

***Analysis of Bacterial, archaeal, and viral dispersal between distantly separated acid mine drainage systems through metagenomic analysis***

**Keywords:** Acid mine drainage, metagenomics, biogeography

**Background**

Acid mine drainage (AMD) is a serious mining-related environmental problem that causes acidification and metal contamination of waters and rivers. The exposure of sulfide minerals such as pyrite to oxygen and water produces sulfuric acid and releases heavy metals into the draining waters (1). It has been shown that microorganisms significantly contribute to this process by catalyzing the limiting step of the involved reaction (1, 2). **I propose to study the rates of dispersal of AMD microorganisms, which will provide insight into understanding the processes that initiate and accelerate AMD formation.**

Microbial communities found in AMD systems are ideal samples to study due to their low diversity and complexity. The most abundant organisms include *Leptospirillum* group II, *Leptospirillum* group III, *Acidithiobacillus ferrooxidans* and *Ferroplasma acidamarinus* (3).

AMD has been widely reported in several countries. The Richmond Mine at Iron Mountain, California, USA is an unusually well studied AMD system (e.g., 2, 3, 4). Metagenomic analysis of microbial biofilms from the Richmond Mine allowed reconstruction of near complete genomes of *Leptospirillum* group II (4) and *Leptospirillum* group III (5), among other organisms. On the other side of the equator, Chile is one of the biggest producers of copper in the world, and, due to extensive mining activity, AMD has been found in several abandoned mines along the country. **I propose to compare AMD microbial communities at North and South American sites as a function of their geographical separation, using genomics of total community DNA, i.e., metagenomics.**

It has been reviewed that diversity of microorganisms correlates with distance separation (biogeography) (6). Because AMD organisms are adapted to extreme environments that are separated by long distances, their rates of migration are limited (7). **I propose to determine the biogeographic and evolutionary dynamics that can account for strain diversity in the mentioned AMD systems.** More specifically, I will determine whether diversification occurred due to contemporary environmental conditions or due to geographical separation, or whether both factors are responsible. This information will provide insights into the rates at which bacteria and archaea responsible for AMD colonize new exposed sites.

Prior studies have indicated that similar AMD environments with similar geochemical conditions tend to have similar microbial communities (8, 9). The objective of this proposal, therefore, is to study strain diversification of the most abundant members of these communities. Thus I hypothesize that:

***1) Any two strains of a single archaeal species from two different sites will be more evolutionary distant than a pair of bacterial strains from the same two sites.***

This hypothesis is based on the concept that opportunities for dispersal of acid-adapted microorganisms will be limited (7). Therefore, bacterial and archaeal populations from distantly separated sites should be distinct at the strain level. Archaeal dispersal is more difficult due to the lack of cell wall, leading to more phylogenetic diversity than that of coexisting bacteria, which would disperse more easily.

**2) AMD virus populations will be generally similar at all sites, despite likely rapid evolutionary rates, due to their efficient rates of dispersal.**

This hypothesis is based on findings that viral populations in other systems are similar at sites separated far apart (10).

To test these hypotheses, I propose to sample and analyze microbial communities found in AMD in Chile. Results will be correlated with metagenomic data from the Richmond mine in California available at Dr. Banfield's lab. Access to sites in Chile will be arranged in collaboration with Drs. David Homes and Raquel Quatrini, from the Millenium Institute in Santiago, and Dr. Cecilia Demergasso from the Northern Catholic University in Antofagasta, Chile. In collaboration with these labs and UC Berkeley, I will apply at the Joint Genome Institute (JGI) for a community-sequencing project. I will collect samples from at least 5 AMD sites in Chile for screening. Initial identification and abundance of organisms will be done using fluorescent *in situ* hybridization (FISH), a technique I have worked with extensively in the past. I will also do 16S rDNA clone libraries in order to determine initial phylogenetic relationships. I will select two samples that share the majority of species for metagenomic analysis.

I will request a total of 70 Mb of metagenomic sequence from JGI. Since complete or near-complete genomes of AMD organisms are available, I will use these genomes to assign DNA fragments to specific organisms. I will assemble genomes using software that our lab has extensive experience with. Then, I will analyze sequence variation within sites and between sites in order to: i) determine whether there is more or less variation *within* the populations than *between* them; ii) quantify the extent of diversity within populations of each organism, and iii) for the virus datasets, to determine whether there is evidence for closely related virus populations at the different sites. In summary, I will do a comprehensive genomic and phylogenetic study between microorganisms found in AMD from the US and Chile.

My research experience working at Dr. Banfield's lab, and completing an undergraduate thesis, has well prepared me to do this kind of analysis. Being a coauthor in two publications also demonstrates my ability to work as part of a team. **No existing community research project is similar to my proposed work**, therefore, this will be a big step in understanding the rates of evolution of microorganisms found in AMD and their relationships within the community. **It will serve as a model for studying other microbial communities separated by long distances.** My work will be shown at national and international conferences, for both specialized and general audiences. In addition, **as a mentor for the SMASH program I will teach my work to minority high school students**, hoping to recruit them as future researchers in this field.

## References

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