Hidden microbial diversity at high temperatures

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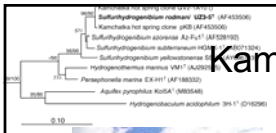
The microbial world still has a lot of surprises

- Genomes – 30-50% coding capacity unknown
- Estimates of <1% grown in the lab
- High biochemical diversity
  - Promise for improving processes
  - Production of high value chemicals/biofuels
- Unknown provides ideal targets for discovery using a host of different approaches including creative culturing, metabolic modeling etc.
- Consortia/more complex systems are now tractable (& help maintain optimal conditions for production)
- Historical- where there is energy, microbes will 'engineer themselves… Silicon chips, PCBs etc..

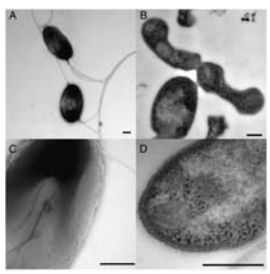
Overview:

- Generally, what do we know about the diversity in high temp systems? (who are some of the key players? 50- 80C thermophily happens)
- Examples of targeting discovery
- Any interesting targets out there?
  - Next wave of pyrosequencing insights
- Any conclusions?

The Mid-Ocean Ridge System



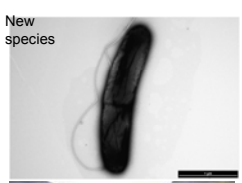
Kamchatka



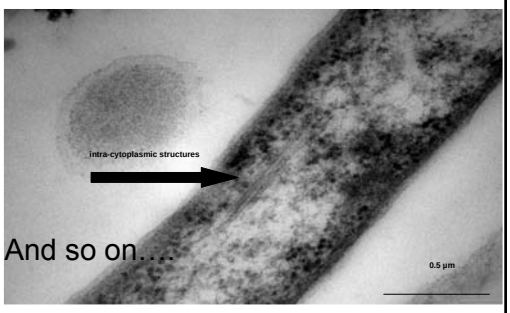
Iceland

No close relative in culture, new genus species

New species



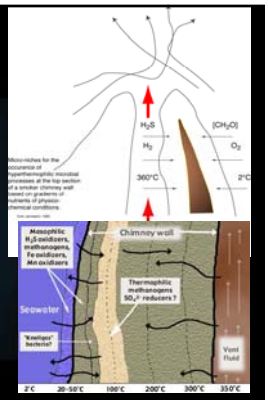
Costa Rica, new species from Rincon volcano

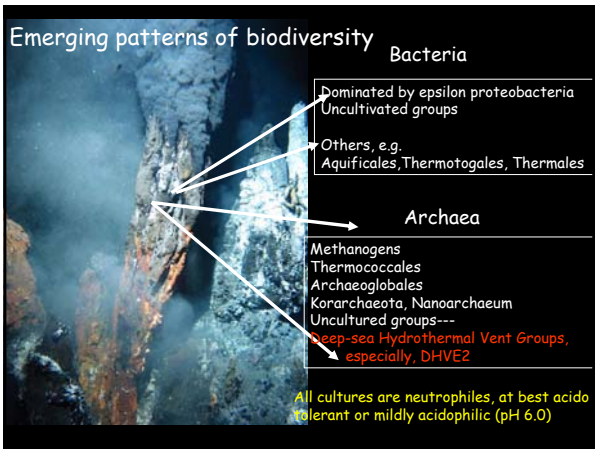


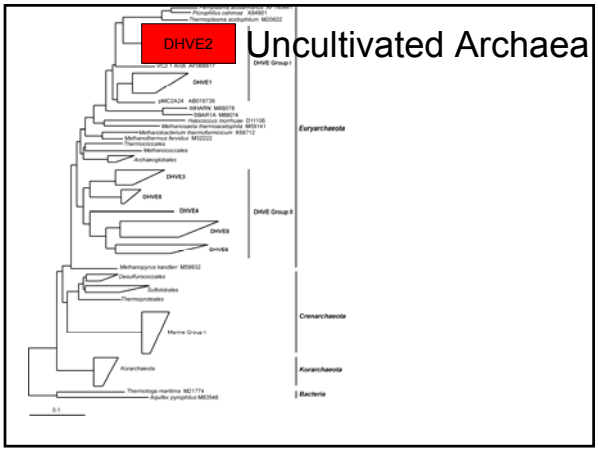
And so on….

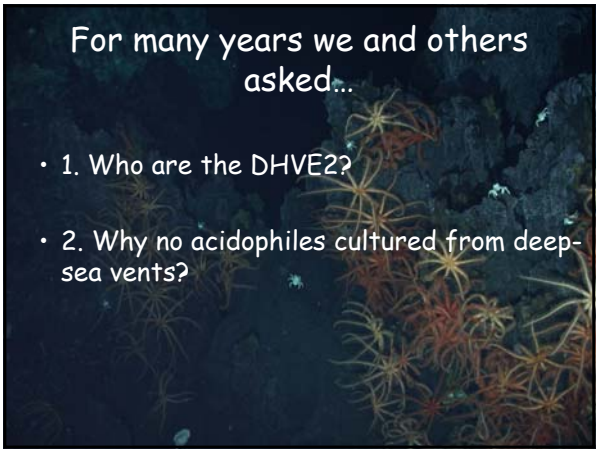
- only explored with undersea vehicles **less than 1 %** of the mid-oceanic ridge zone which accounts for more than 90 % of the earth's volcanic activity

- Yet relatively little is known about microbial diversity (surprising as many of our thermophilic archaeal isolates are from these systems)




Emerging patterns of biodiversity
Bacteria
Dominated by epsilon proteobacteria
Uncultivated groups
Others, e.g.
Aquificales,Thermotogales, Thermales
Archaea
Methanogens
Thermococcales
Archaeoglobales
Korarchaeota, Nanoarchaeum
Uncultured groups---
Deep-sea Hydrothermal Vent Groups, especially, DHVE2
All cultures are neutrophiles, at best acido tolerant or mildly acidophilic (pH 6.0)

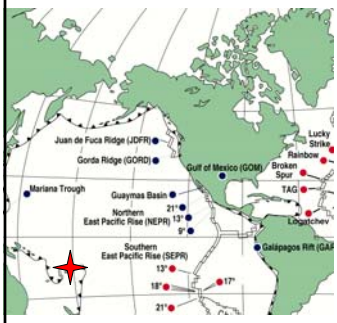

DHVE2
Uncultivated Archaea
Euryarchaeota
Crenarchaeota
Korarchaeota
Bacteria

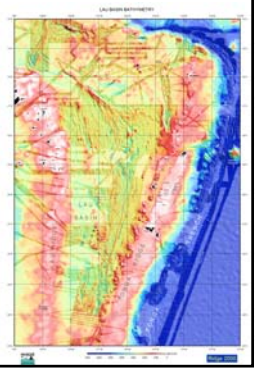

For many years we and others asked…
• 1. Who are the DHVE2?
• 2. Why no acidophiles cultured from deep-sea vents?

Major vent sites, and sites where the DHVE2 have been detected

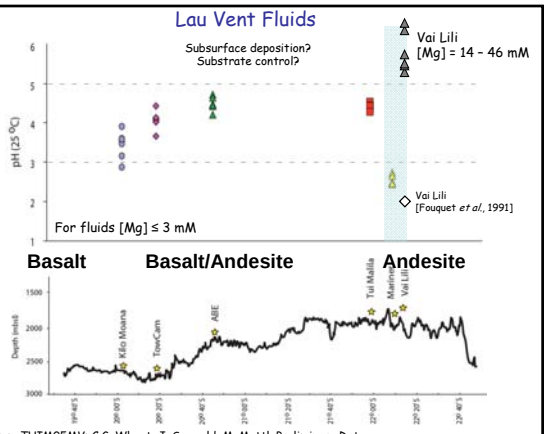
•1. *Who are the DHVE2?*

Eastern Lau Spreading Center





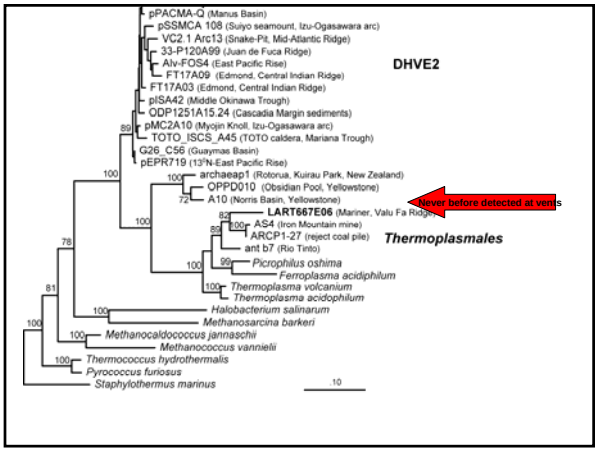
Lau Vent Fluids



Data from TUIM05MV: *C.G.* Wheat, J. Seewald, M. Mottl, Preliminary Data.

Then serendipity enters the search for DHVE2

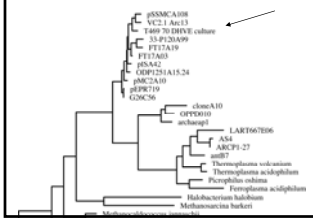
- Mariner- one of the lowest pH measurements for deep-sea vents pH <2.5
- Clone libraries revealed an unusual sequence from an iron-sulfur-rich microbial mat, 60ºC
- never before detected at vents, related to *Picrophilus, Thermoplasma, Ferroplasma*

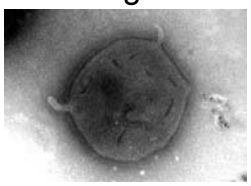


Could we grow the *Thermoplasma*-like phylotype?

Not yet.. But we have somebody else even more interesting…

- The devilheterotrophventblob –DHVE-2 horns
  - *"Aciduliprofundum boonei"*

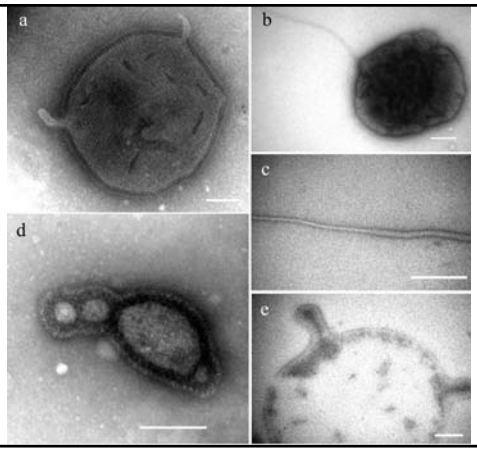


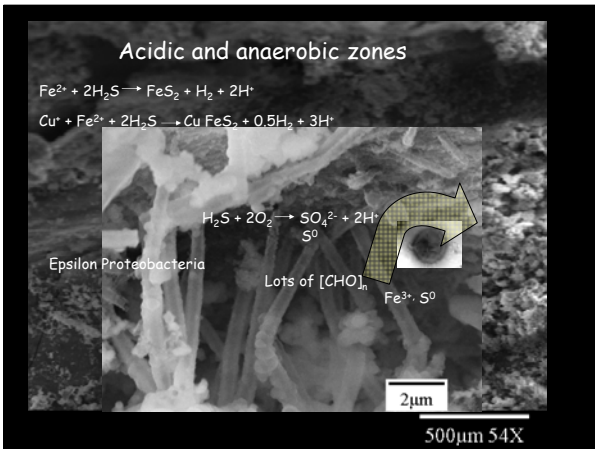


Genome has been sequenced. Draft and now being closed by JGI. Second isolate being sequenced too..

Reysenbach et al. 2006. Nature 442:444-447

Reysenbach and Flores, 2008. Geobiology. 6:331-336





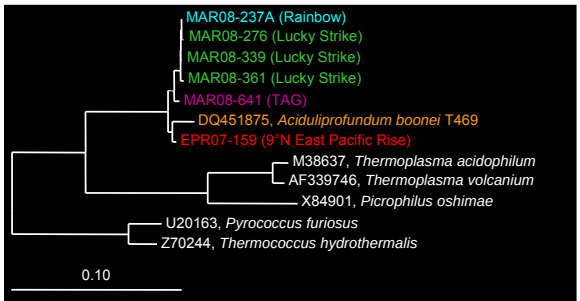
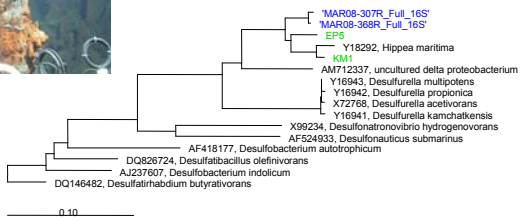
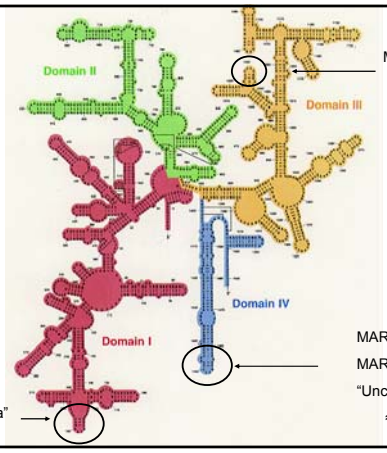


Fig. 2 Neighbor joining tree of cultured representatives of the DHVE2 (colors) based on 1261 unambiguously aligned nucleotides of 16S rRNA gene sequences. *Halobacterium salinarum* was used as the outgroup to root the tree (not shown). Scale bar represents 10 substitutions per 100 nucleotides.

More thermoacidophiles… Deltaproteobacteria





MAR08-307R
≈70 bp

MAR08-307R
MAR08-368R
"Uncultured delta"
≈100 bp

Hippea maritima
"Uncultured delta"
≈80 bp

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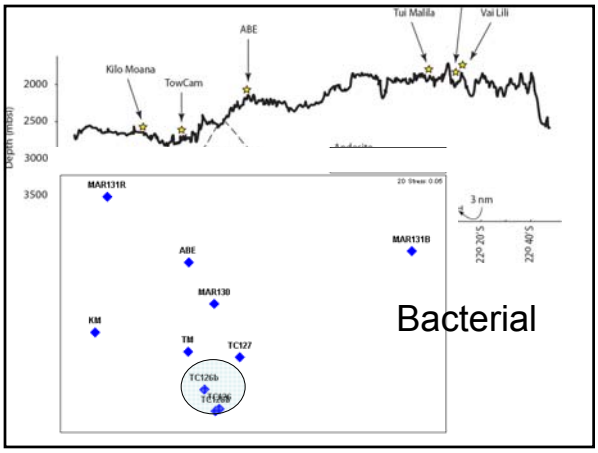
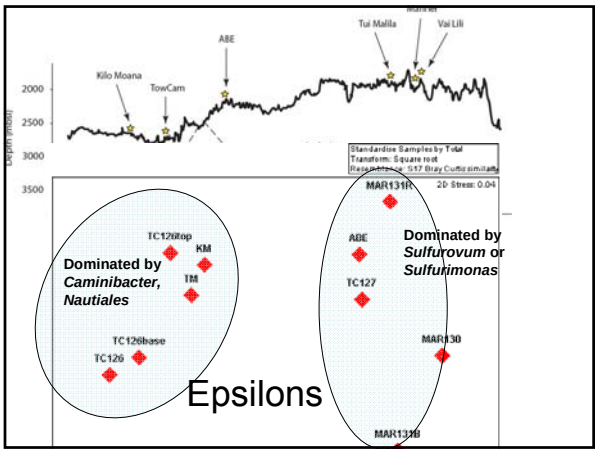
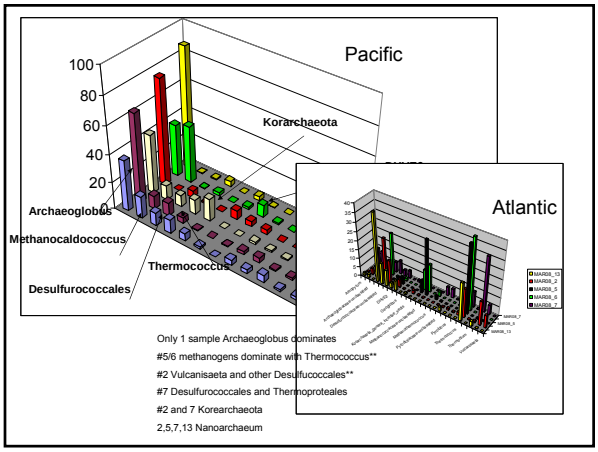
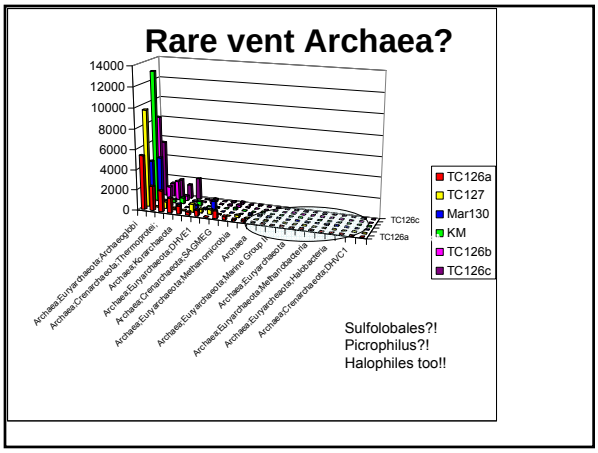


Bacterial


Tui Malila
Vai Lili
ABE
Kilo Moana
TowCam
2000
2500
3000
3500
Depth (mbsl)
Standardise Samples by Total
Transform: Square root
2D Stress: 0.04
MAR131R
TC126top
KM
TM
TC126base
TC126
ABE
TC127
MAR130
MAR131B
Dominated by *Caminibacter, Nautiales*
Dominated by *Sulfurovum* or *Sulfurimonas*
Epsilons


Pacific
100
80
60
40
20
0
Korarchaeota
Archaeoglobus
Methanocaldococcus
Thermococcus
Desulfurococcales
Atlantic
Only 1 sample Archaeoglobus dominates
#5/6 methanogens dominate with Thermococcus**
#2 Vulcanisaeta and other Desulfucoccales**
#7 Desulfurococcales and Thermoproteales
#2 and 7 Korearchaeota
2,5,7,13 Nanoarchaeum


Rare vent Archaea?
14000
12000
10000
8000
6000
4000
2000
0
TC126a
TC127
Mar130
KM
TC126b
TC126c
Sulfolobales?!
Picrophilus?!
Halophiles too!!

Conclusions

- Lots of potential targets still in the environment
- Understanding the ecology… when and where organisms of interest occur, coexist etc.
- Multiple *informed*, coordinated approaches will allow for selective enrichment of metabolisms of interest…
- Consortia for novel metabolisms, synthrophy
- Low biodiversity systems- meta-transcriptome, proteome

Thanks..

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NSF, JGI, ICoMM

The crew, pilots and scientific parties