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1. Abstract

The Mississippi River is one of the Earth's largest and most important waterways, yet we know little about the most common organisms populating this system, its microbiota. This project proposes to provide a more complete understanding of the impact of human activity on the Mississippi River, with the goal of improving water quality and improving public understanding of the importance of this River to the well being of the people and industries in the Mississippi watershed. In this project we will produce an extensive database cataloging the biodiversity and functions of microbial life in the Mississippi River in Minnesota using the new tool of metagenomic analyses. Metagenomics provides us a way to understand, for the first time, more about the microbiology of the River than we currently know through traditional microbiological analyses. The Mississippi River metagenome represents all of the microbial DNA in a water sample from the Mississippi River, regardless of its origin. Information about the diversity and function of microorganisms in the Mississippi, and the types of pathogens present, can be rapidly and directly obtained through the high throughput analysis of the DNA sequences and functional expression of microbial genes in each sample. We will also determine how the sequence and functional data relates to other indicators of water quality, such as hydrological dynamics, and the input of chemical pollutants, pharmaceuticals, and nutrients from run-off. This will allow us to better understand how human and environmental drivers influence this riverine ecosystem. The studies proposed here will put Minnesota at the forefront of this important area of environmental research, providing information on how to dynamically manage this important resource and provide insights into proper remediation efforts and future environmental needs of the Mississippi River. Water is an increasingly strained resource even here in Minnesota where it seems plentiful, and the public needs to know more about how to use this resource wisely. To accomplish this, we will engage the public through exhibits at the Science Museum of Minnesota, the Bell Museum, and the Itasca State Park Nature Center), and by teaching G7-12, undergraduate, and graduate students about metagenomics and analysis of metagenome sequence data. We will also engage the state's citizens in this novel exploration of the Mississippi River.

2. Background

Some of the greatest causes of pollution in the environment are due to the release of sewage, sewage-derived bacteria, and chemicals into waterways. No watershed is immune from human impact, and such is the case for the Mississippi River watershed - both a State and National treasure. The major anthropogenic inputs into the Mississippi River watershed include: nutrient loading from runoff and sewage treatment facilities, antibiotics and pharmaceuticals, and industrial and agricultural chemicals. These materials not only affect the diversity and functioning of microorganisms in the waterway, but also lead to eutrophication and pollution with pathogenic microorganisms. The importance of the

Mississippi River watershed is clearly indicated by the establishment, in 1988, of the Mississippi National River and Recreation Area (MISS), which protects 72 miles and 54,000 acres of the Mississippi River from the cities of Dayton and Ramsey, to downstream of Hastings, MN. The MISS, which is a new unique type of “liquid” National Park, has a Visitor Center located inside the Science Museum of Minnesota, and is staffed by National Park Rangers who are available daily to help people who want to experience the Mississippi River.

The Mississippi River should be viewed as being more than a transportation corridor, it is also an ecosystem whereby >50 cities and 18 million people get their drinking water and a habitat providing for fish and wildlife and recreation for millions of people and an important source of nutrients and organic matter to the Gulf of Mexico. However, despite our State’s intimate relationship with the Mississippi River: including its headwaters and the first navigable locks, we really know little about the impacts of environment, climate, and pollution on the functioning of the Mississippi River ecosystem. Both the McKnight Foundation (http://www.mcknight.org/resources/publications_archive.aspx?catID=2424) and the National Academy of Sciences National Research Council (<http://www.kwalliance.org/Portals/3/Mississippi%20River-Clean%20Water%20Act%20report%20-%20compressed.pdf>) have published reports on the dire condition of the Mississippi River, and have made recommendations on how we can clean-up the Upper Mississippi River. The river was recently described as an “orphan” due to its poor water quality and “lack of coordinated efforts between the U.S. Environmental Protection Agency and states” to manage pollution, much of which is due to pathogenic microorganisms and fecal bacteria ([http://www.kwalliance.org/Portals/3/Mississippi River-Clean Water Act report - compressed.pdf](http://www.kwalliance.org/Portals/3/Mississippi%20River-Clean%20Water%20Act%20report%20-%20compressed.pdf)). To address some of these problems, the microbiological quality of the upper Mississippi River, with respect to fecal indicator bacteria is now subject to a total maximum daily load (TMDL) project (<http://www.pca.state.mn.us/index.php/water/water-types-and-programs/minnesotas-impaired-waters-and-tmdls/tmdl-projects/upper-mississippi-river-basin-tmdl-projects/project-upper-mississippi-river-bacteria.html>) which aims to determine the source(s) of the fecal pollutants and develop an action plan to restore water quality. This is necessary as the river is an important habitat for fish and mussels, collects excess production from terrestrial environments and metabolizes it, while providing nutrients for downstream environments.

Microorganisms are the metabolic driving force, the engine, that runs the planet and the Mississippi river. They are critical to the biological and chemical cycling of elements and materials that keep the planet’s ecosystems in balance and to the health and wellbeing of all plants and animals on earth. However, due to major technical limitations, it is estimated that only about 1% of all microorganisms in any environmental sample can be grown in the laboratory, and thus the majority of microbes are currently unknown to us (Kaeberlein et al. 2002). Consequently, current efforts to monitor water quality in the Mississippi using “indicator” bacteria to measure fecal contamination are overshadowed by our lack of knowledge about 99% of the microorganisms in the water, including pathogens. Results from many studies have shown that the use of indicator organisms to examine microbial water quality may mislead us about the safety of water and its impact on human health (Ishii and Sadowsky 2008). Moreover, recent studies have shown that microbial communities in aquatic environments fluctuate both spatially and temporally due to varied external input sources (Wu et al. 2010).

Recent advances in molecular biology and molecular microbial ecology have provided us a means to identify microbial life in many ecosystems without the need to culture the microorganisms themselves (Ferrer et al. 2009, Schmeisser et al. 2007). The general technology has been referred to as metagenomics (Handelsman 2004). The metagenome refers to the totality of DNA in a given environmental sample, and represents the DNA from every microorganism (fungal, bacterial, viral,

algal, etc.), regardless of its origin. Consequently, the Mississippi River metagenome represents taxonomically and functionally significant DNA from all microorganisms in a given water sample. Metagenomic approaches involve the isolation of the total DNA from an environmental sample, the sequencing of the DNA for taxonomically-relevant genes (e.g. the 16S rDNA genes for bacteria) and/or the cloning of the DNA in a suitable fosmid vector and the ultimate expression of the DNA in a bacterial host (Beja et al. 2000, Rondon et al. 2000, Rondon et al. 1999). The latter technology, which we will also employ, allows for functional analyses and the targeted sequencing of genes encoding physiological processes of interest. All of the steps in this iterative identification process have been previously developed (Rondon et al. 1999) and have been demonstrated to be effective (Rondon et al. 2000). For example, DeLong et al. (2006) used metagenomic analyses of free floating (planktonic) microbes from several different depths in the North Pacific Ocean and these analyses were effective in describing trends in carbon and energy metabolism, and the phylogenetic composition of microbial assemblages in their samples. Similarly, a metagenomic approach has been used to describe the temporal and spatial variability in nearshore planktonic bacterial communities in Lake Michigan (Mueller-Spitz et al., 2009). We hypothesize that the cataloging and analyzing the majority of microbes in Mississippi River water will eventually lead to more confidence in the validity of our regulations and policies, and lead to more targeted remediation efforts by federal, state and local agencies.

Despite Minnesota's rather intimate relationship with the Mississippi River, including its headwaters in Lake Itasca and the first navigable locks, we really know little about the impacts of pollution on the functioning of the River and its microorganisms. To fill this gap, we have already begun this metagenomic research in Minnesota, partially supported by federal stimulus funds. Starting from water samples taken in the summer of 2008, we have prepared fosmid and 16S rDNA libraries that we are now analyzing for function and diversity of bacteria at the headwaters and in the Twin Cities. We are doing the same now for 2010 samples. This proposal will fund further sampling and metagenome analyses of water samples from the Mississippi River at 11 critical junctures and confluences in Minnesota (see Figure 1). At each sampling location and time point we will also obtain information on other indicators of water quality, including industrial and agricultural chemical and pharmaceutical inputs that may impact microbial diversity and functioning. Our preliminary efforts in this area were launched in 2009 using a combination of University funds and federal stimulus funds, and this has resulted in the development of a website (see <http://www.cbs.umn.edu/main/news/inthefield/m3p.shtml>) and an undergraduate teaching curriculum.

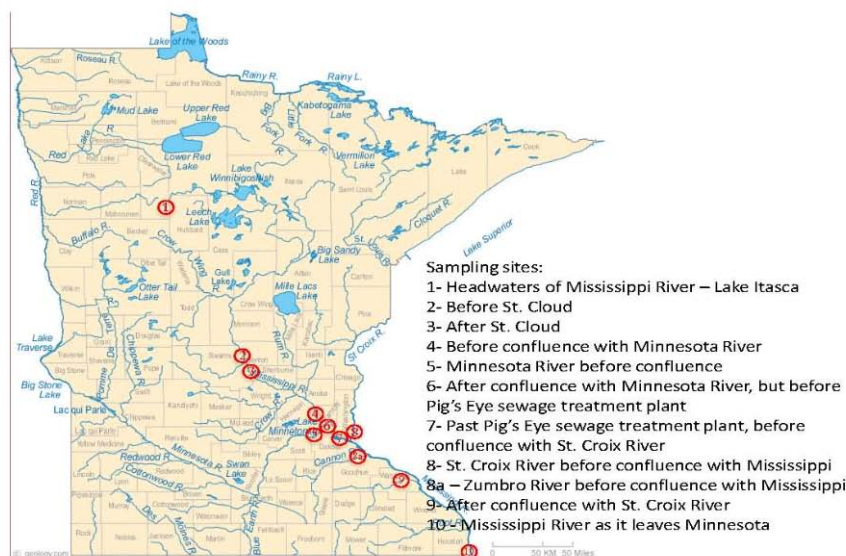


Figure 1. Sampling sites and locations used in this study.

From the beginning, our vision for this project has included not only research, but also education and outreach. We have viewed this project as an incredible opportunity to engage students at the 7-12, undergraduate, and graduate levels in metagenomes and bioinformatic-based data analysis. We now offer two laboratory courses at the undergraduate level, one to provide the background of how to take environmental samples and process them for metagenomic work, and one to give students the opportunity to do individual research projects using the DNA samples and DNA libraries obtained from multiple sampling sites on the Mississippi. This past summer we worked on the incorporation of the metagenomic project research into our non-majors biology courses taken by thousands of students each academic year, while at the same time doing more extensive sampling of the River from the Headwaters to the point where the Mississippi leaves Minnesota. Within a year, we plan professional development programs for teachers to engage students in the grade 7-12 classrooms in this research, and are working with the Science Museum of Minnesota, the Bell Museum, and Itasca State Park to develop public exhibits presenting the project and its potential impact.

3. Hypothesis

This research and project are based on the overall hypothesis that human activity (due to anthropogenic inputs) alters the structure and function of the bacterial microbial community in the Mississippi River. Moreover, we hypothesize that these changes are magnified in the Mississippi River as it accumulates water (from tributaries and major confluences) and chemical pollutants from other rivers as it makes its way from its headwaters at Lake Itasca to the southern boundary of Minnesota near La Crescent. Lastly, we hypothesize that the genomic, chemical, and limnological data obtained from these studies will provide a resource for teachers, students, river managers, and state agencies. The data obtained in these studies will also allow the public to better understand how metagenomics can be used as a classroom teaching tool to learn about genomics and bioinformatics, and by river managers and regulatory agencies as a means to better assess the impacts of human activity on water quality.

4. Methodology

ACTIVITY 1: Analysis of Microorganisms and Metagenomics

This proposal will fund two and a half years of sampling and metagenome and chemical analysis of water samples from the Mississippi River at 11 critical junctures in Minnesota, from Lake Itasca to La Crescent (see Figure 1), focusing on the headwaters and confluences with other major Rivers. We are currently obtaining preliminary data from 10 of these 11 sites sampled this summer, and are requesting funding here for in depth studies of these 11 sites, once per year, for two additional years. At each sampling location we will also obtain information on other indicators of water quality, including industrial and agricultural chemicals and pharmaceuticals, inputs that impact bacterial diversity and functionality.

Metagenome Analysis

The Mississippi River will be sampled once yearly (in early July) at 11 sites from Lake Itasca to La Crescent during years 1 and 2 (see attached map). We will also sample site #4 (Hidden Falls) 6 times per year (monthly from April to September) at two sampling depths (0.3 and 1 meter below the surface). This will allow us to obtain information concerning the temporal and spatial variability of the microbial

populations in the Minnesota River. The exact locations (latitude and longitude) of sampling sites at each location, when possible, will be the same as those used by MPCA and the Met Council to allow comparisons to existing data and those obtained in the future. At each site, two 1 L samples will be taken for water chemistry analysis (see below) and a 40L sample will be taken for metagenomic analyses. The 40 L samples will be collected at least 1.8 meters (6 feet) from shore at a depth of 0.3 meters (1 foot) below the surface, transported in 2- 20 L carboys, and immediately filtered through 90 mm diameter Whatman P5 filter paper to remove debris and organisms that are $\geq 5\text{-}10\ \mu\text{m}$. The water will then be pumped through 142 mm dia., 0.45 μm , poly-ethane sulfonate (PES) filters to trap bacterial microorganisms. This project only focuses on bacteria since microbes larger than 5 μm and less than 0.45 μm are excluded from our analyses. If necessary, samples will be stored overnight at 4°C before filtration. In our experience, 6 to 8 PES filters are needed to filter 40 L of water. Microbial cells will be extracted from the surface of PES filters by gentle agitation for 5 min in PBS buffer. Cells will be pelleted by centrifugation and stored frozen at -80 C. Instead of taking replicate, smaller samples at each site, the large 40 L volume (~ 10 gallons) was chosen to maximize harvest of microorganisms, to overcome problems with heterogeneous samples, and to contain costs.

One half of the cell pellet will be used for preparation of DNA for analysis of taxonomically-significant 16S rDNA. Total DNA will be extracted from cell pellets using Bio101 FP120 Fastprep instrument and MoBio Powersoil DNA extraction kits (Mo Bio Laboratories, Solana Beach, CA) as previously described (Ishii et al. 2006). We have previously found that these kits give maximal DNA extraction, with minimal PCR inhibitors. Once extracted, DNA corresponding to the V6 hypervariable region of the full-length 16S rDNA will be amplified by PCR using primers as described by Wang et al. 2007 and Lazarevic et al. 2009. The taxonomy of microorganisms assigned to the V6 hypervariable regions have been shown to be very similar with the taxonomy assigned to microorganisms obtained via analysis of full-length SSU rRNA (Huse et al. 2008), making this a cost effective approach (Lazarevic et al. 2009) to obtain near complete taxonomic information on the river microbiota. The PCR primers will contain a unique sequence tag (Binladen et al. 2007), such that amplicons from each sample will contain a unique identifier sequence. The amplicons from each of the 11 samples will be pooled together and the multiplexed amplicons will be sequenced on a Illumina/Solexa Sequencer at the National Center for Genomic Research (NCGR) in Santa Fe, New Mexico. Since each amplicon is initiated via a different primer tag, it will allow us to deconvolute sequence data arising from a single sequence run. Sequence data will be obtained by the paired-end read method. Using this approach, about 20 million reads can be obtained from each sequence run, and will result in the collection of approximately 1 - 2 million reads of taxonomically-useful 16S rDNA from each sample.

The 16S rDNA sequence data obtained in our studies will be compared to V6 reference databases as described by Dethlefsen et al. 2008 and Lazarevic et al, 2009. The taxonomic classification of 16S rDNA PCR products will be assigned using the GAST (Global Alignment for Sequence Taxonomy) taxonomic classification tool as described by Sogin et al. (2006), and by analyses done using reference database of V6 rDNA sequences (RefHVR_V6) from SILVA (Pruesse et al., 2007), the taxonomy from known cultured isolates, the Entrez Genome and the Ribosomal Database Project (Cole et al. 2009), Greengenes (DeSantis et al., 2006) and the software program ARB (Ludwig et al. 2004). The taxonomic signature of microorganisms in each sample will be compared within and across samples at all time points and statistically analyzed as described by Dethlefsen et al. 2008 and by using tools available from ARB. Sequences occurring < 2 times will be filtered to reduce the number of unique sequences and potential misreads. Phylotype clusters and diversity estimates will be also obtained by using the MOTHUR software program (Schloss and Handelsman, 2005). Local alignments and graphical representation of data will be facilitated by MOTHUR and phylogenetic relationships of the sequences

will be examined by maximum-parsimony into the ARB dataset. The resulting phylogenetic relationship that are identified will be tested by maximum-likelihood bootstrap trees (with 1000 iterations) using distance-based subsampling and a minimum distance of 3% between sequences. Operational taxonomic units (OTUs) will be determined and compared by using the sortx subroutine of XplorSeq (Frank 2008). Comparisons of bacterial constituents of the river, between sites and sampling dates, will be determined by examining the numbers and types of phyla (or operational taxonomic units) at each sample site. We will also determine species diversity, species richness, and evenness using rarefaction analysis (Robertson et al. 2009). When completed, these analyses will give us a comprehensive picture of the microbial structure of Minnesota portion of the Mississippi River, two times per year, in each year of the study. We will also obtain information about changes in the community structure of microorganism (both saprophytes and pathogens) in the river over time. The phylogenetic and comparative analyses obtained from these studies will be added to a relational database, that were developing now with help from the US Department of Energy's Joint Genome Institute (JGI) so that information can be readily downloaded and utilized by researchers, students, regulatory agencies and stakeholders to examine the type of microorganisms present at each sampling site over the study period. In addition, we will upload the data sets that we obtained from pre-project studies (done in the summer of 2010), which will add an additional year of analysis for comparison purposes.

In addition to phylogenetic information, our metagenomic analyses will also examine the functionality of the bacterial community of the Mississippi at each sampling site. To do this, we will send one half of the frozen cells from each site (as described above) to the Clemson University Genome Institute (<http://www.genome.clemson.edu/>) for the construction of functional gene libraries. This will be done only for the samples obtained once per year at each site. The libraries, consisting of randomly sheared metagenomic DNA, will be constructed in fosmid vector pEPIFOS-5 and transformed into *E. coli* DH10 as the host. Each fosmid will have an average insert size of ~39 kb (we have made libraries for year -1 already and have these data), enough to encode to about 20-40 bacterial genes. We will obtain ~10,000 clones (containing about 390,000 kb of DNA) from each sample. Library clones will be picked into 384 well microplates using a Qbot colony picking robot and screened, by students, for functionally active genes involved in resistance to antibiotics and heavy metals, and those that encode for degradation of recalcitrant organic compounds. Functionally active fosmid clones will be sequenced at the Biomedical Genomics Center at the University of Minnesota, and this sequence data will be assembled into contigs and analyzed by Blast and IMG-ACT software and websites (<http://img.jgi.doe.gov/cgi-bin/pub/main.cgi>). The IMG-ACT is a database of microbial genomes and metagenomic data that is maintained by DOE's Joint Genome Institute. The Mississippi River DNA sequences in this website will be accessed and annotated (identified) by students at the University of Minnesota and throughout the state. This will allow students to participate in the Mississippi Metagenome project, learn about bioinformatic and metagenomics, and help discover novel microbial genes that are related to growth and survival in the Mississippi River. It will also allow students throughout the state (see below) to gain an appreciation for how human activity influences the functioning of the river ecosystem. Because student access to the website and annotation of genes will be monitored by us, we can obtain metrics on how many students are participating in our educational and outreach programs, on a near instantaneous basis.

Water Analysis

In addition, to metagenomic and phylogenetic information, we will also obtain data on the presence and concentration of chemical compounds known to influence human health and water quality. At each site (Figure 1) we will examine for the presence of the following compounds and chemicals:

pharmaceuticals – acetaminophen and caffeine; **antibiotics** - tylosin, erythromycin, and trimethoprim; **pesticides** - atrazine, acetochlor, and metolachlor; **personal care products** - DEET, triclosan, and nonylphenol; and **endocrine disrupters** - trenbolone and estradiol. The data obtained from these analyses will be entered into the same relational database described above so that students and researchers can examine the possible relationship between the chemical constituents of the Mississippi River at each site and the presence and types of microbes recovered at each site. Water samples at each site and during each sampling event will also be analyzed for standard limnological parameters, including: water pH and temperature, turbidity, and nutrient (N,P, and C) concentrations, and inorganic trace elements via ICP analysis. The measurements of the river's physical parameters will be done by co-PI James Cotner and by the University of Minnesota Research Analytical Lab. Nutrient composition in water samples will be determined using a Alpkem RFA 300- Rapid Flow Analyzer by the Research Analytical Laboratory in the Department of Soil, Water & Climate, at the University of Minnesota. The water samples obtained in July will also be analyzed for *E. coli* concentration by using the EPA-recommended membrane filtration technique and mTEC agar medium.

Surface water samples for analysis of pharmaceuticals, antibiotics, pesticides, personal care products, and endocrine disrupters will be collected as outlined in the USGS National Field Manual for the Collection of Water-Quality Data (USGS 2009). All samples used for these analyses will be collected in glass bottles and will be analyzed by our collaborator Dr. William Koskinen at the USDA-ARS Soil and Water Management Unit at the University of Minnesota. Samples will be refrigerated during transport from sample sites to the University of Minnesota, filtered through glass-fiber filters (0.7 μm) to remove particulates, and processed within 2 days of collection. HPLC-grade solvents (acetonitrile, methanol, and water) will be used in these studies and standard analyte compounds will be obtained from Chem Service Company. Analytical methods utilized to measure the various analytes will be those previously developed in Dr. Koskinen's laboratory (Marek and Koskinen 2007) and adapted from those described by Snyder and his colleagues (Benotti et al. 2009). Dr. Shane Snyder (now at the University of Arizona), who developed many of the analytical methods we will use, has also agreed to collaborate with us on this project. Briefly, target compounds and added surrogate compounds will be extracted from the filtered water using solid-phase extraction (SPE) or liquid-liquid extraction (LLE) methods, followed by solvent reduction. Samples will be extracted in duplicate and water spikes (check standards) and ultrapure water blanks will serve as controls. For SPE, the samples will be adjusted to pH 3 and passed through either a preconditioned Oasis Hydrophilic-Lipophilic-Balance (HLB) cartridge or a HLP cartridge that will be placed on top of a mixed mode, HLB-cation exchange (MCX) cartridge (Waters Inc., Milford, MA). After sample passage, cartridges will be eluted using CH_3OH . The SPE procedure was previously automated by using a Zymark Autotrace system and CH_3OH extracts will be evaporated to dryness using a Zymark Turbovap. For LLE, acidified samples will be extracted, multiple times, with dichloromethane (DCM) and extracts will be evaporated just to dryness under nitrogen using a Zymark Turbovap. Samples will contain internal standards appropriate for each analysis. Samples will be analyzed by using capillary-column GC/MS before and after derivatization using TMS.

Depending on the compound under analysis, extracted chemicals will also be analyzed using a Waters Alliance high performance liquid chromatography/mass spectrometer with electrospray interface operating in positive-ion (LC/MS-ESI(+)) mode, or by using a Agilent 6890 gas chromatograph with capillary column coupled to a mass selective detector (GC/MS) operating in selected ion mode. The operating conditions for LC, GC, and MS analyses (i.e. column specifications, carrier gas and mobile phase details, etc.) will be as is detailed in Benotti et al. 2009 and Vanderford and Snyder 2006. Positive identification of the compound of interest will be determined by peaks having a signal-to-noise ratio of 3:1 in the sample matrix eluting and expected retention times of $\pm 5\%$. Positive samples will also have

the presence of the confirmation ion(s) within $\pm 20\%$ of the ratio of the qualifying ion(s) as found in the reference standard. The concentrations of the target compound(s) in water samples will be calculated from 5-point calibration curves utilizing internal standard quantification. Many of the chemicals to be analyzed in this study are also being examined in a current LCCMR-funded study of the Zumbro River led by Deborah L Swackhamer at the University of Minnesota. This will allow cross comparison of results obtained at different sites along this same waterway. Water chemical and physical data obtained from each site and sampling time will added to the relational database using to store metagenomic data, allowing for analysis of the potential correlative relationship between microbial community structure and the presence of chemical contaminants.

Results and Deliverables for ACTIVITY 1

This activity will produce the following results and deliverables:

1. Sampling of the Mississippi River and analysis of samples for microbial species diversity and functionality at each sampling location.
2. Correlations of structural (sequence of diversity) and functional metagenome data to physical and chemical data at each location.
3. Initial development of relational web database consisting of metagenomic and physical chemical data.
4. Uploading of metagenomic data into IMG-ACT for searching and retrieval by researchers, students, river managers, regulatory agencies, and the public to better understand how human activity influences the microbiology of the Mississippi River.

ACTIVITY 2: Professional Development of 7-12 teachers

In this result we will develop a hands-on professional development program for G7-12 teachers, offered both in the Twin Cities and in Northwest Minnesota (Itasca) to provide greater access to this opportunity statewide. This professional development program will focus on preparing teachers to include Mississippi metagenomics studies in their science curriculum in a way that meets state standards for science inquiry.

Learning science requires building a foundation of skills and knowledge. However, science itself is essentially an inquiry-based endeavor. This is recognized nationally in the National Science Education Standards (National Research Council 1996; <http://www.nap.edu/openbook.php?isbn=0309053269>) and in the state of Minnesota's science standards for K-12 students (http://www.education.state.mn.us/MDE/Academic_Excellence/Academic_Standards/Science/index.html). In addition, the fast pace of the biological sciences requires constant attention to bring advances in biology to teachers, students, and the public so they are able to understand new discoveries and their social implications. Our program provides opportunities for G7-12 teachers and students and for the general public to become engaged in the Minnesota Mississippi Metagenomics project. Our project goals for the following individuals are outlined below:

G7-12 teachers and students:

Good inquiry-based science in the G7-12 classrooms is often complicated for schools due to cost - school districts often have difficulty finding adequate funding, difficulties that teachers have in obtaining commitments for professional development programs by scientists in the field, and the necessary “hook” to keep students engaged. The use of the data from the Minnesota Mississippi Metagenome project (M3P) in the classroom addresses each of these issues. Firstly, the M3P provides a platform for students to work on this project whether in a wet-lab setting, or by doing online genome analyses requiring only a web browser. Thus, the costs can be scaled to the budget of the district. Secondly, our program will provide workshops for teachers that will allow them to interact with scientists and build a learning community with scientists and each other. Thirdly, the M3P provides a “hook” for engaging students by fostering the excitement of discovering something no human has known before. (How cool is that?). The other “hook” is that the Mississippi River is the largest and most historic river in the United States and, through this project, the students have an opportunity to contribute to knowledge about, and care of, this incredible environmental resource.

The College of Biological Sciences at the University of Minnesota has extensive experience in the K-12 activities and creation of professional development programs for teachers. One our programs, Investigative Plant Biology for Elementary Teachers, is entering its 19th year, funded by the Minnesota Office of Higher Education which administers federal professional development grants. Another program, The Science Education Partnership in Greater Minnesota engaged teachers from Northwest Minnesota and undergraduates at the University in a 6 year long program supported by the Howard Hughes Medical Institute to improve science education in area schools. Another program in progress is a 5 year long partnership with the Austin (MN) Public Schools, the College of Education and Human Development, the College of Science and Engineering, and the College of Biological Sciences to provide teachers in Austin with a structured professional development and Master’s degree program to improve science education in K-12 in Austin. From these and other activities, we have developed standards for providing successful professional development for teachers that lead to changes in their classroom practices and changes in student achievement. We are incorporating these standards into the design of programs to bring Mississippi Metagenomics into G7-12 classrooms.

One of our first actions in this activity will be to engage school district administration. We have found that real change in a classroom requires commitment from the district and school administration, providing the teacher with the support and encouragement that leads to improvement in the classroom. Our second action will be to identify teacher leaders who will help us develop the specifics of the program. Teacher leaders have been instrumental in all our successful programs because they have the in-classroom experience needed to ensure that activities are focused at the right grade level and to the correct abilities of the teacher and the school. Finally, teachers need to know more than just what they will present to their students: they need an authentic experience with the science so that they become “the scientist in the classroom”. From our perspective and experiences, these three actions are foundations for achieving success.

The professional development plan of this project is to engage 20 teachers per year, in 2012 and 2013, via workshops to bring Mississippi Metagenomics to their classrooms. Teachers participating in this project will receive graduate credit, a stipend, books for their own reference, teaching materials for their classrooms, and continuing support. Each cohort will begin with a one week, full time workshop. The 2012 workshop will be held at the University of Minnesota -Twin Cities Campus and will recruit districts and teachers in the metro area. The 2013 workshop will be held at the University of Minnesota Itasca Biological Station and Laboratories (<http://www.cbs.umn.edu/itasca/>) which has laboratories,

housing, and dining facilities, as well as the same instrumentation (e.g., high throughput genomic and robotic facilities) as are present on campus. This will allow teachers at both locations to experience the real science of metagenomics. In addition, both the campus and Itasca sites have access to the Mississippi River for sampling activities.

In this result we will also develop dedicated and jointly administered websites making the metagenome diversity and functional and chemical data accessible to middle and high school students, undergraduate and graduate students, researchers, and the public. As discussed above, students and teachers participating in this activity will also be involved in annotating the functionally active fosmid clones that will be sequenced at the Biomedical Genomics Center at the University of Minnesota. They will also analyze Mississippi River metagenome data that reflects microbial diversity issues, the presence of pathogens, and the relationship between microbial data and chemical constituents in the river. School participants in this project will chiefly use the IMG-ACT software and websites (<http://img.jgi.doe.gov/cgi-bin/pub/main.cgi>). The IMG-ACT is a database of microbial genomes and metagenomic data that is maintained by the DOE. We will also utilize existing national web resources, such as Dolan DNA Learning Center (<http://www.dnalc.org/>) and Mothur databases (Schloss et al. 2009) to facilitate archiving, retrieval, and analysis of metagenome data. This will allow students to participate in the Mississippi Metagenome project, learn about bioinformatic and metagenomics, and help discover novel microbial genes that are related to growth and survival in the Mississippi River. It will also allow students throughout the state to gain an appreciation for how human activity influences the functioning of the river ecosystem. Since student access to the website and annotation of genes will be monitored by us, we will obtain near instantaneous metrics on how many students are participating in our educational and outreach programs.

Teachers attending our workshops will begin with a review of molecular biology, an introduction to metagenomics, and a discussion about water quality and the Mississippi River. While it is expected that the inquiry activities that students do will use genomic data and bacterial clones generated by the data gathering research side of the project described above, it is important that the teachers and workshop participants have an idea of how this data and strains were obtained. Thus, the next activity for the workshop will be environmental sampling and discussions of processing beyond the scope of the classroom (e.g. DNA sequencing and production of fosmid libraries). Several good videos and websites (e.g., materials at the Dolan DNA Learning Center, <http://www.dnalc.org/>) that can be used in the 7-12 classroom are available to help with these discussions. After a discussion of inquiry, the teachers will then begin their activities using both the DNA sequences in the IMG database and fosmid library cultures that were generated during the first project activity and during this current year with federal stimulus funding. We have been teaching metagenomics of the Mississippi River in our undergraduate labs for 2 semesters and this has provided us with insights into what is feasible in the one week workshop. These same inquiry-based activities that the teachers will be engaged in will be modified to be used in their classrooms. Teachers will be asked for their input on the development of curricular action plans for their students that will be worked on throughout the academic year. These curriculum plans will include mechanisms to meet state science standards using metagenomics as the focus. Three required follow-up meetings in the academic year will provide teachers with a learning community for support, continued access to the scientists in the project, and to gauge additional needs for the incorporation of metagenomics in their classrooms. These follow-up meetings will also provide teachers with the increased and continued intellectual support identified by Huffman, et al (2003) as instrumental for changing teaching practice. Throughout this project we will also develop educational materials, such as webinars and PowerPoint presentations that can be used by teachers for instructional purposes.

Results and Deliverables for ACTIVITY 2:

1. Provision of professional development workshops, in the summer 2012 and 2013.
2. Production of a web accessible, searchable database with downloadable datasets for use in the 7-12 and undergraduate classrooms, as well as by researchers in Minnesota and elsewhere. This will occur via a partnership of researchers with G7-12, undergraduate, and graduate students and educators, and citizens working on this database.
3. Production of curriculum packets, webinars, books, materials, presentations, and approaches that can be incorporated into G7-12 classrooms.
4. Annotation of gene identity and function in IMG-JGI website by G7-12 students.
5. Development of trained teachers that incorporate this cutting edge science into their classrooms and pedagogical materials for other teachers to use throughout the state (and nation).

ACTIVITY 3: Project data dissemination.

Project data, teacher information, and research concerning the Minnesota Mississippi Metagenomics project will be disseminated via five main routes. Dissemination activity is paramount to the success of this project since the Minnesota Mississippi Metagenomics project has the potential to engage the public in the excitement of state-of-the art research, application of this research to real problems in our state, and discussions about the implications of policy decisions on our natural resources. We have chosen three venues through which to reach the general public: the Science Museum of Minnesota, the Bell Museum of Natural History on the University of Minnesota campus, and Itasca State Park. Each of these facilities attracts many families throughout the year to their exhibits: the Science Museum, which had 856,000 visitors in 2009 (<http://www.smm.org/static/annualreport/2009annualreport.pdf>), the Bell Museum of Natural History at the University of Minnesota, who hosted 45,000 visitors (personal communication), and Itasca State Park which had 470,000 visitors in 2008 (http://www.dnr.state.mn.us/faq/mnfacts/state_parks.html). At the Science Museum, we will work with exhibit staff (via our collaborator Patrick Hamilton, Director, Environmental Sciences and Earth-System Science, Science Museum of Minnesota) to incorporate information and database access for this project both on their EarthBuzz website (<http://www.sciencebuzz.org/buzz-tags/earth-buzz>) and in kiosks in their exhibit about Minnesota and the environment. Earth Buzz, which was developed through SMM's Future Earth Initiative (NSF DRL 0741760) and Climate Change Education (NASA NNX09AL39G) grants, are portable, web-enabled kiosks set up in public locations that highlight the work of SMM's research partners. Earth Buzz provides both onsite and online audiences ready access to environmental science and Earth-system science news through the use of templates that permit quick change of content without revising graphic styles and designs. Earth Buzz kiosks have been installed in seven museums and other public venues around the U.S., and three more installations are scheduled by the end of December 2010. As part of the Minnesota Mississippi Metagenome Project, the SMM will build an Earth Buzz kiosk and install at public venue in the Twin Cities area and support the Earth Buzz project manager who will devote their time over the 2.5 year duration of the project. The SMM staff will coordinate the generation of stories and blogs about the Minnesota Mississippi Metagenomics project for the Museum's Earth

Buzz network, mentor graduate students involved in the research project on how to write science blogs for general public audiences, and stay abreast of the research project in order to prepare relevant stories and blogs for Earth Buzz. At the Bell Museum, we will be working with the museum curators to add materials at existing aquatic dioramas to explain the use of metagenomics in measuring water quality. And finally, at Itasca State Park, we will work with the Park Naturalist to design and build two exhibits, one for the Nature Center at the East entrance to the Park, and one along the trail to the Mississippi headwaters, that will explain and engage the visitors in this research on the Mississippi. Together these venues have the ability to engage a large population of the public concerning metagenomics, the Mississippi River, the microbial constituents of the river, including pathogens, and how human activity influences the structure and function of this important waterway and ecosystem. Results from this project will also be disseminated via more traditional routes, including in reports made to the LCCMR, the generation of teaching materials, in periodic update reports made to cooperators, in seminars given throughout the state and nation, and in scientific publications in peer-reviewed scientific and teaching journals. Lastly, project data and approaches, including all teaching and learning activities will be disseminated via a dedicated web site that will be built specifically for the project. The web site, which will reside on servers at the University of Minnesota, will contain information about the goals of this project, links to all the data and databases need for data analysis, information and resources for teachers, and updated references and project publications.

Results and Deliverables for ACTIVITY 3:

1. Development of Minnesota Mississippi Metagenome Website.
2. Production of public exhibits at SMM, Lake Itasca, and the Bell Museum.
3. Production of curriculum packets, webinars, books, materials, and presentations for G7-12 students and teachers.
4. Dissemination of project data and results via webinars, seminars and workshops, and publications.

5. Results and Deliverables

The results and deliverables from this project are numerous and for ease of reading these have been enumerated under each activity. These include: Sampling of the Mississippi River and analysis of water samples for microbial species diversity and functionality at each sampling location, the correlation of structural (sequence of diversity) and functional metagenome data to physical and chemical data at each location, the development of relational web database consisting of metagenomic and physical chemical data. We will upload the metagenomic data into IMG-ACT for searching and retrieval by researchers, students, river managers, regulatory agencies, and the public to better understand how human activity influences the microbiology of the Mississippi River. We will also provide professional development workshops in the Summer of 2012 and 2013, and produce a web accessible, searchable database with downloadable datasets for use in the 7-12 and undergraduate classrooms, as well as by researchers in Minnesota and elsewhere. To aid the teachers participating in this project we will produce curriculum packets, webinars, books, materials, presentations, and approaches that can be incorporated into G7-12 classrooms. Students in G7-12 classrooms will annotate genes that are functionally relevant by using the DOE IMG-ACT website. This project will produce trained teachers that incorporate this cutting edge

science into their classrooms and pedagogical materials for other teachers to use throughout the state (and nation). Other dissemination activities for this project include the development of Minnesota Mississippi Metagenome Website, and the production of public exhibits at SMM, Lake Itasca, and the Bell Museum. Dissemination of project data and results will also occur via webinars, seminars and workshops, and the production of curriculum packets, books, materials, and presentations for G7-12 students and teachers.

6. Timetable

The project will take place over a period of 2.5 years, beginning on or about July 1, 2011. We have already obtained preliminary metagenomic data (not chemical data) this summer (2010) for 10 of the 11 sites discussed in this proposal. This information will be included with data generated during this project.

Activity	Date							
	2011		2012				2013	
	7/2011	9/2011	1/2012	7/2012	9/2012	12/2012	7/2013	12/2013
Sampling of the Mississippi River	X	X		X	X			
Analysis of metagenomes	X	X	X	X	X	X	X	X
Correlation of physical, chemical and microbiological data			X	X	X	X	X	X
Production of curriculum packets, webinars, books, teaching materials	X	X	X	X	X	X	X	X
Professional development workshops				X			X	
Incorporating metagenomic approaches into G7-12 classrooms			X	X	X	X	X	X
Annotation of Gene Identity in Fosmids (functional clones)			X	X	X	X	X	X
Production of website and database			X	X	X	X	X	X
Production of public exhibits	X		X	X	X			
Dissemination of project results in seminars, publications, webinars, and reports to LCCMR and State Agencies				X	X	X	X	X

References

- Beja, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S. B. Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, E. N. Spudich, and E. F. DeLong. 2000. Bacterial rhodopsin: Evidence for a new type of phototrophy in the sea. *Science* 289:1902-1906.
- Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Standford, B.D., and S. A. Snyder. 2009. Pharmaceuticals and endocrine disrupting compounds in U.S. drinking water. *Environ. Sci. Technol.* 43:597-603.
- Binladen, J., Gilbert, M.T.P., Bollback, J.P., Panitz, F., Bendixen, C., et al. 2007. The use of coded PCR primers enables high-throughput sequencing of multiple homolog amplification products by 454 parallel sequencing. *PLoS ONE* 2(2): e197. doi:10.1371/journal.pone.0000197
- Cole, J.R., Wang, Q., Cardenas, E., Fish, J., Chai, B., et al. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 37: D141-145.
- DeLong, E., C. M. Preston, T. Mincer, V. Rich, S. J. Hallam, N. Frigaard, A. Martinez, M. B. Sullivan, R. Edwards, B. R. Brito, S. W. Chisholm, and D. M. Karl. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496-503.
- DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L. et al. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72: 5069-5072.
- Dethlefsen, L., Huse, S., Sogin, M.L., and D. A. Relman. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 6(11): e280. doi:10.1371/journal.pbio.0060280
- Ferrer, M., Beloqui, A., Vieites, J.M., Guazzaroni, M.E., Berger, I., and A. Aharoni. 2009. Interplay of metagenomics and in vitro compartmentalization. *Microbiol. Biotechnol.* 2:31–39.
- Frank, D.N. 2008. XplorSeq: a software environment for integrated management and phylogenetic analysis of metagenomic sequence data. *BMC Bioinformatics* 9: 420.
- Handelsman, J. 2004. Metagenomics: Application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews* 68:669-685.
- Huffman, D., F. Lawrenz, and K. Thomas. 2003. Relationship between Professional Development, Teachers' Instructional Practices and the Achievement of Students in Science and Mathematics. *School Science and Mathematics*, Vol. 103
- Huse, S.M., Dethlefsen, L., Huber, J.A., Welch, D.M., Relman, D.A., and M. L. Sogin. 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genet* 4:e1000255.

- Ishii, S., and M. J. Sadowsky. 2008. *Escherichia coli* in the environment: implications for water quality and human health. *Microbes Environ.* 23:101-108.
- Ishii, S., Yan, T., Shively, D. A., Byappanahalli, M. N., Whitman, R. L., and M. J. Sadowsky. 2006. *Cladophora* (Chlorophyta) spp. Harbor Human Bacterial Pathogens in Nearshore Water of Lake Michigan. *Appl. Environ. Microbiol.* 72: 4545-4553
- Kaeberlein, T., K. Lewis, and S.S Epstein. 2002. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* 296:1127-1129.
- Lazarevic, V., Whiteson, K., Huse, S. et al. 2009. Metagenomic study of the oral microbiota by Illumina high-throughput sequencing. *J Microbiol Meth* 79: 266–271.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., et al. 2004. ARB: a software environment for sequence data. *Nucleic Acids Res* 32: 1363-1371.
- Marek, L. J., and W. C. Koskinen. 2007. Multiresidue analysis of seven anticoagulant rodenticides by high-performance liquid chromatography/electrospray/mass spectrometry. *J Agric. Food Chem.* 55: 571-576.
- Mueller-Spitz, S. R., G. W. Goetz, and S. L. McLellan. 2009. Temporal and spatial variability in nearshore bacterioplankton communities of Lake Michigan. *FEMS Microbiol. Ecol.* 67:511-522.
- National Research Council. 1996. National Science Education Standards. National Academy Press. Washington D.C.
- Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, et al. 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 35: 7188-7196.
- Robertson, C.E., Spear, J.R., Harris, J.K., and N. R. Pace. 2009. Diversity and stratification of archaea in a hypersaline microbial mat. *Appl Environ Microbiol* 75: 1801–1810.
- Rondon, M. R., P. R. August, A. D. Bettermann, S. F. Brady, T. H. Grossman, M. R. Liles, K. A. Loiacono, B. A. Lynch, I. A. MacNeil, C. Minor, C. L. Tiong, M. Gilman, M. S. Osburne, J. Clardy, J. Handelsman, and R. M. Goodman. 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Applied and Environmental Microbiology* 66:2541-2547.
- Rondon, M. R., S. J. Raffel, R. M. Goodman, and J. Handelsman. 1999. Toward functional genomics in bacteria: analysis of gene expression in *Escherichia coli* from a bacterial artificial chromosome library of *Bacillus cereus*. *PNAS* 96:6451-6455.
- Schloss, P. D., and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl. Environ. Microbiol.* 71:1501-1506.

- Schloss, P. D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75:7537–7541.
- Schmeisser, C., Steele, H., and W.R. Streit. 2007. Metagenomics, biotechnology with non-culturable microbes. *Appl. Microbiol. Biotechnol* 75: 955–962.
- Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., et al. 2006. Microbial diversity in the deep sea and the underexplored “rare biosphere”. *PNAS* 103:12115–12120.
- USGS. 2009. National field manual for the collection of water-quality data; techniques of water-resources investigations, Book 9, Handbooks for water-resources investigations. U.S. Geological Survey, USA. 8 April 2009. Available online at <http://water.usgs.gov/owq/FieldManual/>
- Vanderford, B.J. and S.A. Snyder. 2006. Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry. *Environ. Sci. Technol.* 40:7312–7320.
- Wang, Q., G.M. Garrity, J.M. Tiedje and J.R. Cole. 2007. A naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73:5261-5267.
- Wu, C. H., Sercu, B., Van De Werfhorst, L. C., Wong, J., DeSantis, T. Z., et al. 2010. Characterization of coastal urban watershed bacterial communities leads to alternative community-based indicators. *PLoS ONE* 5(6): e11285. doi:10.1371/journal.pone.0011285

7. Budget

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET 2.5 year project - Links to Activity shown in parenthesis (e.g., Activity 1 = A-1)

BUDGET ITEM	AMOUNT
Personnel:	
Postdoctoral student (100% time, 84% salary, 16% fringe, 2 years, 1 person) (A-1, A-3)	\$95,200
Technician (100% time, 73% salary, 27% fringe, 2 years, 1 person) (A-1)	\$95,900
Website staff (2% time, 73% salary, 27% fringe, 1 year, 1 person) (A-1, A-2, A-3)	\$1,414
Instructor (5% time, 76% salary, 24% fringe, 2 years, 1 person) (A-2)	\$12,000
Advanced graduate student (25% time, 77% salary, 23% fringe, 1 year, 1 person) for assessment of student achievement in 7-12 classrooms in this program. (A-2)	\$9,167
Contracts: Two teachers (grade 7-12) as co-leaders of professional development workshop/program for teachers (\$3000 per teacher X 2 teachers X 2 years) (A-2)	
Exhibit staff at Science Museum of Minnesota (30% time, 73% salary, 27% fringe, 2 years, 1 person) (A-3)	\$54,800
Equipment/Tools/Supplies:	
Text and reference books, information materials (e.g., posters) for classrooms \$190 X 20 teachers X 2 years (A-2, A-3)	\$7,600
Laboratory supplies \$385 per teacher X 20 teachers X 2 years (A-2, A-3)	\$15,400
Laboratory supplies for filtering, cultures, and genome preps for river samples (A-1)	\$1,111
Exhibit materials: metal and wood stands, glass frames, photographs (3 sites X \$5000 per site) (A-3)	\$15,000
Acquisition (Fee Title or Permanent Easements):	
	\$0
Travel:	
In-State Travel for 10 samplings per year X 2 years @1800 mi *\$0.50/mi (A-1)	\$900
Room & board for 4 people X 3 days/year X 2 years for sampling: \$1664 for lodging; \$1120 for food (A-1)	\$2,784
Participant travel (30 mi/day X 5 day * 20 teachers/yr * 2 year * 0.50/mi) (A-2)	\$3,000
Participant room and board for Itasca workshop held 2012 (2011 workshop held in the Twin Cities for Metro area teachers so no room and board needed): (\$362.50 each X 20 teachers) (A-2)	\$7,250
Instructor/co-leader travel to Itasca in 2012 (2 cars: 430 miles round trip * \$0.50/mile) (A-2)	\$430
Room and board for instructor and co-teachers during teacher professional development program in 2012 (2011 workshop held in Twin Cities so no room and board needed): 1 week at Itasca (\$362.50 per person) for instructor and 2 co-teachers (A-2)	\$1,088
Additional Budget Items:	
Fees for participants for graduate credit: Tuition is waived for the project, but there will be an administrative fee of \$100 per registrant + \$157 fees (total \$282) X 20 teachers X 2 years (A-2)	\$11,280
Stipend (\$1500 per teacher for 1 week workshop/lookup work X 20 teachers X 2 years) (A-2)	\$60,000
Sample analysis: 22 samples for each analysis: (genome preparation @\$5000/sample = \$110,000, genome analysis @\$4562/mixed sample X 8 mixed samples = \$36,496, physical analysis @ \$20/sample X 44 samples = \$880, chemical analysis @\$75/sample X 44 samples = \$3300). Genome preparation and all genomic, physical, and chemical analyses are done most cost effectively in specialty labs that charge by the sample. (A-1)	\$150,676
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND \$ REQUEST	\$557,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State \$ Being Applied to Project During Project Period: <i>Federal Stimulus funds paying the undergraduate course instructor and laboratory support personnel, for July and August, 2011.</i>	\$ 16,670	Secured
Other State \$ Being Applied to Project During Project Period: <i>Portion of Phillips salary for management of teacher professional development programs; College match.</i>	\$ 4,533	Secured
In-kind Services During Project Period: \$0	\$ -	
Remaining \$ from Current ENRTF Appropriation (If applicable): \$0	\$ -	
Funding History: <i>Federal Stimulus funding to launch this project at the undergraduate level; \$400,000 beginning September, 2009; this is the funding reported in "Other Non-State\$" above; \$400,000-16,700 = \$383,300</i>	\$ 383,300	

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a. Budget Narrative

ACTIVITY 1: Result: Analysis of Microorganisms **Budget: \$346,571**

Outcomes (Specific deliverables): Completion date December 31, 2013

The outcomes of this activity are:

1. Sampling of the Mississippi River and analysis of samples for microbial species diversity and functionality at each sampling location.
2. Correlations of structural (sequence of diversity) and functional metagenome data to physical and chemical data at each location.
3. Initial development of relational web database consisting of metagenomic and physical chemical data.
4. Uploading of metagenomic data into IMG-ACT for searching and retrieval by researchers, students, river managers, regulatory agencies, and the public to better understand how human activity influences the microbiology of the Mississippi River.

To achieve these outcomes, we have budgeted for personnel, travel, and sample analysis.

Personnel: a postdoctoral research associate and a technician for two years to complete the sampling of the Mississippi (11 sites in 2011 and 2012) and analysis of these samples. The specific duties of each are:

- Postdoctoral associate (2 years salary and fringe, 100% time): Conduct research on the impacts of human activity on microbial structure and function through DNA sequence acquisition, data analysis, functional screens for microbial activity, correlating analysis of chemical/physical/genomic DNA, writing reports and publications and being involved in outreach/extensions/educational activities in Activity 2 and 3.
- Technician (2 years salary and fringe, 100% time): Provide technical support to the work of the postdoctoral student and to the outreach/extensions/educational activities in Activity 2 and 3 through support and management of sample acquisition and processing, data collection and analysis, sample management, DNA preparations, and submission of samples to collaborators/partners.

Travel: In-state travel expenses, including room and board for some sites, are provided for the postdoctoral student and technician to obtain the 44 samples used for this project (2 sampling times, 11 sites, 2 years.)

Sample analysis: Two samples per year will be collected at each of 11 sites. One sample per year will be fully analyzed through preparation of fosmid libraries and sequencing of 16S rDNA from the filtered water, as well as physical/chemical analyses of the sample water. The other sample will be analyzed for 16S rDNA and physical/chemical markers, but no fosmid libraries will be produced. The reason for the difference is that screening of the fosmid libraries that will be used for functional analysis of genes present in each clone is a time-consuming process and each clone can be analyzed for hundreds of traits. Thus, the number of fosmids generated in just one sample time/year in these two years will be all that

can be analyzed in the time frame of this project. Costs are detailed in the budget pages. Of note is that we will use specialty labs for some of the sample preparation and analyses. Genome preparation and all genomic, physical, and chemical analyses are done most cost effectively in specialty labs that charge by the sample. Specific costs include: genome preparations for functional analyses, robot supplies for high throughput plating of libraries, genome DNA sequence analysis, physical analyses – supplies and fees for nutrient analyses (N,P, C) physical parameters (pH, turbidity), and chemical analyses for 14 organic chemicals (pesticides acetochlor, atrazine, metolochlor; antibiotics tylosin, erythromycin, trimethorpim; pharmaceuticals acetaminophen, caffeine; personal care chemicals DEET, triclosan; and endocrine disruptors 17 a,b – trenbolone [growth hormone], estradiol.)

ACTIVITY 2: Result: Professional Development of 7-12 teachers. Budget: \$140,629

Outcomes (Specific deliverables): Completion dates variable (see full proposal)

1. Provision of professional development workshops, in the summer 2012 and 2013.
2. Production of a web accessible, searchable database with downloadable datasets for use in the 7-12 and undergraduate classrooms, as well as by researchers in Minnesota and elsewhere. This will occur via a partnership of researchers with G7-12, undergraduate, & graduate students and educators, and citizens working on this database.
3. Production of curriculum packets, webinars, books, materials, presentations, and approaches that can be incorporated into 7-12 classrooms.
4. Annotation of gene identity and function in IMG-JGI website by 7-12 students.
5. Development of trained teachers that incorporate this cutting edge science into their classrooms and pedagogical materials for other teachers to use throughout the state (and nation).

To achieve these outcomes, we have budgeted for personnel, course costs and materials, travel, and assessment.

Personnel: (2 yr salary and fringe - 5% time)

- One faculty member at 5% time will develop and teach professional development workshops for G7-12 teachers in Summer 2012 and 2013
- Two G7-12 teachers for 2 years to act as co-leaders for the professional development workshops. They will be engaged in planning, delivery and follow-up activities, providing the expertise at the G7-12 level of what teachers need to for this material to be incorporated into their classrooms.
- Website staff to provide the web interface for easier access for G7-12 teachers and students to the databases being used for database searching and additions
- One graduate student (1 year salary and fringe - 25% time) from the College of Education and Human Development will assess student achievement in G7-12 classrooms involved in the project. This assessment will attempt to provide formative evaluation of our activities as well as a summative measure of the effectiveness of the program and of the subsequent incorporation of the instructional material and approach into the G7-12 classrooms.

Course costs and materials:

- University course fees for teachers participating in the teacher development program to provide them with graduate credits (tuition will be waived by the college, but it cannot waive fees.)
- Relevant books and instructional materials for teachers and their classrooms.
- Laboratory supplies for teachers for the initial incorporation of some functional analyses of the fosmid clones in their classrooms.

Room and board, travel:

- Travel funds will be provided for teachers to attend workshops at the University of Minnesota-Twin Cities in the 2012 program. It is expected that the 2012 program will concentrate on recruiting teachers from the Twin Cities Metro Area.
- Room, board and travel expenses will be provided for instructor, co-teachers, and teacher participants for the 2013 program to be held at the Itasca Biological Station. The 2013 program will recruit teachers from Greater Minnesota so will provide a residential setting for the program.

Stipends:

- Teachers participating in professional development programs will be provided with a stipend for participation. Since many teachers have outside jobs during the summer months, this stipend provides them the ability to attend professional development activities.

ACTIVITY 3: Result: Project data dissemination. Budget: 69,800**Outcomes (Specific deliverables): Completion dates variable (see full proposal)**

1. Development of Minnesota Mississippi Metagenome Website.
2. Production of public exhibits at SMM, Lake Itasca, and the Bell Museum.
3. Dissemination of project data and results via webinars, seminars and workshops.
4. Production of curriculum packets, webinars, books, materials, and presentations for G7-12 students and teachers.

To achieve these outcomes, we have budgeted for personnel and exhibit materials in this section of the budget and will use some of the same personnel budgeted in Activity 2 to contribute to this Activity. For ease of budgeting, the budget for the web accessible database has been incorporated into Activity 2; the web development needed for the professional development of the teachers for use of this project in their classrooms is the same as that needed for project data dissemination (however, also see below in production of public exhibits). To meet the 4th outcome, we have also budgeted for teacher professional development and assessment of the teacher program effectiveness in Activity 2.

New budget items in this section include exhibit personnel and materials:

Personnel:

An exhibit staff member at the Science Museum of Minnesota, SMM, (30% time for 2 years) to incorporate the project's materials into EarthBuzz, a National Science Foundation and SMM funded

program, which has an interactive web site about Minnesota and the environment. This will be on kiosks at SMM and throughout MN, as well as accessible through a web-browser anywhere.

Exhibit Material:

Material costs have been budgeted to construct exhibits/displays at Itasca State Park and the Bell Museum. Materials are suitable for indoor or outdoor display, as appropriate for the chosen sites.

Other Funding:

Other funds being applied to the project during the project period include both non-state and state funds. The non-state funds that will be applied are \$16,670 remaining from a \$400,000 federal stimulus grant administered by the University to launch the research and undergraduate Mississippi Metagenomics program. These funds will provide some supplies for the sampling process. The state funds are \$4,533 in matching salary costs for the time the Co-Director of the Biology Program who will provide the planning and oversight of the teacher professional development program and communication between the teachers and the scientists throughout the project. State funds are also being used for the salaries of Michael Sadowsky and Jane Phillips, who each will devote part of their effort to manage this project.

8. Credentials

NAME: MICHAEL J. SADOWSKY
TITLE: McKnight Distinguished University Professor, Director BioTechnology Institute, Director Graduate Studies Microbial Ecology Program
DEPARTMENT: Department of Soil, Water, and Climate, and Biotechnology Institute, University of Minnesota
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EDUCATION:

1979 - 1983 Ph.D. University of Hawaii, Honolulu, Hawaii - Microbiology
1977 - 1979 M.S. University of Wisconsin-Oshkosh, Wisconsin - Biology/Microbiology
1973 - 1977 B.S. University of Wisconsin-Madison, Wisconsin - Bacteriology

POSITIONS HELD:

2009 - present Director, BioTechnology Institute
2006 - 2009 Co-Director, Microbial and Plant Genomic Institute
2004 - present Distinguished McKnight University Professor
1996 - 2004 Professor, Department of Soil, Water, and Climate, Biological Process Technology Institute, and Department of Microbiology, University of Minnesota
1993 - 1996 Associate Professor, Department of Soil, Water, and Climate and Department of Microbiology, University of Minnesota
1989 - 1993 Assistant Professor, Department of Soil, Water, and Climate and Department of Microbiology, University of Minnesota
1986 - 1989 Microbiologist, U.S. Department of Agriculture-ARS; Beltsville, Maryland,
1985 Molecular Biologist, Allied Corporation; Plant Sci. Lab., Syracuse, New York.

HONORS AND AWARDS:

Fellow, American Association for the Advancement of Science, 2008, Fellow - American Academy of Microbiology, 1999, Editor, Molecular Plant-Microbe Interactions 2009-present, Editor, Applied and Environmental Microbiology, 1999-2004; Young Investigator Award, American Society for Microbiology, 1990; Editorial Board, Symbiosis, 1997-present; Editorial Board, Microbe and Environment 2000-present; Associate Editor, Applied Environmental Microbiology 1989-1999, 2007-present. CFANS Distinguished Graduate Teaching Award, 2009, CFANS Distinguished Diversity and Inclusion Award, 2008, Time Magazine Innovator Article, 2006.

SELECTED PUBLICATIONS (16 out of 162):

1. **Badgley, B. D., J. Ferguson, A. Vanden Heuvel, G. T. Kleinheinz, C. M. McDermott, T. R. Sandrin, J. Kinzelman, E. Junion, M. N. Byappanahalli, R. L. Whitman, and M. J. Sadowsky.** 2010. Multi-scale temporal and spatial variation in genotypic composition of *Cladophora*-borne *E. coli* populations in Lake Michigan. *Water Res.*: **In Press.**

2. **Hamilton, M.H., A. Z. Hadi,, J. F. Griffithd, Satoshi Ishii, and M. J. Sadowsky.** 2010. Large scale analysis of virulence genes in *Escherichia coli* strains isolated from Avalon Bay, CA. *Water Res.*: **In Press**.
3. **Unno, T., J. Jang, D. Han, J. Ha Kim, M. J. Sadowsky, O.-S. Kim, J. Chun and H.-G. Hur.** 2010. Use of barcoded pyrosequencing and shared OTUs to determine sources of fecal bacteria in watersheds. *Environ. Sci. Tech.* : **In Press**
4. **Unno, T., D. Han, J. Jang, S.-N. Lee, J. H. Kim, G. Ko, B. G. Kim, J.-H. Ahn, R. A. Kanaly, M. J. Sadowsky, and H.-G. Hur.** 2010. High Diversity and Abundance of Antibiotic Resistant *Escherichia coli* Isolated from Humans and Farm Animal Hosts in Jeonnam Province, South Korea. *Sci. Total Environ.* **In Press**
5. **Ishii, S., Tao. Yan, H. Vu, D. Hansen, R. E. Hicks, and M. J. Sadowsky.** 2010. Factors controlling long-term survival and growth of naturalized *Escherichia coli* in temperate field soils. *Microbes and Environ.* 25: 8-14.
6. **Vanden Heuvel, A, C. McDermott, R. Pillsbury, T. Sandrin, J. Kinzelman, J. Ferguson, M. Sadowsky, M. Byappanahalli, R. Whitman, and G. T. Kleinheinz.** 2010. The Green Alga, *Cladophora*, Promotes *Escherichia coli* Growth and Contamination of Recreational Waters in Lake Michigan. *J. Environ. Qual.* 39:333-344.
7. **Li, Q., J. L. Seffernick, M. J. Sadowsky, and L. P. Wackett.** 2009. Thermostable cyanuric acid hydrolase from *Moorella thermoacetica* ATCC 39073. *Appl. Environ. Microbiol.* 75: 6986 - 6991.
8. **Weidhaas, J., T. MacBeth, R. Olsen, M. J. Sadowsky, D. Norat, and V. Harwood.** 2009. Identification of a *Brevibacterium* marker gene specific to poultry litter and development of a quantitative PCR assay. *J. Appl. Microbiol.* doi: 10.1111/j.1365-2672.2009.04666.x
9. **Khoruts, A., J. Dicksved, J. K Jansson, and M. J. Sadowsky.** 2009. Changes in the Composition of the human fecal microbiome following bacteriotherapy for recurrent *Clostridium difficile*-associated diarrhea. *J. Clin. Gastro. In Press*: doi: 10.1097/MCG .0b013e3181c87e02.
10. **Byappanahalli, M. N., R. Sawdey , S. Ishii, D. A. Shively, J. A. Ferguson, R. L. Whitman, and M. J. Sadowsky.** 2009. Seasonal stability of *Cladophora*-associated *Salmonella* in Lake Michigan watersheds. *Water research* 43:806-814.
11. **Unno, T., D. Han, J. Jang, S.-N. Lee, G. Go, H.Y. Choi, J.H. Kim, M.J. Sadowsky, and H.-G. Hur.** 2009. Absence of *Escherichia coli* phylogenetic group B2 strains in humans and domesticated animals from Jeonam Province, Korea. *Appl. Environ. Microbiol.* 75:5659-5666.
12. **Hansen, D., S. Ishii, M. J. Sadowsky and R. E. Hicks.** 2009. *Escherichia coli* populations in great lakes waterfowl exhibit spatial stability and temporal shifting. *Appl Environ Microbiol.* 75: 1546–1551.
13. **Ishii, S., and M. J. Sadowsky.** 2008. *Escherichia coli* in the Environment: Implications for Water Quality and Human Health. *Microbes and Environments.* 23:101-108.
14. **Hansen, D. L., J. J. Clark, S. Ishii, M. J. Sadowsky, and R.E. Hicks.** 2008. Sources and sinks of *Escherichia coli* in benthic and pelagic fish. *J. Great Lakes Res.* 34:228-234.
15. **Ishii, S., D. L. Hansen, R. E. Hicks, and M. J. Sadowsky.** 2007. Beach sand and sediments are temporal sinks and sources of *Escherichia coli* in Lake Superior. *Environ. Sci. Technol.* 41:2203-2209.
16. **Yan, T., and M. J. Sadowsky.** 2007. Determining sources of fecal bacteria in waterways. *Environ. Monitor. Assess.* 129:97-106

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Education:

B.A., Wittenberg University, Springfield, Ohio, 1981, Biology.
 M.Sc., Kent State University, Kent, Ohio, 1984. Biology.
 Ph.D., University of Michigan, Ann Arbor, 1990 Biology.
 Post-doctoral research fellow, Great Lakes Environmental Research Laboratory and University of Michigan, Biological Limnology and Oceanography, 1990-1992.

Professional Experience:

2008 to present Professor, Department of Ecology, Evolution and Behavior,
 University of Minnesota
2001 to 2008 Associate professor, Department of Ecology, Evolution and Behavior,
 University of Minnesota
1998 to 2001 Assistant professor, Department of Ecology, Evolution and Behavior,
 University of Minnesota
1992 to 1998 Assistant professor, Department of Wildlife and Fisheries Sciences
 and Department of Oceanography, Texas A&M University.

Publications (5 most relevant):

Cory, R.M., J.B. Cotner, K. McNeill, A.M. Amado, M. Jacobson, and B.P. Peterson. Fluorescent dissolved organic matter helps unravel the carbon cycle in Earth's largest lake. To be submitted to *Limnology and Oceanography*.

Boreen, A. L., B. L. Edhlund, J. B. Cotner, and K. McNeill. 2008. Indirect photodegradation of dissolved free amino acids: The contribution of singlet oxygen and the differential reactivity of DOM from various sources. *Environmental Science and Technology* 42: 5492-5498.

Cory, R.M., J.B. Cotner and K. McNeill. 2009. Quantifying interactions between singlet oxygen and aquatic fulvic acids. *Environmental Science and Technology* 43: 718-723.

Amado, A.M., J.B. Cotner, A.L. Suhett, F. de A. Esteves, V. F. Farjalla. 2007. Complementary interactions among dissolved organic carbon substrates for photochemical and microbial degradation processes in aquatic ecosystems. *Aquatic Microbial Ecology* 49: 25-34.

Cotner, J B, B A Biddanda, W Makino, and T Stets. 2004. Organic Carbon Biogeochemistry of Lake Superior. *Aquatic Ecosystem Health and Management* 7: 451-464.

Publications (5 others):

- Biddanda, B., M. Ogdahl and J.B. Cotner. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. *Limnology and Oceanography* 46: 730-739.
- Stets, E.G., and J.B. Cotner. 2009. Littoral zones as sources of biodegradable dissolved organic carbon in lakes. *Canadian Journal of Fisheries and Aquatic Science* 65 :2454-2460.
- Biddanda, B.A., and J.B. Cotner. 2002. Love handles in aquatic ecosystems: Role of dissolved organic carbon drawdown, resuspended sediments and terrigenous inputs in the carbon balance of a Great Lake (Michigan). *Ecosystems* 5: 431-445.
- Cotner, J.B., J.W. Ammerman, E.R. Peele, and E. Bentzen. 1997. Nutrient-limited bacterioplankton growth in the Sargasso Sea. *Aquatic Microbial Ecology* 13:141-149.
- Cotner, J.B., and B.A. Biddanda. 2002. Small players, large role: Microbial influence on auto-heterotrophic coupling and biogeochemical processes in aquatic ecosystems. *Ecosystems* 5, 105-121.

Synergistic Activities

- American Society of Limnology and Oceanography Board of Directors (Member at large; 2008-present)
- Director of Undergraduate Studies for Dept. EEB, University of Minnesota (2008-present)
- PI for Global Change Ecology, NSF-REU Site, Itasca Biological Station and Laboratories (2008-)
- Co-Chair (with Samantha Joye) of the American Society of Limnology and Oceanography's Aquatic Sciences Meeting in Feb 2003, Salt Lake City.
- Mentor for American Society for Limnology and Oceanography Committee for Under-represented Minorities in Limnology and Oceanography. (1995)

Collaborators (in the past 48 months)

Bopaiah Biddanda, Grand Valley State University, James Elser, Arizona State University, Dan Engstrom, Minnesota Science Museum, Thomas Johengen, University of Michigan, Peter Lavrentyev, Akron University, Kris McNeill ETH Switzerland, Rose M. Cory, University of North Carolina, Noel Urban, Michigan Tech University, Galen McKinley, University of Wisconsin, Kevin Theissen, University of St. Thomas, Kyle Zimmer, University of St. Thomas, Andre Amado, University of Natal-Brazil, James Waples, University of Wisconsin-Milwaukee, Val Klump, University of Wisconsin-Milwaukee, Kirk O. Winemiller, Texas A&M University.

Advisors and advisees

Robert T. Heath, Kent State University, M.Sc.; Robert G. Wetzel (deceased), Ph.D.; Wayne Gardner, Univ. Texas, Postdoctoral research supervisor.

Advisees and Related Experience:

Advisees: Casey Moore, M.Sc., Kelly Gloger, M.Sc., Michael Suplee, M.Sc., Ph.D., David E. Shormann, Ph.D., Yesim Buyukates, M.Sc., Edward Hall, Ph.D., Edward Stets, Ph.D., Brian Johnson (M.Sc.), Jon Kenning (Ph.D.)

9. Dissemination and Use.

Dissemination and use of the data generated in this project is outlined under each activity and objective. Briefly, in this project we will develop a relational web database consisting of metagenomic and physical chemical data. Project data will also be uploaded into DOE's IMG-ACT database system for searching and retrieval by researchers, students, river managers, regulatory agencies, and the public. We will also provide a series of professional development workshops in the summer of 2012 and 2013, which will be used to train teachers in metagenomics. We will also produce a web accessible, searchable database (as part of the Minnesota Mississippi Metagenome Website), with downloadable datasets, for use in the 7-12 and undergraduate classrooms, as well as by researchers in Minnesota and elsewhere. To aid in teaching and data dissemination we will produce curriculum packets, webinars, books, materials, presentations, and approaches that can be incorporated into 7-12 classrooms by teachers. Lastly, we will produce public exhibits at the SMM, Lake Itasca, and the Bell Museum to teach the public about the usefulness of metagenomics and how human activity influences the structure and function of the Mississippi River ecosystem. Results from this project will also be disseminated via more traditional routes, including in reports made to the LCCMR, the generation of teaching materials, in periodic update reports made to cooperators, in seminars given throughout the state and nation, in targeted presentations made to state agencies (mostly MPCA and MDA) and in publications in peer-reviewed scientific and teaching journals. Lastly, project data and approaches, including all teaching and learning activities will be disseminated via a dedicated web site that will be built specifically for the project. The web site, which will reside on servers at the University of Minnesota, will contain information about the goals of this project, links to all the data and databases need for data analysis, information and resources for teachers, and updated references and project publications

10. Project Team/Partners. The project will be carried out under the direction of Drs. Michael Sadowsky (PI) and co-PI James Cotner. Funded project partners will include Pat Hamilton of the Science Museum of Minnesota, Itasca State Park, and the Bell Museum, Dr. William Koskinen (USDA-ARS) who will be involved in sample analysis for chemicals and the NCGR who will do DNA sequence analysis on a fee basis. We will also collaborate with the National Park Service at the SMM, Adam Birr at the Minnesota Department of Agriculture, and Barb Peichel at MPCA for dissemination activities.

11. Long-term Strategy and Future Finding Needs. This request seeks funding for the first 2.5 years of this program. This will provide the basis for a long-term, continuing study of the health of the Mississippi River that will include all the states bordering the Mississippi and eventually all the states in the Mississippi watershed. Since the River starts in Minnesota at Itasca, this new in depth study and broad impact program begins in Minnesota. Additional funding for more long term and more extensive analyses (of the upper and lower Mississippi River) will be obtained from the National Science Foundation, other states, and other foundations. This National project will be organized similar to the MN project, but involve researchers, students, and the public all the way to New Orleans.