

**From West Point to the Battery:  
Bacterial Diversity Along the Lower Hudson Estuary**

A Final Report of the Tibor T. Polgar Fellowship Program

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## ABSTRACT

The goal of this study is to categorize bacterial diversity along the salinity gradient of the lower Hudson River Estuary. Previous research has shown that lower salinities support higher diversity, while more saline environments host lower, but more specialized diversity. Mid-river samples were taken in the middle of July from the Battery, Piermont, and West Point, New York. Both subsurface and deepwater samples were taken as the salinity changes by both distance from the mouth of the river and depth. Samples were vacuum filtered to collect the bacteria on 0.2um pore size filters and bacterial DNA was extracted using mechanical and chemical techniques. The 16S region of the bacterial genomes were then extracted and replicated via a PCR reaction, and the sequences were separated by inserting them in vectors in *E.coli* and allowing the *E. coli* cells to form colonies. Colonies with the insert were then selected and the 16S regions amplified by colony PCR. Sequencing of this region was accomplished through cycle sequencing and sequences were read using an ABI automatic sequencer machine. Sequences were analyzed by comparing them to a national database (NCBI BLAST analysis) and also by completing a phylogenetic analysis of the aligned sequences. BLAST analysis of the Battery and West Point samples revealed a higher diversity in the West Point samples (fresh water), with the presence of gamma proteobacteria in the West Point subsurface sample. Phylogenetic analysis provides evidence of eight major bacterial groups among all four samples, but does not provide evidence for location-specific bacteria clusters. Further analysis will include sequencing clones from the Piermont samples as well as analyzing more clones from the Battery and West Point locations. Useful future research includes analyzing seasonal components to bacterial diversity as well as examining the morphological characteristics of Hudson River bacteria.

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## INTRODUCTION

### *Bacterial Diversity in the Lower Hudson Estuary*

Understanding the diversity and ecological processes of the Hudson River Estuary is essential to the continued improvement and protection of this unique ecosystem. The high diversity in the Hudson River is partly due to significant influxes of salt that wash upriver from the Atlantic Ocean (Stanne et al. 1996). Incoming salt water usually influences approximately 60 miles of the river; however, in drier years, the salt front has reached points as far north as Poughkeepsie, approximately 85 miles upriver (Stanne et al. 1996). Although much is known about the diversity and distribution of higher-level taxa along the river's salinity gradient, very few studies have attempted to categorize bacterial communities along it.

Bacteria are essential components of ecosystems, providing essential services and stability to natural systems. Most of the essential biogeochemical nutrient cycles rely on bacteria, including the carbon and nitrogen cycles. In the Hudson River, for example, bacteria serve as the largest carbon producers (Findlay et al. 1991). Bacteria also help stabilize ecosystems through their high diversity levels. Diversity has been linked to ecosystem stability, productivity, and resistance to stress; therefore, the high diversity of microbes serves as a stabilizing force in environmental systems (Torsvik and Ovreas 2002).

The study presented here aims at providing a baseline index of bacterial diversity along the salinity gradient in the lower Hudson Estuary. Previous studies of aquatic bodies encompassing differing salinities have revealed significant changes in bacterial species composition (Benlloch et al. 2002). In the study by Benlloch et al. (2002), lower

salinity levels supported multiple assemblages of bacteria, while higher salinities supported one dominant yet diverse bacterial group. Additionally, comparisons of freshwater and oceanic bacterial communities revealed an overall absence of one major type of bacteria, the  $\beta$ -*Proteobacteria*, in salt water (Methe et al. 1998).  $\beta$ -*Proteobacteria* have been uncovered in most freshwater systems; however, their presence or absence in aquatic systems with both freshwater and saltwater components has yet to be determined. Based on the aforementioned studies by Benlloch et al., it is hypothesized that the more saline environments house lower, but more specialized bacterial diversity, while the lower saline environment (West Point) supports higher, but less specialized diversity.

#### *Study Approach*

Presently, two main approaches are used to study bacterial diversity – culturing bacterial cells and obtaining genetic sequences from bacterial cells. Although culture techniques better correlate biological diversity with functional diversity, less than one percent of bacteria can be cultured with current techniques, making it difficult to obtain information on total diversity (Torsvik and Ovreas 2002). Sequencing the highly conserved 16S region of bacterial DNA allows a significant portion of bacterial genomes to be amplified and eventually sequenced (Amann and Kuhl 1998). Colony cloning techniques by PCR reactions involving 16S rRNA are better at detecting overall diversity and also have the capability of detecting bacteria in low quantities. Thus, this study utilized colony cloning and sequencing techniques for categorizing bacterial samples.

## METHODS

### *Sample Collection*

Sampling took place on July 12, 2003. Mid-river samples were taken along the Hudson River in New York State at West Point (N 41° 23.809, W 073° 56.938), Piermont (N 41° 02.263, W 073° 52.865), and the Battery (N 40° 41.77, W 74° 01.76). At each location, subsurface (15 cm below surface), and deepwater samples were taken. The deepwater sample depths were dependent on the depth where salinity was at its maximum. Replicate samples were taken at each site. Samples were collected using a sterilized mechanical release collection bottle, which was rinsed with distilled water between samples to reduce cross contamination of samples. Samples were stored on ice in sterilized Nalgene 500 ml bottles. At each location, temperature, water quality (appearance), and salinity (measured with a conductivity meter) were recorded.

### *Filtration and DNA Extraction*

Bacteria were collected via vacuum filtration using sterilized Nalgene disposable filter units. Water samples were first pre-filtered to remove large particles, then poured through filters with 0.2 um pore sizes to trap bacteria. Samples were then stored at -20° C until further processing.

DNA was extracted from the bacterial cells using both mechanical and chemical extraction techniques. Filters were combined with extraction buffer (100mM Tris-HCL, 100mM sodium EDTA, 100mM sodium phosphate, 1.5 M NaCl), proteinase K, and lysozyme (final concentrations 10 mg/ml). The samples were then shaken at 225 rpm for 30 minutes at 37° C. Next, 1.5 ml of 20% SDS solution was added to the samples, after

which the samples were incubated for 2 hours at 65° C. Samples were then centrifuged at 6400 rpm for 10 minutes, and the supernatant was collected. The pellet was resuspended in 4.5 ml extraction buffer and 0.5 ml SDS, incubated for 10 minutes and centrifuged for 10 minutes. This process was repeated once, following which the supernatant was pooled and combined with an equal amount of chloroform: isoamyl alcohol mix (24:1). The mixture was centrifuged at 6400 rpm for 10 minutes, and 0.6 vol of isopropanol was added to the supernatant. DNA precipitated out overnight, and the pellet was collected after centrifuging for 30 minutes at 10,000 rpm. The pellet was washed with 70% ethanol, collected again after centrifugation at high speed, and the remaining ethanol was evaporated by letting the pellet air dry in a sterile environment.

### *Cloning Techniques*

The 16S rRNA region of the bacterial cells was isolated and replicated via a standard PCR reaction (33 cycles of 94° C for 1 minute, 55° C for 1 minute, and 72° C for 1 minute). Bacteria-specific 16S primers were used for the reaction. The targeted genome region of the bacterial samples were then implanted in *E. coli* cells using a TOPO TA cloning kit (Invitrogen), following product protocols. Cells were incubated overnight at 37°C, white colonies were selected (white colonies indicate the presence of the 16S bacterial insert), and reference plates were made of the white colonies.

### *Colony PCR and Cycle Sequencing*

The 16S inserts were isolated and replicated via colony PCR using M13 forward and reverse primers (30 cycles, see above settings). The PCR products were purified by

combining 1/10 vol ammonium acetate and 2 vol 100% isopropanol, incubating at  $-20^{\circ}$  C, and centrifuging at 3500 rpm for 30 minutes. The pellet was then combined with 2 vol 70% isopropanol and centrifuged at 3500 rpm, and then dried with a speed vacuum.

Products were sequenced via standard cycle sequencing protocols ( 30 cycles of  $95^{\circ}$  C for 15 seconds,  $50^{\circ}$  C for 15 seconds, and  $60^{\circ}$  C for 4 minutes) and cleaned with ethanol and isopropanol. Sequences were read on an ABI automatic sequencer (Sessitsch et al. 2001).

### *Data Analysis*

Sequences were compared to known sequences in the NCBI database (see [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), <http://rdp.cme.msu.edu/html/index.html>) using a standard BLAST search (default settings). Sequence alignment was completed using Clustal-X (Thompson et al. 1994), and phylogenetic analysis was performed using PAUP software.

## **RESULTS**

### *Sampling Data*

Temperature, salinity, and water quality (appearance of water) were recorded for both subsurface and deepwater samples at all three locations (Table 1). With the exception of the Battery deepwater sample, water temperatures across the remaining samples were equivalent. Salinity ranged from 0.53 mS (millisiemens) to 33.90 mS. West Point, being just north of the salt front, had a constant, extremely low salinity, placing it in a freshwater category. Both the deepwater sample at Piermont and the Battery were more saline than their corresponding subsurface samples, due to the angle of

