**Abstract**

Our ability to research freshwater microbes has been transformed by the availability of single amplified genomes (SAGs) and metagenomes. Before these advances, we were limited to easily cultivatable microbes. Now, using differential coverage, genomes can be binned using a innovative technique from 192 metagenomes from two very different freshwater lakes. These genomes from metagenomes (GFMs) and SAGs allow for unprecedented understanding of the genomics of the lake community. In conjunction with a long-term metagenomic time series one can use these genomes to track populations, looking at how they evolve and how their populations change over time. In addition, these genomes also allow for the study of metabolic potential, giving insight into the functions these microbes may be performing in the lake. Genome streamlining, a lifestyle found to be common in ubiquitous marine microbes, can also be studied in lakes with this dataset.

**Specific Aims**

Overarching Goal: To learn about the ecology and evolution of microbes in lakes using genomics, including how populations change over time, and both metabolic potential and genomic features of lake bacteria.

**AIM 1:**

Bacterial evolution in nature has not previously been well studied because many microbes cannot be cultivated and it is nearly impossible to track natural populations on a whole genome scale. Most such efforts have compared natural populations using whole genomes from cultivated organisms isolated from a single kind of environment. There have also been a few studies that have mapped metagenomes to genomes from cultivated organisms. However, neither of these methods considers the uncultivated majority or attempts to look at the bacterial populations in a single site on a broader scale. In lakes, many of the abundant microbes have yet to be cultivated and isolated, such as the ubiquitous and abundant acI lineage of actinobacteria. Using our metagenomic time series, which spans multiple years in two very different lakes (Trout Bog and Mendota), we can use coverage based binning and sequence features to retrieve reference genomes, which are directly relevant to our dataset and not necessarily from cultivatable lineages. We also have single amplified genomes (SAGs), representing a variety of major freshwater lineages, many of which were recovered from Lake Mendota. We can map metagenomic reads back onto these genomes from metagenomes (GFMs) and onto the SAGs and in order to study the population structure and dynamics over time.

I plan to focus specifically on two cosmopolitan freshwater groups: the LD12 tribe, which is the only tribe of the alfV lineage that is found in lakes, and the acI lineage. In our controlled freshwater taxonomy vocabulary, a lineage is defined as a monophyletic group by phylogenetics within a phylum; whereas a tribe is monophyletic by phylogenetics and has ≥ 97% 16S rRNA gene sequence identity (Newton *et al.*, 2011). My approach is novel because coverage based binning techniques have only recently been developed. The metagenomic time series is also unprecedented in its length and coverage, including a total of 192 samples sequenced using Illumina HiSeq technology. To date, I have worked with collaborators at the Joint Genome Institute (JGI) on the evolutionary dynamics, specifically single nucleotide polymorphisms (SNPs) and gene gain and loss, in a smaller set of manually binned genomes, for which there is a manuscript currently in review.

**To determine the evolutionary dynamics of sequence discrete populations of bacteria in freshwater lakes, we will map reads from our metagenomic time series to composite GFMs and SAGs and examine abundance patterns, SNP patterns, gene gain and loss, and recombination.**

*H1.1 GFMs from lakes with more stable biogeochemical conditions over time, have less variation in relative abundance.*

*H1.2: As predicted due to their low diversity and low rates of recombination, the LD12 tribe experiences genome-wide sweeps over time.*

*H1.3: The acI lineage, which has much greater diversity in comparison with the LD12 tribe, undergoes gene-specific sweeps over time and has higher rates of recombination.*

*H1.4: Each population undergoes gene-specific or genome-wide sweeps when under selective pressure, depending on their rates of recombination.*

**AIM 2:**

There is little known about the functions these freshwater bacteria are performing in the environment. For example, without cultivation, experiments cannot be performed to directly evaluate substrate uptake. Microautoradiography and fluorescence *in situ* hybridization (MAR-FISH) can be used to observe which cells are taking up a radio-labeled substrate but it is a laborious and expensive experimental method. In order to reduce the number of substrates to test and to focus on key candidate lineages there must be some information known about metabolic potentials. Gene and pathway annotations/predictions in the GFMs and SAGs will provide clues for such functional potentials in specific key lineages. We can use the evolutionary patterns and population dynamics found above to target functions applying evolutionary pressures to microbes in the environment. Our lab has previous been involved in looking at functional capabilities of the acI-actinobacteria lineage using SAGs (Garcia *et al.*, 2013; Ghylin *et al.*). We can also search for temporal correlations between nutrient conditions in the lakes and relative abundances of the sequence discrete populations determined in Aim 1. Learning more about the metabolic functions that specific groups of microbes are performing in the environment can help us to better understand the biogeochemical cycling being carried out.

**To generate hypotheses about which bacterial groups are performing specific metabolic functions in the community, we will characterize the functional potential of bacteria using GFMs and SAGs.**

**AIM 3:**

Genome streamlining has been observed in freshwater and marine settings but little is understood about the features that make this lifestyle successful in aquatic environments. As previously mentioned, freshwater systems have very few reference genomes and many of those previously studied were cultivatable. It has also been suggested that previous difficulty in culturing these bacteria may be due to their streamlined nature(Giovannoni *et al.*, 2005). Furthermore, the genomes from cultivatable bacteria may not be representative of those microbes that are most common or abundant. To learn if streamlined genomes are common among freshwater microbes, we need to analyze the genomes from uncultivated organisms. We propose to characterize the genome features of the same GFMs and SAGs from Aims 1 and 2 to find evidence and possible mechanisms of genome streamlining. This substantial data set provides the opportunity to compare the whole genomes, many which may be streamlined. We can also observe if streamlined genomes are more common among abundant bacteria in the lake, and discover features that are different between streamlined and non-streamlined genomes from the same lake. Analysis of genome streamlining will help us to know how important genome reduction is for successful populations in aquatic environments. This work will improve our understanding of genomic streamlining as a microbial lifestyle and how it functions in a community setting.

**To determine if genome streamlining is a general characteristic of abundant freshwater bacteria and is predicted by certain genomic features, we will search for these features in GFMs and SAGs from two different lakes.**

*H3.1: As genome streamlining increases, the portion of genes allowing for a diversity of carbon substrate utilization decreases*

*H3.2: Genome streamlining correlates negatively with growth rate.*

*H3.3: As genome streamlining increases, the portion of genes associated with motility or signal transduction decreases.*

*H3.4: Streamlined genomes are traits of highly successful, abundant freshwater microbes.*

**Background and Significance**

The identification and study of microbes has long depended on our ability to culture them. However many microbes are not amenable to growing in the lab environment(Amann *et al.*, 1995). The development of molecular techniques, such as high throughput sequencing and genomics, has enabled us to start to studying which microbes reside in an environment and what they are doing. Many approaches have allowed scientists to peek into the uncultivated world, such as 16S rRNA gene sequencing, metagenomics, and sequencing of single cells. Each method has different advantages and disadvantages, but can be used to answer specific questions about the microbes in an environment.

In freshwater lakes, the 16S gene has often been used to determine which microbes are in the lake. This method has been used to define the taxonomy of bacteria commonly found in lakes and to develop a highly curated 16S database(Newton *et al.*, 2011). While this approach, with a good reference database, can help to answer the question of which microbes inhabit the lake, it does not inform what genomic features or genes these microbes may have. As this method uses a conserved marker, it does not capture more recent evolutionary trends such as horizontal gene transfer and mutations in genes under evolutionary pressures.

Another tool that can yield genomic information about the uncultured microbes is metagenomics (Gilbert & Dupont, 2011). This method involves extracting all DNA from lake water samples and sequencing it. One limitation of this technique is that it is difficult to track back the reads or even assembled contigs to individual organisms or closely related populations. A recently developed technique uses differential coverage to separate out contigs that belong to the same organism(Wrighton *et al.*, 2012; Albertsen *et al.*, 2013). This method requires two or more different metagenomes from the same ecosystem. One such method was used to separate out 49 draft genomes from an acetate amended aquifer using three metagenomes collected from different times(Wrighton *et al.*, 2012). From these genomes, metabolic potential was inferred from annotated genes. Another such study used the differential coverage as the first metric for binning genomes from an activated sludge bioreactor(Albertsen *et al.*, 2013), where tetranucleotide was used first in Wrighton et al. In the activated sludge example, the bins were refined using tetranucleotide frequency, GC content, length, and single copy gene analysis. While both of the previous examples represent less complex microbial communities than those found in lakes, the lake community is a good next test set as compared to complex communities such as those found in soil. However, one must keep in mind that with more complex systems, assembly becomes more difficulty and rare populations may be missed due to insufficient sequencing coverage.

Another recently developed technique for investigating the genomes of uncultured organisms is whole genome amplification from single cells. Each cell is sorted by fluorescent activated cell sorting, lysed, amplified with whole genome amplification, sequenced and then assembled. From this process, single amplified genomes, SAGs are created. The genes are then predicted and annotated using standard computational genome analysis methods (Woyke *et al.*, 2009). This method was applied to produce 201 SAGs from never before sequenced lineages (Rinke *et al.*, 2013). These phyla had been known to exist in nature due to 16S sequencing but have never been isolated. These genomes markedly expand the reference database to include these phyla and resolve broader evolutionary patterns of related phyla.

SAGs can also be used in conjunction with metagenomic reads to track populations. Two SAGs from marine flavobacteria were used as references for metagenome sequences from the Global Ocean Sampling (GOS) expedition (Woyke *et al.*, 2009). Each read from the metagenome is checked to see if it matches against the reference and is considered recruited if it matches above a specified identity threshold. These SAGs recruited reads from the GOS expedition much better than cultured marine flavobacteria. The authors recruited reads from different GOS sites and saw that the populations of flavobacteria closely related to these SAGs were likely dispersed by a known ocean current. This study is an example of how important it is to consider uncultured organisms and to have genome references that are relevant to the environment in question. It is also an example of tracking populations using SAGs as references for metagenomes.

One important limitation with SAGs is that the whole genome amplification process has random bias and can miss whole sections of the genomes. A common method to estimate how complete these genomes are uses single copy conserved genes shared among bacteria or a particular bacterial lineage (Rinke *et al.*, 2013; Albertsen *et al.*, 2013; Garcia *et al.*, 2013).

Bacterial diversification and speciation is of great interest in microbial ecology (Cordero & Polz, 2014). The ecotype model is one popular theory proposed to explain bacterial speciation in nature (Cohan & Perry, 2007). However, support for this theory comes only from models and inference since direct observations in support of it were not available (Chan *et al.*). Our collaborators at JGI have manually binned several genomes, based on tetranucleotide, phylogenetic gene distribution, and differential coverage, from the Trout Bog hypolimnion metagenomes (Chan *et al.*). They then mapped the metagenome reads back to the genomes and identified single nucleotide polymorphisms (SNPs). All of the SNPs in the genomes they explored went to fixation, genome-wide over three years. This provides direct evidence for the ecotype model of speciation, which predicts genome-wide sweeps due to low rates of recombination.

Little is known about what functions bacteria are performing in nature. This is especially true for microbes that cannot be cultured, since experiments cannot be performed to test for functions. To find possible bacterial functions in nature, there have been a number of studies characterizing metabolic potential from annotated genomes. One influential paper, characterizes the functional potential of 32 marine isolates from the lineage Roseobacter and was authored by a former member of the McMahon Lab (Newton *et al.*, 2010). This study systematically characterizes these genomes and their functional potential. A similar functional characterization has been done for our SAGs from the acI lineage (Garcia *et al.*, 2013; Ghylin *et al.*).

Experimental evidence for lineage-specific traits has also been generated for some freshwater groups. To directly test for substrate uptake some have used microautoradiography and fluorescence *in situ* hybridization (MAR-FISH) to evaluate incorperation of 14 radiolabeled dissolved organic compounds in 30 common freshwater bacterial groups at different levels of taxonomic resolution(Salcher *et al.*, 2013). This represents great strides in what is known about bacterial functions in the lake but is limited by the small number of substrates could be tested and by known FISH-defined phylogenetic groups.

Genome streamlining may be a key factor in why many of the microbes in the lake are so difficult to culture (Giovannoni *et al.*, 2014) and may also contribute to the success of common and abundant lineages. This feature has also been observed in endosymbionts and in the ubiquitous marine bacterium SAR11 (Grote *et al.*, 2012; Viklund *et al.*, 2012; Giovannoni *et al.*, 2005). One of the first freshwater bacterial genomes to be published was that of the streamlined acI-B1 tribe (Garcia *et al.*, 2013). In another study, which published a number of genomes from freshwater isolates, the genomes were compared and a variety of lifestyles were predicted based on growth rate, carbon usage, and signal transduction and motility genes (Livermore *et al.*, 2013).

**Preliminary Results**

*Genomes from Metagenomes (GFMs)*

While at JGI, I worked with collaborators who were developing a program called Metabat to bin genomes from metagenomes. Their program uses differential coverage across the 46 metagenomes for the epilimnion of Trout Bog, the 47 metagenomes from the hypolimnion of Trout Bog, and the 97 metagenomes of Lake Mendota to bin together contigs. It also takes into account sequence features such as k-mer frequency when binning. The bins are statistically selected by the program and require no manual intervention to choose bins as is done with other methods. From this program, I recovered 87 GFMs from Trout Bog epilimnion, 167 GFMs from Trout Bog hypolimnion, and 502 GFMs from Lake Mendota. I then filtered the output to include only genomes which were 50% complete by single copy conserved genes found in 90% of all bacteria, and for genomes where 90% of those single copy genes were unique. After this filtering, there are 36, 70, and 104 GFMs from Trout Bog epilimnion, Trout Bog hypolimnion, and Lake Mendota, respectively.

In order to classify these genomes, I used a program called Phylosift (Darling *et al.*, 2014). It finds 37 conserved evolutionary marker genes using hidden markov models, aligns them, and gives a probability of classification for each maker gene. It is designed to be used on metagenomes and give the user an idea of which organisms are in a metagenome by each marker. I created a parsing program in python to take the probabilities and classifications for each marker gene and interpret their results as from one organism. My program gives a classification based on a probability cutoff and a percent matching cutoff. At each level of Linnaean classification, it removes any hits below the probability cutoff and then if the hits left match above the percent matching threshold, it will save that classification and proceed to the next Linnaean classification level. I used 100%, 90%, and 80% for each cutoff in all possible combinations. Some examples of such classifications from Lake Mendota are shown in Table 1.



Most genomes are not classified to the next level because it did not match above the matching percentage cutoff. However, some genomes, such as those classified to the cyanobacteria phylum, do not proceed to the more refined levels because their classification in the NCBI database does not follow the Linnaean structure. They are missing several levels and contain some levels of “no rank”. This is a downside to my program since it requires each Linnaean level and matching at that level to proceed to the next. However, this was necessary because otherwise one genome could have a classification where, for example, the genus did not belong to the order. For organisms of interest that were not sufficiently classified by this system, we can always go back and manually find the classification. Table 2 is a summary of how many genomes were classified to phylum and class and the number of phyla and classes represented from each lake/layer.



Shown in Table 3 is the breakdown of the number of genomes classified for a phylum by lake and layer. Though there are a number of phyla only represented in one of the two lakes, most of them are very low in numbers and could be due to difference in assembly between the lakes. There are also a number of phyla only represented in the hypolimnion of Trout Bog, but these too could be difference due to assembly. The two phyla that have several genomes represented from Lake Mendota and none from Trout Bog include cyanobacteria and planctomycetes. The presence of cyanobacteria in Lake Mendota and not in Trout Bog is unsurprising since the latter is a dystrophic lake and therefore is darkly stained and allows for little light penetration. This is also unsurprising since Lake Mendota is eutrophic and is known to have cyanobacterial blooms throughout the summer (Beversdorf *et al.*, 2013). Also, from a preliminary analysis of our unpublished 16S data for both of these lakes, cyanobacteria have 6x the relative abundance in Lake Mendota than in Trout Bog. However, planctomycetes is relatively the same portion of reads in the 16S data for both Lake Mendota and Trout Bog. Possible explanations include that planctomycetes did not assemble from Trout Bog due to differences in diversity, or that the differential coverage signal was not strong enough to get GFMs binned. Both of these explanations highlight that these GFMs may not capture all the populations in the lakes.

*AcI evolution*

 In collaboration with a postdoc in the McMahon lab, Dr. Sarahi Garcia, I have been working on an evolutionary analysis of SAGs from the acI lineage of actinobacteria. The acI lineage is ubiquitous and often abundant member of freshwater lakes (Garcia *et al.*, 2013). We analyzed 14 genomes from this lineage, which represent 5 of the 13 tribes as defined by 97% 16S similarity. Figure 1 shows a tree I made with RAxML (Stamatakis, 2014) from an alignment of 400 conserved single copy genes made by PhyloPhlAn (Segata *et al.*, 2013), which also resolves the genomes into the tribes previously defined by 16S similarity. I first used BLASTP and MCL to cluster orthologous genes. We hoped to use this strategy to determine the core genome of the acI lineage and the core for some of the tribes within this lineage.

However, we encountered a problem determining which genes were shared among all members of the lineage since the SAGs are varying levels of completeness. A current project, which developed out of this issue, is to determine if each cluster of orthologous genes is core for a group, using the completion estimates for the genomes in the group. I am currently working on this project in collaboration with PhD student Ben Oyserman in the McMahon lab. For the core determination and genome completion project I have developed a script to randomly delete portions of the genome to simulate the missing portions of a SAG. This program takes a complete genome, the percentage to delete, and a block size to delete. It then deletes that block size when possible or the number of bases left to be deleted when there is less than a block left to delete. It will finish when it has deleted the number of bases that make up the percentage you entered. Next, we hope to test this method on a simulated set of SAGs and a test set of SAGs from JGI.



To continue on the acI project, Dr. Garcia and I decided to look at tracking these populations in nature by mapping our metagenomic reads back to the SAGs. I wrote a script to automate the process of running BLAST for each SAG against all of the metagenomes. We also proved to ourselves how the 95% average nucleotide identity(ANI) cutoff would track each SAG by shredding the SAGs and using BLAST to map these simulated metagenomes back to the most compete SAG from each tribe. Once we proved the cutoff, I filtered out BLAST hits below the 95% ANI limit from the recruitment of metagenome reads to SAGs. I then tracked relative abundance across the strains (Figure 2). I developed a script to parse the BLAST results and calculate the ANI of all the hits and the relative abundance. I also calculated the 16S similarity and wrote a program to find average amino acid identity(AAI) for comparing the genomes to one another, see Figure 3. This experience will help me for tracking the populations of other SAGs and GFMs using the metagenomic reads.



**Experimental Plan**

**Aim 1:**

**To determine the evolutionary dynamics of sequence discrete populations of bacteria in freshwater lakes, we will map reads from our metagenomic time series to composite GFMs and SAGs and examine abundance patterns, SNP patterns, gene gain and loss, and recombination.**

I will start by identifying GFMs and SAGs (henceforth referred to as genomes when both are used) with interesting abundance patterns based on the metagenomic mapping already done by our collaborators at JGI. Reads will be mapped to each genome at 95% sequence identity as this has previously been shown to correspond to sequence discrete populations when mapping metagenomic reads to reference strains (Caro-Quintero & Konstantinidis, 2012; Chan *et al.*). However, I will test a subset of GFMs and all SAGs to check that this generally holds true across this dataset. The term population will be used to mean sequence discrete population for the remainder of this paper. Relative abundance for all the populations will be calculated using the coverage normalized by the size of the metagenome and size of the genome. The relative abundance inferred from the mapping is crucial for identifying if the bacterial populations are **persistent/transient** or **abundant/rare** in the lake. A population will be considered **abundant** or **rare** by its mean relative abundance across all time points. A ****population of bacteria will be considered **persistent** or **transient** by thecoefficient of variation (CV), which is standard deviation of relative abundance across all time points standardized by mean. The term **persistent** will mean a population found be constant in abundance across time; where the term **transient** will be applied to populations with large variation in abundance across time. Once I have calculated both the mean and coefficient of variation (CV) for each population, I will be able to assign cutoffs relevant to this dataset. An example plot of mean verses CV is shown in Figure 5 and the quadrants are labeled as **persistent/transient** and **abundant/rare.**

I will also use statistical inference to see if any of the abundance profiles vary predictably by season, biogeochemical conditions of the lake, or relative abundance of another population. One might expect that the GFMs that are transient have some dependence that allows for blooms or causes the decline in population. Some possible causes for such blooms and busts include changes in biogeochemical conditions, changes in another population’s relative abundance, or an increase in predation such as a population specific phage. With our dataset, we are unable to test the predation aspect. However, we can test dependence on biogeochemical conditions or the relative abundance of another population. The expectation of dependence is reversed for a persistent community member, which should tolerate a variety of lake conditions.

Since the two lakes in question are very different in their overall composition, one would also expect that the variation in average GFM relative abundance over time might be different for these two lakes. In fact, preliminary abundance profiles for the GFMs from the two lakes have different patterns when coverage cutoffs are applied across samples. GFMs from Mendota generally have fewer time points that meet the coverage cutoff. A possible explanation for the variation in relative abundances for the GFMs is that a more biogeochemical stable lake has a more stable composition of GFMs (Hypothesis1.1).

We can also search for genomes with interesting SNP patterns, as these have already been identified and quantified across time by our collaborators at JGI. I intend to look at the difference in numbers of SNPs between populations and the changes over time. Some possible patterns of intrapopulation variation, based on evolutionary models, are gene-specific or genome-wide sweeps. Sweep generally is defined as when all the SNPs in a population, either in a gene or whole genome, become the same variant over time. Populations experiencing rates high recombination compared to selective pressure would show gene-specific sweeps; where an advantageous gene variant sweeps the population. This is opposed to the ecotype model where genome wide sweeps occur with a higher selective pressure and lower rates of recombination since recombination cannot act to spread an advantage through the population in time. I will look for gene-specific or genome-wide sweeps in the persistent populations, as these metrics do not apply well to transient populations.

For the transient populations, we can look to see if different or the same variants are dominant for each bloom. I will look for this by examining the SNP patterns for transient populations. In Lake Mendota, I expect to find cyanobacteria in the transient group of genomes, as we know the cyanobacteria undergo blooms throughout the summer (Beversdorf *et al.*, 2013). I will be interested to see how the different genera of cyanobacteria bloom over the summer. I will search for the phycocyanin gene in these GFMs since it can be used refine classifications for cyanobacteria (Miller *et al.*, 2013) I will also look for toxin genes which are known to be found in particular genera of cyanobacteria.

In order to look at the gene gain and loss patterns over time, the genes must be called and annotated for all the genomes. The GFMs are currently submitted for annotation in the JGI pipeline and are awaiting annotation. With annotations in hand, I can look for gene gain and loss over time using the metagenomes mapped to the genomes at 95% sequence ID and using methods similar to those used by or collaborators at JGI with the manually binned genomes (Chan *et al.*). This method normalizes the coverage by the gene length and excludes coding regions shorter than 450 bp. To find the copy number of each gene per cell, I will divide the coverage of each gene by the average coverage for all the other genes in the genome, as was done previously. Genes will be considered lost from the genome if they rise above or below a certain threshold over all the time periods and are considered significant by the Metastats software (White *et al.*, 2009)

I also intend to look at recombination for these genomes in the environment. I will use a method for looking at the recombination of these populations, which also uses the metagenomic mapping. I will use this method to test the levels of recombination within these populations. Previous studies have shown that the lineage acI has much larger diversity in lakes than the tribe LD12 (Newton *et al.*, 2011). Due to this difference, I expect that acI will show higher rates of recombination and gene-specific sweeps where the LD12 tribe will show lower rates for recombination, as was found previously seen (Zaremba-Niedzwiedzka *et al.*, 2013), and genome-wide sweeps (Hypothesis1.2, Hypothesis1.3). I also expect that this trend will apply to the other populations studied (Hypothesis1.4).

**Aim 2:**

**To generate hypotheses about which bacterial groups are performing specific metabolic functions in the community, we will characterize the functional potential of bacteria using GFMs and SAGs.**

To make searching for metabolic potential in such a large number of genomes feasible we need to develop a systematic way of looking at these genomes. Currently Shaomei He, a scientist in the McMahon lab, is developing a scheme to do this. The method will be based on the system JGI’s IMG uses to assess functional pathways and infer phenotypes (Chen *et al.*, 2013). The process will involve looking at multiple annotations for functions and finding a consensus among the systems.

Once a process is developed to methodically classify metabolic potential in genomes, I can then apply this system to the GFMs and SAGs. I plan on first identifying metabolic potential for genomes in aim 1 that showed interesting gene gain or loss patterns. I will also focus on specific phyla of interest to comparing the different GFMs to see if there is any pattern among the larger evolutionary group.

I will also use a transporter specific process to identify uptake potential. I will use the TCDB database (Saier *et al.*, 2014) to find transporters for the substrates tested with MAR-FISH (Salcher *et al.*, 2013). I will use BioV collection of scripts developed to work with the TCDB database to find homologs to known transporters in the GFMs and SAGs from groups studied by MAR-FISH. I will first specifically search for transporters that could perform the substrate uptake found by MAR-FISH. I can also use this method to determine if all bacteria in the groups tested take up the substrate or just a subset. I will also use the TCDB database to search for homologs to known transporters in GFMs and SAGs not tested in previous studies. These predictions can be used in conjunction with the metabolic potential predictions as a starting point for designing further MAR-FISH experiments.

Once the metabolic functions and transporters are predicted for the GFMs and SAGs, we can then look for functions specific to one group. Functions predicted to be found in specified groups will be the basis for MAR-FISH experiments. Currently Alex Linz, a MDTP student in the McMahon lab, is working on learning MAR-FISH and getting the lab set up for running these types of experiments.

**Aim 3:**

**To determine if genome streamlining is a general characteristic of abundant freshwater bacteria and is predicted by certain genomic features, we will search for these features in GFMs and SAGs from two different lakes.**

Genomes will be considered more streamlined if they are both smaller and have a smaller proportion of intergenic spacer DNA to coding DNA. Each GFM and SAG will be evaluated by these criteria. Based on previous characterization of lake genomes, we expect certain genomic features may separate streamlined genomes from non-streamlined genomes (Livermore *et al.*, 2013). I will characterize these factors for the GFMs and the SAGs. The three features previously found to differentiate bacterial lifestyles in lakes are diversity of carbon substrate usage, growth rate as predicted by codon bias, and proportion of signal transduction and motility genes in the genome.

With regards to carbon substrate utilization, I will use the carbon usage predicted for these genomes in aim 2. I will then cluster the results to see if the pattern of carbon substrate utilization groups by genome streamlining. I expect that the diversity of carbon substrate utilization will decrease as genome streamlining increases (Hypothesis3.1). This can also be done with the other functions predicted in aim 2 to see if any other functions correlate with streamlining.

The second dimension that I will characterize is predicted growth rate. Since codon bias was found to be an accurate single predictor of growth rate, I will use it to calculate predicted growth rate (Vieira-Silva & Rocha, 2010). I will create a program to calculate codon bias and growth rate when given a genome with its genes called. I started to create a similar program to calculate codon bias during my rotation in the Vetsigian lab. I will add the additional function of growth rate estimation using the method developed by Vieira-Silva et al. I will plot both streamlining values against growth rate to see how these relate. I expect that the growth rate will negatively correlate with streamlining (Hypothesis3.2), as the genome becomes more streamlined the growth rate decreases.

The final previously characterized dimension is that I will search for in these genomes is the proportion of signal transduction and motility genes for each of these genomes. I will use signal transduction and motility groups from the Cluster of Orthologous Genes (Tatusov *et al.*, 2000) assignments within IMG. I will normalize by the total number of genes with COG assignments. I plan to plot these values against the genome streamlining values to see how these correlate, if at all. I expect that genome streamlining will correlate negatively with proportion of genes assigned to the motility and signal transduction COGs, as streamlining increases the proportion of genes for motility and signal transduction decreases (Hypothesis3.3).

Based on previous calculations of effective genome size using metagenomes, I expect that genome streamlining is common among abundant freshwater bacteria (Hypothesis3.4). In previous calculations of effective genome size, both Lake Mendota and Trout Bog, had effective genome sizes of less than 3 megabases (Eiler *et al.*, 2013). To test this hypothesis, I will see if the abundance categorizations from aim 1 correlate with genome streamlining.

**Timetable**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Year | 2nd | 3rd | 3rd | 3rd | 4th | 4th | 4th | 5th | 5th |
| Season | Su | Fa | Sp | Su | Fa | Sp | Su | Fa | Sp |
| AIM 1 |  |  |  |  |  |  |  |  |  |
| Rel. Abund. |  |  |  |  |  |  |  |  |  |
| SNP patterns |  |  |  |  |  |  |  |  |  |
| Gene gain/loss |  |  |  |  |  |  |  |  |  |
| Recombination |  |  |  |  |  |  |  |  |  |
| AIM2 |  |  |  |  |  |  |  |  |  |
| Metabolic Potential |  |  |  |  |  |  |  |  |  |
| AIM 3 |  |  |  |  |  |  |  |  |  |
| Carbon Diversity |  |  |  |  |  |  |  |  |  |
| Growth Rate |  |  |  |  |  |  |  |  |  |
| Sign. Trans. + Mot. |  |  |  |  |  |  |  |  |  |
| Other features |  |  |  |  |  |  |  |  |  |
| Paper Writing |  |  |  |  |  |  |  |  |  |
| Mentoring |  |  |  |  |  |  |  |  |  |
| Conferences | ISME |  |  |  |  |  | ISME |  |  |

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