International collaboration: tool to improve knowledge in molecular techniques to access microbial community.

Marla Sonaira Lima; Katherine McMahon; David da Motta Marques; Emily Louise Kara; Georgia Loudon Wolfe

Abstract: *Graduate student Marla Sonaira Lima visited McMahon`s Lab of Dr. Katherine McMahon at University of Wisconsin, USA, to learn and develop the technique with members of McMahon research group. The intention of the site exchange travel fellowship supported by GLEON (Global Lake Ecological Observatory Network) was to learn and adopt methods to access microbial population in surface water ecosystems, using molecular techniques as Automated Ribosomal Intergenic Spacer Analysis (ARISA) combined with Automated Phycobilin Interspacer Analysis(APISA). ARISA is used to identify bacterial population structure and APISA is used to identify Cyanobacteria population structure. These techniques were used to improve methods to access microbial community by researchers in Motta Marques` Lab in Brazil. During the international collaboration, Lima tested the use of RNA Later (preservation of DNA and RNA) in water samples for ARISA technique. Also she worked using ARISA and APISA techniques on Lake Mendota, Madison, WI, USA investigating relationships between free-living and particle-associated bacteria with Cyanobacteria.*

Introduction

Graduate student Marla Sonaira Lima from Limnology and Ecotechnology Laboratory has been investigating microbial community dynamics in surface water in Brazil, working towards a Masters degree since 2009. Motta Marques` research at Federal University of Rio Grande do Sul has been contributing to the understanding of aquatic microbial communities since 2005. The aim of this international cooperation was to learn the ARISA and APISA methods, in order to apply these techniques in a large subtropical lake Magueira, as part of Marla's dissertation, and at last to establish these techniques at the home institution after Marla's return from UW-Madison.

This internship was part of the Global Lake Ecological Observatory Network. The lab work witch involved learning of molecular fingerprints techniques as ARISA and APISA was supervised by Dr. Katherine McMahon.

Dr. Katherine McMahon is an Associated Professor of Civil and Environmental Engineering department at University of Wisconsin, Madison (UW-Madison). She is affiliate with the program Applied Microbial Ecology which program is developed in McMahon Lab.

During the 45 days of site exchange travel fellowship the graduate student has developed two kinds of studies applying the fingerprint methods. For the first two weeks, the activities carried out were related to the use of *RNA Later* - a preservative of DNA and RNA - in surface water sample for ARISA analysis. For the other three weeks, the student investigated the relationship between free-living and particle-

associated bacteria with Cyanobacteria. At the end, the graduate student presented her results to McMahon's group research.

Exchange research activities

Testing RNA Later for Arisa

The RNA later is a tissue storage reagent and rapidly permeates most tissues to stabilize and protect RNA and DNA in fresh specimens. It eliminates the need to immediately process or freeze samples, providing further analysis.

The aim of this study was to check whether there is variation between the bacterial genetic diversity technique accessed by ARISA between surface samples from Lake Mendota, treated with and without RNA Later. We hypothesized that would not have differences between each treatment.

Sample preparation-The samples were collected in a single point in Lake Mendota, during the summer, on June 21th of 2010. We collected 250ml of water for each sample, for each treatment. We did three replicates for each treatment. For the samples with the preservation were added 250ml of *RNA Later* plus 250ml of sample from the lake. The filtration was performed with a 0,2um filter, filtering 500ml of samples with RNA Later and 250ml of sample without RNA Later.

Analysis- The analysis followed the "Lake Microbial Community Analysis using Automated Ribosome Intergenic Spacer Analysis (ARISA)" protocol, available in McMahon`s Lab. To analyze the results for ARISA we used GeneMarker software to check the results of the denaturing capillary electrophoresis and make the bin, after that we used R2.11.1 software to calculate: relative abundance data, presence and absence data and the raw size data.

All statistical analyses were carried out using Primer 6. We carried out a CLUSTER using Bray-Curtis similarity, to see how the samples were hierarchically organized, them similarity percentages (SIMPER) to look for species contributions using relative abundance data and presence absence data. At last, we carried out a test of diversity looking for total of species and species richness.

Results- We found almost 50% difference in DNA quantification between samples with and without *RNA Later* (Table 1). This could mean that *RNA Later* preservation is being more efficient in less lost of quantity of DNA then samples without RNA Later that should be process very quickly after collection.

Table 1. DNA quantification of sample from surface water of Lake Mendota.

Sample ID	ng/ul
1-RNA Later	31.01
2-RNA Later	34.53
3-RNA later	33.8
1 - none RNA Later	18.4
2-none RNA Later	18.59
3-none RNA Later	16.83

The cluster (Fig.1) showed that all samples share 75% of similarities and within each group of treatment they are 85% similar.

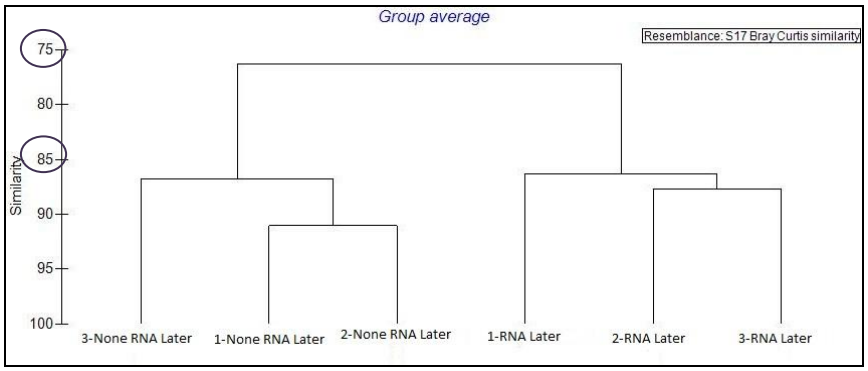

Figure 1.Cluster analysis between samples with and without RNA later.

The test looking for species contribution confirmed the cluster, showing that within each treatment samples were very similar for relative abundance and presence absence data. And between treatment samples have 23.66 % of dissimilarity of relative abundance and 14.09% dissimilarity in presence absence. Also we found three Operational Taxonomic Units (O.T.U) occurring only in samples without RNA later.

Conclusion- In terms of the relative abundance, the O.T.U varies for each treatment, but in terms of composition, number and species richness, all treatment was very similar.

Each treatment has a strong relationship within its group, but shares similarity of bacterial community together. So the use of RNA later could be very useful when the sample will take long time to be process after be collected.

Relationship between free-living and particle-associated bacteria with Cyanobacteria

Different kind of bacteria may or may not be associated with Cyanobacteria. A number of studies have shown that bacteria attached to particles may be phylogenetically different from free-living bacteria (Bidle K. et al.1995; Crump BC et al. 1999; DeLong EF et al. 1993; Rath J 1998). There may be selective forces present in particle microenvironments that select community with phylogenetic composition that differs from the composition in the surrounding water (Riemann et al. 2001).

Recently, the use of different molecular tools has supported the perception of free-living and particle-associated bacterial assemblages as metabolically and phylogenetically distinct (Riemann et al. 2001).

Hollibaugh et al. 2000 observed similarities as well as dissimilarities between the composition of free-living and particle-associated bacterial assemblages in samples collected at different locations in San Francisco Bay.

The main goal of this study was to analyze the community composition of free-living and particle-associated bacterial assemblages that occur during the course of a freshwater phytoplankton bloom. We hypothesized that would be find difference in the identity of O.T.U between those associated with clusters of Cyanobacteria and those free-living. Also those free-living bacteria may be correlated with Cyanobacteria, but this correlation does not occur with the same Cyanobacteria that the particle-

associated bacteria are associated. At last, fingerprint methods as ARISA and APISA could be used to compare dynamic between bacteria assemblages and Cyanobacteria.

Sample preparation- 10 samples were collected across a transect in the surface water in Lake Mendota, during the summer, on June 29th of 2010. For each sample, 250ml were filtered on 0.45um filter to retain bigger particles and the pre-filtrate (with small particle) were concentrated on 0.2um filter. The total of 20 filters were stored in -80^{o}C until DNA extraction. We follow the "Lake Microbial Community Analysis using ARISA protocol", available in McMahon`s Lab, to identify bacterial population structure. Also available in McMahon's Lab was the protocol of APISA to identify Cyanobacteria population structure.

Data Analysis- We carried out similarity percentages (SIMPER) test, in Primer 6, to look for species contributions, trying to find what on 0.45um filter that did not occur on 0.2um filter pre-filtered and vice-versa. Also looking for what O.T.U. contributes more for the dissimilarity. We used presence and absence data from Arisa filter 0.45um (Ar0.45) and Arisa filter 0.2 pre-filtered (Ar0.2p) to carry out those analysis.

Pearson Correlation, using relative abundance data, was used between Ar0.45 against Apisa filter 0.45um (Ap0.45), using a cut off of 0.62 one-tailed, P=0.05. As well between Ar0.2p against Ap0.45, using a threshold of 0.72, two tailed, considering for P=0.05. Then we carried out a Principal Component Analysis, using O.T.U from Arisa as species and Apisa data as environment.

Results- We found for ARISA data a total of 196 O.T.U for Ar0.45 and a total of 181 O.T.U for Ar0.2p. For APISA data we found a total of 19 O.T.U for Ap0.45 and 12 O.T.U for Ap0.2p. The simper test showed us that for each filter (Ar0.45 and Ar0.2p) exclusive species occurred: seven unique O.T.U for Ar0.45 and six unique O.T.U for Ar0.2p. The average of the relative abundance for each group represent 1.7% and 0.8% of total community respectively.

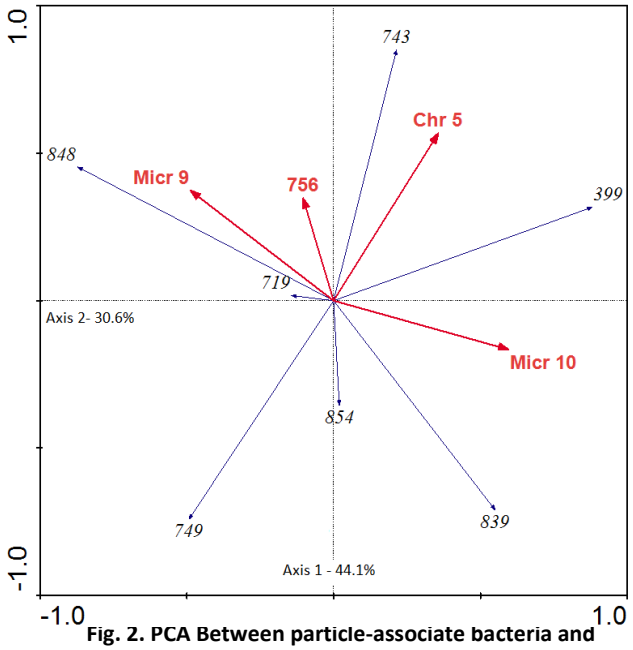

Fig. 2. PCA Between particle-associate bacteria and Cyanobacteria. Legend: In red- O.T.U of Cyanobacteria. In Blue, O.T.U of particle- associate bacteria.

The Pearson correlation between particle-associate bacteria and Cyanobacteria showed three correlations into the threshold of 0.62, P=0.05. Being two O.T.U correlate with more than one Cyanobacteria. Also we could identify three Cyanobacteria, according Miller, T. (unpublished data), two Microcystis and one Chrysophycea. For the correlation between free-living and Cyanobacteria we found three O.T.U of free-living with strong positive and/or

negative correlation into the threshold of 0.72, P=0.05. The PCA between particle-associate bacteria and Cyanobacteria (Fig. 2) shows the percentage of explanation of 44.1% in the first axis of the relation presented on the graph. Axis 2 explains 30.6% of the variation. Also, as show in Fig 3, the PCA between free-living bacteria and Cyanobacteria accounted for 73.7% of total variation.

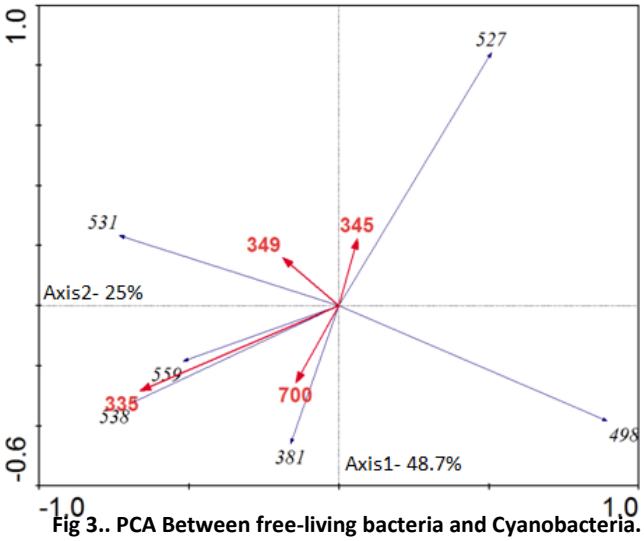

Fig 3.. PCA Between free-living bacteria and Cyanobacteria. Legend: In red- O.T.U of Cyanobacteria. In Blue, O.T.U of particle- associate bacteria.

Discussion - We were able to identify only some of the free-living and attached bacteria for whole community. For the rest of the community that we could not identify, as only free-living or only particle-associated because they occur in both kind of filter (Ar0.45 and Ar0.2p), we may suppose that this community may have a phylogenetic overlap, indicating that free-living are also members of particle-associated bacteria assemblages.

Another hypothesis is that the particle-associated bacteria lysed during filtration, passed through the filter Ar0.45, and were subsequently indentified in the filter Ar0.2p.

Also it seems that those Identified as free-living and particle-associated bacteria are correlated with different Cyanobacteria supposing that we have O.T.U with different strategy. In addition, free-living and particle-associated bacteria could be adapted to different niches.

Finally, in this work we were able to identify possible relationships between Bacteria and Cyanobacteria dynamics using both methods, even if we have obtained answers to a few percent of the community.

Conclusion about the Site Exchange

The experience as a site exchange student was very important for the graduate student Marla Lima. It helped her to improve not only her knowledge in the proposed techniques but also increased her experience with American language, academic institution and culture. With this experience the student could also develop her scientific network in her area of expertise. Also was a great opportunity to make friendship.

Acknowledgements

This work was supported in by the Global Lake Ecological Observatory Network Student Travel Fellowship. The travel fellowship has been funded by grants awarded from Gordon & Betty Moore

Foundation Award # 1182 and U.S. National Science Foundation Grants DBI-0639229. Also had the support by McMahon Laboratory at University of Wisconsin (UW-Madison).

Special thanks for Dr. Katherine McMahon that received me very well in the lab and for the opportunity. For McMahon`s research who help me with this work specially Emily Kara, Georgia Woolf, Lucas Bedersdorf and Todd Miller.

Thank you for Paul Hanson from the Center for Limnology for the opportunity. Also thanks for Marilyn Larsen, Carol Schraufnagel and Trish Haza who helped me to get all documents I needed to make this experience happened.

References

Bidle K, Fletcher M (1995) Comparison of free-living and particle-associated bacterial communities in the Chesapeake Bay by stable low-molecular-weight RNA analysis. Appl Environ Microbiol 61:944–952.

Crump BC, Armbrust EV, Baross JA (1999) Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. Appl Environ Microbiol 65:3192–3204.

DeLong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934.

Rath J, Wu KY, Herndl GJ, DeLong EF (1998) High phylogenetic diversity in a marine-snow-associated bacterial assemblage. Aquat Microb Ecol 14:262–269.

Riemann L., Windi A. (2001) Community Dynamics of Free-living and Particle- associated Bacterial Assemblages during a Freshwater Phytoplankton Bloom. Microbial Ecology 42:274-285.