Subsurface Microbial Worlds of the Nevada National Security Site (NNSS) and Death Valley Flow System

Duane Moser, Ph.D.

Division of Earth and Ecosystems Sciences,

Desert Research Institute (DRI)

Nevada Site Specific Advisory Board

Educational Session on August 16, 2017

Desert Research Institute

SCIENCE. ENVIRONMENT. SOLUTIONS.

Scientific Collaboration

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

Presentation Overview

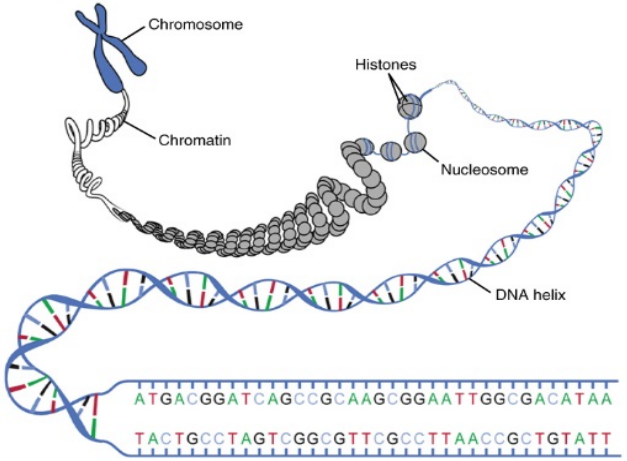
- Introduction
- Life underground and tools used to study it
- Local windows into the ‘deep biosphere’
- ‘Microbial dark matter’
- Strange tale of *c.* Desulforudis audaxviator

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

Illumina HiSeq 2500

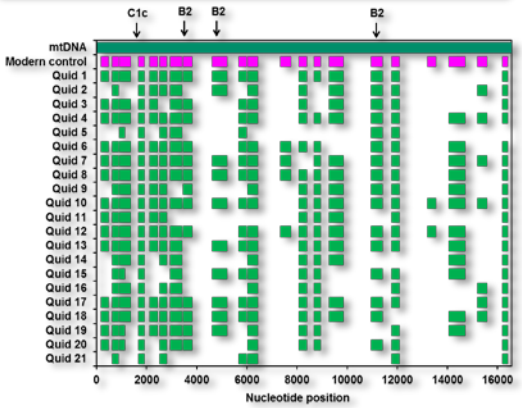


Philschatz.com

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

Molecular Archaeology

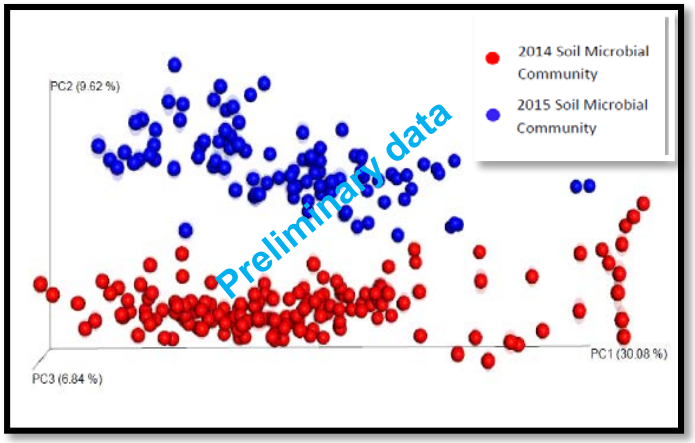


Scott Hamilton-Brehm, Sue Edwards, Jeff Wedding, et al., 2017. PLOS 1, in review

Wildfire and Soil Erodibility

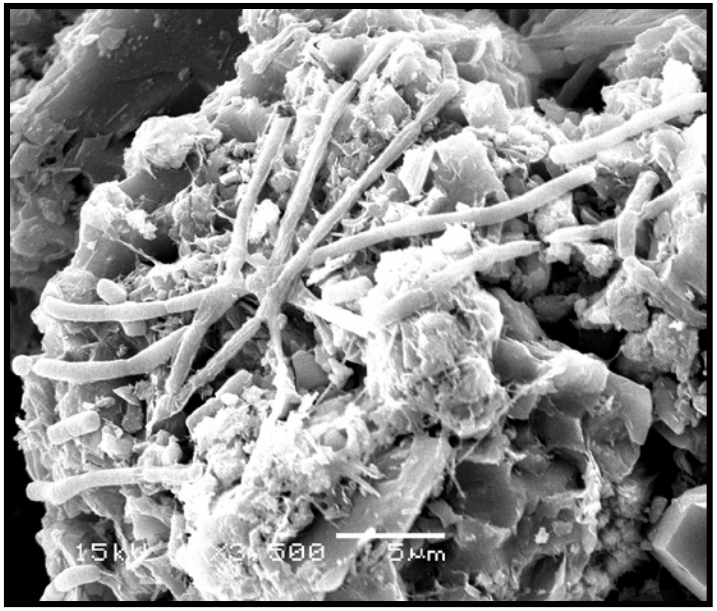
Microorganisms as indicators of soil health and recovery after disturbance?

White Rock Fire Study, Bunkerville, NV

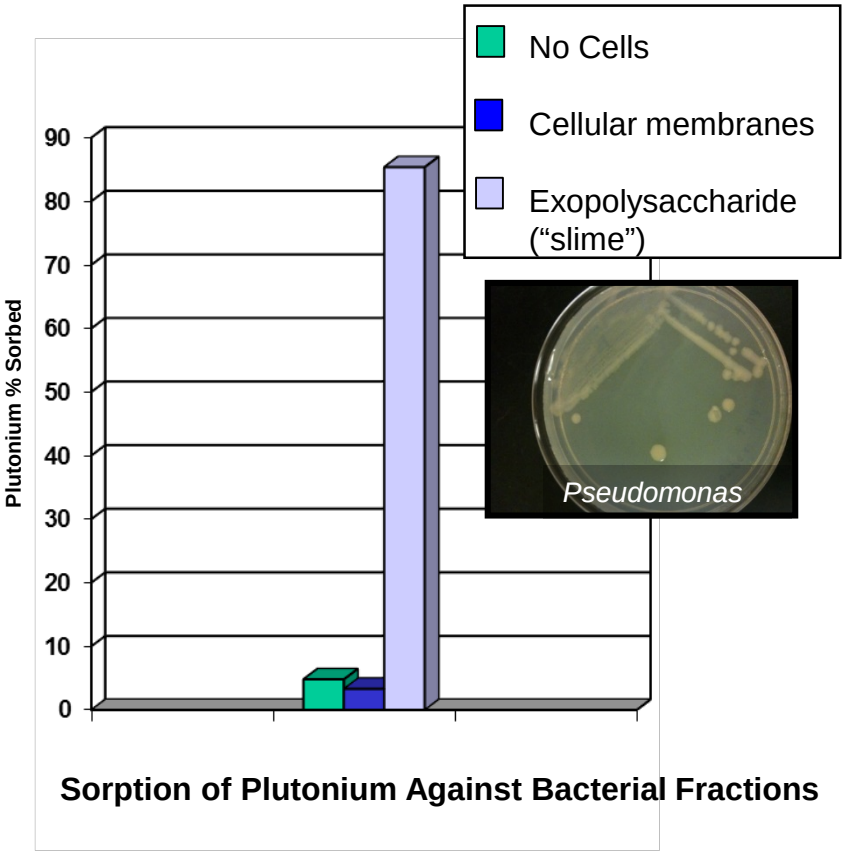


Environmental Contamination

Radionuclides



Microbial Biofilm on NNSS Volcanic Tuff



Sorption of Plutonium Against Bacterial Fractions

Deep Biosphere (Part 1)

Mponeng Mine, South Africa, 2.5 miles deep

Deep Biosphere (Part 1)

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SCIENTISTS & THINKERS

70 of 100 Previous Next

Tullis Onstott

By Carolyn Sayre

Wondering whether extraterrestrial life exists? Tullis Onstott, 53, has found it—sort of. The fact that the living things he's discovered make their homes nearly two miles below the earth' disqualify them from the extra part of ex them up and fly them off, and there's no places they might survive. That's the bea Onstott, a professor of geosciences at Pr doing. Until we collect living aliens, he's probably there to be found. Scientists on discovering even simple otherworldly or assumed that all life should be like earth warmth, light and liquid water. In recen biology has shown itself to be tougher th have discovered organisms—simple one and thriving in scalding ocean vents.

Experts have investigated less thorough within the earth—places where living thi safe redoubt. Uncover such critters on E prove that they might exist elsewhere too and his team published such a find. Afte dirt, piggybacking off gold miners in Sou identified self-sustaining bacteria living surface that draw their energy from che radioactive splitting of water, essentially and sulfur compounds. So when we do f alien life-form, you may get the sense th

Next: Svante Pääbo >>

ARTICLE TOOLS: Print, Email, Sphere, AddThis, RSS

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

Li-Hung Lin,[1,2*] Pei-Ling Wang,[3] Douglas Rumble,[4] Johanna Lippmann-Pipke,[5] Erik Boice,[6] Lisa M. Pratt,[6] Barbara Sherwood Lollar,[7] Eoin L. Brodie,[8] Terry C. Hazen,[8] Gary L. Andersen,[8] Todd Z. DeSantis,[9] Duane P. Moser,[9] Dave Kershaw,[10] T. C. Onstott[1]

Geochemical, microbiological, and molecular analyses of alkaline saline groundwater at 2.8 kilometers depth in Archaean metabasalt revealed a microbial biome dominated by a single phylotype affiliated with thermophilic sulfate reducers belonging to *Firmicutes*. These sulfate reducers were sustained by geologically produced sulfate and hydrogen at concentrations sufficient to maintain activities for millions of years with no apparent reliance on

Environmental Genomics Reveals a Single-Species Ecosystem Deep Within Earth

Dylan Chivian,[1,2*] Eoin L. Brodie,[2,3] Eric J. Alm,[2,4] David E. Culley,[5] Paramvir S. Dehal,[1,2] Todd Z. DeSantis,[2,3] Thomas M. Gihring,[6] Alla Lapidus,[7] Li-Hung Lin,[8] Stephen R. Lowry,[7] Duane P. Moser,[9] Paul M. Richardson,[7] Gordon Southam,[10] Greg Wanger,[10] Lisa M. Pratt,[11,12] Gary L. Andersen,[2,3] Terry C. Hazen,[2,3,12] Fred J. Brockman,[13] Adam P. Arkin,[1,2,14] Tullis C. Onstott[12,15]

DNA from low-biodiversity fracture water collected at 2.8-kilometer depth in a South African gold mine was sequenced and assembled into a single, complete genome. This bacterium, *Candidatus Desulforudis audaxviator*, composes >99.9% of the microorganisms inhabiting the fluid phase of this particular fracture. Its genome indicates a motile, sporulating, sulfate-reducing, chemoautotrophic thermophile that can fix its own nitrogen and carbon by using machinery shared with archaea. *Candidatus Desulforudis audaxviator* is capable of an independent life-style well suited to long-term isolation from the photosphere deep within Earth's crust and offers an example of a natural ecosystem that appears to have its biological component entirely encoded within a single genome.

A more complete picture of life on, and even in, Earth has recently become possible by extracting and sequencing DNA from an environmental sample, a process called environmental genomics or metagenomics (*1–8*). This approach allows us to identify members of microbial communities and to characterize the abilities of the dominant members even when isolation of those organisms has proven intractable. However, with a few exceptions (*5, 7*), assembling complete or even near-complete genomes for a substantial portion of the member species is usually hampered by the complexity of natural microbial communities.

In addition to elevated temperatures and a lack of O_2, conditions within Earth's crust at depths >1 km are fundamentally different from those of the surface and deep ocean environments. Severe nutrient limitation is believed to result in cell doubling times ranging from 100s to 1000s of years (*9–11*), and as a result subsurface microorganisms might be expected to reduce their reproductive burden and exhibit the streamlined genomes of specialists or spend most of their time in a state of semi-senescence, waiting for the return of favorable conditions. Such microorganisms are of particular interest because they permit insight into a mode of life independent of the photosphere.

One bacterium belonging to the *Firmicutes* phylum (Fig. 1A), which we herein name *Candidatus Desulforudis audaxviator*, is prominent in small subunit (SSU or 16S) ribosomal RNA (rRNA) gene clone libraries (*11–14*) from almost all fracture fluids sampled to date from depths greater than 1.5 km across the Witwatersrand basin (covering 150 km by 300 km near Johannesburg, South Africa). This bacterium was shown in a previous geochemical and 16S rRNA gene study (*11*) to dominate the indigenous microorganisms found in a fracture zone at 2.8 km below land surface at level 104 of the Mponeng mine (MP104). Although Lin *et al.* (*11*) discovered that this fracture zone contained the least-diverse natural free-living microbial community reported at that time, exceeding the ~80% dominance by the methanogenic archaeon IUA5/6 of a comparatively shallow subsurface community in Idaho (*15*), we were nonetheless surprised when the current environmental genomics study revealed only one species was actually present within the fracture fluid. Furthermore, we found that the genome of this organism appeared to possess all of the metabolic capabilities necessary for an independent life-style. This gene complement was consistent with the previous geochemical and thermodynamic analyses at the ambient ~60°C temperature and pH of 9.3, which indicated radiolytically generated chemical species as providing the energy and nutrients to the system (*11*), with formate and H_2 as possessing the greatest potential among candidate electron donors, and sulfate (SO_4^{2-}) reduction as the dominant electron-accepting process (*11*).

DNA was extracted from ~5600 liters of filtered fracture water by using a protocol that has been demonstrated to be effective on a broad range of bacterial and archaeal species, including recalcitrant organisms (*16*). A single, complete, 2.35-megabase pair (Mbp) genome was assembled with a combination of shotgun Sanger sequencing and 454 pyrosequencing (*16*). Similar to other studies that obtained near-complete consensus genomes from environmental samples (*5, 17*), heterogeneity in the population of the dominant species as measured with single-nucleotide polymorphisms (SNP) was quite low, showing only 32 positions with a SNP observed

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

Duane P. Moser,[1*] Thomas M. Gihring,[1†] Fred J. Brockman,[1] James K. Fredrickson,[1] David L. Balkwill,[2] Michael E. Dollhopf,[2‡] Barbara Sherwood Lollar,[3] Lisa M. Pratt,[4] Erik Boice,[4] Gordon Southam,[5] Greg Wanger,[5] Brett J. Baker,[6] Susan M. Pfiffner,[7] Li-Hung Lin,[8]§ and T. C. Onstott[8]

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Alkaline, sulfidic, 54 to 60°C, 4 to 53 million-year-old meteoric water emanating from a borehole intersecting quartzite-hosted fractures >3.3 km beneath the surface supported a microbial community dominated by a bacterial species affiliated with *Desulfotomaculum* spp. and an archaeal species related to *Methanobacterium* spp. The geochemical homogeneity over the 650-m length of the borehole, the lack of dividing cells, and the absence of these microorganisms in mine service water support an indigenous origin for the microbial community. The coexistence of these two microorganisms is consistent with a limiting flux of inorganic carbon and SO_4^{2-} in the presence of high pH, high concentrations of H_2 and CH_4, and minimal free energy for autotrophic methanogenesis. Sulfide isotopic compositions were highly enriched, consistent with microbial SO_4^{2-} reduction under hydrologic isolation. An analogous microbial couple and similar abiogenic gas chemistry have been reported recently for hydrothermal carbonate vents of the Lost City near the Mid-Atlantic Ridge (D. S. Kelly et al., Science 307:1428-1434, 2005), suggesting that these features may be common to deep subsurface habitats (continental and marine) bearing this geochemical signature. The geochemical setting and microbial communities described here are notably different from microbial ecosystems reported for shallower continental subsurface environments.

Numerous studies have revealed the presence of microbial communities occupying oceanic and terrestrial deep subsurface settings (12, 14, 23, 27, 53, 54). Due to its enormous volume, this habitat may host the majority of Earth's prokaryotes (76), and according to some estimates (25, 76), the collective biomass of subsurface microbiota may rival that of flora and fauna at the surface. It is generally accepted that life on Earth requires liquid water (9). The upper 4 km of the terrestrial crust contains 9.5×10^6 km^3 of groundwater, of which about 56% lies below 0.75 km in depth (6). Since reports of microorganisms over this depth interval continue (4, 53, 54, 70) and the basic requirements for life appear to be met (e.g., liquid water, habitable space, and permissive temperatures), it follows that a significant proportion of the biosphere may be microbial and associated with deep terrestrial hydrologic systems (76).

Although a reasonable understanding of the energetic foundations for marine and terrestrial subsurface sedimentary ecosystems to a depth of 500 m has been achieved (20, 55), mechanisms for the energetic support of igneous and metamorphic rock systems remain incompletely addressed. Best known are igneous rock-hosted aquifers at depths of <450 m, where chemolithoautotrophic subsurface ecosystems, founded upon biological methanogenesis or acetogenesis and supported by geological H_2 production and inorganic C, are thought to be limited in photosynthetically derived organic C (12, 56, 68). Relatively little is known, however, about the distribution of such communities at much greater depths or whether microbial ecosystems of other deep rock types (e.g., sedimentary and metamorphic) operate with similar energy and C sources.

Microbiological sampling opportunities in the deep subsurface are extremely limited, and a persistent challenge remains the differentiation of indigenous from introduced microorganisms (52). Several studies, however, have shown that in mine boreholes, introduced microbes fail to persist (47, 57), especially when the unidirectional flushing of hot, anaerobic fracture water reestablishes a redox environment similar to that of the predrilling conditions (47). South African mines are unusual in that they routinely attain depths of >3 kilometers below land surface (kmbls), thereby accessing thermophilic, anaerobic environments distinct from more frequently studied,

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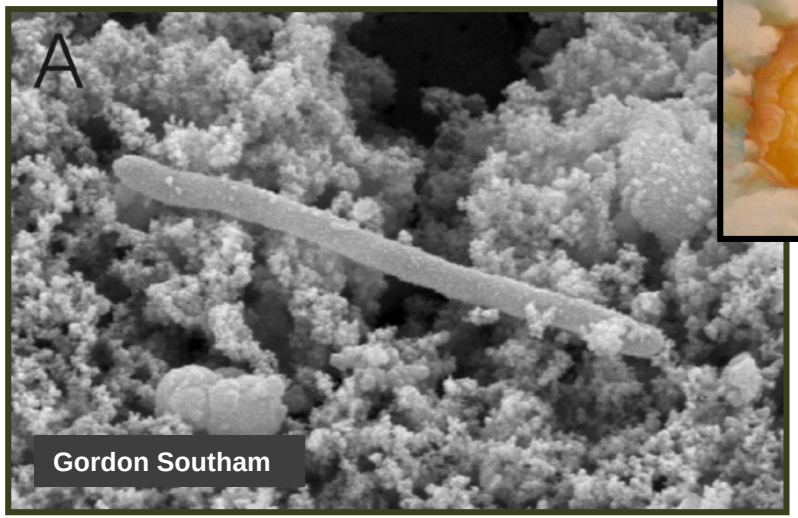
Deep Biosphere (Part 1)

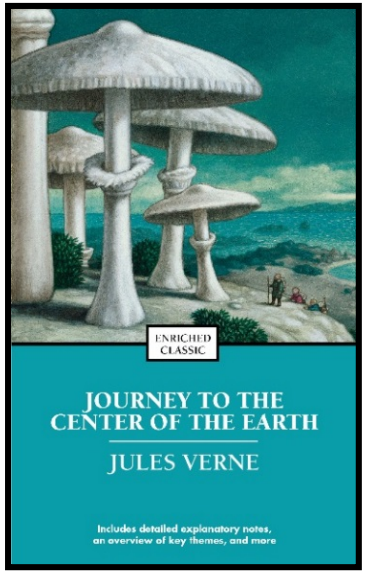
Sample Collection at Evander Mine, South Africa

Deep Biosphere (Part 1)

C. Desulforudis audaxviator



Gordon Southam



Simon and Schuster

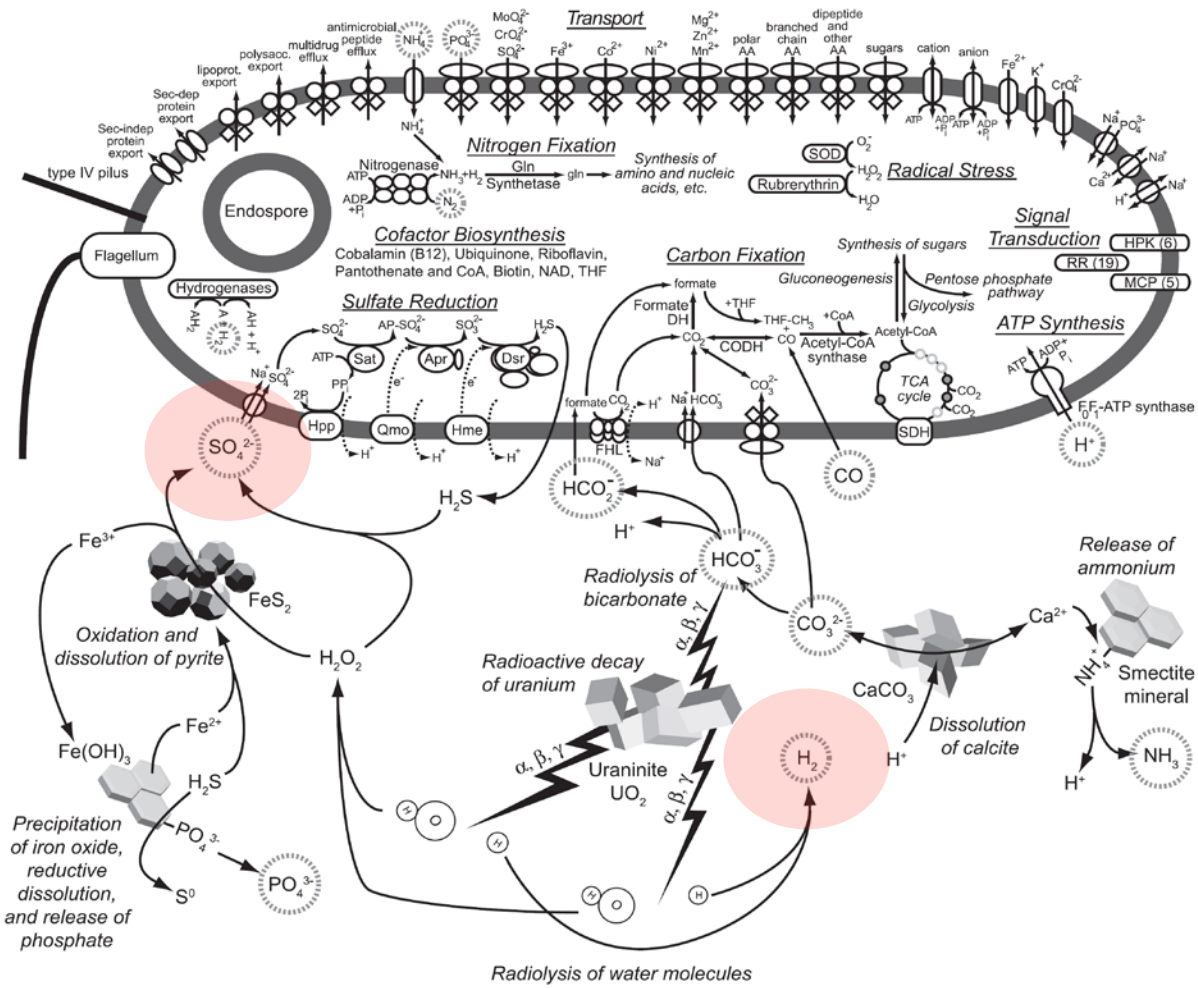
"Based on its rod-like morphology, its apparent use of the dissimilatory sulfate reduction pathway for energy production, and because of the journey this "audax viator" (bold traveler) undertook to live in the extreme depths of the Earth, we have named this organism *Candidatus* Desulforudis audaxviator".

"Desulforudis" = 48,200 Google hits

Chivian, D., E.J. Alm, E.L. Brodie, et al., Science, 2008.

Deep Biosphere (Part 1)

C. Desulforudis audaxviator Predicted Lifestyle

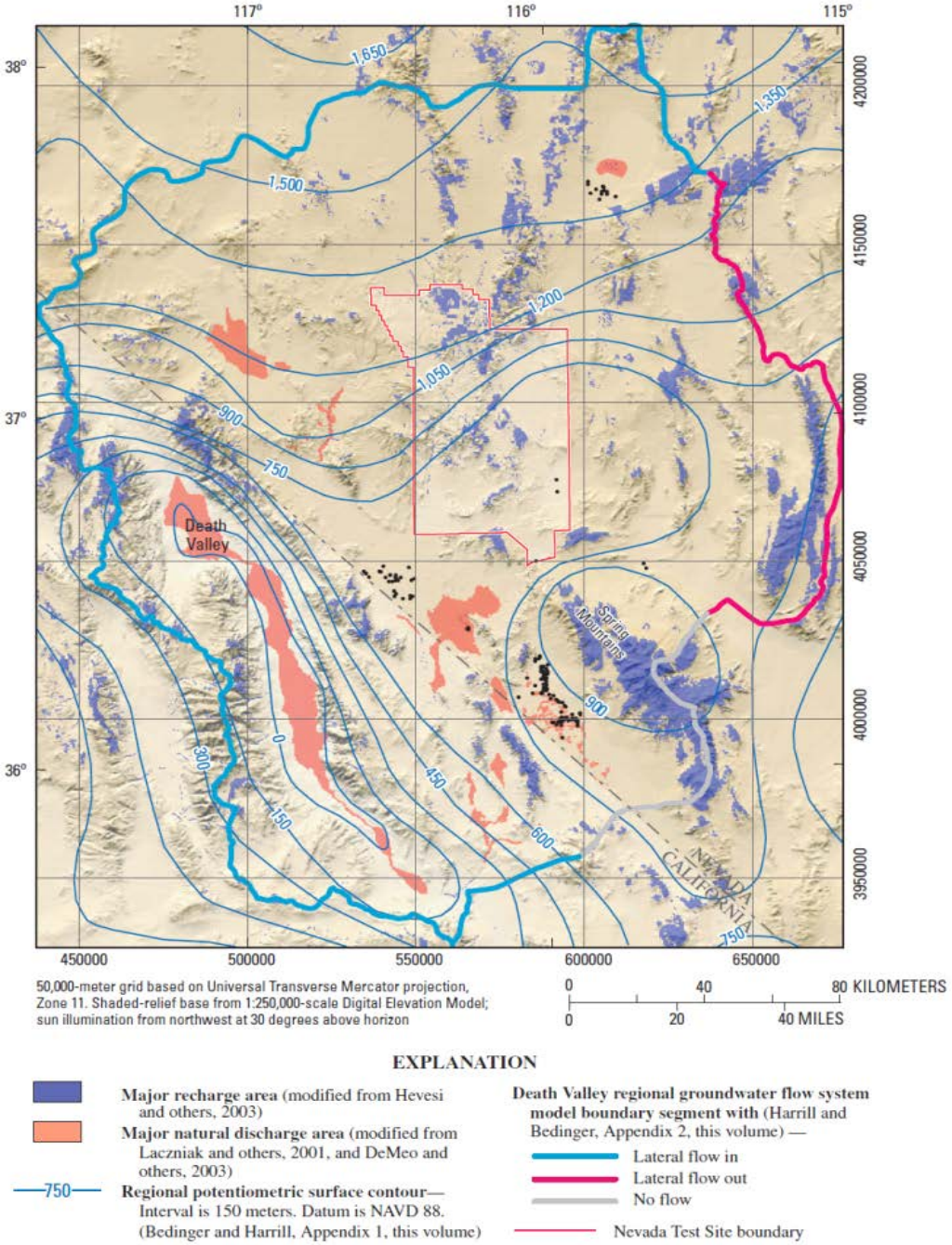


L. Brodie, et al., Science, 2008

Deep Biosphere (Part 2)

Devils Hole, Jonathan Eisen

The DVRFS



Belcher and Sweetkind, 2004, USGS Professional Paper 1711

Natural Windows - Springs

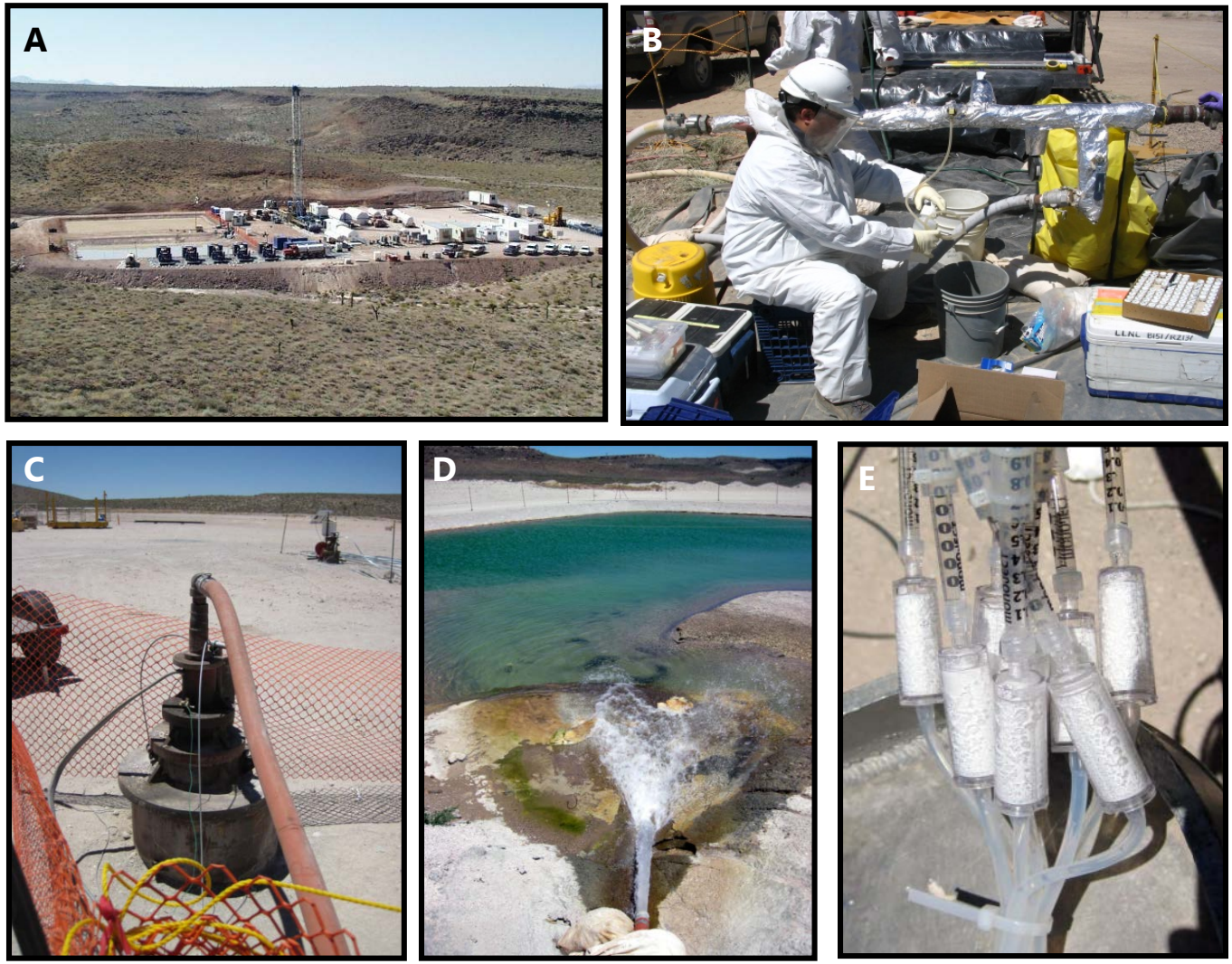
Manmade Windows - Boreholes

Nevares Deep Well 2, Death Valley

Down-Hole Video at 302 Feet

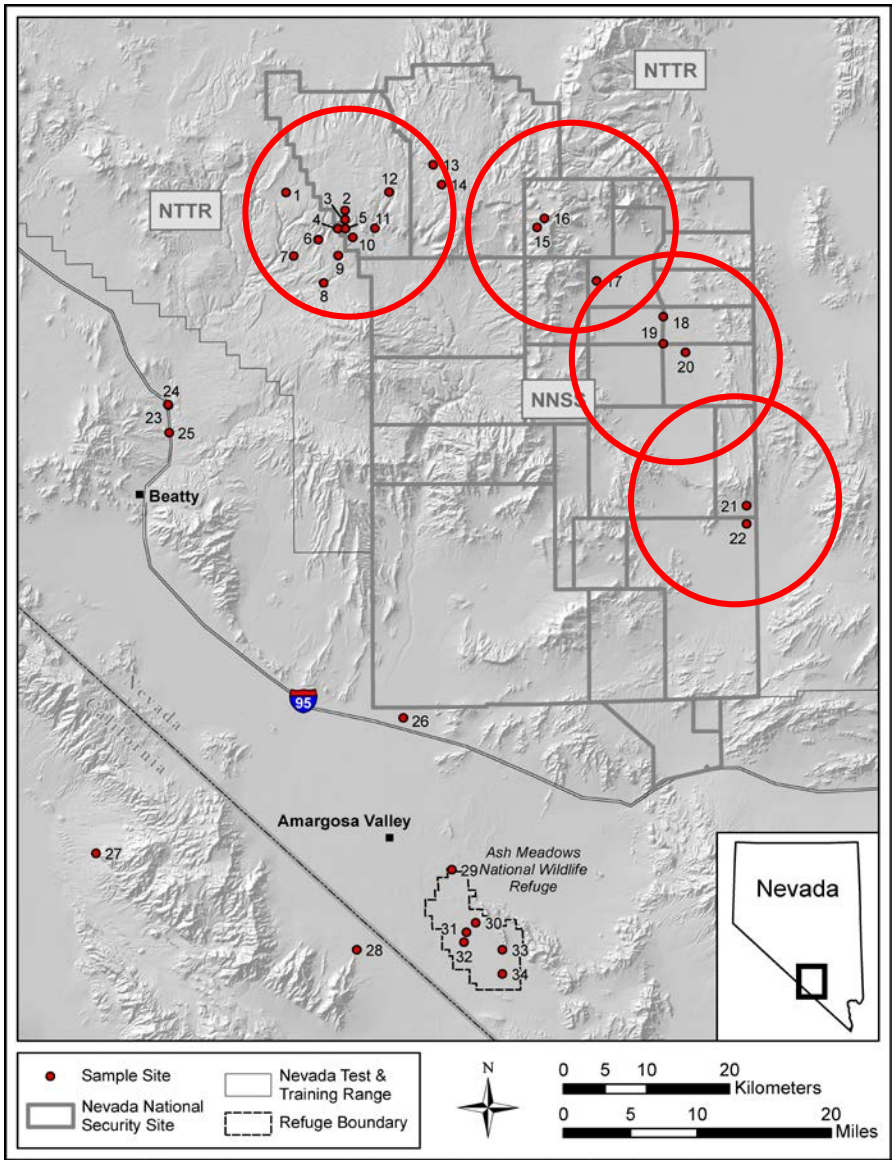
Down-Hole Video at 310 Feet Showing Fracture

Microbiological Sampling at the NNSS



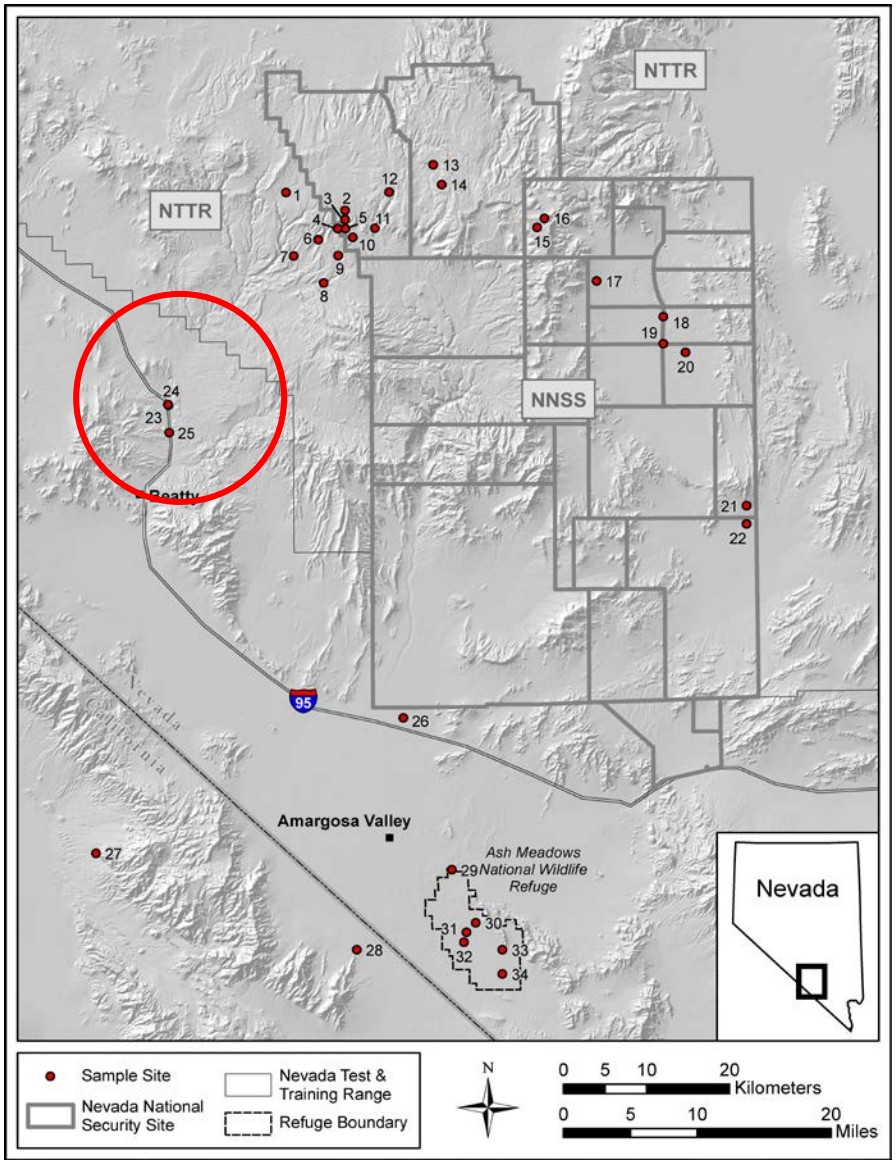
Examples of NNSS and DVRFS sampling sites. A) ER-EC-13 being drilled, from Bill Wilborn 2011 Community Environmental Monitoring Program Presentation; B) Hotwell sampling; C) ER-EC-13 during hydrologic pumping test; D) groundwater discharge during pumping test; and E) 0.2 micron filters used for microbial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

DVRFS Microbiology Sites



Key Code	Sample Name
1	PM 3 #1 & 2
2	ER 20-5 #1 & 3
3	ER 20-7
4	ER 20-11
5	ER-EC-11
6	ER-EC-15
7	ER-EC-13
8	ER-EC-14
9	ER-EC-12
10	ER 20-8
11	ER 20-4
12	UE-20N#1
13	U19ad PS#1A
14	U19V PS#1ds
15	U12n.Vent#2
16	U12n.10
17	UE-2ce
18	U-4t PS#3
19	UE-3E #4
20	U-3cn5
21	ER 11-2
22	ER 5-5
23	OV1
24	OV2
25	Bailey's Hot Spring
26	4PD
27	Nevares
28	BLM-1
29	Fairbanks Spring
30	Indian Spring
31	Crystal Pool
32	Crystal Reservoir
33	King's Pool
34	Big Spring

DVRFS Microbiology Sites



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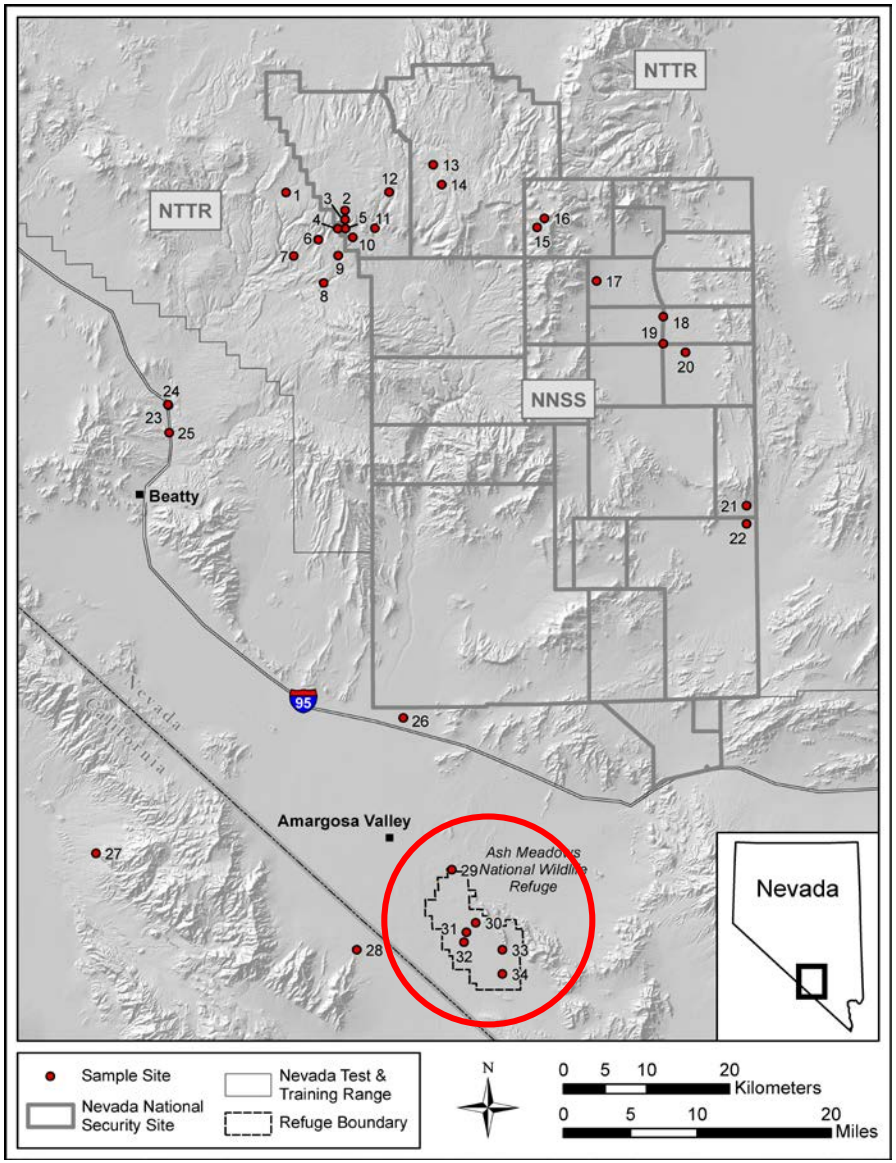
DVRFS Microbiology Sites



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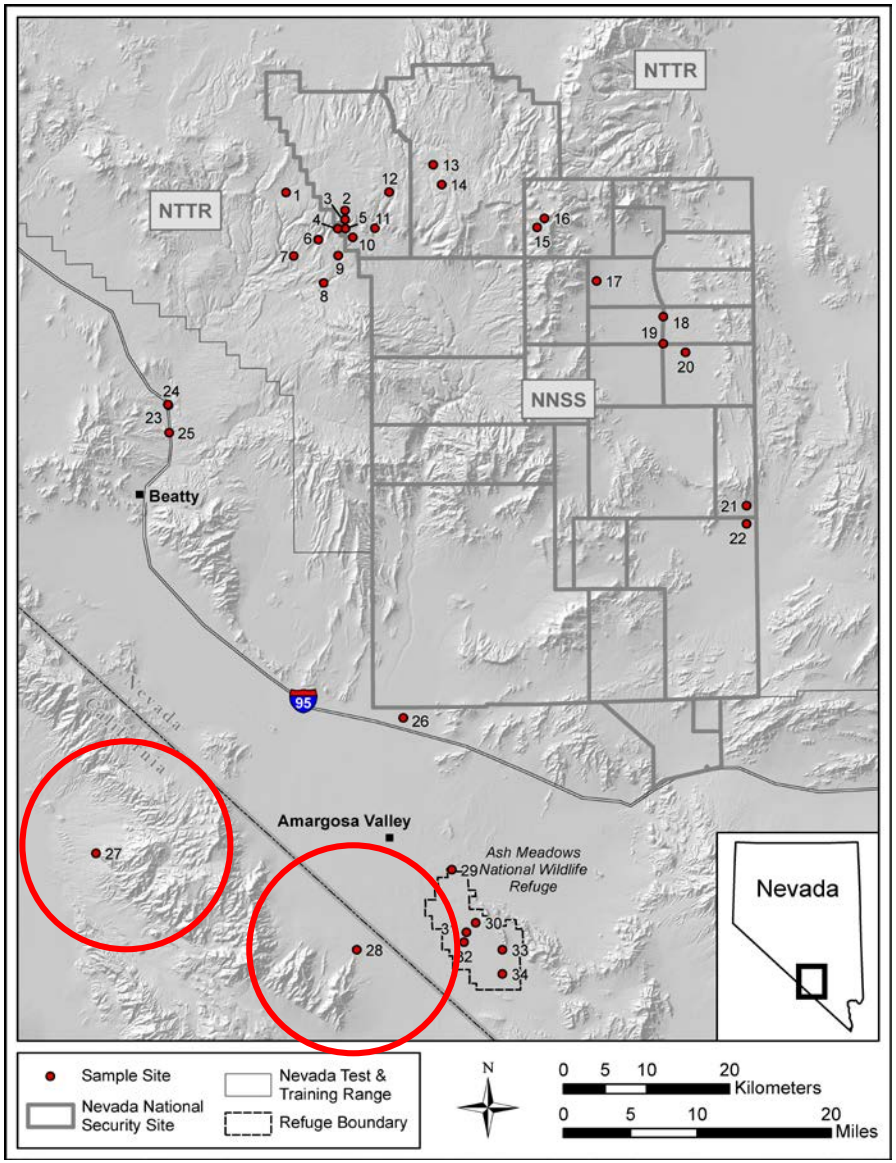
DVRFS Microbiology Sites



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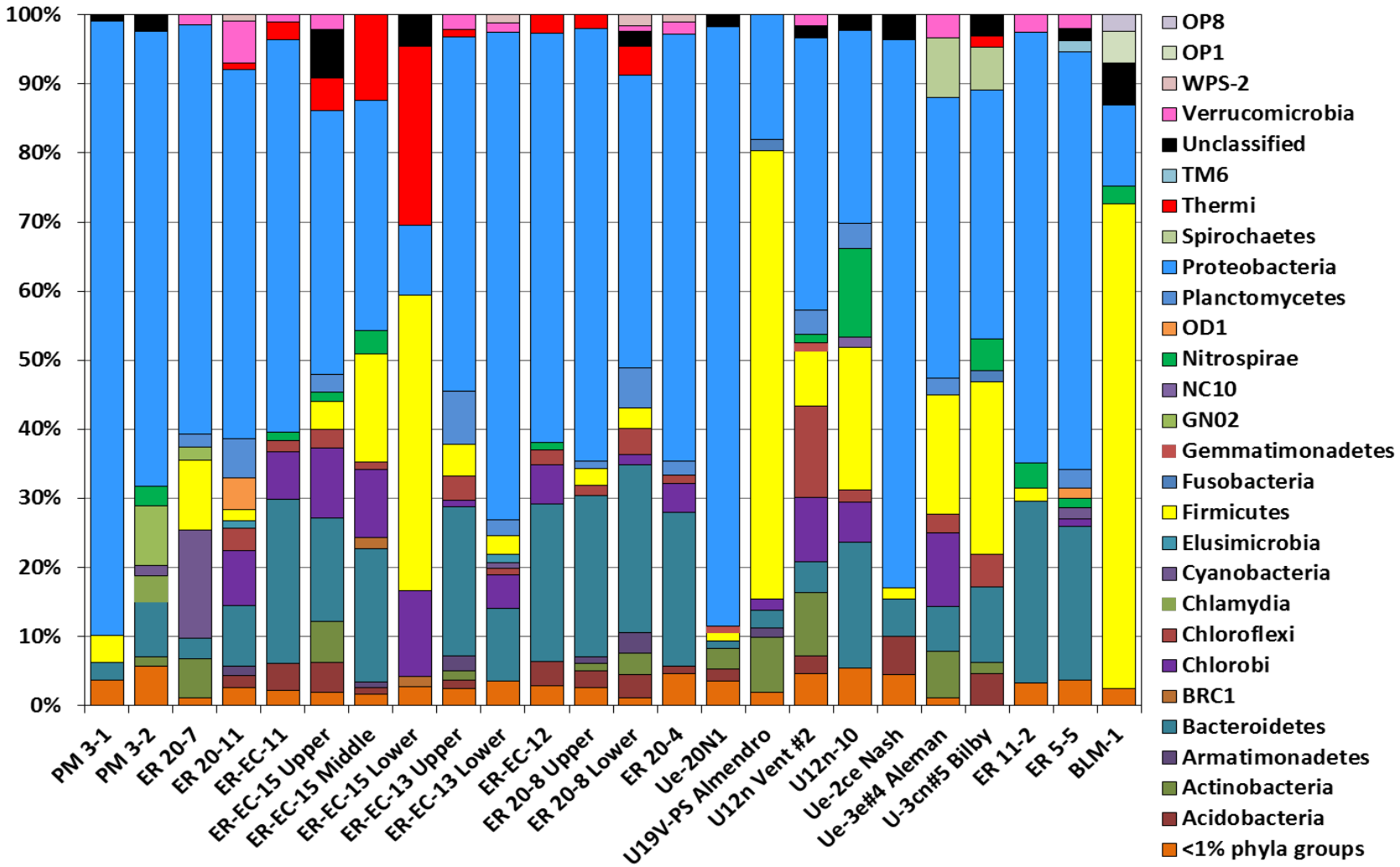
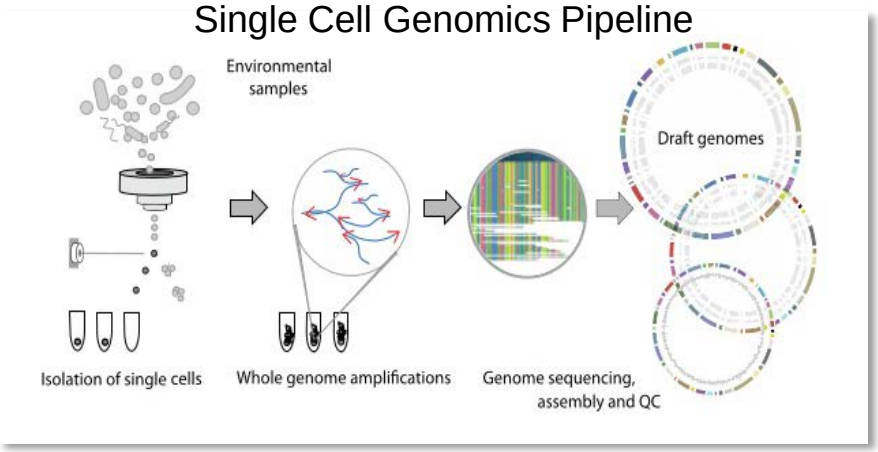
Microbial Communities Across DVRFS

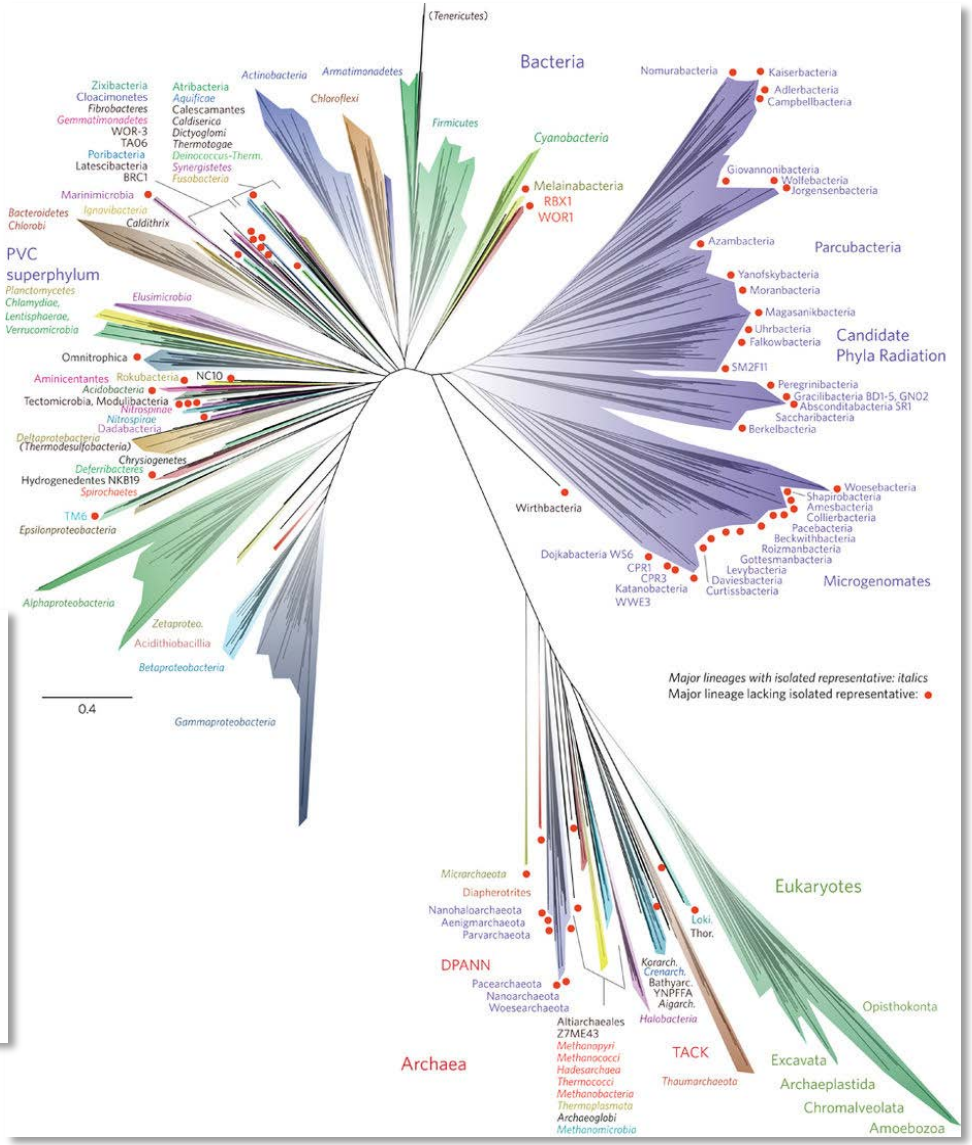


Deep Biosphere (Part 3)

The Strange Case of "Microbial Dark Matter"

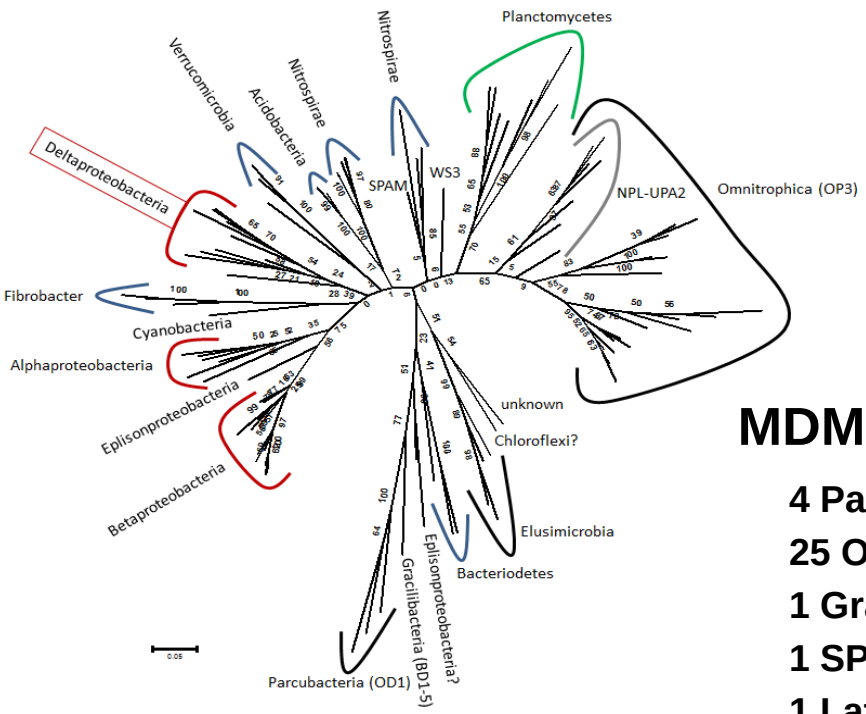
- Microbial phyla with no cultured representatives
- Majority of life's diversity
- Requires advanced tools for study





Hug et al. 2016. Nature Microbiology
Rinke et al., 2013. Nature

DVRFS Microbial Dark Matter (MDM)



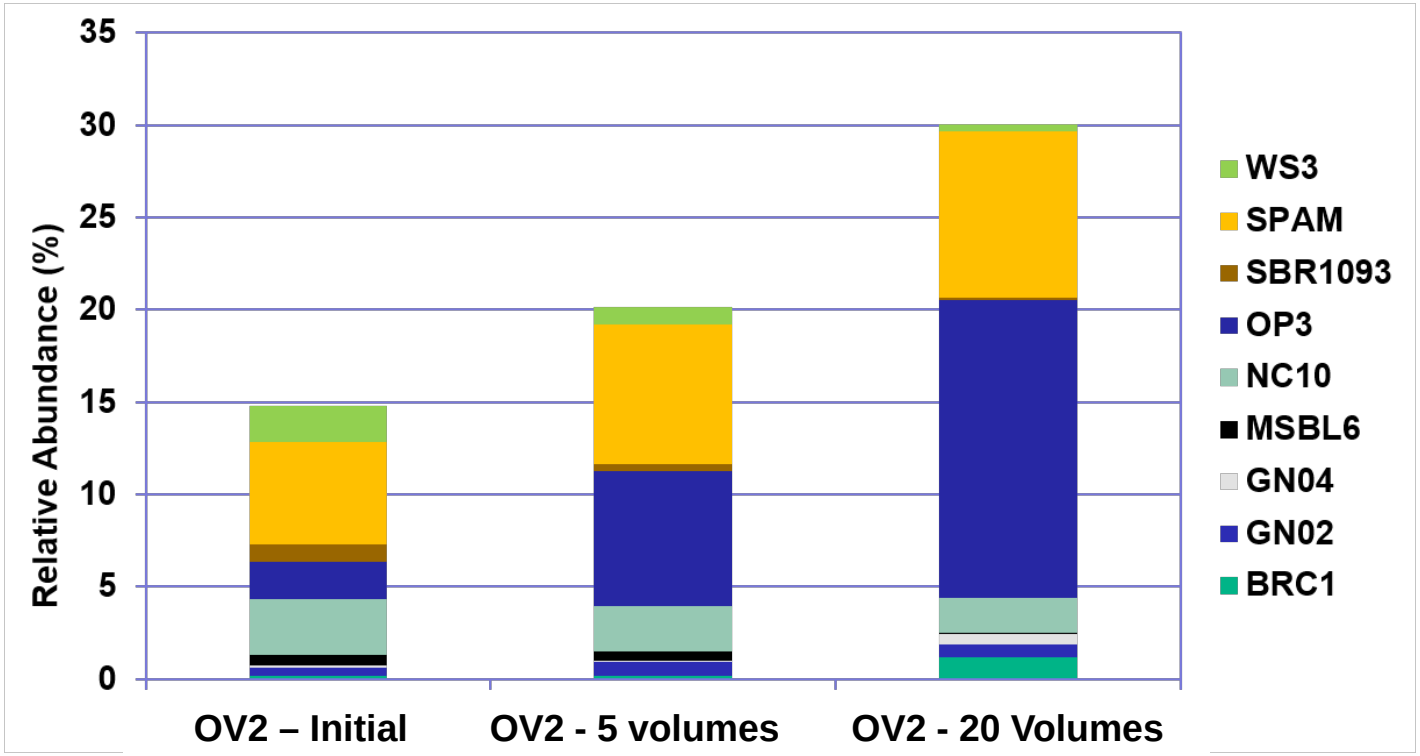
Full MDM work-ups

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

MDM summary Crystal Spring (42.3% MDM)

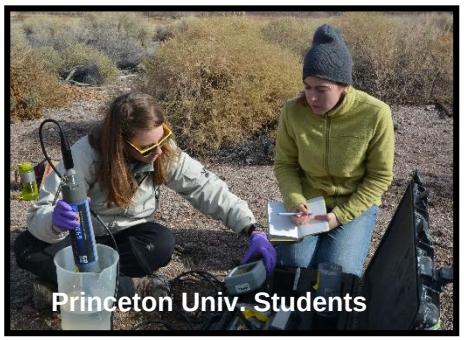
- 4 Parcubacteria (OD1)
- 25 Omnitrophica (OP3)
- 1 Gracillibacteria (BD1-5)
- 1 SPAM
- 1 Latescibacteria (WS3)
- 4 Elusimicrobia
- 8 Woesearcheota
- 1 Pacearcheota
- 1 unclassified

A MDM Hotspot - Well OV-2



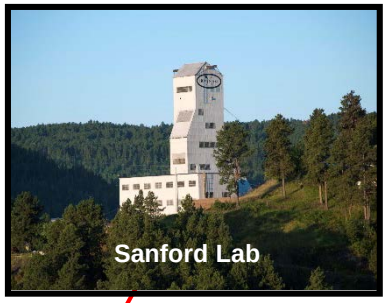
Moser, Kruger, Sackett (DRI)

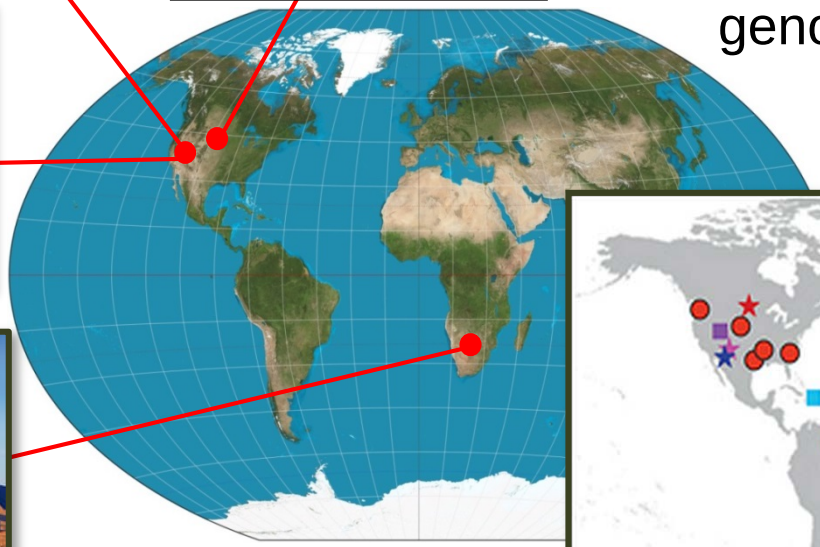
John Klenke, NWRPO


Princeton Univ. Students

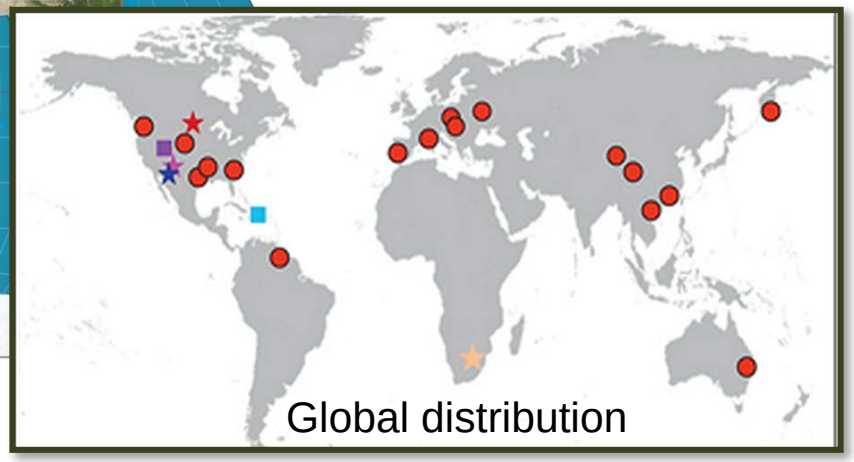
Scott Hamilton-Brehm, Levi Kryder, NWRPO

Solagigasbacteria: the 'Lone Giant'





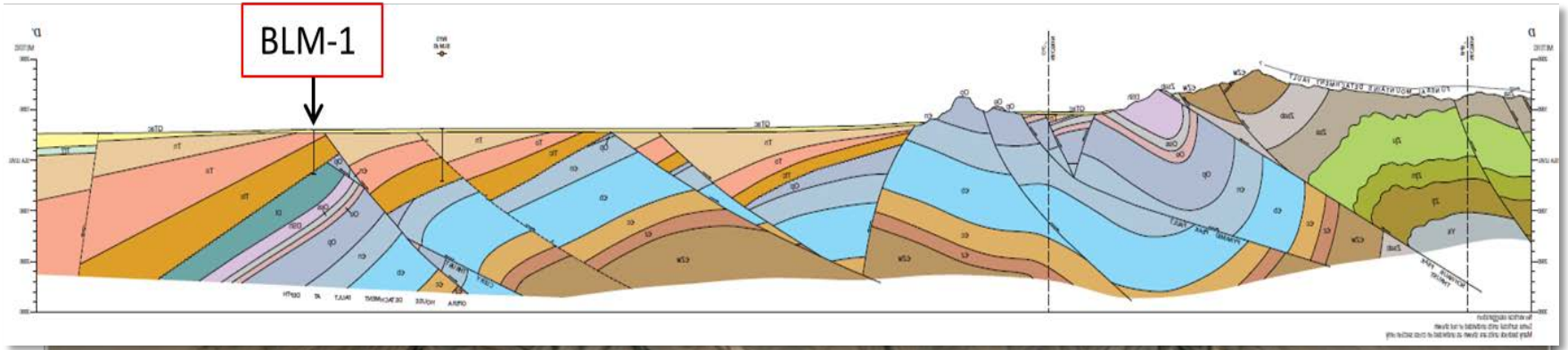
- Formerly known as "SPAM"
- 6 – 8 million base pair genome
- Terrestrial only
- 16 of 19 single amplified genomes from Well OV-2



Becraft et al., Microbial Ecology, in revision

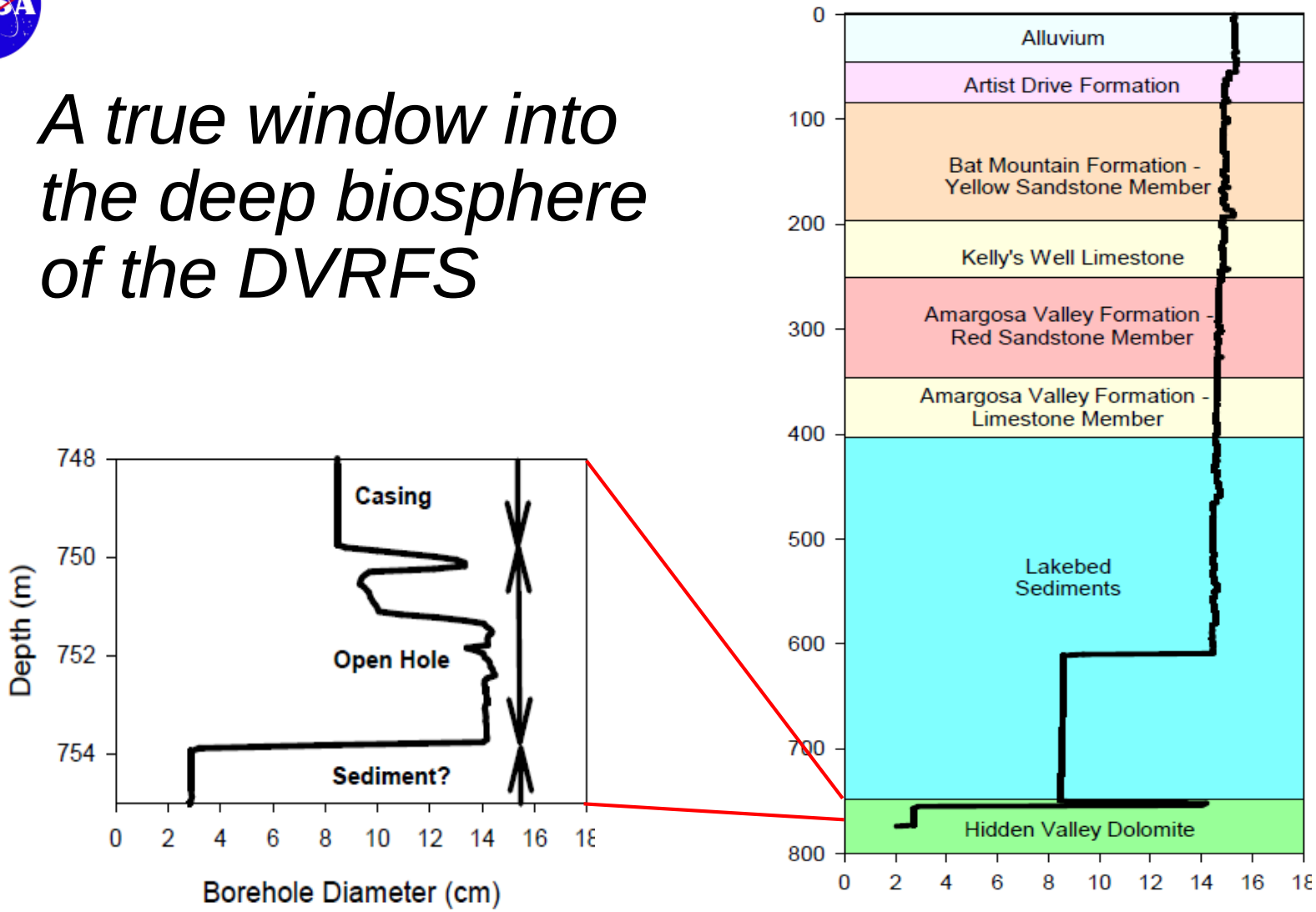
Well BLM-1

Well BLM-1



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

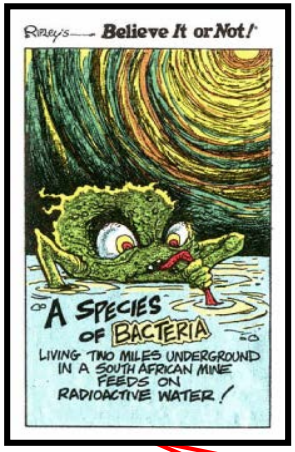
An attempt to study hard-rock microbial communities and their activities on subsurface fracture surfaces

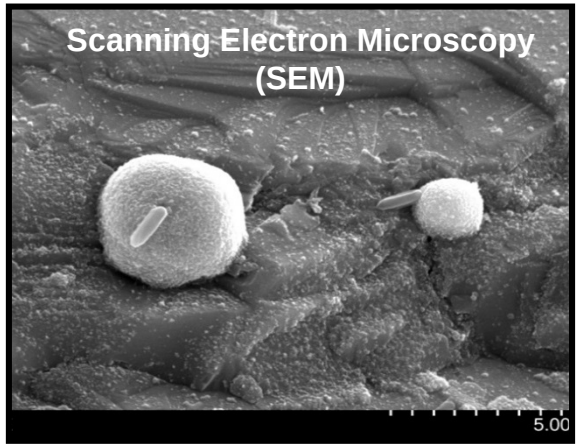
Well BLM-1 Downhole Incubations

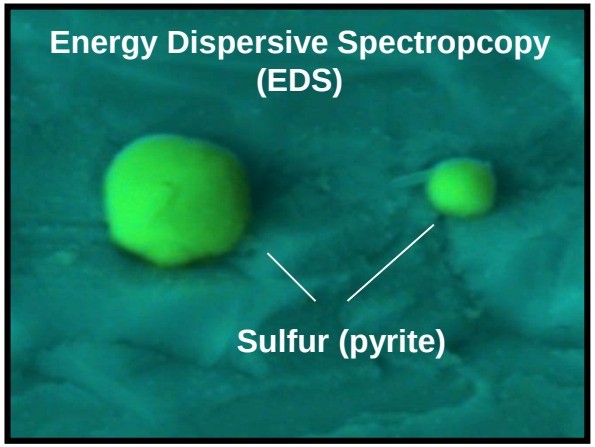
Deployment of Materials

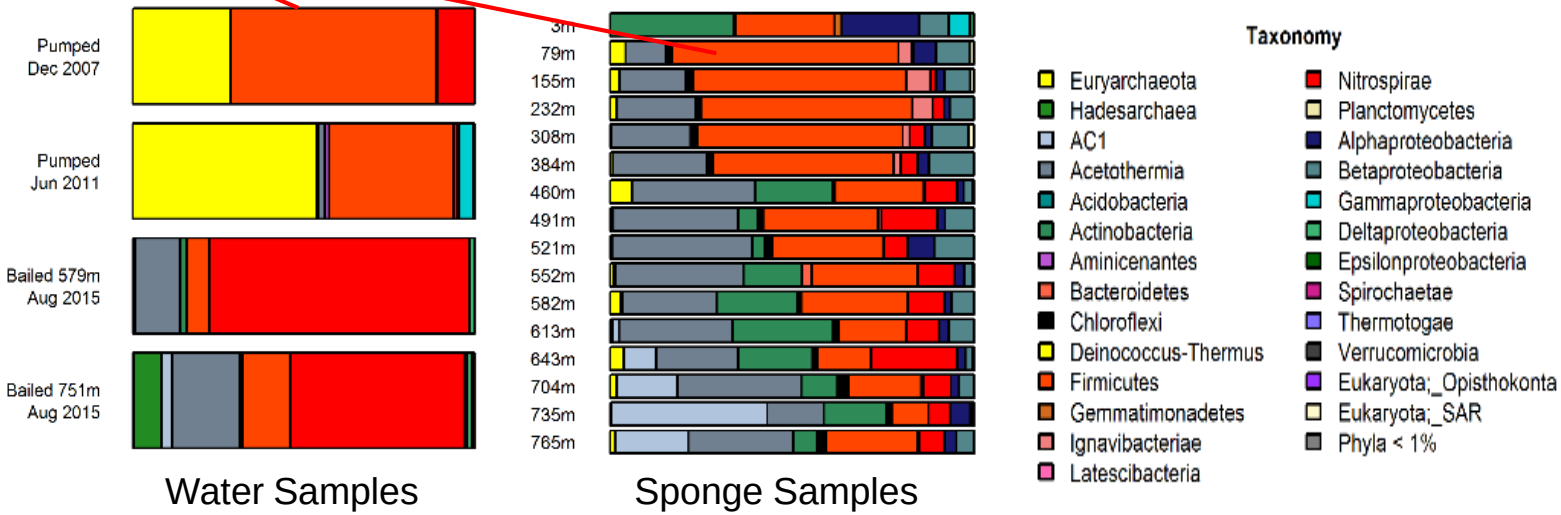
Retrieval of Materials

Well BLM-1 Downhole Incubations: Results





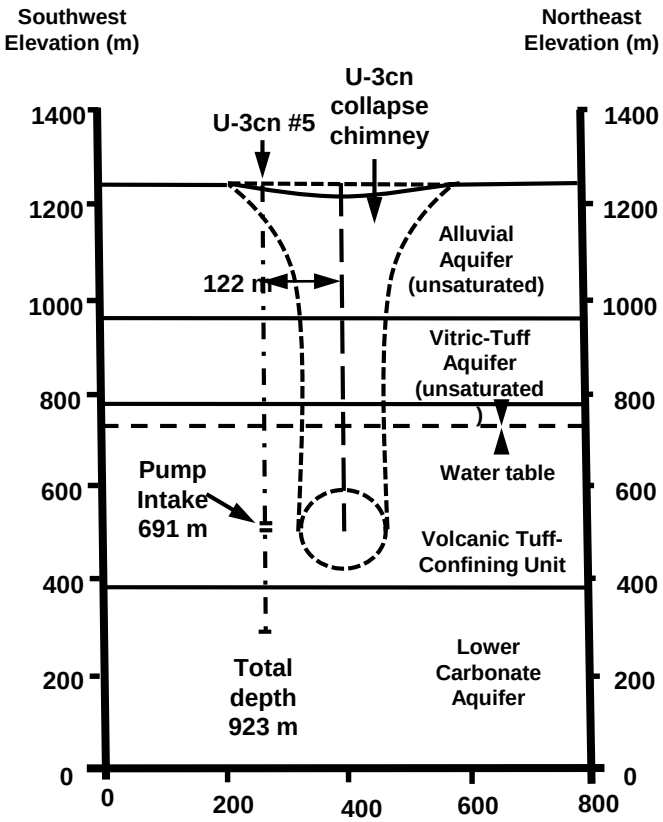




Upper: Microbial cells on calcite after in situ incubation. Lower: Examples of microbial community structure at phylum level for water (Left) and incubated sponges (middle). Lighter orange is primarily *C.* Desulforudis

Culturing Microorganisms

Traditional Microbiology – Anaerobic Microbes from NNSS Subsurface

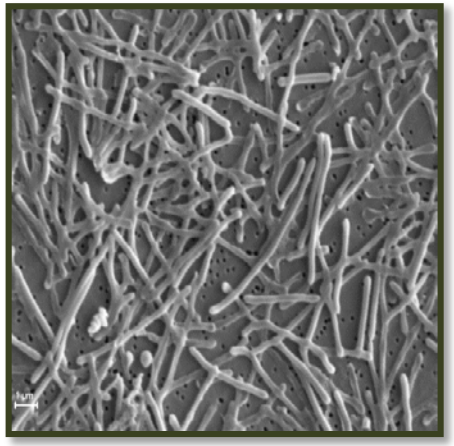


Modified from DOE/NV/-500-UC-700

New Microorganism: *Thermoanaerosceptrum 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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- *Tom Kieft (New Mexico Tech*
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- *Mitch Sogin (Marine Biology Lab)*
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- *Jessica Jaret (JGI)*
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- *Yami Jangir (USC)…..*

Other
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- *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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Devils Hole, National Park Service

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.

Devils Hole, National Park Service