6-2016

Building a Freshwater Bacterial Flora Database for Remote Sensing Applications

Daniel Zimmerman
St. John Fisher College, djz03192@students.sjfc.edu

Laura Moore
St. John Fisher College, lam04586@students.sjfc.edu

J. A. Concha
Rochester Institute of Technology

N. Raqueño
Rochester Institute of Technology

Maryann Herman
St. John Fisher College, mherman@sjfc.edu

See next page for additional authors

Building a Freshwater Bacterial Flora Database for Remote Sensing Applications

Abstract
The identification and classification of microbial flora in bodies of fresh water has the potential of enhancing our understanding of this ecosystem and improving water management and bioremediation. This effort may be facilitated by the use of remote sensing technologies. For the last 3 years our undergraduate students have collected water samples in the Lake Ontario Rochester Embayment and Irondequoit Bay with the goal of constructing a database of bacterial species and water parameters (e.g. organic matter and chlorophyl content). Such a database is necessary to establish potential correlations between the presence of certain bacterial species and water parameters that can be measured using satellite imagery collected by the Landsat 8 OLI and TIRS sensors. In the past we reported initial efforts at mapping the distribution of bacterial species using 16S rRNA. Here we present our results for the summer of 2015 and present a compounded analysis of 3 consecutive summers. Of approximately 450 bacterial isolates, we have cultured and identified more than 40 different species spanning over 20 genera. Several fish and human pathogens were identified, and antibiotic-resistance profiles determined. Year to year variation of the flora's composition at individual locations has emerged as the main challenge in establishing reproducible patterns that may be linked to satellite measurements.

Keywords
fsc2016

Disciplines
Biology

Comments
Presented at American Society for Microbiology 116th General Meeting. Boston, MA.

Authors
Daniel Zimmerman, Laura Moore, J. A. Concha, N. Raqueño, Maryann Herman, and Fernando Ontiveros

Building a Freshwater Bacterial Flora Database for Remote Sensing Applications

D. Zimmerman*, L.A. Moore*, J.A. Concha†, N. Raqueño†, M.A.B. Herman, PhD*, F. Ontiveros, PhD*
†Rochester Institute of Technology, 1 Lomb Memorial Dr., Rochester, NY 14623 *St. John Fisher College, 3690 East Ave, Rochester, NY 14618

The identification and classification of microbial flora in bodies of fresh water has the potential of enhancing our understanding of this ecosystem and improve water management and isolating candidate organisms for bioremediation. This effort may be facilitated by the use of remote sensing technologies. For the last 3 years we have collected water samples in the Lake Ontario Rochester Embayment & Irondequoit Bay with the goal of constructing a database of bacterial species and water parameters (e.g. organic matter and chlorophyll content). Such a database is necessary to establish potential correlations between the presence of certain bacterial species and water parameters that can be measured using satellite imagery collected by the Landsat 8 OLI and TIRS sensors. In the past we reported initial efforts at mapping the distribution of bacterial species using 16S rDNA. Here we present new results for the summer of 2015 and present a compounded analysis of 3 consecutive summers. Of approximately 400 bacterial isolates, we have cultured and identified more than 50 different species spanning over 20 genera. Several fish, plant and human pathogens were identified, and antibiotic-resistance profiles determined. Year to year variation of the flora's composition at individual locations has emerged as the main challenge in establishing reproducible patterns that may be linked to satellite measurements.

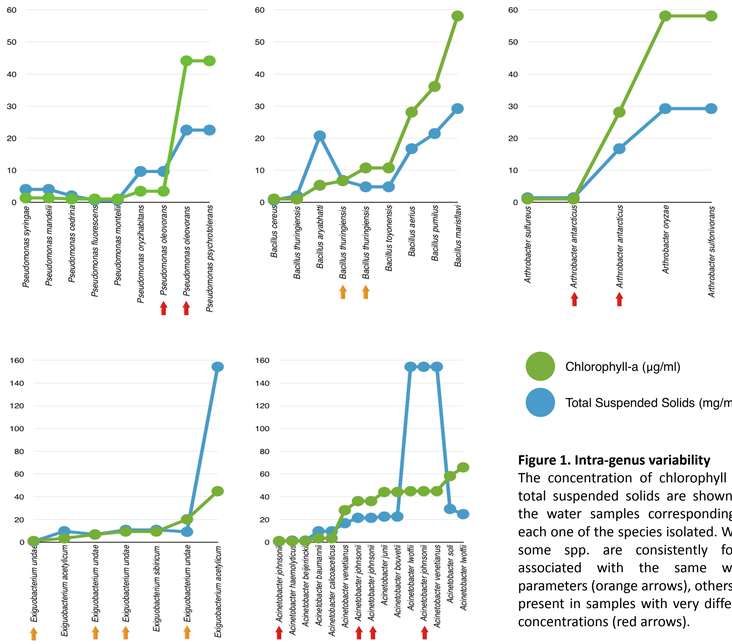

Figure 1. Intra-genus variability
The concentration of chlorophyll and total suspended solids are shown for the water samples corresponding to each one of the species isolated. While some spp. are consistently found associated with the same water parameters (orange arrows), others are present in samples with very different concentrations (red arrows).

Figure 2. Sampling locations and bacterial genera found over a period of 3 years. Water samples were filtered for the isolation of live organisms and total suspended solids (TSS) and chlorophyll-a were measured.

Results

Given the goal of building a database that would provide statistical support for establishing useful correlations between water parameters (e.g. chlorophyll, total suspended solids *TSS*), we set to isolate and identify a large number of samples over a long period of time and at different locations. We collected the samples at the water surface on the same days that the LANDSAT 8 carrying the OLI sensors flew over the sampled area. This was with the objective of being able to compare satellite imagery with ground-truth data. The water samples were filtered and the resulting colonies were isolated, grown in pure cultures and stored for future sequencing/identification. A simple analysis in which the concentrations of water particles and pigments are compared between species of the same genus is shown in Fig. 1. Member of the genus *Acinetobacter* were isolated from samples showing a wide range of concentrations, while members of the *Pseudomonas* and *Exiguobacterium* genera mostly appear in samples with overall low concentrations of the parameters measured. Figure 2 displays the sites of collection and the genera found at different locations on different years. Our bacterial library is composed of approximately 450 bacterial isolates, spanning 23 genera and over 80 distinct species. Several plant and animal pathogens have been found, including members of *Aeromonas, Enterobacter, Pasteurella, Bacillus, Erwinia, and Pantoea.* Among these, strains of *Pantoea agglomerans and Pseudomonas oryzhabitans* showed resistance to ampicillin and erythromycin. We are currently in the process of building meaningful correlations between the presence of certain species and the parameters that satellite's sensors can measure. Table 1 displays a real-data example of the database under construction.

Chlorophyll (ug/L)	TSS (mg/L)	SPECIES ISOLATED				
< 1.00	< 2.00	*Microbacterium maritypicum*	*Bacillus thuringiensis*	*Pseudomonas cedrina*	*Budvicia diplopodorum*	
1.00	1.40	*Arthrobacter sulfureus*	*Flavobacterium psychrolimnae*	*Arthrobacter antarcticus*	*Acinetobacter haemolyticus*	*Acinetobacter beijerinckii*
1.00	1.72	*Pantoea agglomerans*	*Curtobacterium plantarum*			
1.00	0.67	*Acinetobacter johnsonii*	*Pseudomonas fluorescens*	*Bacillus Cereus*	*Pseudomonas monteilii*	*Exiguobacterium undae*
3.43	9.60	*Exiguobacterium acetylicum*	*Acinetobacter baumannii*	*Pseudomonas oryzihabitans*	*Pseudomonas oleovorans*	*Acinetobacter calcoaceticus*
8.02	39.10	*Comamonas testosteroni*	*Pseudomonas psychrotolerans*	*Acinetobacter lwoffii*	*Exiguobacterium acetylicum*	*Exiguobacterium sibiricum*
9.36	10.80	*Pantoea vagans*	*Exiguobacterium sibiricum*	*Exiguobacterium undae*		
10.70	4.80	*Bacillus thuringiensis*	*Bacillus toyonensis*	*Chryseobacterium greenlandense*		
20.10	9.11	*Rhodococcus erthropolis*	*Arthrobacter antarcticus*	*Rhodococcus jialingiae*	*Xanthomonas dyei*	*Aeromicrobium fastidiosum*
28.10	16.70	*Arthrobacter antarcticus*	*Acinetobacter venetianus*	*Flavobacterium psychrolimnae*	*Bacillus aerius*	
36.10	21.40	*Erwinia billingiae*	*Pseudomonas psychrotolerans*	*Pseudomonas oleovorans*	*Acinetobacter johnsonii*	*Bacillus pumilus*
44.10	22.50	*Pseudomonas oleovorans*	*Acinetobacter junii*	*Pseudomonas oryzihabitans*	*Acinetobacter bouvetii*	*Pseudomonas psychrotolerans*
44.86	154.29	*Acinetobacter lwoffii*	*Exiguobacterium acetylicum*	*Acinetobacter johnsonii*	*Acinetobacter venetianus*	

Table 1. Database *example*. Water parameters are linked to presence of individual species.

Methods

Water samples were collected at the water surface from 12 locations on the Lake Ontario-Rochester Embayment. Water samples were filtered through a 0.2 µm Millipore membrane to separate bacteria. Membranes were placed on R2A plates and incubated at room temperature for 24 hours. Bacterial colonies were then streak plated onto fresh R2A plates and allowed to grow for 24 hrs. Colonies were sub-cultured until pure colonies were obtained. Pure cultures were stored in a 10% skim milk/50% glycerol nutrient solution and frozen at -80°C. DNA purification kit was used to extract and purify the amplified DNA. Gel electrophoresis was performed to visualize DNA and PCR was performed to amplify the 16s rRNA gene for DNA sequencing. Pathogens were tested for antibiotic resistance to 5 common antibiotics using Kirby-Bauer diffusion assay.

References

Landsat imagery courtesy of NASA Goddard Space Flight Center and the U.S. Geological Survey.
Taylor CB. 1942. Bacteriology of Fresh Water: III. The Types of Bacteria Present in Lakes and Streams and Their Relationship of the Bacteria Flora of Soil. The Journal of Hygiene. 284-296
Bozo-Hurtado L, Garcia-Amado MA, Christoserdov A, Varela R, Narvaez JJ, Colwell R, Suarez P. 2013. Identification of Bacteria in Enrichment Cultures of Sulfate Reducers in the Cariaco Basin Water Column Employing Denaturing Gradient Gel Electrophoresis of 16s Ribosomal RNA Gene Fragments. Aquatic Biosystems. 9-17
Lobitz, B., Beck, L., Huq, A., Wood, B., Fuchs, G., Faruque, G., and Colwell, R. 2000. Climate and infectious disease: Use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. PNAS. 97, 1438.

Acknowledgements. This project was funded by the School of Arts and Sciences at St. John Fisher College, the National Geographic Society and the Chester F. Carlson Center for Imaging Science at the Rochester Institute of Technology.