

Final report, LEPF 177-02, "Molecular assessment of the potential for *in situ* PCB bioremediation," George Bullerjahn, PI

The LEPF award "Assessment of the potential for *in situ* PCB bioremediation by RT-PCR molecular profiling," was completed in the year 2003 and led to the findings summarized below. Specifically, the project set out to develop and fine-tune an assay for the presence of PCB-degrading organisms in the sediments and benthos of Lake Erie. Whereas the assay we developed does detect PCB-degrading bacteria within the microbial consortia of hypoxic Lake Erie (Hoostal et al. 2002), the RT-PCR assay yielded false positives due to the complexity of the bacterial community present. Thus, we felt the best approach was to characterize the major members of the sediment community of Lake Erie, determining which members could likely play roles in PCB and PAH degradation. To achieve this, our initial plan was to generate 16S rDNA libraries to assess total diversity via high-throughput DNA sequencing. Overall, such work has led to the current hypothesis that abundant Actinobacteria are involved in PCB degradation. Thus, the work summary for this project is presented in the attached paper (Hoostal et al. 2002), and studies stemming from this sequencing work have led to current work presented at the 2006 IAGLR Annual Meeting (Cupp et al. 2006), and a future peer-reviewed publication on Lake Erie bacterial diversity (Cupp et al. in preparation).

Our data suggest that the geochemistry of Lake Erie sediments is strongly influenced by the large actinobacterial populations present. Given the ability of many of these bacteria to mediate anaerobic and aerobic degradation of PCBs, our future focus is to examine how actinobacterial populations change in mesocosms following spiking of sediment cores with biphenyl and PCBs. Such work can help define those species involved in bioremediation, and help develop molecular (PCR-based) probes for their detection *in situ* in the Lake. Some of this work is a pilot study described in a pending proposal to the NSF entitled "MIP: Linking the metabolic potential and diversity of the microbial community with seasonal shifts to hypoxic conditions."

Results to date

Eubacterial Library Diversity:

Bacterioplankton mediate a large portion of the carbon turnover in freshwater lakes. Unfortunately, the function these organisms play in their environment is largely unknown due to the lack of knowledge on their physiology. The ability to culture members from both marine and freshwater ecosystems is very low with cultivation success for approximately 0.1% of marine and up to 1.4% of freshwater bacterioplankton (Bruns, 2003; Kogure, 1980; Page, 2004). With this inability to properly culture diverse populations, sequence analysis of 16S rRNA gene was performed to assess the composition of these bacterial communities. Understanding the community composition of lakes will reveal the role of these microorganisms in biogeochemical processes. The Ribosomal Database Project

(RDP) Classifier was used to analyze the sequences into subsequent phyla and class designations (Figure 1). Neighbor-joining analysis using the Kimura-2 parameter from the Mega 3.1 software was used to cross-examine the sequences obtained. The phylogenetic studies were capable of assigning taxonomic position to most (90%) sequences that remained unclassified by the RDP Classifier.

Actinobacteria. From the eubacterial libraries obtained from Lake Erie, the most prevalent phyla were the Actinobacteria comprising of almost half of all sequences (40-47%; Figure 1). Two separate clusters of Actinobacteria with high bootstrap support and similarity were found at depth in July (prior to seasonal hypoxia) and August (during seasonal hypoxia). It is well known that the Actinobacteria constitute many different habitats, one of which includes a wide variety of freshwater ecosystems of differing trophic status (Crump, 1999; Glöckner, 2000; Hahn, 2003; Lindstrom, 2002; Zwart, 1998; Zwart, 1998). This group is often found in soils and sediments and was previously known as the high-G+C gram-positive bacteria obviously due to the high guanine and cytosine nucleotides found in its genome. These gram-positive bacteria have been found to account for up to 60% of freshwater bacterioplankton (Glöckner, 2000) perhaps due to the fact that certain strains of Actinobacteria are resistant to grazing from bacterivorous microorganisms (Hahn, 2003; Pernthaler, 2001). This resistance, along with its facultative anaerobic respiration, may have allowed for the abundance and proliferation of this group during hypoxia. Several clusters of freshwater Actinobacteria can be evident in most ecosystems. Three sequences (LE46F20m73, LE46F20m716, and Le46F20m720) were found to be closely related to an Actinobacterial isolate *Mycobacterium hodleri*. This species is a fast-growing Actinomycete that is able to degrade polycyclic aromatic hydrocarbons (PAHs) and other environmentally hazardous elements (Kleespies, 1996).

Proteobacteria. The *Proteobacteria* are divided into five distinct classes, while three of these five (α , β , and γ) are often found in marine and freshwater habitats (Glöckner *et al*, 1999). Not surprisingly, these three groups were all represented in the present libraries. Twenty-three percent of all sequences identified through phylogenetic analysis were assigned to the α -*Proteobacteria*. Relative abundance of this group normally found in freshwater ecosystems varies from 0-20% of all bacteria (Glöckner *et al*, 1999). The *Proteobacteria* cluster, like the Actinobacteria clusters, did not yield any isolated strains from the GenBank database, showing these groups are most similar to uncultured environmental samples than to any cultured strain. This group was a major constituent of in the August library, but was reduced drastically in July showing a dynamic shift of community members from July to August. The cultured isolate this group clustered with was a marine α -*Proteobacterium* SKA48 (Simu, 2003). The inability to assign a specific metabolic function to this cluster is due to a lack of physiological characterization of many environmental samples. From GenBank BLAST searches, several other sequences were found to be closely related to

isolated strains, including the α -*Proteobacteria*. Two groups were found to be very similar to other α -*Proteobacterial* Crater Lake isolates, *Sphingomonas* sp.HTCC503 and *Rhodobacter* sp.HTCC515 (Page, 2004). The aerobic Gram-negative *Sphingomonas* species are often isolated from contaminated soils for their ability to use polycyclic aromatic hydrocarbons (PAHs) as a source of energy and carbon (Bastiaens, 2000; Khan, 1996; Mueller, 1990; Pinyakong, 2000) and have also been shown to degrade pesticides (Nagata, 1999) and herbicides (Adkins, 1999). Two sequences from Lake Erie (LE46F20m719 and LE46F165m738) resembled this isolate and were only found in July libraries. Whereas Lake Erie is still recovering from the phosphorus loadings and other anthropogenic impacts induced on the ecosystem during the 1970s and 1980s, it still receives the most industrial pollution of all the Great Lakes. Thus, it should not be surprising to find potential pollutant-degrading organisms in the sediments and water column.

Studies on the chemoautotrophic *Rhodobacter* species from freshwater habitats have lagged behind those of marine environments. The *Rhodobacter* group consists of purple bacteria capable of anaerobic anoxygenic photosynthesis by the use of bacteriochlorophyll *a*, but does not evolve oxygen like cyanobacteria (Yurkov and Beatty, 1998). Alternatively, they grow heterotrophically as unpigmented nonphotosynthetic aerobes. One sequence (LE46F20m737) from July was found to be closely related to this group. Increased water clarity due to filtering of the zebra mussels may drive the proliferation of this photosynthetic purple bacterium in Lake Erie sediment. One sequence similar to a *Caulobacter* sp. FJI423 was found from the July library. This Gram-negative aerobic α -*Proteobacteria* is often found in both marine and freshwater ecosystems. This prosthecate group is able to adhere to objects by the use of holdfasts and have been found in hypolimnia at anaerobic depths, which suggest their survival at cooler, anaerobic locations (Staley, 1987).

Six percent of all sequences were assigned to the class β -*Proteobacteria* with representatives found in all libraries with a higher percentage of sequences found at depth. The β -*Proteobacteria* contain chemoheterotrophs or chemoautotrophs which commonly derive their nutrients from the decomposition of organic material (Dexter-Dyer, 2003). Two sequences from August (LE46F22m810 and LE46F22m828) closely resembled a species of *Nitrosovibrio* that is a chemoautotrophic ammonia-oxidizing nitrifier. *Nitrosovibrio* converts ammonia into nitrite that is later converted into nitrate by the nitrite-oxidizing bacteria (Bock, 1989) in the nitrogen cycle. Several species of *Nitrosovibrio* are able to survive in oxygen limitation and even anoxia for periods of time (Kowalchuk, 1998). Since these sequences were found at depth in August, this may facilitate the survival of this species in the Central Basin during hypoxia. Only 1% of sequences was assigned to γ -*Proteobacteria* and thus was underrepresented in the libraries. These less abundant groups often have high cell specific activities (Bernard, 2000; Zubkov, 2001) and may indicate their low presence. This gram-negative group commonly contains facultative anaerobes. All sequences

obtained came from depth during hypoxia in August, indicating their preference for anaerobic conditions, and may indicate soils and sediments that may have stirred and resuspended into the lower water column during sampling.

Overall, it appears that there is indeed a dynamic community shift of Proteobacteria in Lake Erie during seasonal hypoxia. Nutrient data taken at this time indicate no significant difference of ammonia, nitrate, or total phosphorus concentrations between July and August, indicating that the bacterial community shift may be explained by the presence or absence of oxygen. The Uncultured α -Proteobacteria Cluster is much more pronounced and there is an increase in number and diversity of cyanobacteria. The continued presence and high abundance of Actinobacteria throughout the summer warrants further work focusing on their role in Lake geochemistry, given their known role in bioremediation processes.

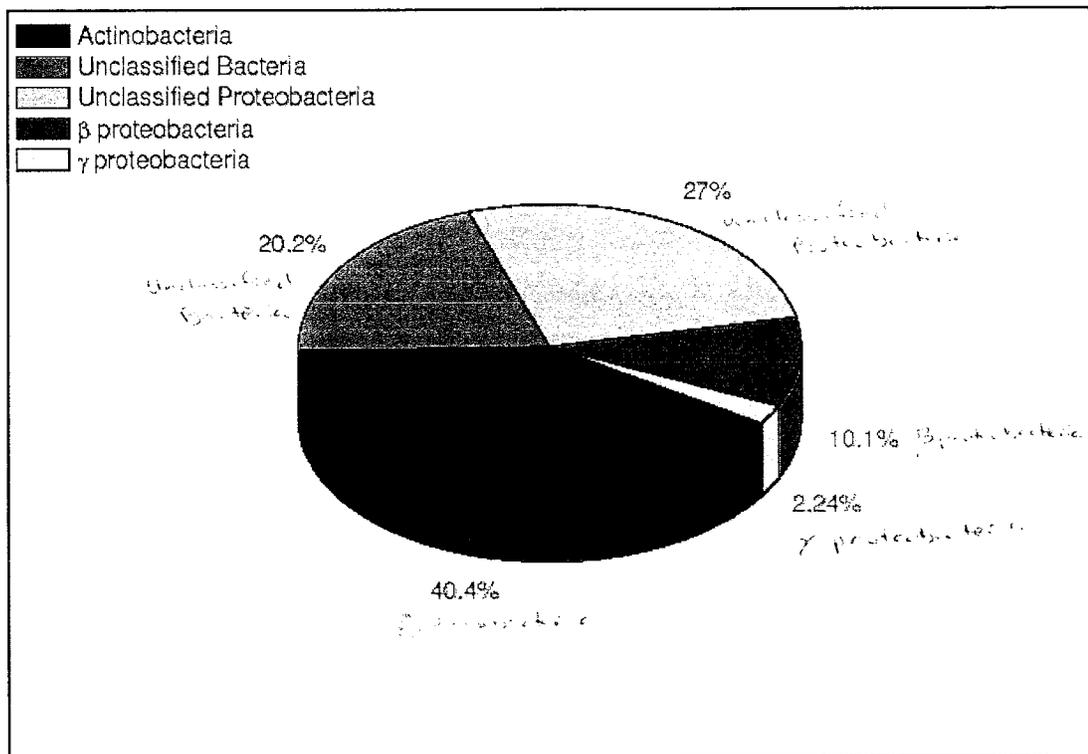


Figure 1: Bacterial assignment of clones from Station 84 August 2002 at 22 meters using eubacterial primers for a partial 16S rRNA sequence. Phyla were determined using the RDP Classifier with a confidence of at least 80%. Phylogenetic analysis by neighbor-joining methods subsequently placed the unclassified Proteobacteria (green) within the α -Proteobacteria and the unclassified bacteria (red) were composed of both α - and β -Proteobacteria

Budget

The budget reporting as appended shows that the funds were spent slightly differently than proposed in the original grant proposal. The proposal requested \$2,018 to increase a graduate student stipend to PhD level. During the award period, we (Bullerjahn and co-PI McKay) were awarded an NSF grant (OCE-9911592) to study nutrient bioavailability in the Great Lakes. This award provided full PhD support for the student engaged in the work described here, as she was working on both projects. The \$2,018 was redirected to supplies, due to the costs of sequencing the 16S rDNA library.

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