Extreme Spatial and Temporal Variability of Microbial Mat Communities From Mariana Island Arc Hydrothermal Vents

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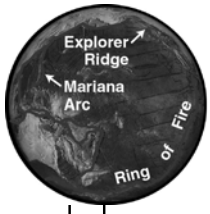
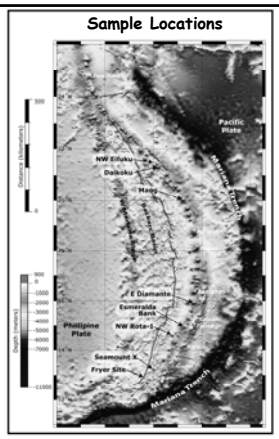
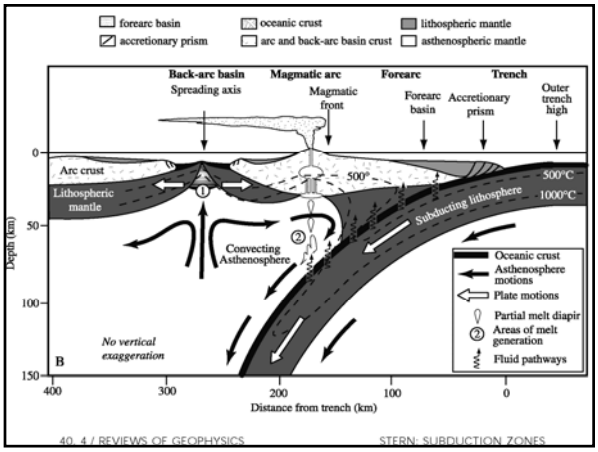
SROF Project
2003-2006



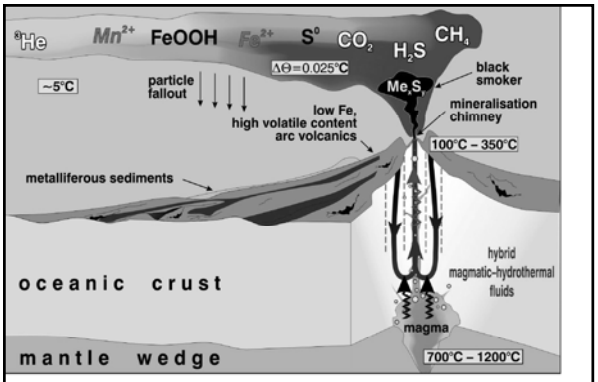
Unlike most vent systems, the Mariana arc/backarc is a convergent plate boundary

Sample Locations





40, 4 / REVIEWS OF GEOPHYSICS STERN: SUBDUCTION ZONES



Schematic cartoon of a hybrid magmatic-hydrothermal venting system with water column plume. Note that the hydrothermal circulation is augmented by magmatic fluid in gaseous (yellow bubbles) and liquid (black squiggly arrows) phases. From "Fluids from Arcs: a submarine magmatic-hydrothermal perspective" G.J. MASSOTH, C.E.J. DE RONDE, E.T. BAKER, J.E. LUPTON, R.J. ARCULUS, & R.W. EMBLEY SOTA 2003

Microbial Metabolic Menu

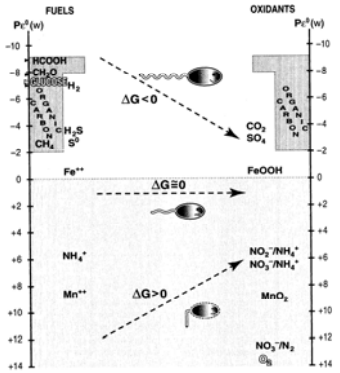
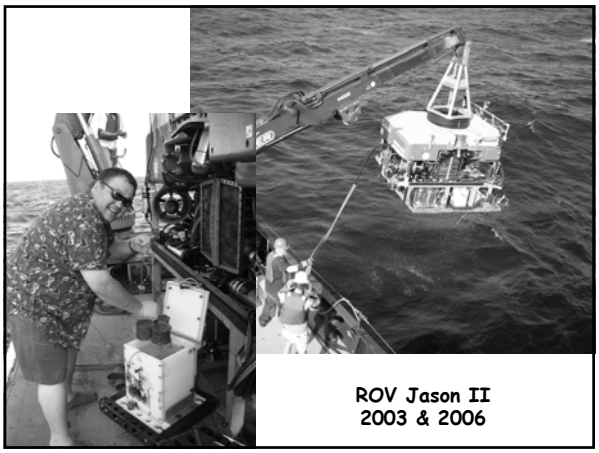


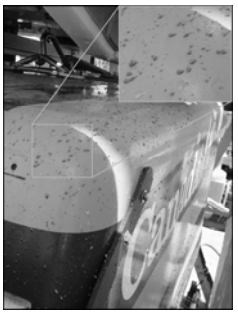
Figure courtesy of Ken Nealson

ROV ROPOS
2004

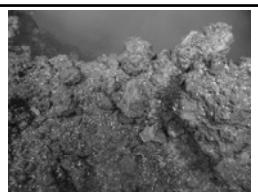


ROV Jason II
2003 & 2006

Observing Active Volcanism at a Submarine Arc Volcano



Sulfur and Rocks Collected Inadvertently by ROPOS



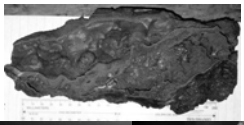
Brimstone Pit, NW Rota-1

Submarine Ring of Fire Major Results/Observations

- **First Observations of Deep Submarine Arc Volcanic Event**
- **Photosynthetic/Chemosynthetic Ecosystems Interaction**
- **Shallowest Massive Sulfide Formation at 344 m (220^0 C)**
- **Champagne Vent, 1600 meter site with intense CO_2 Venting – Bubbles of Liquid CO_2**
- **High Variability of Biological Communities among & between Volcanoes**

Discovery of Active Smokers at only 344 meters

East Diamante Caldera



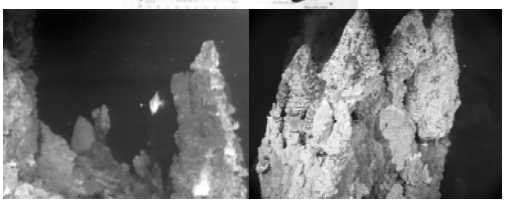


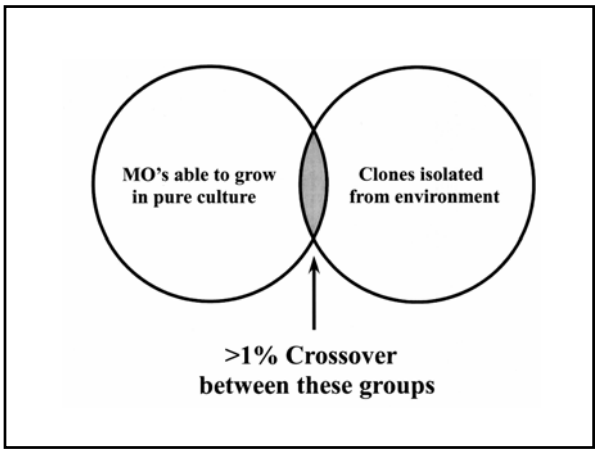
Table 1. Microbial mat samples (n=25) were collected and analyzed using T-RFLP, Q-PCR, and then targeted cloning & sequencing.

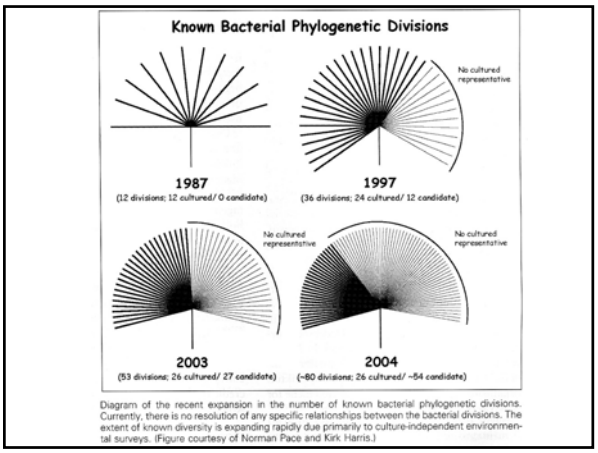
Year	Sample	Vent Site	Location	Spreading Center/ Arc Seamount	Latitude/Longitude	Sample Type	Sample Description	Depth (m)	Temp (°C)
2003	J2-42-1W	Fryer Site	Backarc	SMBSC†	12°57.190 N 143°37.125 E	Push Core	Yellow/White mat	2860	77
2003	J2-42-2W	Fryer Site	Backarc	SMBSC†	12°57.190 N 143°37.125 E	Push Core	Yellow/Green mat	2860	77
2004	R782-b5	Shimmering Vent	Volcanic arc	NW Rota-1	14°36.072 N 144°46.530 E	Suction Sample	Orange mat	516	15
2004	R782-b7	Shrimp Mound	Volcanic arc	NW Rota-1	14°36.072 N 144°46.530 E	Suction Sample	Yellow mat	518	15
2004	R783-b56	Iceberg	Volcanic arc	NW Rota-1	14°36.048 N 144°46.578 E	Suction Sample	White mat	529	58
2004	R786-b567	Fault Shrimp	Volcanic arc	NW Rota-1	14°36.036 N 144°46.644 E	Suction Sample	White mat	584	20
2004	R788-b7	Mat City	Volcanic arc	E Diamante	15°56.322 N 145°40.518 E	Suction Sample	White mat	206	ambient
2004	R788-b5	Five Towers	Volcanic arc	E Diamante	15°56.556 N 145°40.884 E	Suction Sample	Orange mat	344	220
2004	R788-CC	Five Towers	Volcanic arc	E Diamante	15°56.556 N 145°40.884 E	Chimney Chunks	Multi-colored rocks	344	220
2004	R789-b5	Egg Drop Soup	Volcanic arc	Maug Crater	20°01.206 N 145°13.308 E	Suction Sample	Orange mat	149	ambient
2004	R790-b56	Cave Vent	Volcanic arc	Maug Crater	20°01.404 N 145°13.356 E	Suction Sample	Orange mat	145	28
2004	R791-b56	Bacto Balls	Volcanic arc	NW Eifuku	21°29.328 N 144°02.436 E	Suction Sample	Fe mat	1716	ambient
2004	R791-b7	Yellow Top	Volcanic arc	NW Eifuku	21°29.304 N 144°02.424 E	Suction Sample	Fe mat	1674	~6-8
2004	R792-b57	Champagne	Volcanic arc	NW Eifuku	21°29.256 N 144°02.508 E	Suction Sample	White mat	1608	72-103
2004	R792-CC	Champagne	Volcanic arc	NW Eifuku	21°29.256 N 144°02.508 E	Chimney Chunks	White rocks	1608	72-103
2004	R793-b1	Yellow Cone	Volcanic arc	NW Eifuku	21°29.292 N 144°02.526 E	Suction Sample	Fe mat	1587	11
2004	R793-b57	Yellow Top	Volcanic arc	NW Eifuku	21°29.310 N 144°02.424 E	Suction Sample	Fe mat	1678	~6-8
2004	R795-b56	Fish Spa	Volcanic arc	Daikoku	21°19.476 N 144°11.532 E	Suction Sample	White sediment	390	ambient
2006	J2-184-W	Fe-Mats	Volcanic arc	Seamount X	13°15.098 N 144°01.089 E	Suction Sample	Fe mat	1305	nd
2006	J2-184-B	Snail Mat	Volcanic arc	Seamount X	nd	Suction Sample	White mat	1188	nd
2006	J2-190-W	Fe-Mounds	Volcanic arc	Esmeralda Bank	14°57.364 N 145°14.478 E	Suction Sample	Fe sediment	291	40
2006	J2-190-CC	Fe-Mounds	Volcanic arc	Esmeralda Bank	14°57.364 N 145°14.478 E	Chimney Chunks	Fe crust	291	40
2006	J2-191-W	Iceberg	Volcanic arc	NW Rota-1	14°36.052 N 144°46.579 E	Suction Sample	White mat	530	25
2006	J2-197-W	Bubble Bath	Volcanic arc	Daikoku	21°19.505 N 144°11.488 E	Suction Sample	White mat	411	52
2006	J2-197-B	Fish Spa	Volcanic arc	Daikoku	21°19.484 N 144°11.585 E	Suction Sample	Brown sediment	390	ambient

† southern Mariana Backarc Spreading Center

Importance of a Molecular Microbiological Approach

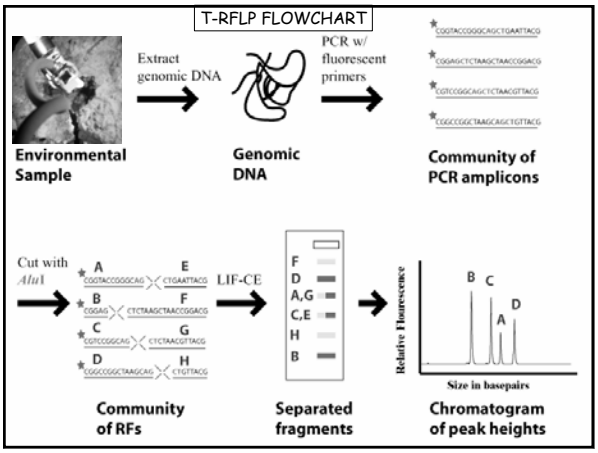
- **Traditional culturing** techniques isolate ~1% of the total bacteria in marine ecosystems, thereby severely underestimating diversity and community structure.
- Because nutrient-rich **culture media** have been historically used during enrichment procedures, bacteria which may be dominant in natural communities are selected against in favor of copiotrophic (weedy) bacteria.
- **SSU rRNAs** and their respective genes are excellent descriptors of microbial taxa based on phylogeny.

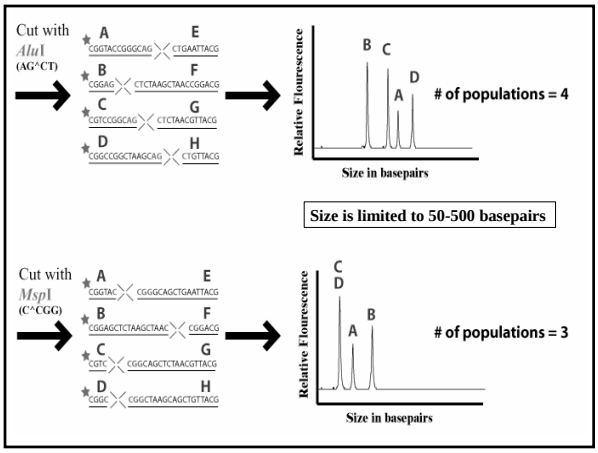


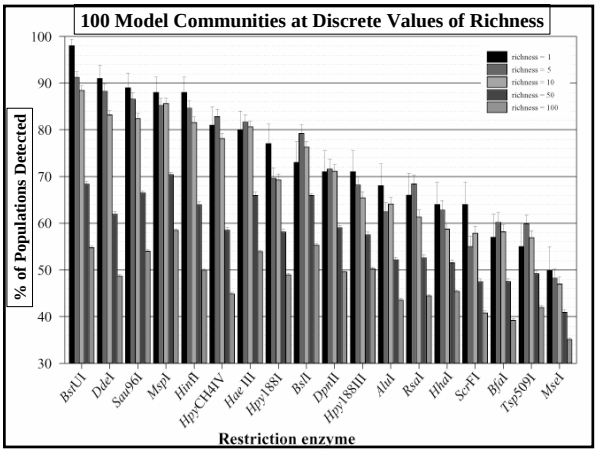


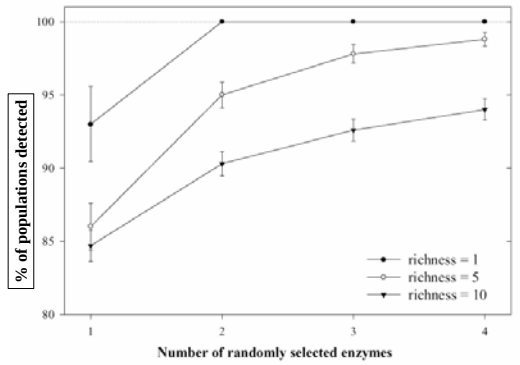
Why ribosomal RNAs?

- Found among **all** living organisms (for 3.8 of the last 4.5 billion years). Integral part of protein synthesis machinery.
- Cell component analyses provide culture-independent means of investigating questions in microbial ecology (lack of morphology).
- rRNAs offer a type of sequence information that makes them excellent descriptors of an organism's evolutionary history.
- No detectable horizontal gene transfer, especially important for *Bacteria* and *Archaea*.
- Large and growing database; RDP contains >480K SSU rRNA genes.





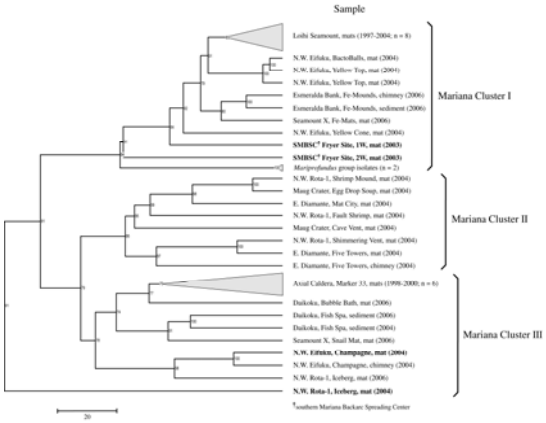


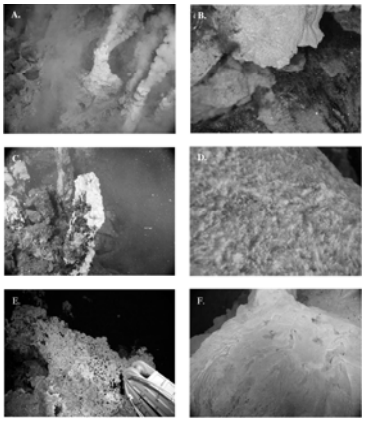


Take Home Message:
Accurately resolve ribotypes in communities with low to intermediate richness!

Integrating Molecular Methods

- Terminal-restriction fragment length polymorphisms (T-RFLPs) were used to track populations based on ribotypes (in lots of samples).
- A/B Q-PCR needed to determine domain level proportions.
- Clone library and phylogenetic analyses needed to identify these phylotypes within the community (in few samples).

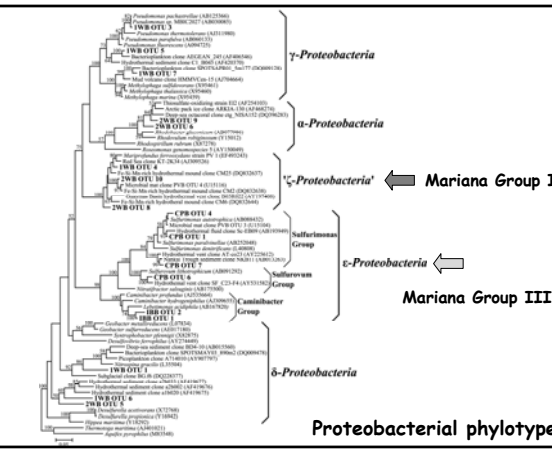




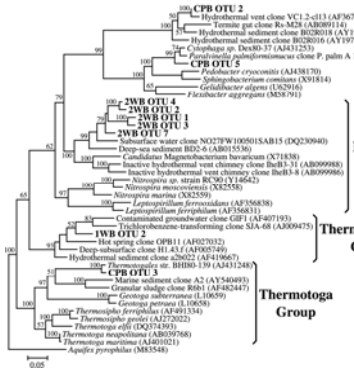
Mariana Group III
Mostly Sulfur-cycling
ε-*Proteobacteria*

Mariana Group II
Mostly Heterotrophs
like CFBs

Mariana Group I
Mostly Iron-cycling
ζ-*Proteobacteria*



Proteobacterial phylotypes



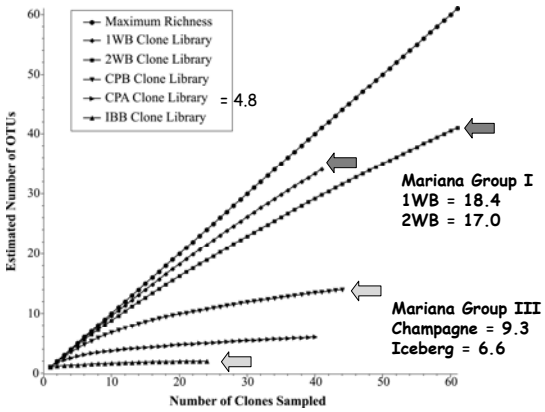
Non-Proteobacterial phylotypes

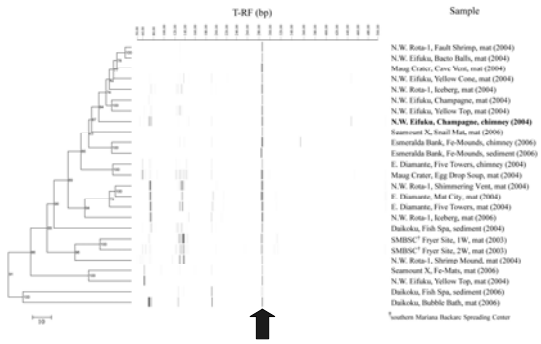
Biomass Proxy

Estimate of Complexity (aka Richness)

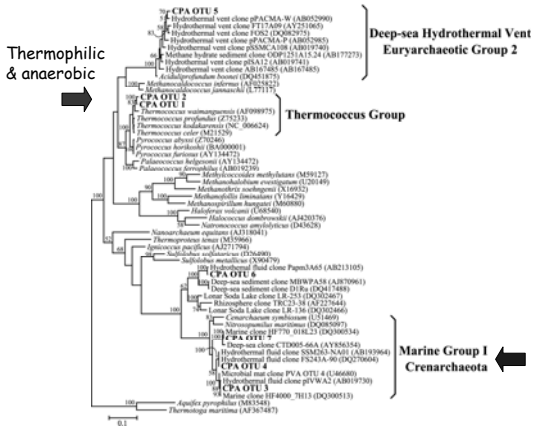
Year	Sample	Vent Site	ng DNA/g Sample (wet weight)	Percent Archaea	Ave. T-RFs Bacteria	Ave. T-RFs Archaea
2003	J2-42-1W	Fryer Site	2250	11.3±0.49	18.4±4.2	18.3±6.5
2003	J2-42-2W	Fryer Site	2466	10.4±0.26	17.0±1.5	16.9±5.4
2004	R782-b5	Shimmering Vent	147	2.2±0.16	16.6±3.6	9.8±3.5
2004	R782-b7	Shrimp Mound	2450	4.4±0.37	12.8±4.0	8.3±3.0
2004	R783-b56	Iceberg	2370	5.5±0.45	6.6±4.7	9.0±3.8
2004	R786-b567	Fault Shrimp	6080	2.3±0.34	12.8±3.0	4.0±1.4
2004	R788-b7	Mat City	11540	10.1±1.10	13.5±3.8	9.8±2.3
2004	R788-b5	Five Towers	190	2.3±0.17	16.8±3.7	11.3±4.0
2004	R788-CC	Five Towers	1044	0.2±0.02	14.3±3.3	10.3±4.0
2004	R789-b5	Egg Drop Soup	156	2.3±0.62	18.3±4.8	5.5±1.5
2004	R790-b56	Cave Vent	4380	11.3±1.37	12.8±2.4	3.3±1.9
2004	R791-b56	Bacto Balls	477	4.0±0.19	16.4±5.2	2.0±1.0
2004	R791-b7	Yellow Top	784	4.4±0.78	17.1±4.0	2.4±0.9
2004	R792-b57	Champagne	3300	0.8±0.07	9.3±6.5	4.8±2.3
2004	R792-CC	Champagne	1350	0.5±0.03	6.4±3.9	3.5±2.3
2004	R793-b1	Yellow Cone	218	2.0±0.24	15.4±4.6	5.5±2.3
2004	R793-b57	Yellow Top	517	2.9±0.67	12.3±6.8	7.0±2.6
2004	R795-b56	Fish Spa	7106	2.8±0.40	12.4±3.6	11.8±2.4
2006	J2-184-W	Fe-Mats	1486	2.9±0.28	10.4±4.3	5.9±2.5
2006	J2-184-B	Snail Mat	5817	1.5±0.16	12.9±2.9	3.3±1.7
2006	J2-190-W	Fe-Mounds	3722	6.0±0.17	13.5±5.5	6.1±1.1
2006	J2-190-CC	Fe-Mounds	1631	6.3±0.50	11.5±4.3	6.1±2.0
2006	J2-191-W	Iceberg	617	0.4±0.15	6.0±2.2	7.9±2.6
2006	J2-197-W	Bubble Bath	1608	1.9±0.32	8.4±4.5	7.1±4.7
2006	J2-197-B	Fish Spa	5240	12.7±0.62	14.4±4.3	10.1±2.4

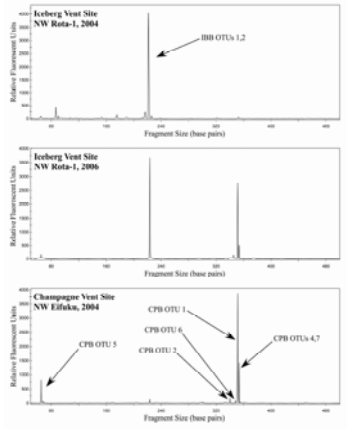
Difference is Bacteria





Marine Group I: Cosmopolitan Archaeoplankton





Mariana Group III
ε-*Proteobacteria*
Mostly Caminibacter Group

58 to 25°C Shift

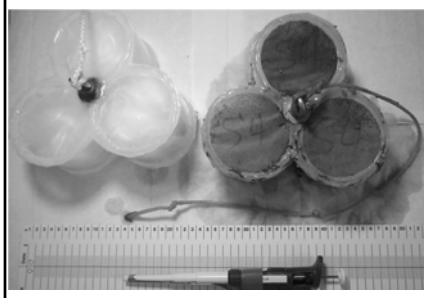
Mariana Group III
ε-*Proteobacteria*
Both

Mariana Group III
ε-*Proteobacteria*
Mostly Sulfurimonas Group
~73°C

SUMMARY

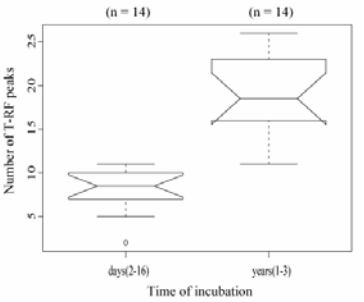
- Microbial community diversity is much greater along the Mariana Arc/Backarc than at either a hotspot volcano or at mid-ocean ridge along similar same spatial scales.
- This is most likely the result of the complexity of vent effluent chemistry and the ephemeral nature of hydrothermal venting along the Mariana Arc/Backarc.
- Cluster analysis of T-RFLP fingerprints reveals the microbial communities formed three distinct clusters designated Mariana Clusters I, II, and III.
- The Mariana Arc/Backarc hydrothermal vent systems support a Bacterial biodiversity hotspot which may be indicative of convergent plates boundary vent systems worldwide.

Microbial Growth Chambers (MGCs)



- Silica wool as inert substrate with ~3m^2 surface area
- Enclosed by 202 micron Nytex filter
- Can withstand 100°C
- Placed within flow for short- and long-term durations

Notched Box-and-Whisker Plot: Axial Seamount



- Entire dataset pooled into 2 bins
- Notches do NOT overlap indicating that the differences are highly significant
- Yields a first order approximation for bacterial colonization rates.

Quote: Baas Becking (1934)

(Referring to bacteria) - "everything is everywhere: but the milieu selects ... in nature and in the laboratory".

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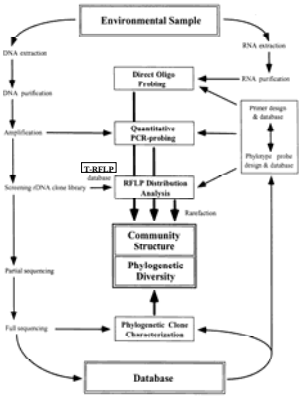
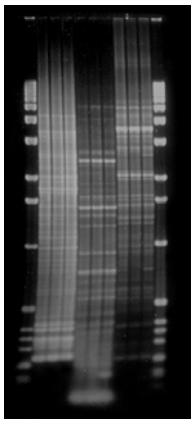
Submarine Ring of Fire
Diakoku Volcano 2006





BOX, ERIC, & REP PCR

Lanes represent: Strains RL1, ES1, & ES2

- Minimal genetic variability among strains of Fe-oxidizing bacteria.
- Additional strains will determine if endemic or cosmopolitan at genomic level.