Thank you to the U.S. Department of Energy’s Environmental Management Nevada Program for providing the opportunity to sample a number of Underground Test Area Activity wells on the NNSS for these unique microbial studies and to provide results to rural communities in southern Nevada.
• Introduction
• Life underground and tools used to study it
• Local windows into the ‘deep biosphere’
• ‘Microbial dark matter’
• Strange tale of c. Desulforudis audaxviator
Our Labs

- Dr. Brittany Kruger, Staff Scientist
- Dr. Scott Hamilton-Brehm, Staff Scientist
- Dr. Katerina Papp, Postdoctoral Fellow
- Joshua Sackett, Ph.D. Student, University of Nevada Las Vegas (UNLV)
- Daniel Walsh, Ph.D. Student, UNLV
- Lidia Hristova, Undergraduate, UNLV
- Nicole Thomas, Undergraduate, UNLV
Today’s Biology Tool Kit

DNA – Big Data

- 16S rRNA gene ~1,500 base pairs (bp)
- 16S rRNA gene library ~50,000 sequences
- Bacterial genome ~3,000,000 bp
- Human genome ~3,000,000,000 bp
- Metagenome – millions of genes – gigabases of data
Wildfire and Soil Erodibility

Microorganisms as indicators of soil health and recovery after disturbance?

White Rock Fire Study, Bunkerville, NV

Preliminary data

Microcoleus, J. Elster
Environmental Contamination

Radionuclides

Microbial Biofilm on NNSS Volcanic Tuff

Sorption of Plutonium Against Bacterial Fractions

- No Cells
- Cellular membranes
- Exopolysaccharide ("slime")

Pseudomonas
Deep Biosphere (Part 1)

Mponeng Mine, South Africa, 2.5 miles deep
Deep Biosphere (Part 1)

Long-Term Sustainability of a High-Low, Energy-Low-Diversity Biome

T. Onstott

Environmental Genomics Reveals a Single-Species Ecosystem Within Earth

Deep Biosphere (Part 2)

Desulfiomaculum and Methanobacterium spp. Dominate a 4- to 5-Kilometer-Deep Fault


Deep Biosphere (Part 1)

Sample Collection at Evander Mine, South Africa
Based on its rod-like morphology, its apparent use of the dissimilatory sulfate reduction pathway for energy production, and because of the journey this "audax viator" (bold traveler) undertook to live in the extreme depths of the Earth, we have named this organism *Candidatus* Desulforudis audaxviator*.

“Desulforudis” = 48,200 Google hits
Deep Biosphere (Part 1)

C. Desulforudis audaxviator Predicted Lifestyle

Deep Biosphere (Part 2)

Windows Into the Death Valley Regional Flow System (DVRFS)
The DVRFS
Natural Windows - Springs

- Devils Hole Pupfish
- Crystal Spring
- Big Spring
- Ash Meadows
- Devils Hole
Manmade Windows - Boreholes

Nevares Deep Well 2, Death Valley

Down-Hole Video at 302 Feet

Down-Hole Video at 310 Feet Showing Fracture
Microbiological Sampling at the NNSS

Examples of NNSS and DVRFS sampling sites. A) ER-EC-13 being drilled, from Bill Wilborn 2011 Community Environmental Monitoring Program Presentation; B) Hotwell sampling; C) ER-EC-13 during hydrologic pumping test; D) groundwater discharge during pumping test; and E) 0.2 micron filters used for microbial sampling of groundwater.
### DVRFS Microbiology Sites

#### Key Code

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<th>Sample Name</th>
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Microbial Communities Across DVRFS
Deep Biosphere (Part 3)

The Strange Case of “Microbial Dark Matter”

• Microbial phyla with no cultured representatives
• Majority of life’s diversity
• Requires advanced tools for study

Rinke et al., 2013. Nature

Single Cell Genomics Pipeline

Rinke et al., 2013. Nature
DVRFS Microbial Dark Matter (MDM)

Full MDM work-ups

- Devils Hole
- Crystal Spring
- Cold Creek
- NNSS PM-3-1
- OV-2
- BLM-1

MDM summary Crystal Spring (42.3% MDM)

- 4 Parcubacteria (OD1)
- 25 Omnitrophica (OP3)
- 1 Gracillibacteria (BD1-5)
- 1 SPAM
- 1 Latescibacteria (WS3)
- 4 Elusimicrobia
- 8 Woesearcheota
- 1 Pacearcheota
- 1 unclassified
A MDM Hotspot - Well OV-2
**Solagigasbacteria: the ‘Lone Giant’**

- Formerly known as “SPAM”
- 6 – 8 million base pair genome
- Terrestrial only
- 16 of 19 single amplified genomes from Well OV-2

Becraft et al., Microbial Ecology, in revision
Well BLM-1
A true window into the deep biosphere of the DVRFS
Well BLM-1: Sampling and Logging

Logging Truck “Big Blue”

Caliper Tool

Discrete Sampler (Bailer)
Well BLM-1 Downhole Incubations

An attempt to study hard-rock microbial communities and their activities on subsurface fracture surfaces

Materials Suspended in Well BLM-1

- Lake Sediments (BLM1)
- HV-Dolomite (BLM1)
- Low Carbon Steel (Casing Material)
- Cartridge (PTFE)
- Dolomite (Wards)
- Calcite (Wards)
- Glass Wool

Sponges Used for Microbial Colonization
Well BLM-1 Downhole Incubations

Deployment of Materials

Retrieval of Materials
Well BLM-1 Downhole Incubations: Results

Upper: Microbial cells on calcite after in situ incubation. Lower: Examples of microbial community structure at phylum level for water (Left) and incubated sponges (middle). Lighter orange is primarily C. Desulforudis.
Culturing Microorganisms

Traditional Microbiology – Anaerobic Microbes from NNSS Subsurface

Modified from DOE/NV/-500-UC-700
New Microorganism: 
*Thermoanaerososceptrum fracticalcis*

"The hot anaerobic staff from the fractured carbonates"

- Named by local high school student
- New genus and species
- Requires complete lack of oxygen
- Thermophile (131°F) – heat-loving
- Exclusively from carbonate aquifers
- Grows only on chemical compound called fumarate
- Genome size 3.6 million bp
- ~91% genetic identity to *c. D. audaxviator*
Future Directions?

Deep Life Drilling in the Death Valley Extensional Zone

International Workshop: February 5 – 9 or March 5 – 9, 2018 at Desert Research Institute

Contact: duane.moser@dri.edu, mark.hausner@dri.edu
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Deep Life Community
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- Barb Sherwood Lollar (U Toronto)
- Tom Kieft (New Mexico Tech)
- Gordon Southam (U of Queensland)
- Mitch Sogin (Marine Biology Lab)
- Rick Collwell (Oregon State U)
- Esta Van Heerden (U of Free State)

NSF Genealogy of Life
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- Tanja Wojke (JGI)
- Jessica Jaret (JGI)
- Jon Eisen (U California Davis)
- Eric Becraft (Bigelow Lab)
- Brian Hedlund (UNLV)

NASA Astrobiology - Life Underground
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- Katrina Edwards (USC)
- Ken Nealson (USC)
- Victoria Orphan (Caltech)
- Moh El Naggar (USC)
- Rohit Bhartia (JPL)
- Magdelena Osburn (Northwestern)
- Greg Wanger (Dallhousie)
- Sean Mullin (Caltech)
- Yami Jangir (USC)…..

Other
- Jen Blank (NASA Ames)
- Dan Gerrity (UNLV)
- Sue Edwards (DRI)
- Jeff Wedding (DRI)
- Ron Hershey (DRI)
- Jim Thomas (DRI)
- Chuck Russell (DRI)
- John Healey (DRI)
- Henry Sun (DRI)
- Brad Lyles (DRI)
- Mavrik Zavarin (Lawrence Livermore Natl. Lab)

...and many more…
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Conclusions

- Microorganisms perform multiple functions of relevance to NNSS stewardship.
- Deep microbial communities are distinct, recognizable, and reflect their geological surroundings.
- Some deep life strains (e.g. C. Desulfordudis) dominate isolated deep habitats worldwide.
- The deep subsurface, and the DVRFS in particular, are world-class repositories for microbial diversity.