Metagenomics of hydrothermal fumaroles to study the evolution of early life at high temperatures

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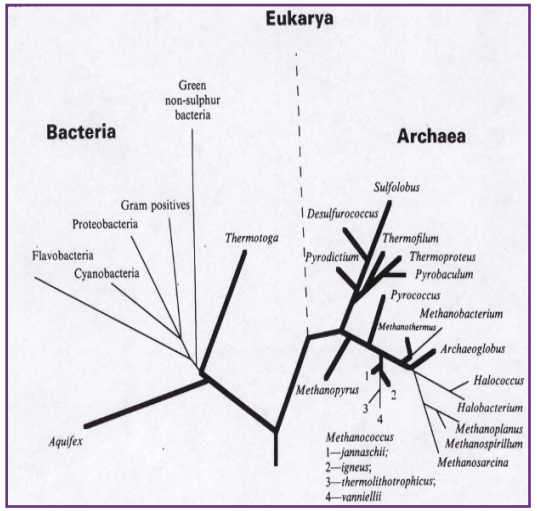
Extremophilic Archaea

In an anthropocentric vision, *extremophiles* are organisms living at conditions inhospitable for human beings.

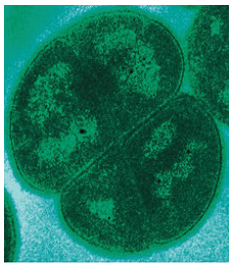
Most, but not all, extremophiles belong to the domain of Archaea and most, but not all, Archaea are extremophiles.



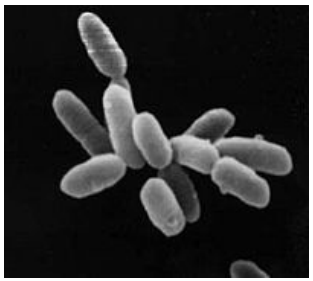
Extremophiles *require* these conditions for living (extremophily *vs* extremotolerance)

No fossil nucleic acids: no molecular signatures
Only phylogenetic analysis can be performed

Phylogenetic analysis with several molecular probes locates (hyper)thermophiles in the basal position of the tree

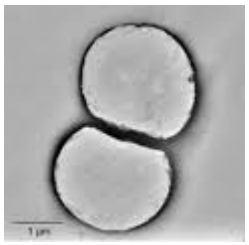


Deinococcus radiodurans
pH 7.0, 37°C
Radiation (5000 Gy), cold, dessication, vacuum, acid, etc

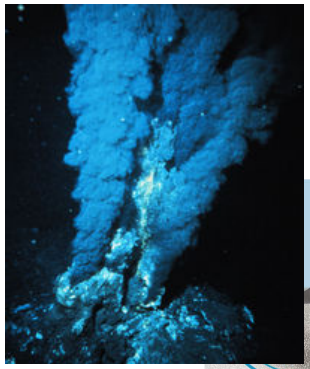


Halobacterium
NaCl 5.5 M

Halomonas salaria
1000 atm; 4°C



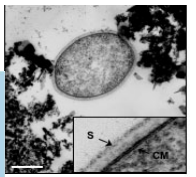
Picrophilus torridus
pH 0.06

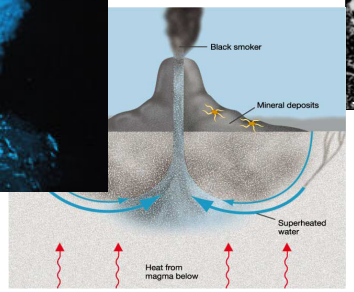


BREVIA 15 AUGUST 2003 VOL 301 SCIENCE

Extending the Upper Temperature Limit for Life

Kazem Kashefi and Derek R. Lovley*





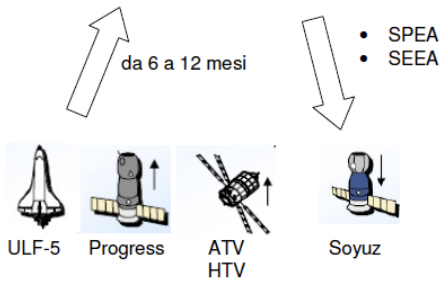
Strain121
120°C
(130°C)

Why the study of thermophilic archaea is relevant in astrobiology?

•Effect of space parameters on cell survival and on the basic processes of life.
temperature extremes, microgravity, radiation, etc.

•Models of pioneering life-forms on other planets
traces of extant or past life on space bodies.

•Evolutionary history of life on Earth
origin, evolution and distribution of life.
nature of the Last Universal Common Ancestor (LUCA)

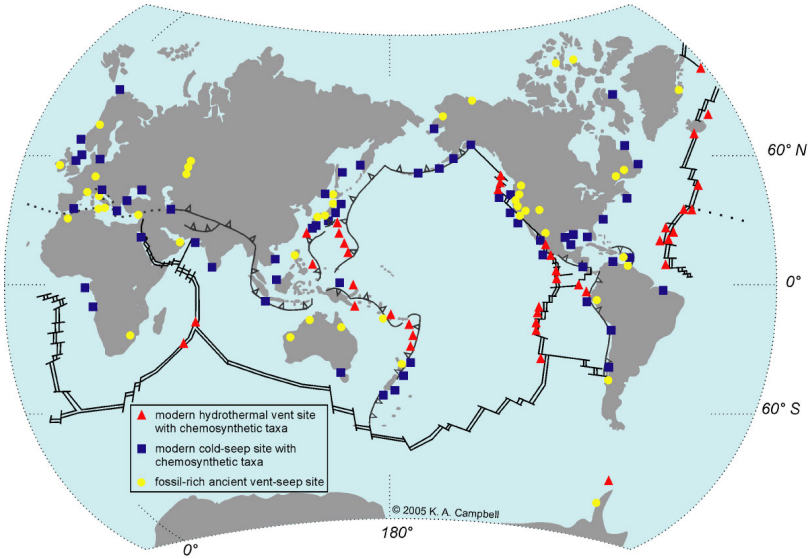


A QUALCUNO PIACE CALDO. Nel Parco di Yellowstone (a sinistra) ci sono sorgenti termali dove a temperature superiori ai 100 °C vivono batteri e archea (come la specie estremofila *Pyrococcus furiosus*, in basso).

L'interesse scientifico attorno a questi organismi ha una ragione: potrebbero farci capire com'è la vita sugli altri pianeti

Experiment ELESEO
Effects of the Long Exposure to the Space environment on Extremophilic Organis

Exploration of hydrothermal vents on Earth for astrobiology



Culture-independent surveys (rRNA genes) have identified a widespread euryarchaeotal lineage, DHVE2

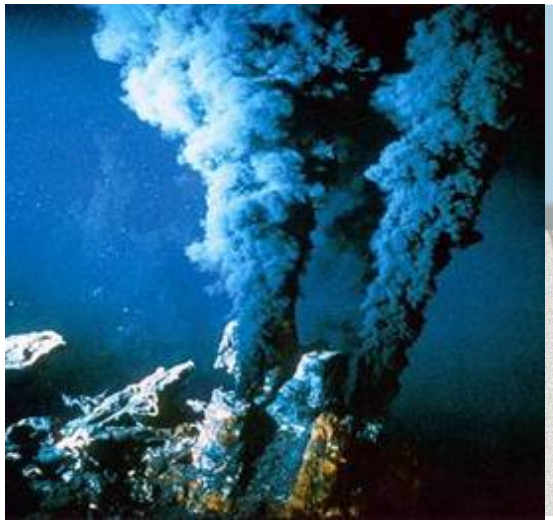
Exploration of these environments on Earth can teach us a lot on how life originated/evolved and it is a cheap alternative/parallel to space exploration

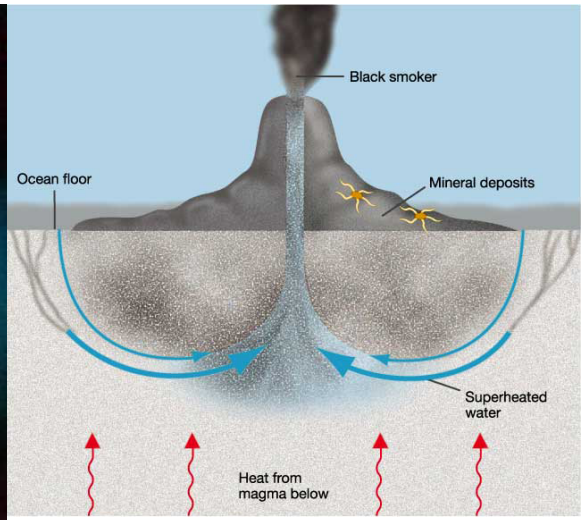
nature Vol 442|27 July 2006|doi:10.1038/nature04921

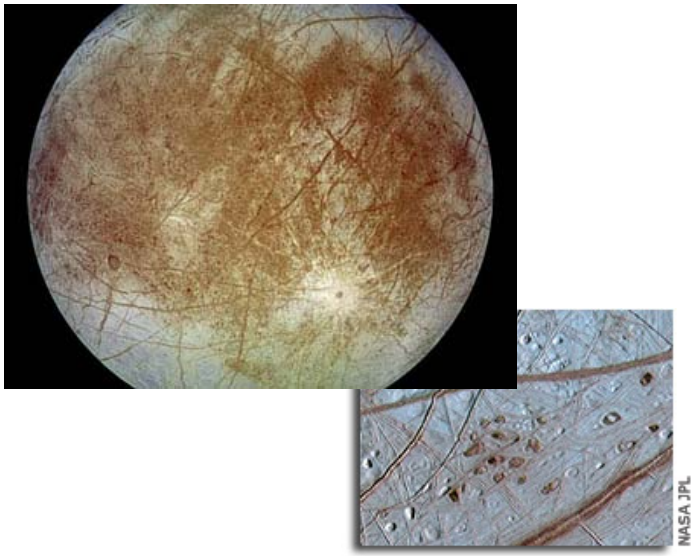
LETTERS

A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents

Anna-Louise Reysenbach[1], Yitai Liu[1], Amy B. Banta[1], Terry J. Beveridge[2], Julie D. Kirshtein[3], Stefan Schouten[4], Margaret K. Tivey[5], Karen L. Von Damm[6] & Mary A. Voytek[3]







Bando di Ricerca n. DC-DTE-2011-033

Esobiologia ed ambienti estremi: dalla **C**himica delle **M**olecole alla **B**iologia degli Estremofili

ECMB

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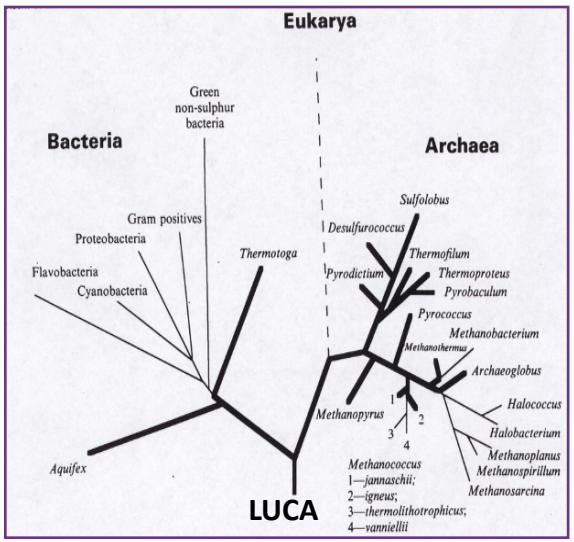
IBBR in ASI-ECMB: System Biology of hyperthermophylic Archaea

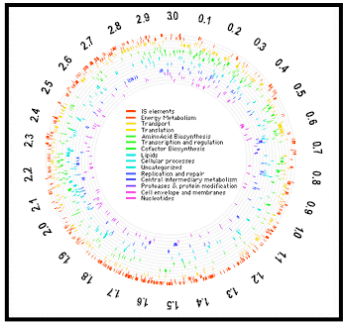
Evolution



mesophiles

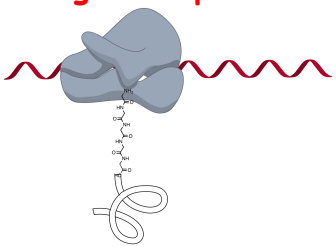
(hyper)thermophiles

Molecular Biology

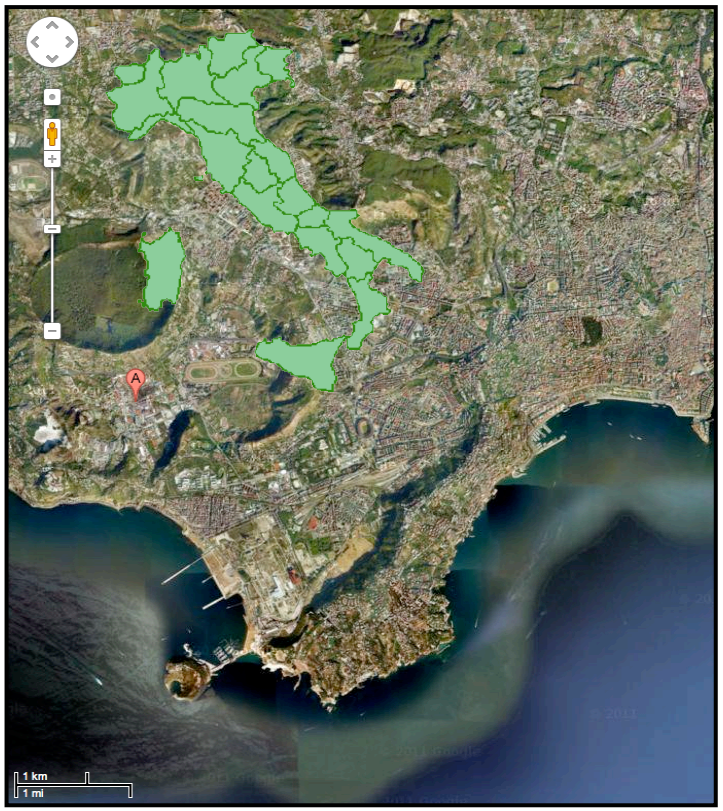


Enzymatic bocatalysis and biotransformations

Regulation of gene expression



Biodiversity of extreme environments



Protein glycosylation

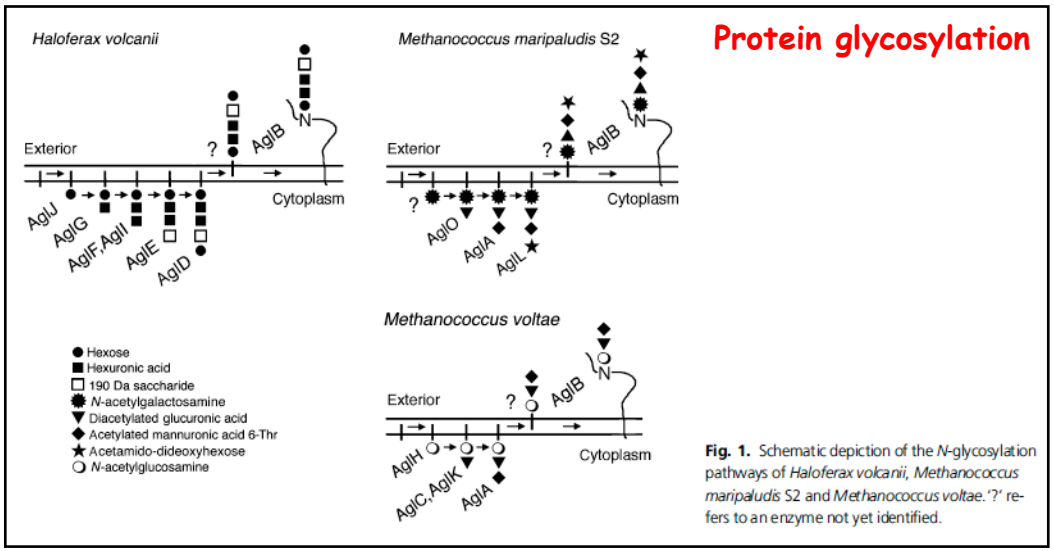


Fig. 1. Schematic depiction of the *N*-glycosylation pathways of *Haloferax volcanii*, *Methanococcus maripaludis* S2 and *Methanococcus voltae*. '?' refers to an enzyme not yet identified.

Sampling site: Solfatara Pisciarelli Agnano (Naples)

ELSEVIER

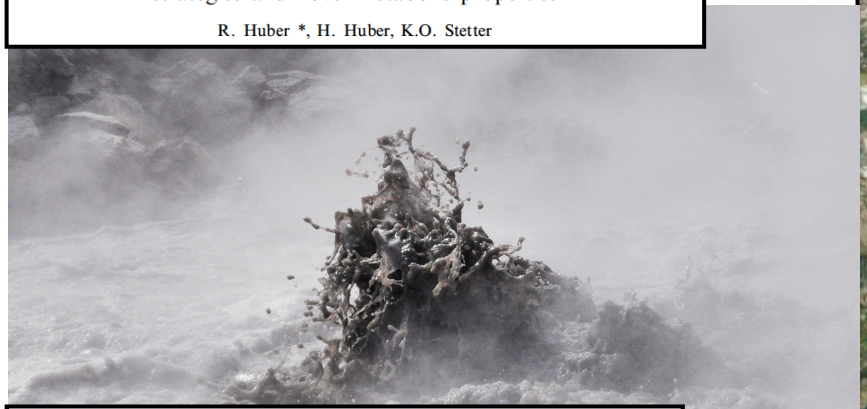
FEMS Microbiology Reviews 24 (2000) 615–623

FEMS MICROBIOLOGY Reviews

www.fems-microbiology.org

Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties

R. Huber *, H. Huber, K.O. Stetter

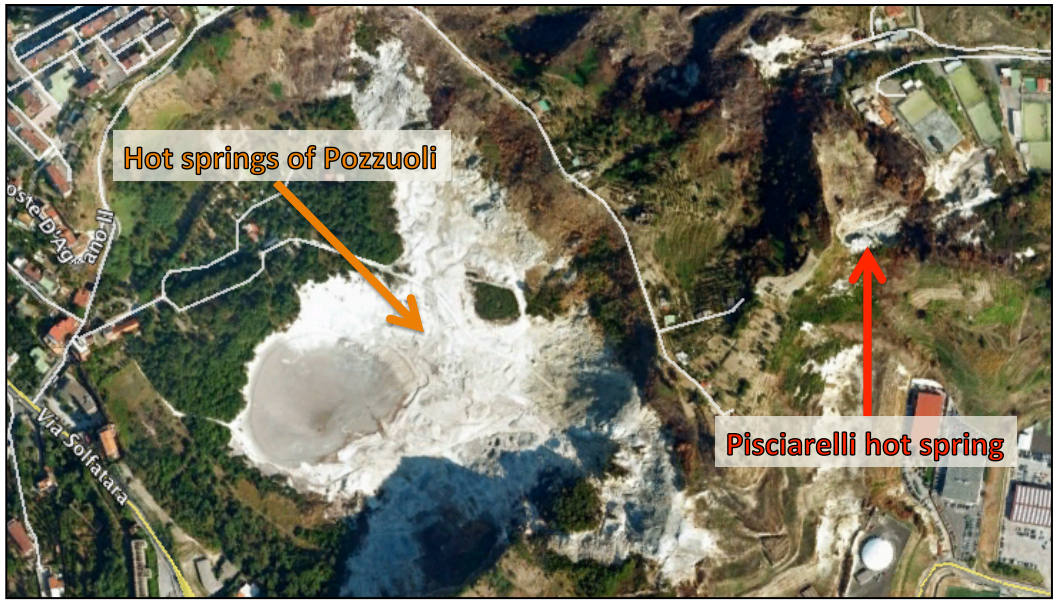


ANNALS OF GEOPHYSICS, 54, 2, 2011; doi: 10.4401/ag-5002

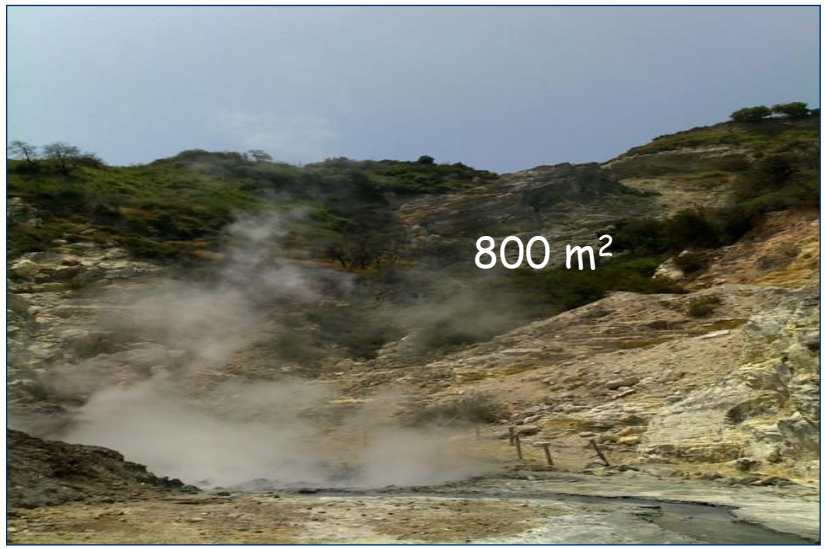
Temperature and pressure gas geoindicators at the Solfatara fumaroles (Campi Flegrei)

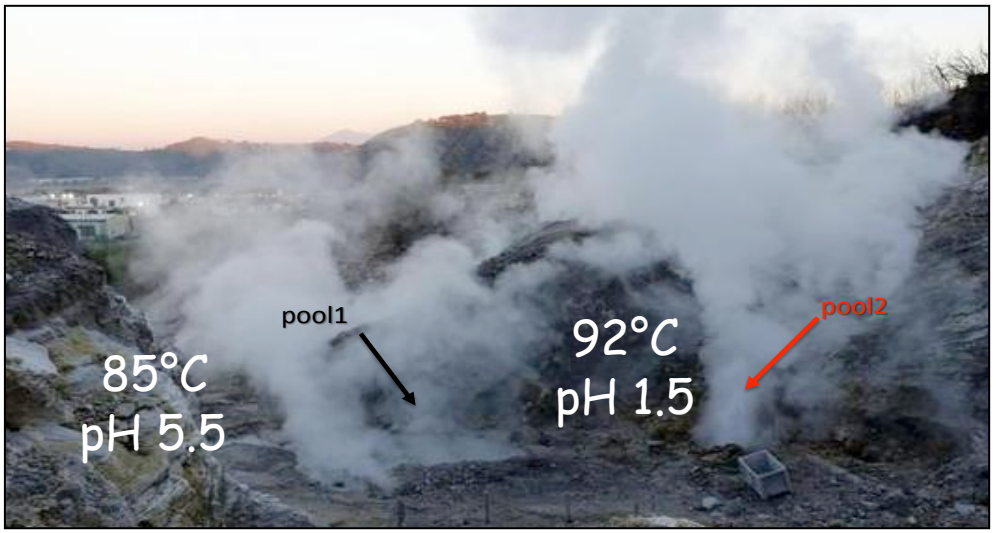
Giovanni Chiodini[1,*], Rosario Avino[1], Stefano Caliro[1], Carmine Minopoli[1]

800 m²
T=60-92°C
pH= 1.5-6.0



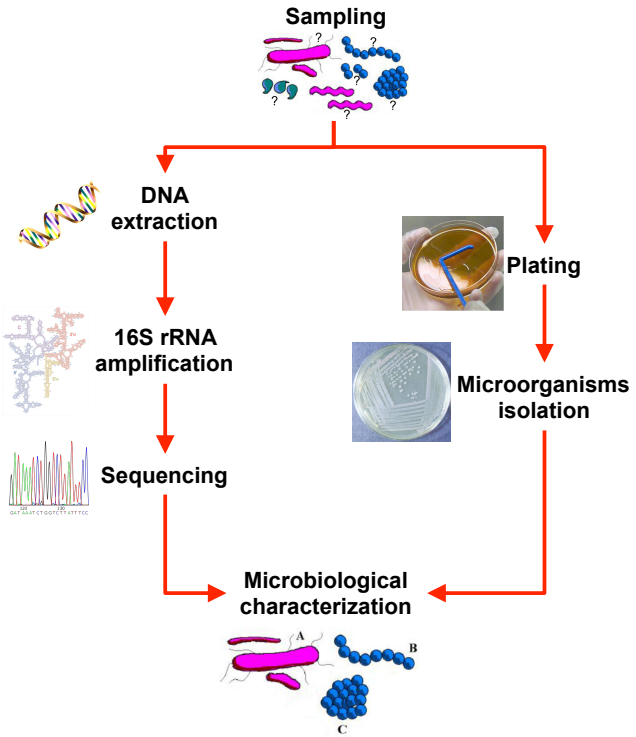
40°50'34"N; 14°9'56"E





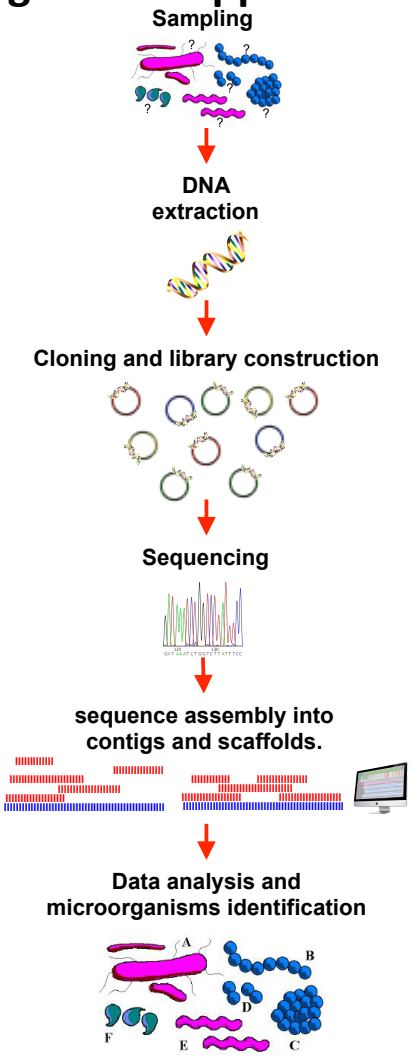
the Approach

Cultivation-dependent approach

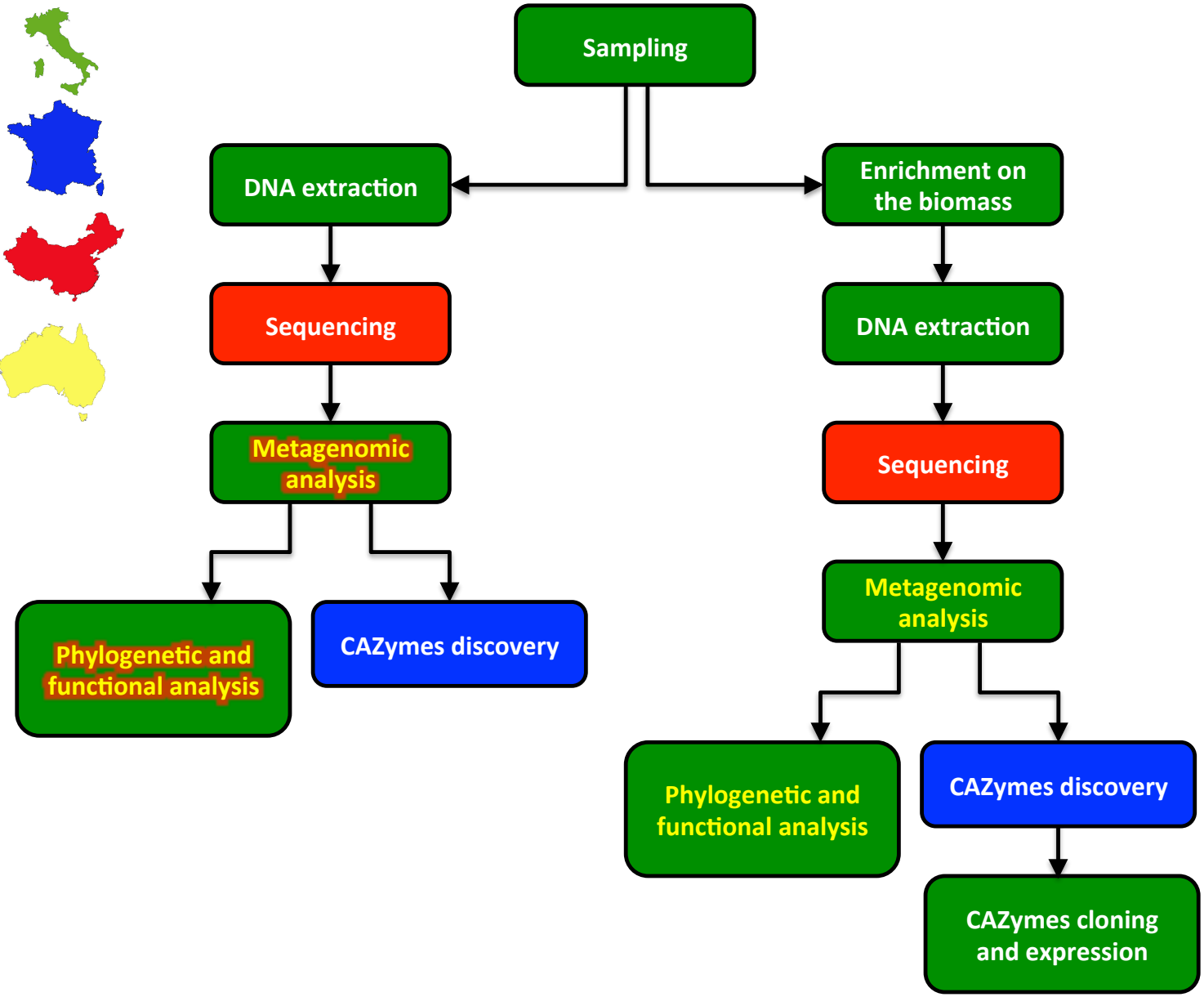


vs

Metagenomic approach



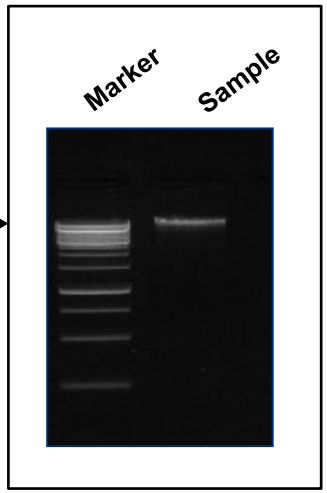
Project flowchart



Sampling and DNA preparation

Sampling in Pool 1 hot spring <u>water and mud</u> **(pH 5.5, 86°C)**

Sampling in Pool 2 hot spring <u>sediment</u> **(pH 1.5, 92°C)**



Pool 1 sample was centrifuged and the total DNA was extracted from the debries.

Pool 2 sample was dissolved in salt medium, centrifuged, and the total DNA was extracted from the debries.

Comparison of metagenomic analyses between Pisciarelli and Yellowstone (hyper)thermophilic environments

OPEN ACCESS Freely available online PLoS one

Metagenomes from High-Temperature Chemotrophic Systems Reveal Geochemical Controls on Microbial Community Structure and Function

William P. Inskeep[1]*, Douglas B. Rusch[2]*, Zackary J. Jay[1], Markus J. Herrgard[3], Mark A. Kozubal[1], Toby H. Richardson[3], Richard E. Macur[1], Natsuko Hamamura[4], Ryan deM. Jennings[1], Bruce W. Fouke[5], Anna-Louise Reysenbach[4], Frank Roberto[6], Mark Young[7], Ariel Schwartz[3], Eric S. Boyd[8], Jonathan H. Badger[2], Eric J. Mathur[3], Alice C. Ortmann[9], Mary Bateson[7], Gill Geesey[8], Marvin Frazier[2]

1 Thermal Biology Institute and Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana, United States of America, 2 J. Craig Venter Institute, Rockville, Maryland, United States of America, 3 Synthetic Genomics Inc., La Jolla, California, United States of America, 4 Center for Marine Environmental Studies, Ehime University, Matsuyama, Japan, 5 University of Illinois, Urbana, Illinois, United States of America, 6 Idaho National Laboratory, Idaho Falls, Idaho, United States of America, 7 Thermal Biology Institute and Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, Montana, United States of America, 8 Thermal Biology Institute and Department of Microbiology, Montana State University, Bozeman, Montana, United States of America, 9 Department of Marine Science, University of South Alabama, Mobile, Alabama, United States of America

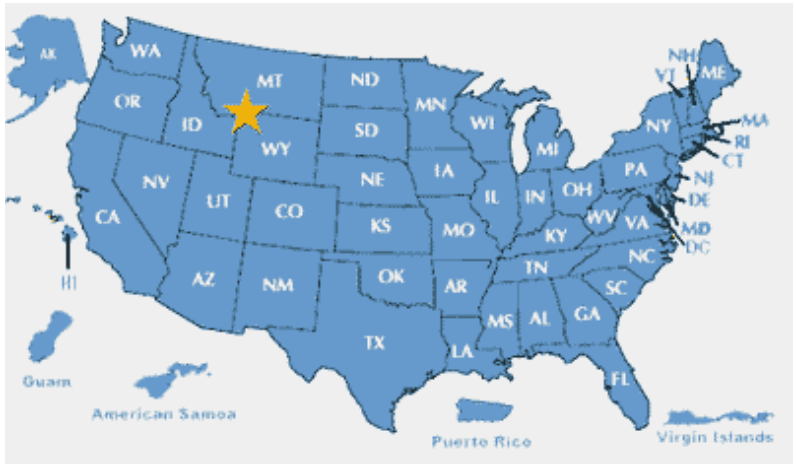
Inskeep et al. 2010; PloS One 5 (3) (January): e9773. doi: 10.1371/journal.pone.0009773

Sample ID	Temp (°C)	pH	Nr. ORFs
Pool1	86	5.5	17640
Pool2	92	1.5	14920
CS*	75	7.8	4823
CH*	75	2.5	2415
JC*	80	6.1	5623
MHS*	71	6.6	1818
NGB*	65	3.0	4834

* 14,000-15,000 Sanger reads/site

CS: Calcite Springs
CH: Crater Hills
JC: Joseph's Coat
MHS: Mammoth Hot Spring
NGB: Norris Geyser Basin

Yellowstone National Park



Take home messages

The Pisciarelli solfatara shows remarkable diversity

The metagenomic approach reported here aim to map the biological diversity of two neighboring mud pools highly different in T and pH.

The Pisciarelli solfatara hosts unknown organisms of uncertain philogenetic origin.

Among known organisms, the analysis of the metagenomic data allowed the identification of metabolic pathways and demonstrated remarkable differences in biological variation between the two pools, also when compared to more physically distant hyperthermal sites.

The high number of complete ORFs from the two pools offers the opportunity to clone and express genes of applicative and basic interest.

The vicinity of the Pisciarelli solfatara allows the constant monitoring of its biodiversity and offers the possibility of performing *in-vivo* studies

The same approach can be applied to other extreme environments

Acknowledgments

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