart-Bouzat, M.G. and **J.L. Bouzat**. Local adaptation of microbial communities to heavy metal stress in polluted sediments of Lake Erie. FEMS Microbiology Ecology 65: 156-168.

Planned submissions for publications:
Technical publication reporting the use of the microarray as a proof-of-concept for the characterization of remediation potential in environmental samples.

Characterization of polluted and unpolluted sediments from the Duck Creek within the Maumee River Area of Concern.

Related Proposal Awarded to Bouzat Lab:
NSF-Doctoral Dissertation Improvement Grant. Diversity of xenobiotics genes may suggest local adaptation and bioremediation potential of microbial communities. September, 2008. PIs: Bouzat and Hoostal; 12,000.00.

Proposal Submission planned for 2009:
Planned submissions to GLNPO and Ohio Sea Grant.

Figures

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1.   MerAXcam  GTGGCGGTGGAACTGGCGCAGGCCTTCGCCCGGCTAGGTAGCCGCGTCACGATCCTGGCA 897
2.   MerAPseu  GTGGCGGTGGAACTGGCGCAGGCCTTTGCCCGGCTGGGCAGCAAGGTCACGGTCCTGGCA 897
3.   MerAPaer  GTGGCCTTGGAGCTGGCGCAAGCCTTTGCCCGGCTGGGTAGCCGCGTCACCATCCTGGCG 897
4.   MerAPflu  GTGGCCTTGGAGCTGGCGCAAGCCTTTGCCCGGCTGGGTAGCCGCGTCACCATCCTGGCG 894
5.   MerAAcal  GTCGCGCTGGAACTGGCGCAAGCCTTCGCCCGGCTGGGCAGCCAGGTCACGATCCTAGCT 900
6.   MerAPagg  GTGGCGCTGGAGCTGGCGCAAGCCTTTGCCCGGCTGGGCAGCAAGGTCACGGCCCTGGCG 900
7.   MerAPstu  GTGGCGCTGGAGCTGGCGCAAGCCTTTGCCCGGCTGGGCAGCAAGGTCACGGTCCTGGCG 900
8.   MerADaci  GTGGCGCTGGAACTGGCGCAAGCTTTTGCCCGGCTGGGCAGCCAGGTCACGATCCTGGCG 900
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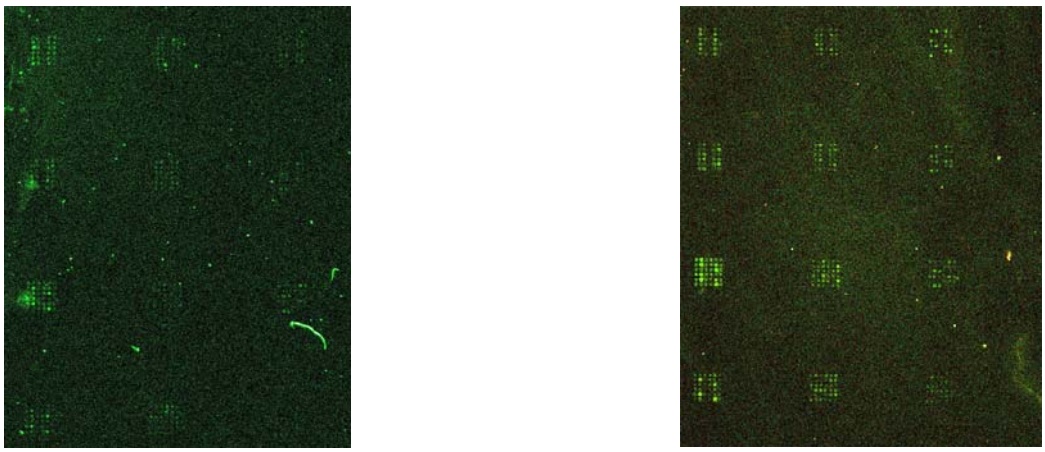
Figure 1. A segment of the *merA* gene from eight bacterial taxa is shown. *MerA* is involved in the detoxification of mercury by bacteria. Stars along the bottom of the aligned DNA sequences correspond to nucleotide bases which are conserved in all eight taxa. The 40 highlighted nucleotides represent a probe used to measure levels of this gene in environmental samples. Probes were designed to maximize the amount of conserved sequences among bacterial taxa, as well as to minimize the potential for false negative readings.



A **B**

Figure 2. Printed slides are shown following the binding of DNA to gene probes. Each spot represents the binding of a specific gene probe to DNA samples. The binding of gene probes to bacterial DNA is demonstrated at two concentrations of bacterial DNA, with the concentration of DNA in B twice the concentration as in A.

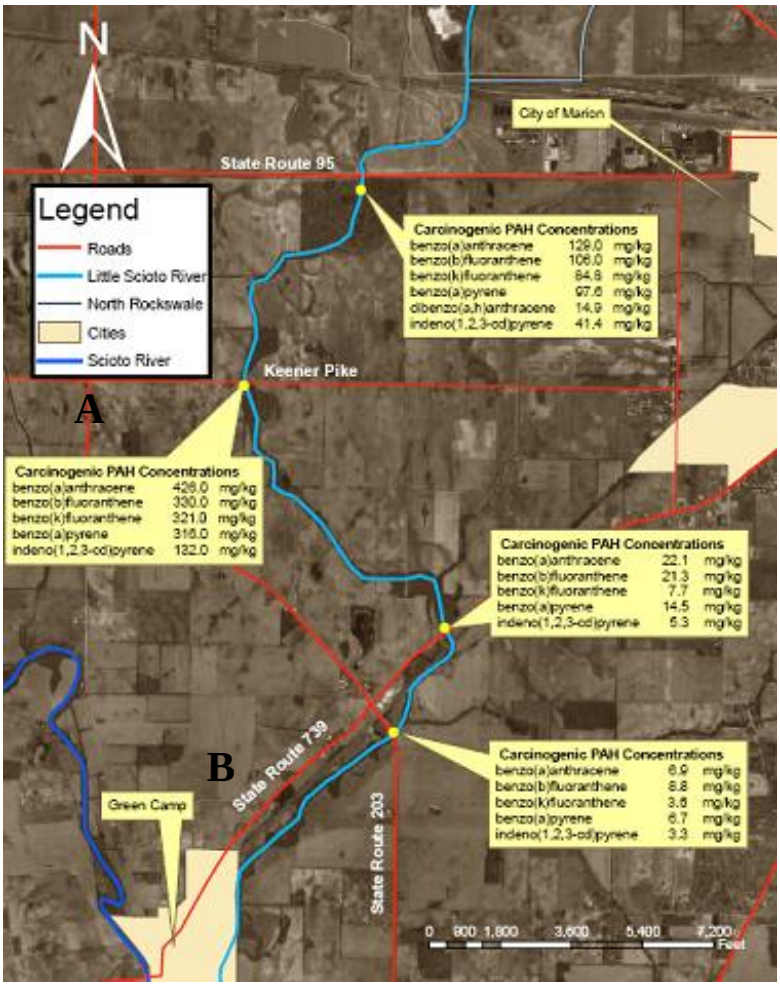


Figure 3. A map of sediment sites within the Little Scioto River characterized by the Ohio EPA (adapted from the Site Inspection Report of the Little Scioto River prepared by the Ohio EPA's Division of Emergency and Remedial Response). Sites designated as A and B represent locations of polluted and unpolluted sediment samples used in the current study

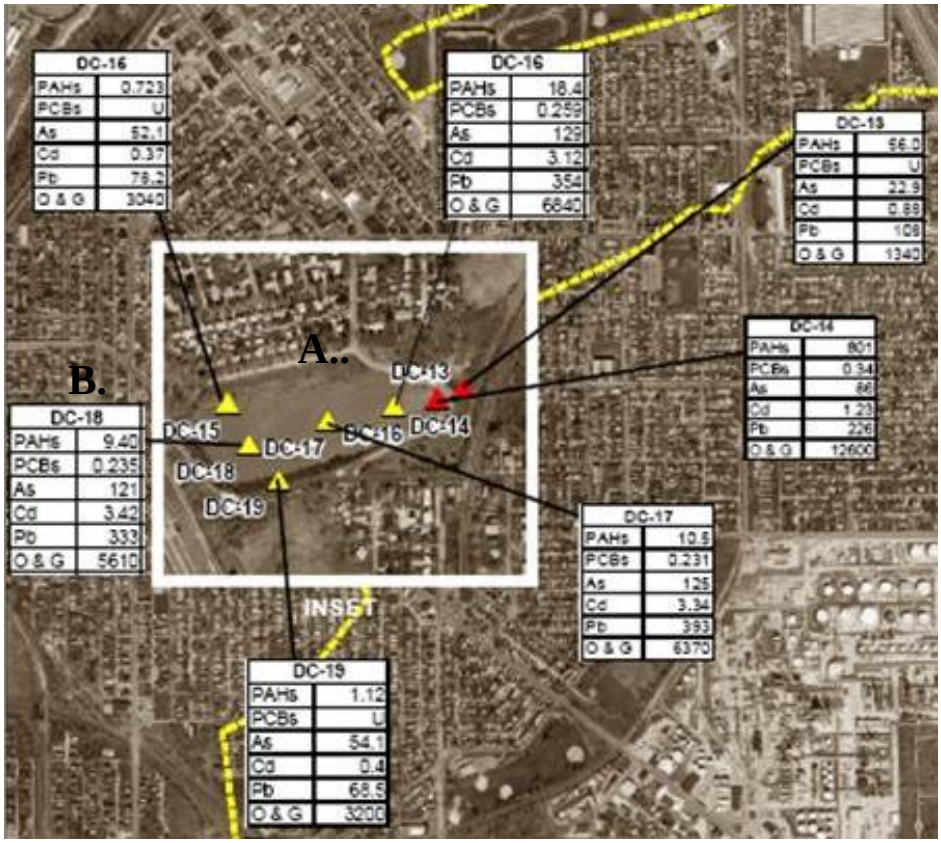


Figure 4. A map of sediment sites within Hecklinger Pond of Duck Creek characterized by the Ohio EPA, adapted from the Sediment Sampling Report for Duck and Otter Creeks Toledo and Oregon, Ohio prepared for the U.S. Environmental Protection Agency's Great Lakes National Program Office in December 2007. Sites designated as A. and B. are respective polluted and unpolluted sediment samples used in the current study.