

Metagenome Analysis Test
Case: Exploring the Hellenic
Volcanic Arc

Rekha Seshadri, Ph.D. MGM-243 Workshop Walnut Creek, CA (9/29/2016)

Summary

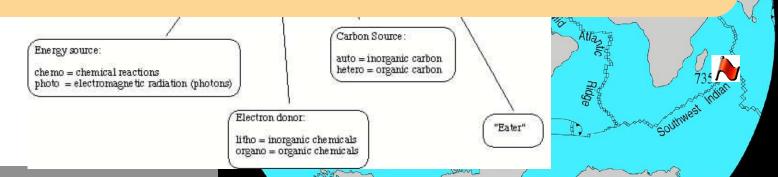


Hydrothermal vent communities represent deep, hot, aphotic biosphere. An entire ecosystem flourishes in an ostensibly hostile environment – how? Geothermal energy converted to biomass. Proxy for studying life on Mars and Europa.

Hellenic Volcanic Arc is geologically unique -relatively recent eruption in1650 AD. Virtually pure CO2 emissions, no macrofauna or complex ecosystem as seen in other sites

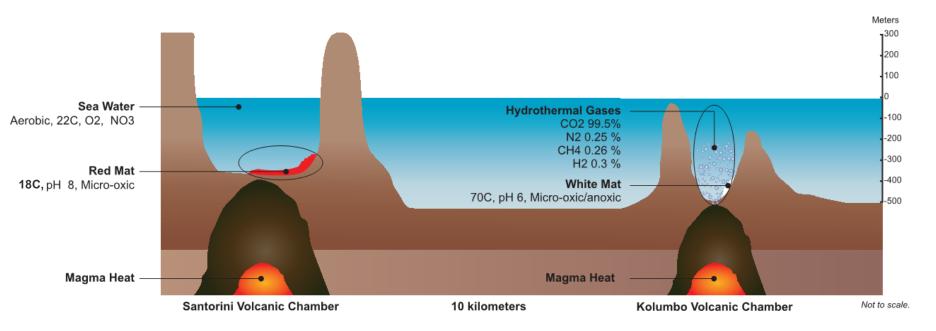
PREDICTION

- Anaerobic communities that derive cellular carbon from CO2 using energy from oxidizing inorganic S, N, Fe compounds
- Some methanotrophy?



Sample Sites





- 3.3 Gbp assembled
- High biomass sample

- 1.44 Gbp assembled
- CO2-intensive bubbling, sulfidic chimney

Objectives

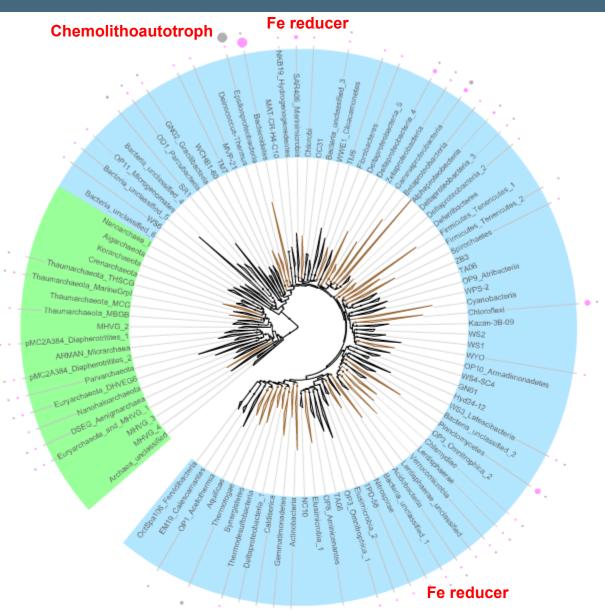


- Who's there summarizing and contrasting taxonomy in Red Mat vs White Mat
- What's going on summarizing and contrasting functions methanotrophy, chemolithoautotrophy, etc
 - Comparative metagenomics with related environments
- Who does what examining binned assemblies for biomarkers

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Who's there: 16S rRNA Phylogeny





White Mat dominated by ζ -Proteobacteria (e.g., *Nitratiruptor*, *Sulfurimonas*, *Sulfurospirillum*) followed by γ -Proteobacterial Methylococceae (methylotroph)

Red mat dominated by Bacteriodetes, Chloroflexi, Planctomycetes, SAR406

Archaea not abundant in either sample

Many unknown genera from candidate phyla or unclassifiable

Asserting physiology from taxonomy is therefore limited

How to do it in IMG?



Scaffold ID

KVRMV2_100000122

KVRMV2_100000200

KVRMV2 100000372

KVRMV2 100000508

KVRMV2 100001192

Length

1414bp

959bp

1459bp

1413bp

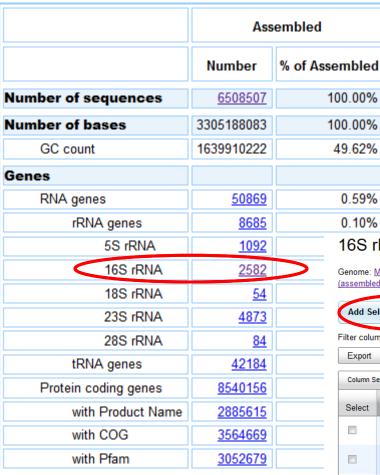
1388bp

9084..10542(-)

2663..4075(+)

1457...2844(+)

Metagenome Statistics



757 16S assemblies > 500 bp selected

16S rRNA Genes (assembled)

KVRMV2 10000037212

KVRMV2 1000005084

KVRMV2 1000011926

rRNA

rRNA

rRNA

0.59%

0.10%

(assembled) Add Selected to Gene Cart Select All Clear All Filter column: Gene ID ▼ Filter text ▼ : Apply Export Page 1 of 10 << first < prev 1 2 3 4 5 6 7 8 9 10 next > last >> 100 ▼ Select Page Deselect Page Column Selector Gene Symbol Coordinates Select Gene ID Locus Type Gene Product Name 16S rRNA, Bacterial KVRMV2_10000012246 rRNA **16S** 47731..49144(+) SSU 16S rRNA. Bacterial KVRMV2 10000020010 rRNA **16S** 10820..11778(+) SSU

SSU

SSU

16S rRNA. Bacterial

16S rRNA, Bacterial

16S rRNA. Bacterial

16S

16S

16S

Genome: Marine sediment microbial communities from Kolumbo Volcano mats, Greece - red mat (*) (MER-FS)

Objectives



- Who's there summarizing and contrasting taxonomy in Red Mat vs White Mat
- What's going on -
 - Comparative metagenomics with related environments
 - summarizing and contrasting expected functions methanotrophy, chemolithoautotrophy, etc

Who does what – examining binned assemblies for biomarkers

Find Genomes>Genome Browser>View "Alphabetically"





- Search for appropriate genomes using genome search or browse list
- Select and add to cart scroll to bottom to "Table Configuration" – select metadata fields and display again



Caveats

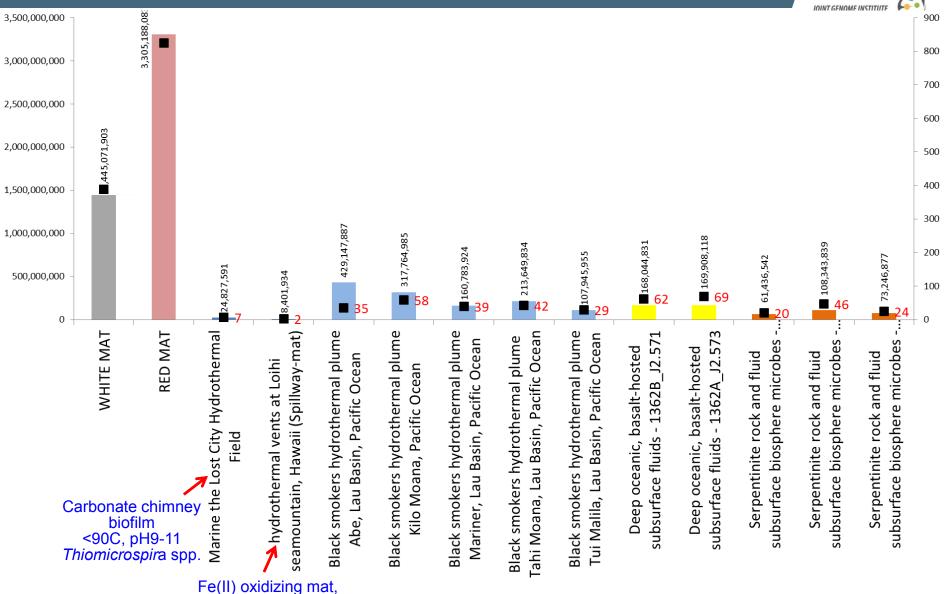


- Differences in sequencing platforms Sanger, 454, Illumina
- Different qualities of assembly (and annotation)
- Many unpublished datasets extensive metadata or particulars not available
- Read data/coverage not available for non-JGI metagenomes

Size & Diversity of Relevant Datasets

23C, pH5.5 Zetaproteo





Compare Genomes>Genome Clustering> Hierarchical Clustering



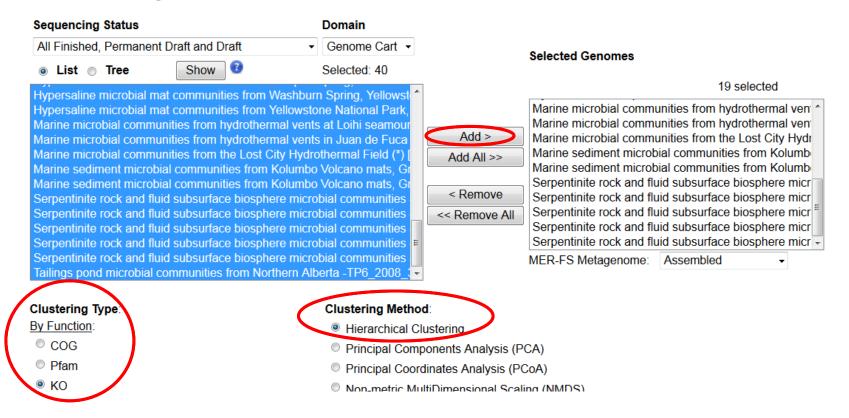
Genome Clustering



You may cluster samples (genomes) based on similar functional profiles.

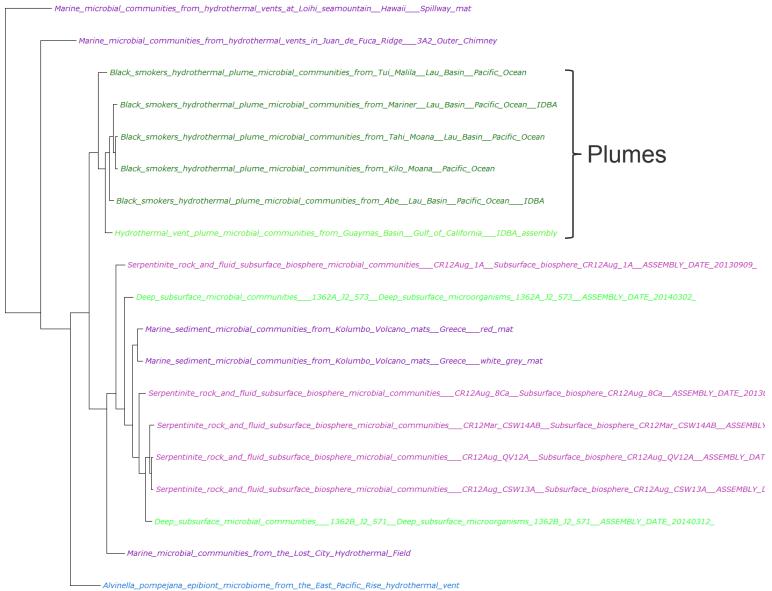
Proximity of grouping indicates the relative degree of similarity of samples to each other.

Please select 2 to 2300 genomes.



Hierarchical Clustering Result





Objectives



- Who's there summarizing and contrasting taxonomy in Red Mat vs White Mat
- What's going on -
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 - Summarizing and contrasting functions methanotrophy, chemolithoautotrophy, etc

Who does what – examining binned assemblies for biomarkers

Profile and Alignment Tools

- Hold down the control key (or command key in the case of the Mac) to select multiple genomes.
 - Drag down list to select all genomes.
 - More genome and function selections result in slower query.



Sequencing Status Domain All Finished, Permanent Draft and Draft Genome Cart ▼ Show Selected: 40 List Tree Marine microbial communities from hydrothermal vents at Loihi seamountain, Hawaii - Spillway-mat (*) [D] Marine microbial communities from hydrothermal vents in Juan de Fuca Ridge - 3A2 Outer Chimney (*) [D] Marine microbial communities from the Lost City Hydrothermal Field (*) [D] Marine sediment microbial communities from Kolumbo Volcano mats, Greece - red mat (*) [D] Marine sediment microbial communities from Kolumbo Volcano mats, Greece - white/grey mat (*) [D] Serpentinite rock and fluid subsurface biosphere microbial communities - CR12Aug 1A (Subsurface biospher Serpentinite rock and fluid subsurface biosphere r **Function Profile** Serpentinite rock and fluid subsurface biosphere

KO:K01895

KO:K13788

KO:K00193

KO:K00194

KO:K00197

KO:K00200 KO:K00201

202 functions and 17 genomes are selected.

- Figure out appropriate markers for processes
- Upload selected function to analysis cart
- View function vs. metagenomes

6

272

4

15

0

366

21

45

34

262

26

56

18

10

16

(MER-FS)

94

189

45

53

19

28

(ass

(assembled)

Function Profile

MER-FS Metagenome:

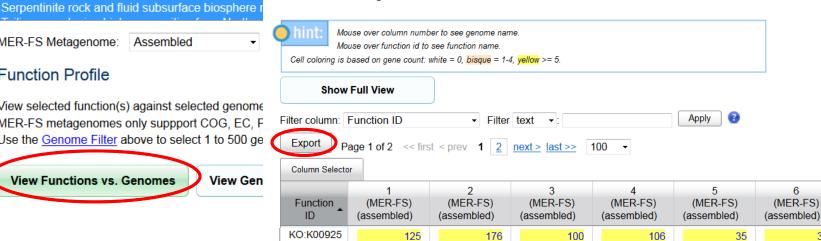
View selected function(s) against selected genome MER-FS metagenomes only support COG, EC, F Use the Genome Filter above to select 1 to 500 ge

Serpentinite rock and fluid subsurface biosphere r

Assembled

View Functions vs. Genomes

View Gen



537

20

47

12

173

<u>16</u>

38

4

2

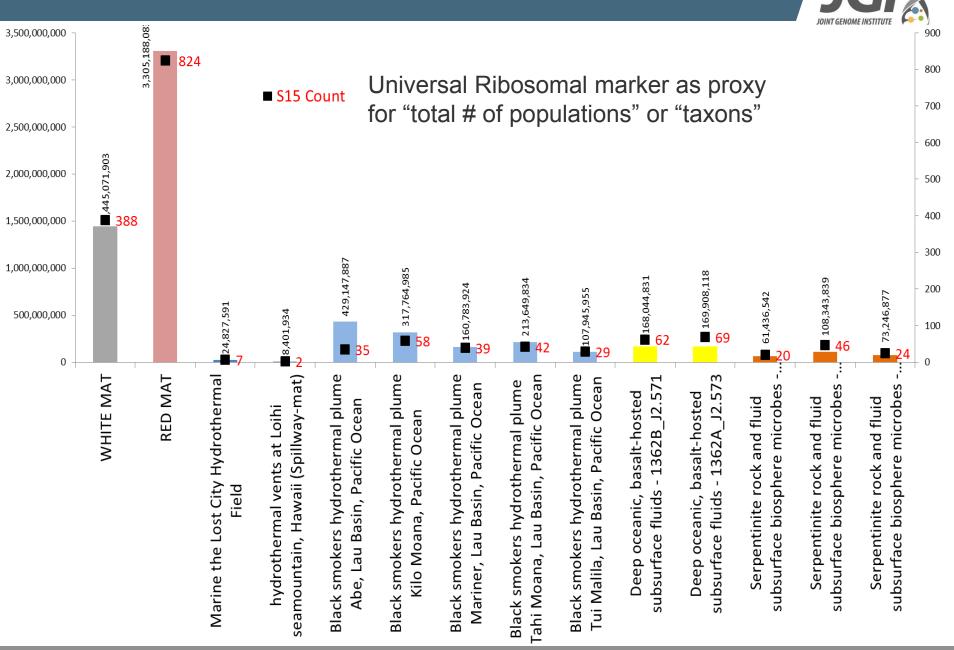
364

10

37

How to Normalize

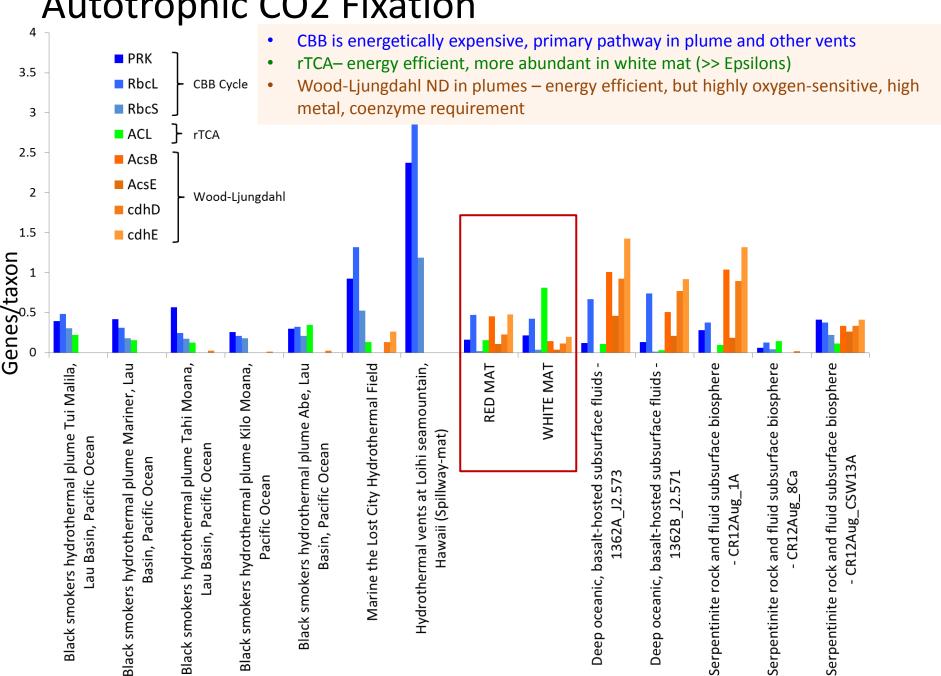




Carbon Source

Autotrophy

Autotrophic CO2 Fixation

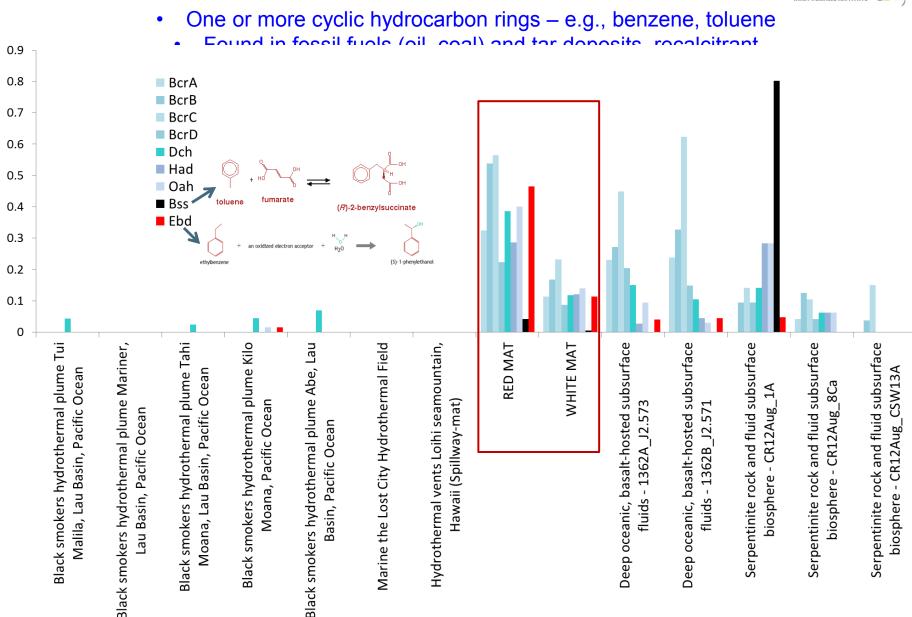


Carbon Source

Heterotrophy?

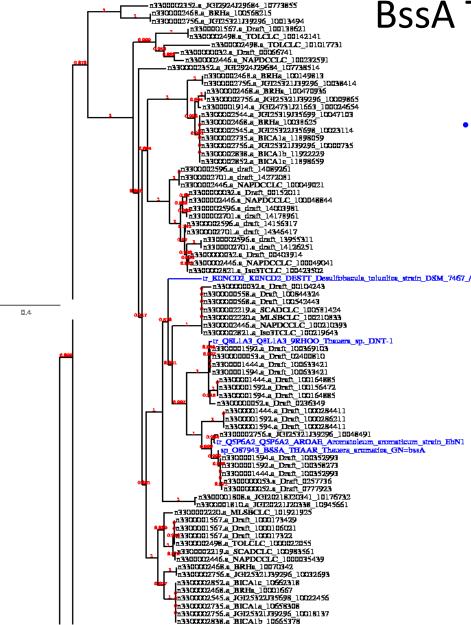
Anaerobic degradation of aromatic hydrocarbons





BssA Tree

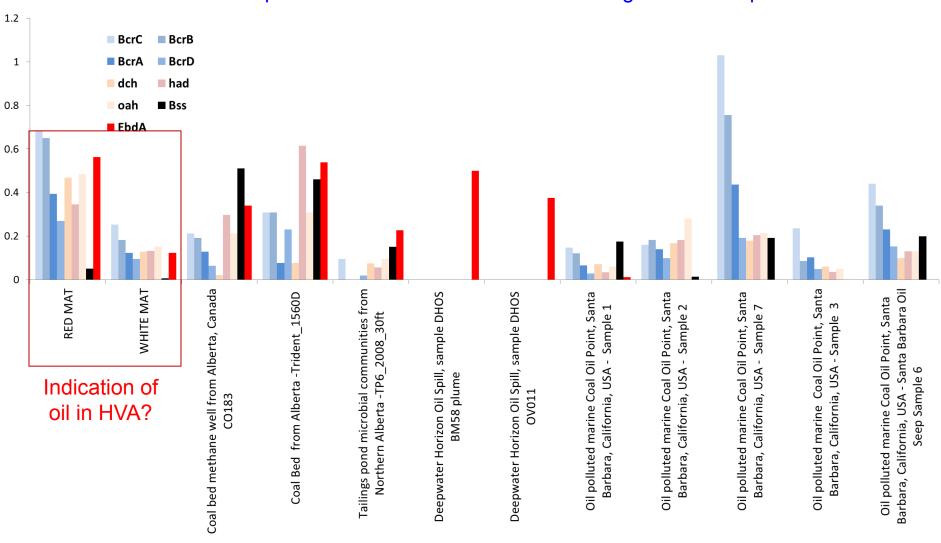
Assignments confirmed – re-did search with marker genes using more stringent Usearch constraints



Compared to Oil-related metagenomes



Markers not ubiquitous - detected in <5% of total metagenomic samples in IMG/MER



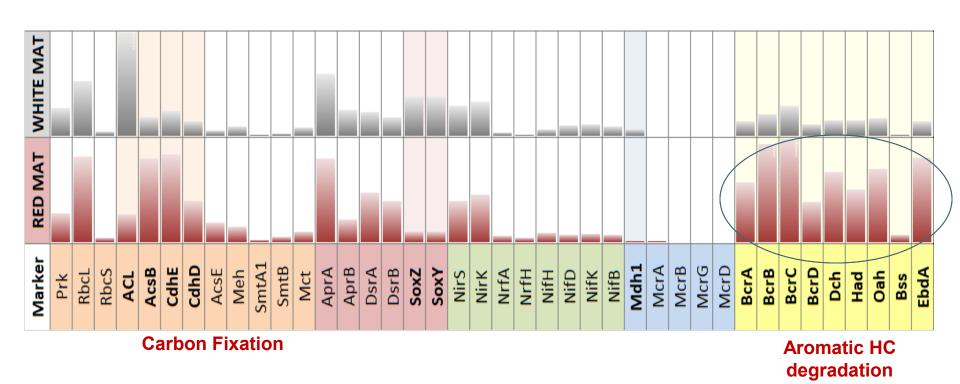
Origin of aromatic hydrocarbons in HVA?



- PAHs previously reported in HVs
- Hydrothermal alteration of contemporary biomass? In Guyamas Basin, OM sediment pyrolized to cycloalkanes, PAH, LMW alkanes, etc
- Abiotic origin of hydrocarbons by thermogenesis (Fisher-Tropsch rxn.) in earth's crust – LMW PAH vented out by dissolving in hydorthermal fluids
- Anthropogenic contaminants in Mediterranean
- Subsurface oil reservoir

Red Mat Vs White Mat Overview

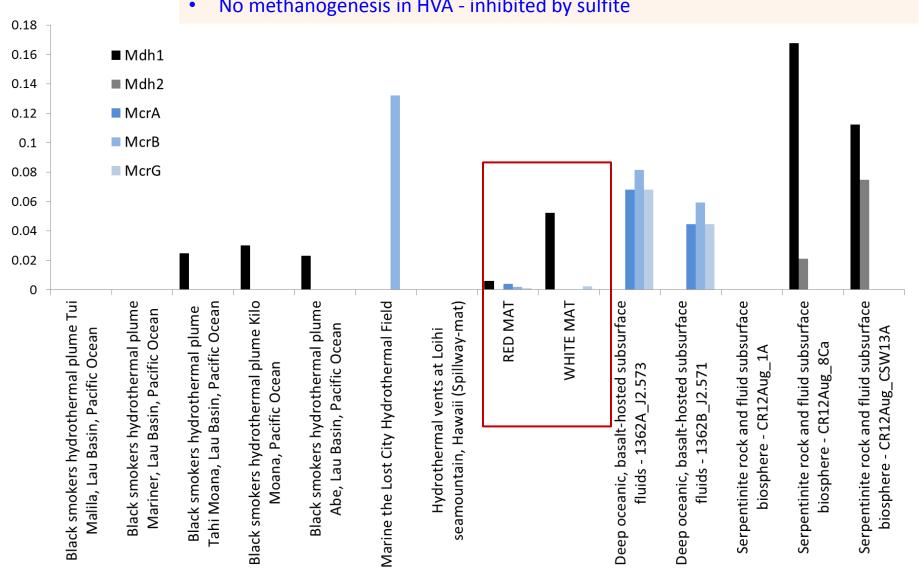




Not just chemoautotrophs, but potentially chemoheterotrophs!

Methane Metabolism

- CH4 as sole carbon and energy source, 0.26% CH4 detected in gas samples
- Methanotrophy primarily in white mat
- No methanogenesis in HVA inhibited by sulfite



Objectives

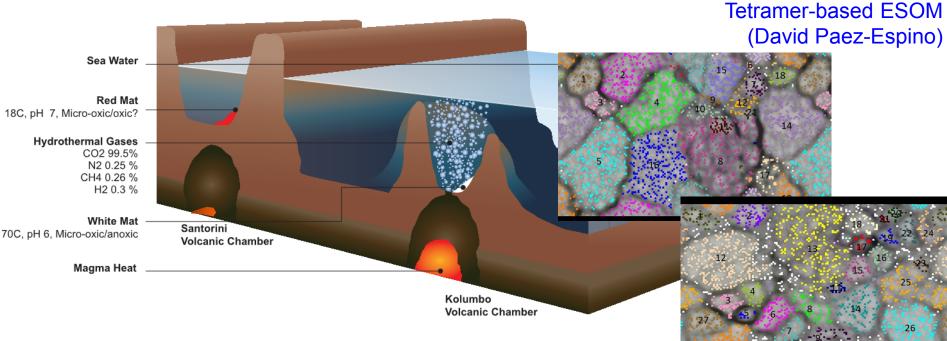


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Who does what – examining binned assemblies for biomarkers

Who does What – Binning & Marker Detection





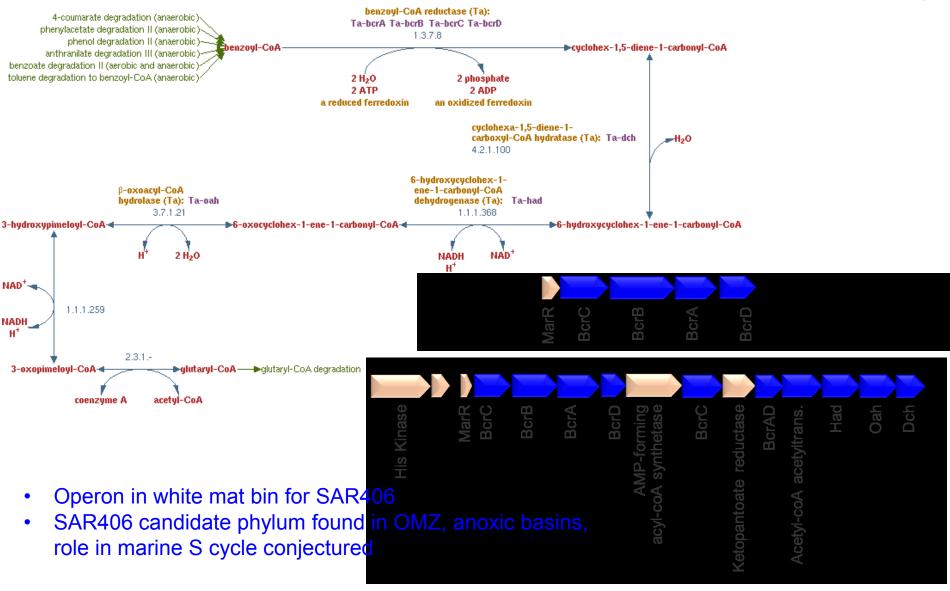
	RED MAT	WHITE MAT
CO2 Fixation	Bacteroides, OP8, Mariprofundus, Halorhodospira	Campylobacteraceae, Delta_NaphS2-like
$SO_4 \rightarrow S^{2-}$	Bacteroides, Halorhodospira	Delta_NaphS2-like
$S_2O_3 \rightarrow SO4$	γ -proteobacteria	Campylobacteraceae, Methylococcaceae_highGC
$NO3 \rightarrow N_2/NH_3$	Bacteroides, SAR406, Gemmatimonas	Bacteroidales_vc21, Campylobacteraceae
$N_2 \rightarrow NH_3$	ND	Coraliomargarita, Campylobacteraceae, Methylococcaceae
$NH_3 \rightarrow NO_3$	ND	ND
$CH_4 \rightarrow CO_2$	ND	Methylococcaceae_38GC, Methylococcaceae_highGC
MAH Deg.	SAR406	Desulfurobacteraceae, Bacteroidales_vc21

E.g., Anaerobic Benzoyl-coA Degradation

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Conclusions



- Evidence of chemolitho- and chemoheterotrophy using energy from oxidation of reduced sulfur, nitrogen, H2, Fe, Mn, and more
- Taxonomic compositions and functional content correspond to physicochemical characteristics of each sample
 - White mat relatively enriched in sulfur oxidation, reverse TCA cycle for CO2 fixation, methanotrophy
 - Evidence of anaerobic degradation of aromatic hydrocarbons predominates in **red mat** – a significant source of carbon in this environment? Origin of these compounds?
- Novel physiological attributes pinned to candidate phyla e.g., benzoate/benzoyl-coA degradation attributed to red mat SAR406 (typically detected in O₂-deficient marine systems)

Contributors



- Natalia Ivanova
- Nikos Kyrpides
- Anastasis Oulas (Hellenic Centre for Marine Research)
- Manolis Mandalakis (Hellenic Centre for Marine Research)
- David Paez-Espino
- Paraskevi Polymenakou (Hellenic Centre for Marine Research)
- Jim Tripp

• Citation: Metagenomic investigation of the geologically unique Hellenic Volcanic Arc reveals a distinctive ecosystem with unexpected physiology. Oulas et. al., Environ Microbiol. 2016. 18(4):1122-36.