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Baseline microbial characterizations of an imperiled aquatic diversity hotspot: Ash Meadows National Wildlife Refuge

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Abstract
Located in the discharge zone of the Death Valley Flow System, Ash Meadows National Wildlife Refuge is a spring-fed desert oasis and biodiversity hotspot about 90 miles northwest of Las Vegas. These critical wetlands are potentially threatened by groundwater pumping, exotic species invasions, and climate change. Although a major component of the lower food web, very little is known about the microbial makeup of this ecosystem. As a first step towards understanding the microbial and biogeochemical aspects of this system, a detailed molecular-based characterization of microbial communities, baseline chemistry, and physical characteristics of various springs of Ash Meadows will be conducted over the summer of 2009. Specifically, springs will be compared using DNA extraction followed by PCR amplification of the 16s rRNA gene, DNA fingerprinting, cultivation, and flow cytometric cell counting.

Baseline Microbial Characterizations of an Imperiled Aquatic Diversity Hotspot: Ash Meadows National Wildlife Refuge

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Abstract

Located in the discharge zone of the Death Valley Regional Flow System (4,5) Ash Meadows National Wildlife Refuge (10) is a spring-fed desert oasis about 90 miles northwest of Las Vegas. These critical wetlands are in constant threat from human water pumping and natural climate change. Although a major component of the lower food web, very little is known about the microbial makeup of this system. Here we propose a detailed molecular-based characterization of microbial communities at the various springs of Ash Meadows to obtain a basis for understanding future changes due to climate change and/or water allocation.

Freshwater and aquatic ecosystems are facing increasing anthropogenic pressures worldwide, especially in arid regions where we risk losing unique aquatic habitats without even knowing the nature and extent of their biodiversity (3). Ash Meadows provides an example of desert oases that are now extremely uncommon in the southwestern United States (10). Ash Meadows National Wildlife Refuge contains over 100 plant and animal species that are classified as state-protected, sensitive, or priority species. (11) It also supports 25 endemic species, twelve of which are currently listed as threatened or endangered: the greatest concentration in the United States (9) and second only to Quatro Cienegas in Mexico (3). Thus, reports indicating that the Southwestern U.S. is likely to experience disproportionately large impacts from global change: increased temperatures and decreased precipitation (2) are cause for concern. In addition to natural threats, Deacon et al. (1) reported that 102 percent of perennial yield of this system is already allocated anc other entities (e.g. SNWA) hope to obtain additional water rights. This paper followed several research models, all predicting that increase withdrawals would have an adverse affect on all the ecosystems supported by the groundwater aquifer. Despite the importance of this site to wildlife, only minimal microbial/microalgal and biogeochemical characterization has been performed to date (6). Thus, to better understand the organisms and processes at the base of the Ash Meadows food web we propose a detailed molecular-based characterization of its aquatic microbial communities.

Objectives

- Determine if microbial communities and aquatic chemistry emanating from spring throats is similar and resemble that from nearby deep wells
- Verify the hypothesis that photosynthetic microbial communities (e.g. cyanobacteria) will be spring-specific due to speciation/community selection driven by isolation.
- Begin to develop a baseline dataset for Ash Meadows of aquatic chemistry and physical measurements.
- Attempt to identify the factors (e.g. nutrients) which control microbial growth in Ash Meadows springs.

Methods

Samples were taken June 6, 2009 as well as July 9, 2009. New DNA samples were collected using a Masterflex portable peristaltic pump and 0.2 µm membrane filters (Super polysulfone, Pall) (8). These samples were then stored at -80°C. Samples from spring throats were obtained by inserting a tube sterilized with a 10% hydrogen peroxide solution. Aquatic chemistry measurements (nutrient, major anions, cations, dissolved organic C, phosphous) were performed at the DRI water laboratory in Reno, NV. Physical measurements (temperature, dO2, conductivity, redox, turbidity) were made using a YSI multiprobe available in the Moser lab. Cell counts were determined for all samples using an Advanced Analytical MicroPro 3000 flow cytometer. DNA was extracted from the filters and benthic samples (DNA Extraction kit, MoBio) and 16S rRNA genes obtained by PCR amplification and cloning (TA cloning, Invitrogen) (7, 8). Terminal restriction length polymorphism (T-RFLP) analysis was performed by the Nevada Genomics Center, Reno, NV. DNA sequencing was performed at Functional Biosciences, Madison, WI and individual reads were merged using Sequencher software. Related organisms were identified using public databases (e.g. NCBI, RDP). ARB and MEGA software were used for sequence management and phylogenetic analysis.

Results

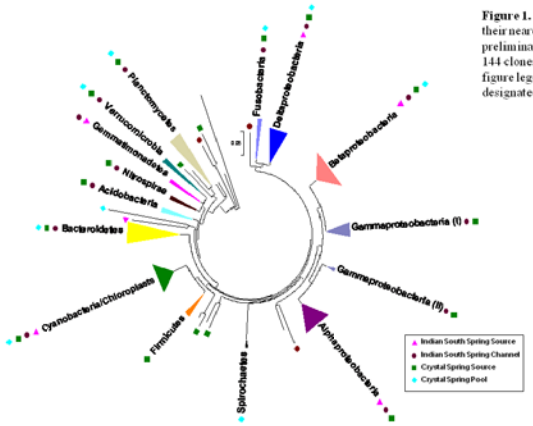


Figure 1. Phylogenetic relationship of clones from Ash Meadows and their nearest neighbor isolates (determined by Greengenes). The preliminary Neighbor-Joining tree indicates topology and represents 144 clones from 4 libraries generated from the sites indicated in the figure legend. Clones not associated with phylum level groups were designated unclassified (see figure 3).

Figure 2. A map of the springs at Ash Meadows

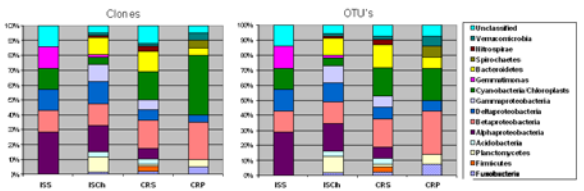
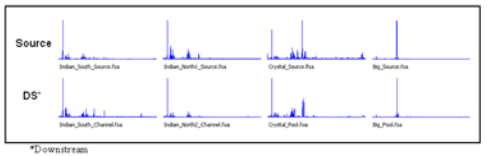


Figure 3. Diversity of Ash Meadows clones at the phylum level. Charts depict per phylum the number of clones and OTUs as a percentage of the total per library. This represents the diversity and relative abundance of phyla within the clone libraries and the diversity of clones (e.g., how many different OTUs) within the individual phyla, respectively.

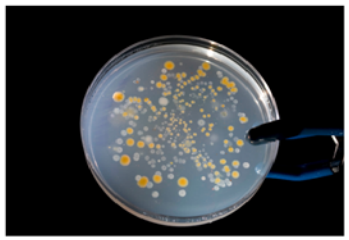


*Downstream

Figure 4. T-RFLP profiles generated from nucleic acids extracted from various springs of Ash Meadows. The profiles demonstrate variations in community structure between a spring's source and its "downstream" (DS) environment as indicated by the different peaks. Downstream was characterized as either the pool (Crystal Spring and Big Spring) or the channel (Indian North Spring and Indian South Spring) resulting from the source.

Figure 5. (Left) Big Spring

Figure 6. (Right) 100µL of water taken from Crystal Spring Pool was plated on ½ R2A media.



Conclusions

- Phylogenetic analysis of the clone libraries suggests that community structure changes from a spring's source to its downstream waters (Figure 3).
  - Indian South Spring: More diverse downstream water
  - Crystal Spring: More diverse source water
- T-RFLP data for the four springs sampled (Figure 4) similarly indicated changes in community structure between source and downstream water.
  - Indian South Spring and Big Spring: More diverse downstream water
  - Crystal Spring and Indian North Spring: More diverse source water

Future Work

- Continue to obtain samples from the Springs at Ash Meadows
  - Further funding for this project has been granted and sampling efforts are slated for fall 2009
- Conduct further phylogenetic analysis of clone libraries
  - Existing: Increase the number of clones analyzed
  - Future: Generate clone libraries from other springs in Ash Meadows
- Continued analysis of T-RFLP data to further elucidate community structure
  - Detailed analysis and comparison with respective clone libraries should indentify most prevalent microbes
- Perform colony PCR and phylogenetic analysis on spring derived isolates
  - Cultures have been inoculated from sampled springs

References

1. **Deacon, J.E., A. E. Williams, C. D. Williams, and J. E. Williams.** 2007. Fueling population growth in Las Vegas: How large-scale groundwater withdrawal could burn regional biodiversity. BioScience 57:688 – 698.
2. **Seager, R., M. Ting, I. Held, Y. Kushnir, J. Lu, G. Vecchi, H. P. Huang, N. Harnik, A. Leetmaa, N. C. Lau, C. Li, J. Velez, and N. Naik.** 2007. Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316:1181-4.
3. **Souza, V., L. Espinosa-Asuar, A. E. Escalante, L. E. Eguiarte, J. Farmer, L. Forney, L. Lloret, J. M. Rodriguez-Martinez, X. Soberon, R. Dirzo, and J. J. Elser.** 2006. An endangered oasis of aquatic microbial biodiversity in the Chihuahuan desert. Proc. Natl. Acad. Sci. USA 103:6565-70.
4. **Winograd, I. J.** 2001. Interbasin groundwater flow in south central Nevada: A further comment on the discussion between Dansgaard et al. (1999a, 1999b) and Thomas (1999). Water Resources Res. 37:431-433.
5. **Winograd, I. J., and F. N. Robertson.** 1982. Deep oxygenated groundwater - anomaly or common occurrence. Science 216:1227-1230.
   Stetzenbach, L. D. 1993. Identification of subsurface microorganisms at Yucca Mountain, Fourth quarterly report. DOE/NV/10872--T69.
7. **Moser, D. P., T. M. Gihring, F. J. Brockman, J. K. Fredrickson, D. L. Balkwill, M. E. Dollhopf, B. S. Lollar, L. M. Pratt, E. Boice, G. Southam, G. Wanger, B. J. Baker, S. M. Pfiffner, L. N. Lin, and T. C. Onstott.** 2005. *Desulfotomaculum* and *Methanobacterium* spp. dominate a 4- to 5-kilometer-deep fault. Appl. Environ. Microbiol. 71:8773-8783.
8. **Moser, D. P., H. W. Martin, and P. J. Boston.** 2002. Microbiological sampling in caves and mines, p. 821-835. *In* O. E. Bitton (ed.), Encyclopedia of Environmental Microbiology. John Wiley and Sons, Inc.
9. http://wildfish.montana.edu/Cases/browse_details.asp?ProjectID=82
10. http://www1.nyu.edu/projects/samples/ashmeadows/
11. Cristi Baldino, pers. Comm.

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