British Geological Survey
NATURAL ENVIRONMENT RESEARCH COUNCIL

Microbes in hyperalkaline systems: comparison of observations from Cyprus Natural Analogue environments

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Introduction

- Cement used in a repository for:
  - structural engineering
  - as a waste isolation matrix
    - *reduced mobility for many radionuclides under alkaline conditions*
- Behaviour and role of microorganisms and biogeochemical processes under alkaline conditions is poorly-understood
- Microbial activity examined from two natural analogue sites:
  - BIGRAD Project, Allas Springs, Troodos, Cyprus
    - pH 10.0-11.9.
    - NERC Consortium Grant (2010-2014) – Manchester Univ., Leeds Univ., Loughborough Univ., Sheffield Univ., British Geological Survey, National Nuclear Laboratory
  - Maqarin Natural Analogue Project, northern Jordan
    - pH 12.4-12.9 (portlandite-saturated)

Alkaliphiles – life at high pH

- Bacterial cytoplasmic membrane very unstable at pH > 8.5

- Alkaliphilic microorganisms: optimal growth at pH > 9

  - The membranes of alkaliphilic Gram-positive bacteria are surrounded by a cell walls rich in peptidoglycan & acidic polymers

  - They maintain a mildly alkaline intracellular pH (~ 8) by pumping H^+ ions across their cell membranes into their cytoplasm

  - Some alkaliphiles can reduce Fe(III) and other metals and therefore they may have an important influence on radionuclide speciation, behaviour and migration

Biogeochemical Gradients and Radionuclide Transport (BIGRAD) Project

Aim

➢ To investigate the diversity of microbial communities in natural hyperalkaline environments and their potential for biotransformation of metal and radionuclide bearing minerals

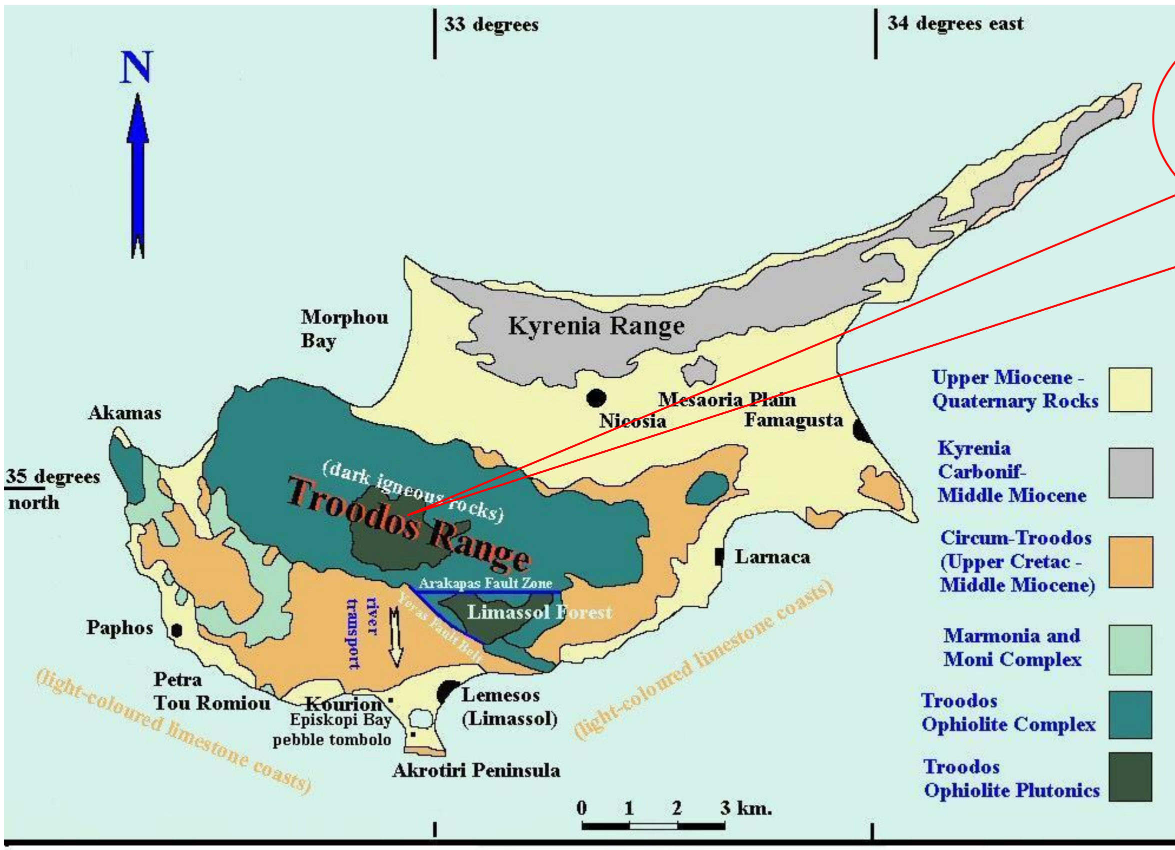
Objectives

➢ To describe the microbial diversity using molecular- based techniques

➢ To establish microbial enrichment cultures growing at high pH (pH 10 or above)

➢ To isolate alkaliphilic microorganisms for use in future biotransformation experiments with metals or radionuclides

Troodos Ophiolite – Allas Springs

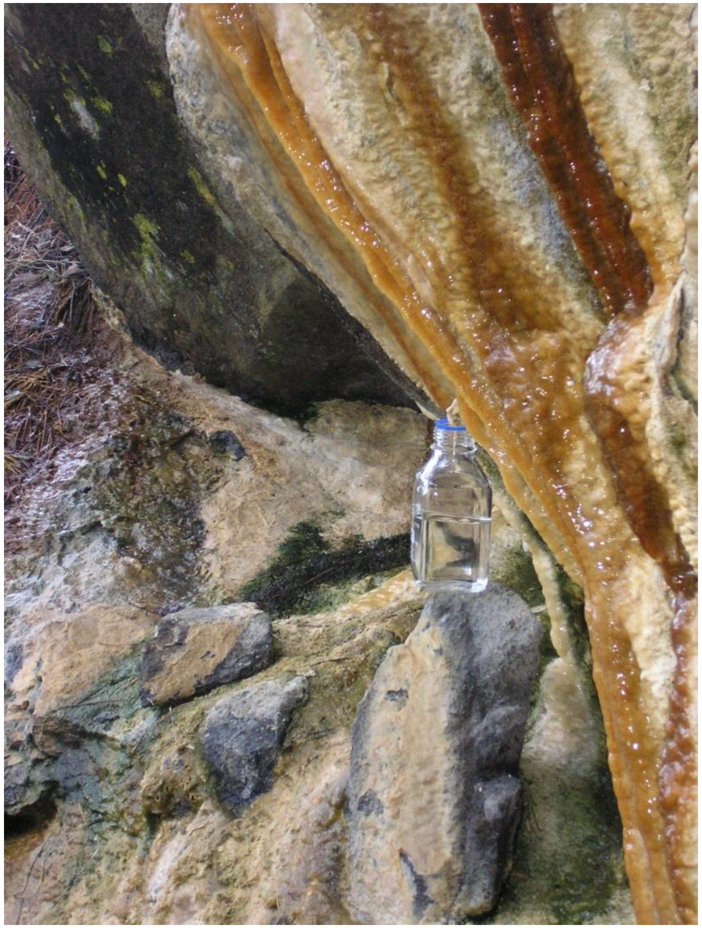


Allas Springs

Allas Springs – A1-1 Site

- Travertine flowstone coating rock surfaces
- Artesian flow through fractured harzbergite (olivine-enstatite peridotite
- Steep N-S fracture – youngest regional fracture pattern
- Extensive tufa and travertine surround the outcrop cementing scree and forest litter cover
- Multicloured calcium carbonate deposits stained by brown gel-like biofilm
- pH 11.9

Sampling spring water for chemistry at A1-1



- Water collected from dripping stalactite ribs in tufa coatings immediately beneath the discharge point in exposed fracture

- Significant biofilm staining on tufa surfaces

- On-site measurement: T, pH, conductivity, dissolved O_2

- On-site sampling, filtering, and preservation of water for major and trace cations and anions, DOC/DIC, ammonium, reduced S, reduced Fe, stable (O/H) isotopes, tritium

- Sampled during CNAP Phase II (2009)

Sampling porewater for chemistry in tufa-cemented forest litter (site A1-3)

•Extensive cementation of forest litter and scree by travertine surrounding the discharge area A1-1

•Pine needles, woody twigs, branches and other plant material embedded and impregnated by calcium carbonate (possibly > 2m thick in places)

•Shallow hole dug (c. 40 cm deep), which rapidly filled with water: sampled by bailing

•Sampled during CNAP II (2009)

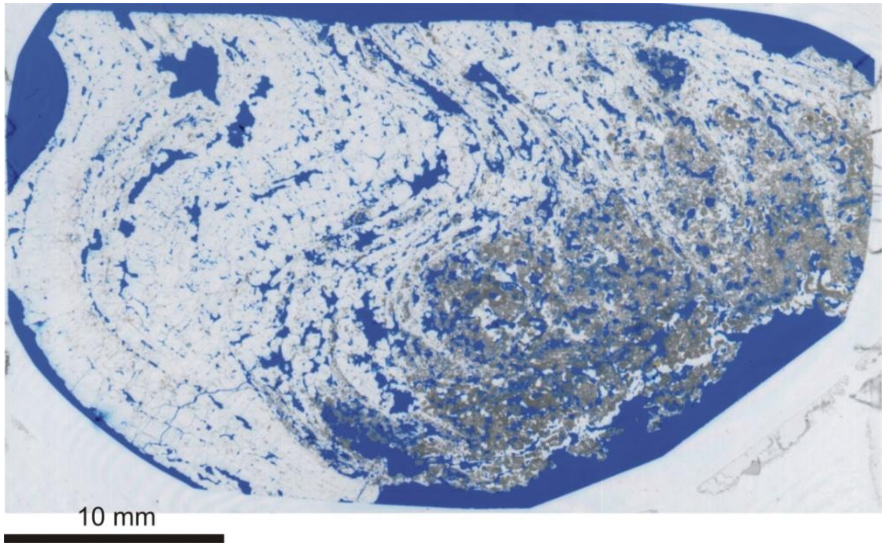
•pH 10.01

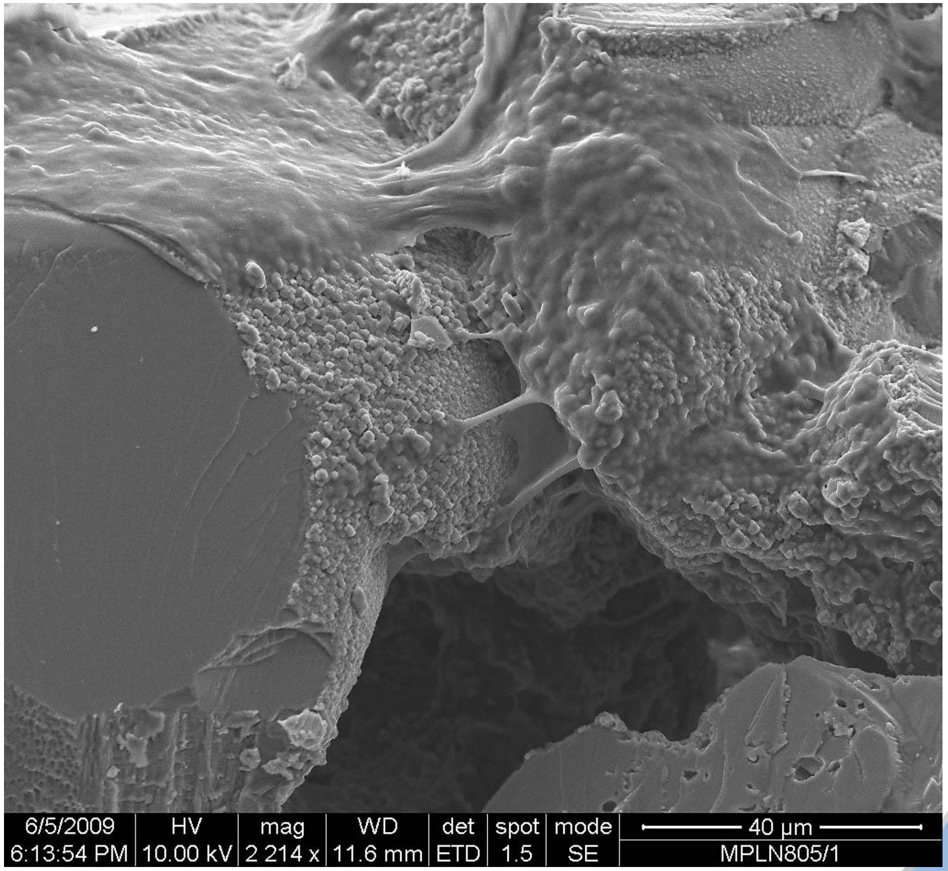
Groundwater chemistry

Sample	Field pH	Lab pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	NO_3^{-}
A1-1	11.9	11.3	37.2	0.101	1435	63.1	n/a	272	2177	101	<1.5
A1-3	10.01	9.31	12.2	0.554	1337	60.1	54.1	96.4	1926	114	10.3
A1-2	9.26	8.82	4.87	48.3	502	23.7	25.8	288	699	50.7	3.90
A1-4	9.78	9.27	11.2	5.73	1214	54.0	61.2	124	1748	78.5	8.33

- pH alkaline 10.0 – 11.9
- Low Ca
- Mildly saline

Petrographical analysis of travertine flowstone

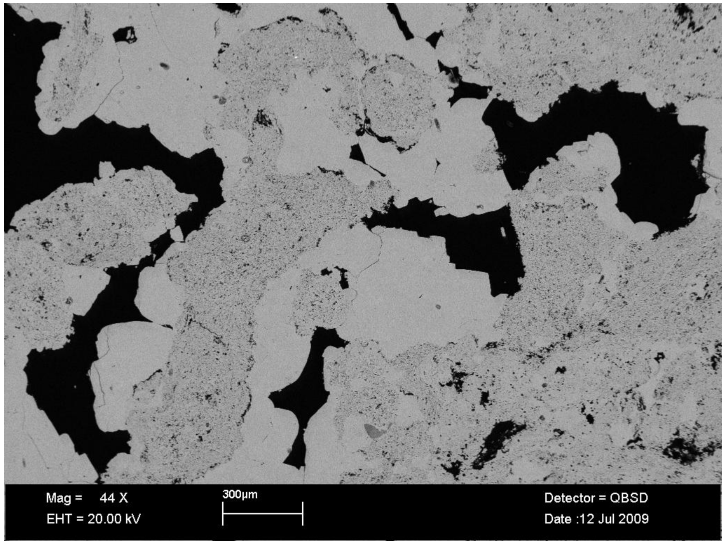


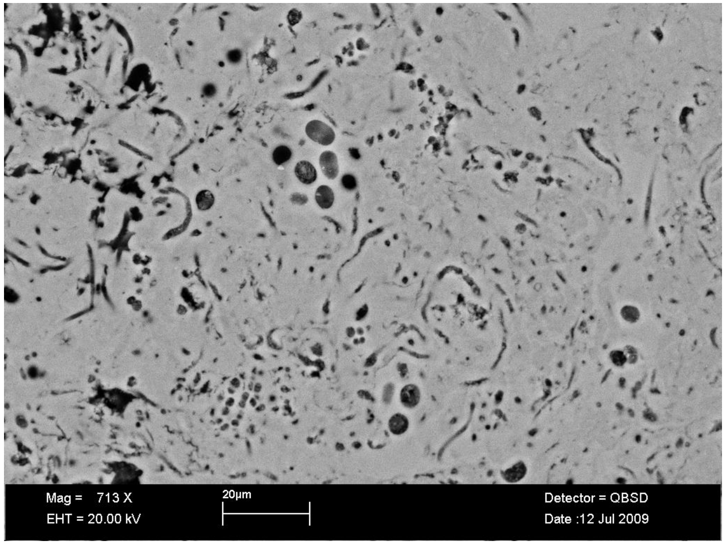


- Finely-banded calcium carbonate – mainly calcite (trig. $CaCO_3$) but with minor dolomite ($CaMg[CO_3]_2$), and aragonite (orth. $CaCO_3$)

- Detailed ESEM observations revealed "glutinous" organic biofilm lining intercrystalline pores and cavities in the travertine

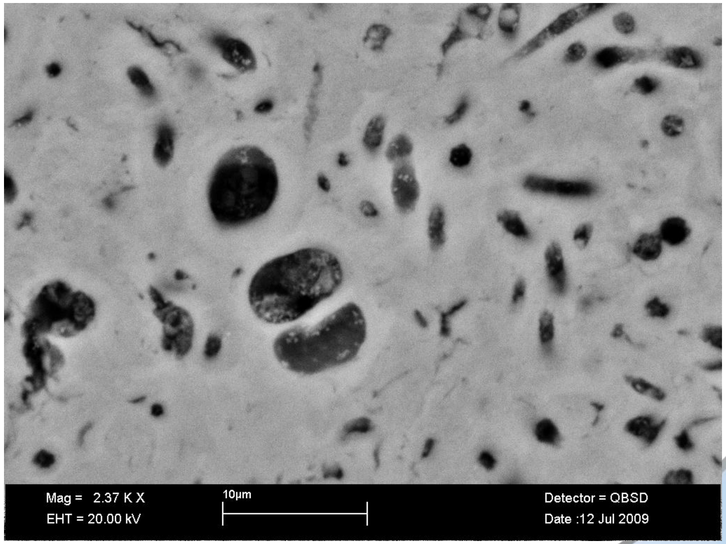
Petrographical analysis of travertine flowstone



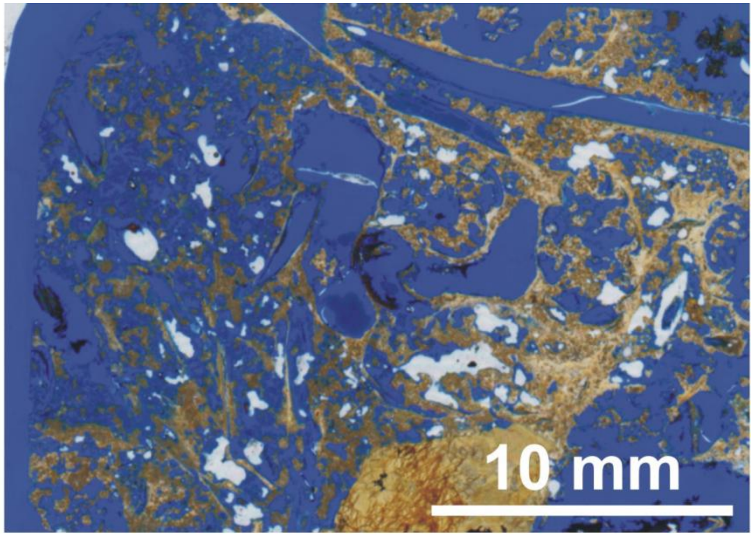


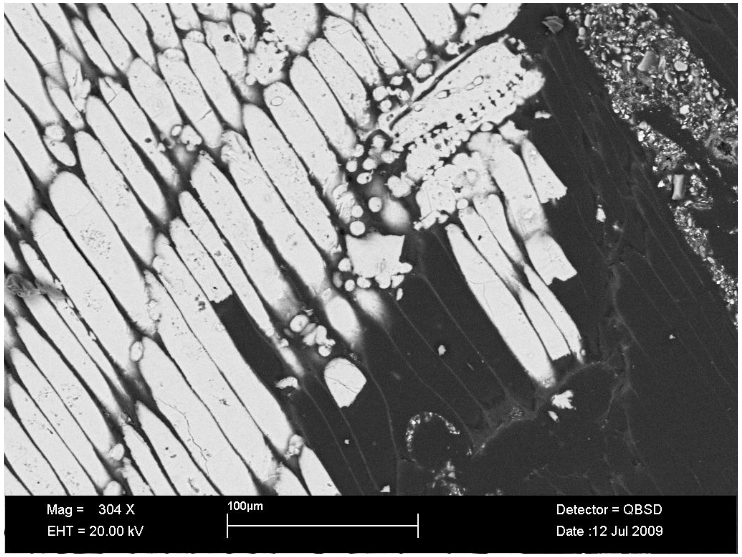
- High-resolution BSEM analysis revealed abundant organic material within the flowstone showing a variety of microbiological structures preserved ("fossilised" or "petrified") within the travertine

•Features observed include: individial bacterial cells, chains and strings of cells, dividing cells and spores



Petrographical analysis of tufa-cemented litter





- Forest litter contains abundant pine needles, and woody plant material (twigs, fragments of tree branches), stems and leaves of deciduous shrubby plants

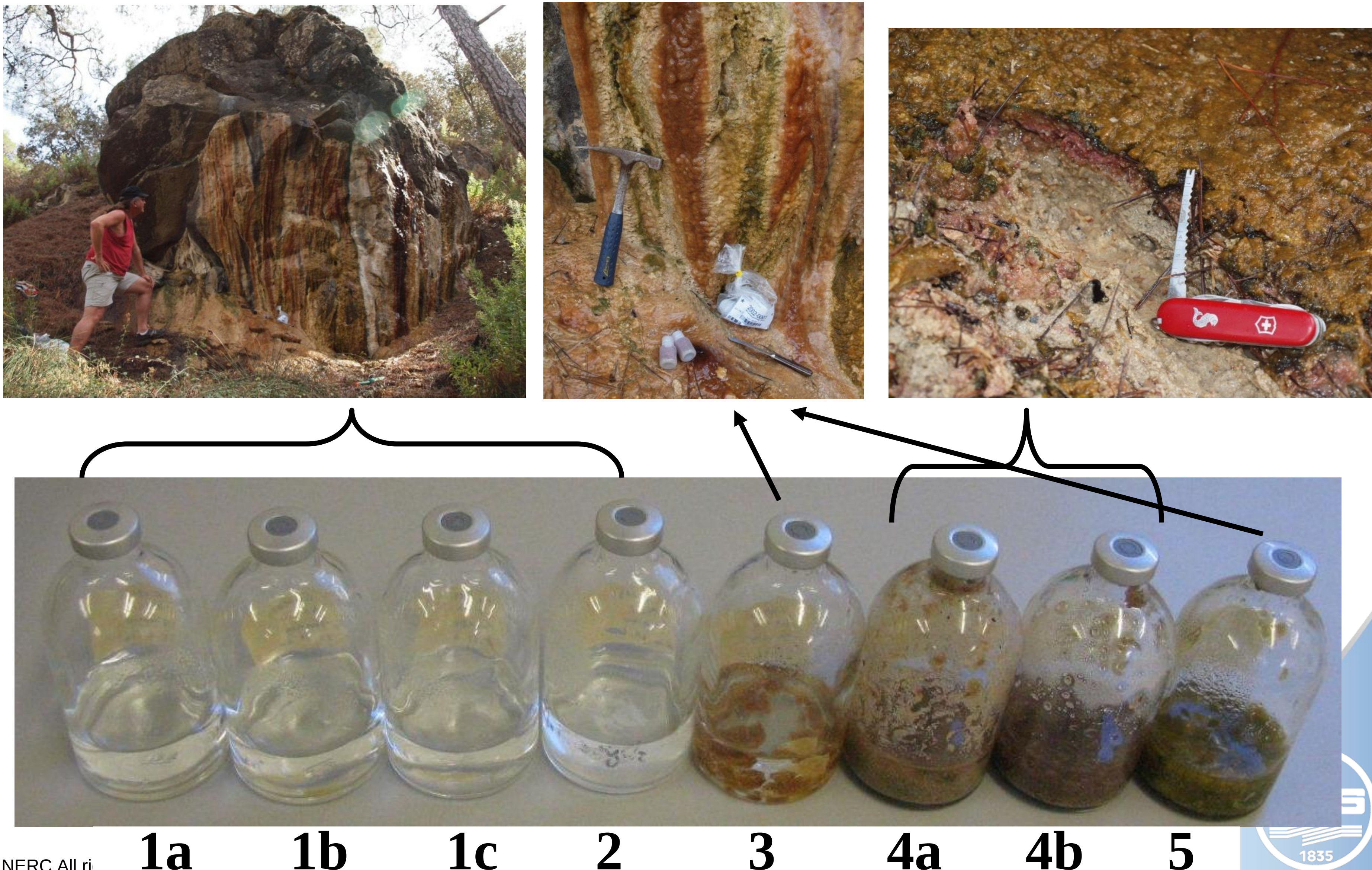
- BSEM analysis revealed:
  - Degradation and breakdown of lignified cell walls and intercellular material in woody and other plant remains

  •Infilling and replacement of intercellular cavities by calcite (and/or aragonite)

  •Partial replacement of lignified cell walls by calcite (and/or aragonite)

•Presence of bacterial cells, chains and strings of cells, dividing cells and spores preserved in calcium carbonate.

Biological sampling and experimental studies
[BIGRAD 2010-2011]

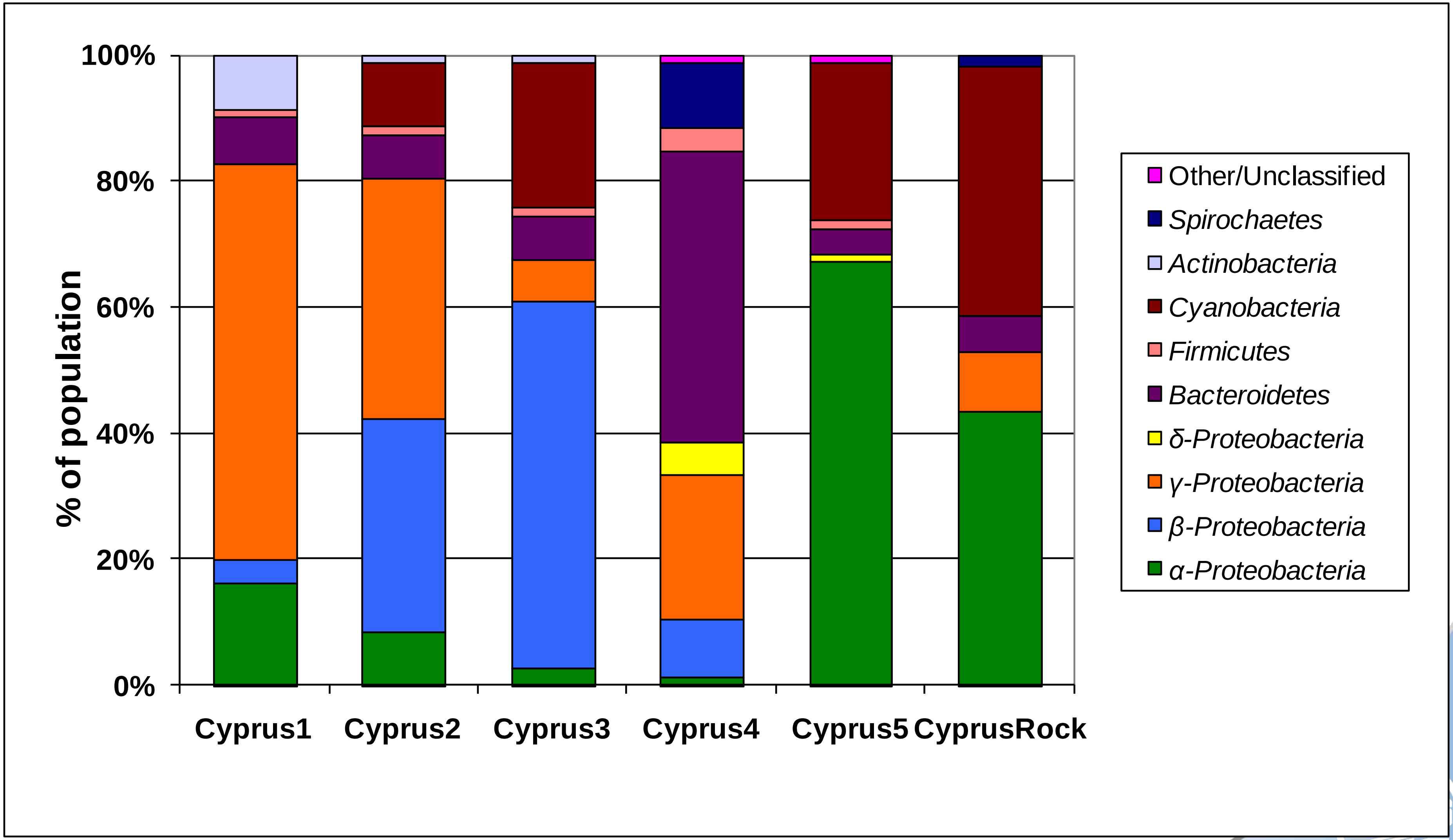


Work so far (at Manchester University)

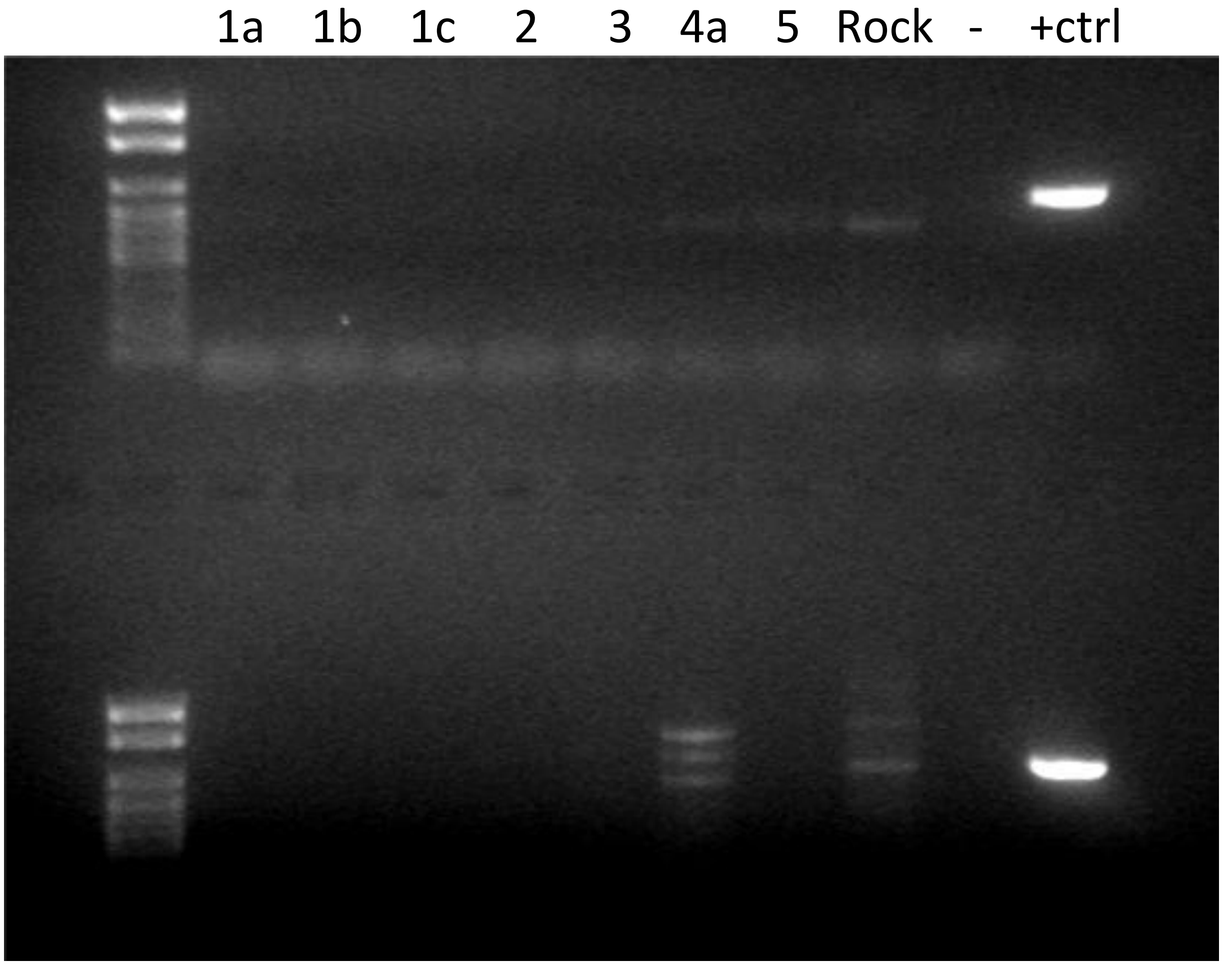
- Measurements of pH, Eh, ICP-AES and major anions.
- Bacterial clone libraries from samples 1a, 2, 3, 4a, 5, Rock
- Archaeal clone libraries from samples 5 and Rock.
- Attempt to isolate iron-reducing bacteria on enrichment medium (Ye *et al*., 2004) solidified with 2% gellan gum.
- Enrichment cultures using the same medium
  - *pH 10, 7mM lactate, 7mM acetate, 7mM Fe(III)-citrate*

1a 1b 1c 2 3 4a 4b 5 Rock

Bacterial 16S rRNA gene clone libraries



PCR for Archaea and Methanogens



<u>Archaea</u>
primers: 21F & 958R
~950 bp

<u>Methanogens</u>
primers: 1AF & 1100AR
~1100 bp

16S clone libraries – closest matches

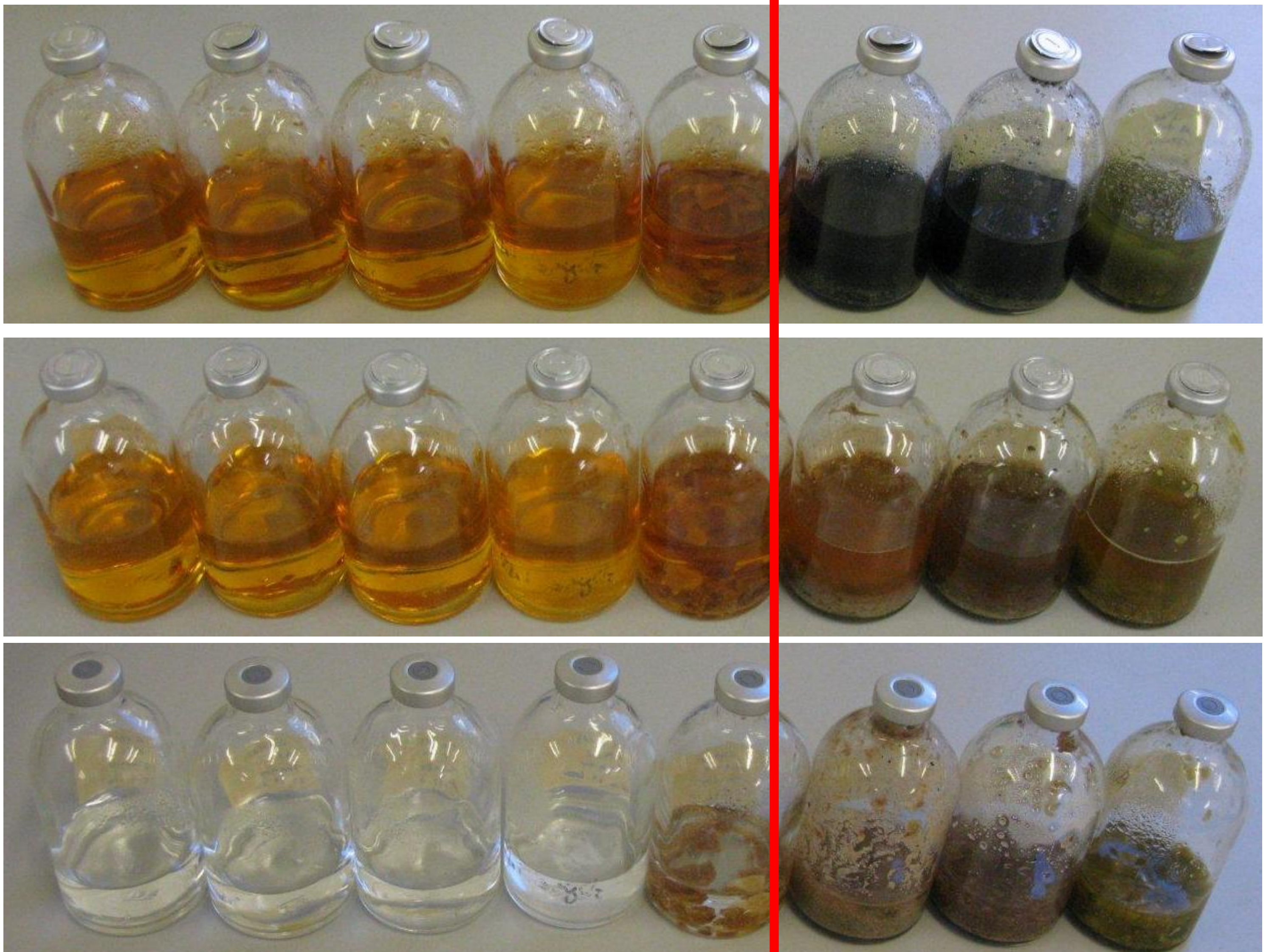
	Cyprus 3	Cyprus 4	Cyprus 5	Cyprus Rock	Closest match	ID %
b-Proteobacteria	52.7				Malikia granosa	97
Cyanobacteria	23.0				Synechococcus sp.	100
Bacteroidetes	4.1			3.8	Flexibacter tractuosus	92.9
g-Proteobacteria	4.1			1.9	Silanimonas lenta	99
Bacteroidetes		25.6			Alkaliflexus imshenetskii	91.3
g-Proteobacteria		21.8			Thioalkalivibrio denitrificans	91.9
b-Proteobacteria		6.4			Azoarcus indigens	94.3
Bacteroidetes		10.3			uncult. Sphingobacterium	94.7
a-Proteobacteria			60.5		uncult. a-Proteobacterium	95.7
Cyanobacteria			15.8	35.8	Leptolyngbya antarctica	99.1
a-Proteobacteria			3.9	15.1	Rhodobacter blasticus	96.8
a-Proteobacteria				5.7	Roseovarius mucosus	99.2
a-Proteobacteria				7.5	Oceanicaulis sp.	96.3

16S clone libraries - summary

- *Cyanobacteria* not present in sample 4, i.e. the soil sample
- Many novel clones/not closely related to cultured microorganisms
- No significant similarity between the clone libraries
- Some sequences related to known alkaliphiles such as *Alkaliflexus imshenetskii*, *Silanimonas lenta*, *Thioalkalivibrio denitrificans*, *Halomonas alkaliphila*, *Pseudomonas alcaliphila*.

Enrichment cultures - 7mM Fe(III)-citrate

Fe(III) + SO_4 reduction



1a 1b 1c 2 3 4a 4b 5

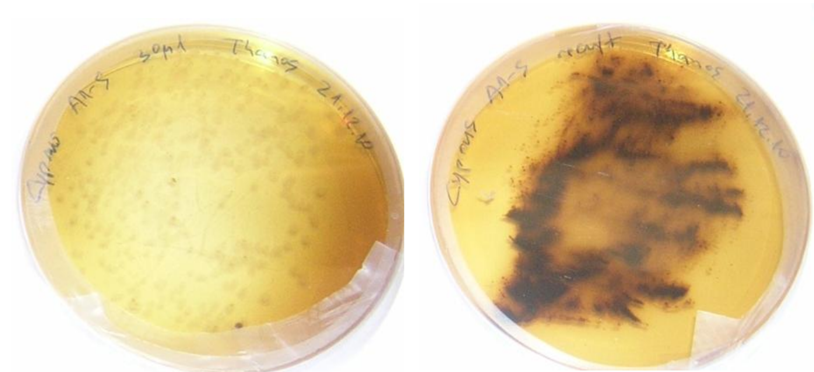
Day 18

Day 0

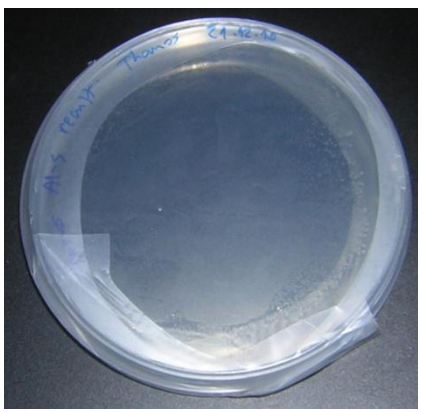
Isolation on solidified Fe and SO_4^{2-} media

Fe(III) sterile control

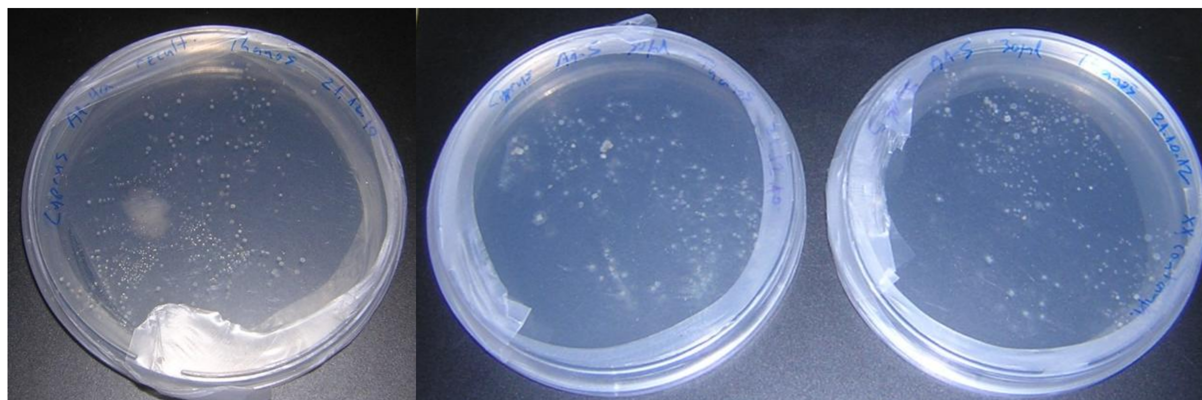
no colonies



colonies & colourless media



SO_4^{2-} sterile control



plates with bacterial colonies

Summary and conclusions – Allas Springs

- Alkaline: pH10.0 – 11.9
- Moderately saline
- High nutrient supply
- 6 bacterial clone libraries prepared.
- Diverse communities detected, including sequences related to alkaliphilic microorganisms.
- 2 archaeal clone libraries are in preparation
- Fe(III) and SO_4^{2-} microbial reduction has been observed at pH 10, using laboratory microcosms (SRB and IRB present abnd viable)
- Some bugs in same family of common soil bacteria (e. *Pseudomonas*)
- Bacteria have also been successfully isolated on solidified media. Their ability to grow at pH > 10 and to reduce metals or other electron acceptors (sulphate) will be tested in BIGRAD

Comparison to Maqarin

- Highly alkaline: pH12.4 – 12.7: [portlandite-saturated]
- Moderately saline
- Very-low nutrient supply
- Diverse microbial community – viable bacteria, including SRB and IRB
- But – very low microbial activity – nutrient-limited


Thank you for your attention