Microbes & Evaporites



Terry McGenity











Microbiology and the foundations & beauty of Salamanca



Eocene Villamayor Sandstone (Golden stone); when quarried it appears "blond"

Garcia-Talegón J et al. (2015) In: Pereira D et al. (eds) Global Heritage Stone. Towards International Recognition of Building and Ornamental Stones. Geol Soc Spec Publ Lond 407:109–120.

Porous, so a terrible foundation stone, but depends on relative humidity (water activity, A_w) – see Jain A et al. (2009) Build Environ 44:1276–1284

Microbiology and the foundations & beauty of Salamanca

- Why does it deteriorate and form an array of colours?
 - physical, chemical & microbiological processes (microbial biofilms and hydrophobicity, photosynthetic activity, Fe-cycling, acid-producers, mineral producers etc.)



•Possible to use microbes to repair biodeterioration, e.g. microbes like *Bacillus pasteurii* precipitate calcium carbonate as cement? – see Gao et al. (2019) Royal Soc Open / doi.org/10.1098/rsos.191318





- What are microbes & how were/are they important for planet Earth?
- Genetic diversity
- Metabolic diversity
- Microbes in extremis

HBSQ00

Salinibacter sp.

5 µm

• Microbes in hypersaline environments & evaporites



• Favourite microbe?

Haloquadratum walsbyi





Bolhuis H et al. (2004) Environ Microbiol 6: 1287-1291. Kessel M, Cohen Y (1982) J Bact 150: 851-860

- What are microbes & how were/are they important for planet Earth?
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•On Earth for at least 3.5 billion years

80% of natural history is about the natural history of microbes



•On Earth for at least 3.5 billion years

•Abundant

Microbes are abundant

All microbes



Whitman WB, Coleman DC & Wiebe WJ (1998) Prokaryotes: the unseen majority. *PNAS* 95: 6578-6583. [revised down slightly by Kallmeyer J et al. (2012) *PNAS* 109: 16213–16216]

- •On Earth for at least 3.5 billion years
- •Abundant
- •Small



Typical bacterial cell is ~ 0.001 mm (100th the width of human hair)

- •On Earth for at least 3.5 billion years
- •Abundant

•Small, with a large, proportionally reactive surface



- •On Earth for at least 3.5 billion years
- •Abundant
- •Small, with a large, proportionally reactive surface
- •Tolerate/require a huge range of physico-chemical extremes





- •On Earth for at least 3.5 billion years
- •Abundant
- •Small, with a large, proportionally reactive surface
- •Tolerate/require a huge range of physico-chemical extremes
- •Colonise every habitable part of the earth





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A cross section of an Escherichia coli cell

Cell wall, concentric membranes, transmembrane proteins, and a flagellum with its motor are shown in green Cytoplasm is shown in blue and purple. Nucleic acids are shown in yellow. Reprinted with permission of David S. Goodsell, The Scripps Research Institute, La Jolla, CA. Pielak GJ (2005) A model of intracellular organization. PNAS USA





white = variable or hypervariable black = highly conserved V1 to V9 = major variable regions •All cells contain ribosomes that perform the same function

Bacterial & Archaeal ribosomal RNA

– 16S (1500 nucleotides) = 18S in eukaryotes

•Highly conserved regions used for aligning

•Variable regions used to establish evolutionary distance

•Nucleotide sequences of rRNA (or its gene) can be compared to construct an evolutionary tree

Microbes in the three Domains of life

- Bacteria
- Archaea
- Most Eukaryotes





W. Ford Dolittle's schematic representation of evolution (not to scale)

An alternative / emerging view is the 2-domain tree



Williams TA, Foster PG, Cox CJ, Embley TM (2013) An archaeal origin of eukaryotes supports only two primary domains of life. *Nature* 504: 231-236

Also,

Asgard Archaea (include the Lokiarchaeota) = Archaea that branch near Eukarya and have Eukarya-like actin & profilin filaments (based on metagenomics)

Spang A et al. (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521:173–179 Akıl C & Robinson RC (2018) Genomes of Asgard archaea encode profilins that regulate actin *Nature* 562: 439–443

An ever-expanding archaeal tree



Albers SV, Meyer BH (2011) The archaeal cell envelope. Nature Reviews Microbiology 9: 414-426



Phylogenetic tree of the domain Bacteria based on 16S rRNA gene sequences

(Keller, M. & Zengler, K. (2004) Tapping into microbial diversity. *Nature Reviews Microbiology.* **2:** 141-150)

Those phylogenetic groups with no cultivated representatives are shown in red (26/53)

Those with few cultivated representatives are in green

Rapid Evolution



Slow Evolution



E. coli evolves resistance to antibiotics in 11 days <u>https://youtu.be/QUfyq8KMzyc</u>

Baym M et al. (2016) Spatiotemporal microbial evolution on antibiotic landscapes. *Science* **53**: 1147-1151

Deep subsurface microbes

Karen Lloyd TED Talk

https://www.ted.com/talks/ karen_lloyd_the_mysterious_microbes_living_deep_insi de_the_earth_and_how_they_could_help_humanity/ transcript?language=en

Massive **genetic diversity** in a drop of water or crumb of soil



- Alter / control the chemistry of the Earth and its atmosphere
- O₂, CO₂, methane,

But also

dimethylsulfide, isoprene, halogenated hydrocarbons etc.

Massive metabolic diversity

Massive **genetic diversity** in a drop of water or crumb of soil



Massive metabolic diversity

- Alter / control the chemistry of the Earth and its atmosphere
- Make & break rocks



Microbial oxidation of pyrite by Acidithiobacillus ferrooxidans

Massive **genetic diversity** in a drop of water or crumb of soil



Massive metabolic diversity

- Alter / control the chemistry of the Earth and its atmosphere
- Make & break rocks
- Form & alter fossil fuels
- Central to global sustainable development
- Constantly evolving
- Compete and cooperate (think in terms of microbial communities)

McGenity TJ (2018) 2038 – When microbes rule the Earth. Environ Microbiol 20: 4213-4220

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Compositions as percentage of total number of atoms

universe		verse	earth's crust	seawater	Living organisms						
	H	91	O 47	Н 66							
	He	9.1	Si 28	0 33	??						
	0	0.057	Al 7.9	Cl 0.33							
	N	0.042	Fe 4.5	Na 0.28							
	С	0.021	Ca 3.5	Mg 0.033							
	Si	0.003	Na 2.5	S 0.017							
	Ne	0.003	К 2.5	Ca 0.006							
	Mg	0.002	Mg 2.2	K 0.006							
	Fe	0.002	Ti 0.46	C 0.0014							
	S	0.001	н 0.22	Br 0.0005							
			C 0.19								
Others <0.1			Others <0.1	Others <0.1							
	Eviador E (1072) The chamical elements of life Opiontific American 207, 52										

Frieden, E. (1972) The chemical elements of life. *Scientific American* **227:** 52

Compositions as percentage of total number of atoms

uni	verse	earth's crust		seawater		hu	human	
Н 91		O 4	O 47		Н 66		Н 63	
He	9.1	Si 2	Si 28		0 33		0 25.5	
0	0.057	Al	7.9	Cl	0.33	С	9.5	
N	0.042	Fe	4.5	Na	0.28	N	1.4	
С	0.021	Ca	3.5	Mg	0.033	Ca	0.31	
Si	0.003	Na	2.5	S	0.017	Р	0.22	
Ne	0.003	K	2.5	Ca	0.006	Cl	0.03	
Mg	0.002	Mg	2.2	K	0.006	K	0.06	
Fe	0.002	Ti	0.46	C	0.0014	S	0.05	
S	0.001	Н	0.22	Br	0.0005	Na	0.03	
		С	0.19			Mg	0.01	
Oth	ers <0.1	Others <0.1		Others <0.1		Oth	Others < 0.01	

Frieden, E. (1972) The chemical elements of life. Scientific American 227: 52

Compositions as percentage of total number of atoms



Cells need energy and building blocks to survive and grow



An encapsulation of metabolic diversity





Example = humans; *E. coli*



Example = *E. coli*


Example = *Thiomargarita namibiensis* (a type of anaerobic chemolithoautotroph, *but* new evidence hints at mixotrophy, and aerobic respiration!)

Sediment core from the Benguela upwelling (courtesy Nat Hicks)

Thiomargarita namibiensis diameter = 400 μm

Schulz HN et al. (1999) Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* **284:** 493-495







Example = Geobacter metallireducens

Geobacter sp. shuttle electrons

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Proposed windows of life for selected parameters



- Survival v growth
- •Pure culture v in-situ activity
- Degrees of uncertainty over methods at extremes

Deep biosphere

•Microbes present and "just about active" in deep-sea sediments

•Record = 2458 mbs in lignite beds – Inagaki et al. (2015) Science 349: 420-424

• Energy from, e.g. H₂, necromass; occasionally hydrocarbons

•Terminal electron acceptors (next slide)





Fig. 1. Subseafloor sedimentary cell counts used for this study. (A) Counted cell concentration vs. depth (mbsf) for the sites used in this study. (B) Site locations overlain on a map of time-averaged sea surface chlorophyll-a (34).

Kallmeyer (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. *PNAS* 109:16213-16216

Microbial element cycling in deep subsurface sediments



Orsi WD (2018) Ecology and evolution of seafloor and subseafloor microbial communities. Nat Rev Microbiol 16: 671–683

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Diversity of hypersaline environments



Salt glaciers in the Zagros Mountains, Iran (NASA)

Diversity of hypersaline environments



Makgadikadi salt pan, Botswana

(NASA; http://thousandwords.ch)



Hypersaline brines are (and would have been in the past) dominated by Archaea, Bacteria and a select few Eukarya



San Francisco Bay salterns (NASA)

Gypsum crust from the bottom of a shallow saltern pond (200 g $\rm I^{-1}$ salinity) in Eilat, Israel, showing layered communities of phototrophic microbes.



Schematic of change in abundance in the water column of a solar saltern with increasing salinity



Haloarchaea become trapped inside halite



Halobacterium NRC-1 growing around / on salt crystals on a dried-out plate Courtesy Matt W. Ford. www.pandasthumb.org



Halite crystal forming around microbial cells in the Berre salt works Courtesy Sabine Castanier

& live inside brine inclusions



Norton & Grant (1988) J Gen Microbiol 134: 1365-1373 Dombrowski (1966) In 2nd Symposium on Salt Vol 1, Rau JL (ed). Northern Ohio Geological Society Fendrihan et al. (2009) Astrobiology 9: 104-112

Also with Dunaliella salina





Thanks to Nora Georgiev, Maria Magliuo & Philippe Laissue





to avoid desiccation and the increasingly chaotropic Mg-rich brine that is left behind

seawater



Percentage evaporation giving rise to different minerals

Proposed windows of life for selected parameters





MgCl₂ is Chaotropic, i.e. it destabilises biological macromolecules (non-linear)

Hallsworth JE, Yakimov MM, Golyshin PN, Gillion JLM, D'Auria G, de Lima Alves F, La Cono V, Genovese M, McKew BA, Hayes SL, Harris G, Giuliano L, Timmis KN, McGenity TJ (2007) Limits of life in MgCl₂-containing environments: chaotropicity defines a habitat. *Environ Microbiol* **9**: 801-813.

Understanding the effects of different salts, e.g. MgSO₄ on microbes (and their gas production)







Mario Toubes at Basque Lake, BC, Canada – Magnesium sulfate rich lake

Distant salterns have similar haliteentombed archaeal communities

•Saltern halite in triplicate from 21 locations (~4 years old)

•Archaeal community analysis (using very high-throughput sequencing)



Clark DR, Mathieu M, Mourot L, Dufossé L, Underwood GJC, Dumbrell AJ, McGenity TJ (2017) Global Ecol Biogeog 26: 1435–1446.



Which microbes survive best in lab-made halite?



Every month (later every two) dissolve crystals in growth medium and check daily for growth

Halobacterium spp. are good at surviving inside halite

•Halobacterium noricense has no apparent loss of viability over 27 months



Gramain, A, Chong Díaz G, Demergasso C, Lowenstein TK, McGenity TJ (2011) Archaeal diversity along a subterranean salt core from the Salar Grande (Chile). *Environmental Microbiology* **13**: 2105–2121.

Co-entombment of *Haloquadratum walsbyi* **& Salinibacter ruber enhances their survival**



Gramain, A, Chong Díaz G, Demergasso C, Lowenstein TK, McGenity TJ (2011). *Environ Microbiol* **13**: 2105–2121.

Haloarchaea are uniformly entombed and survive equally as a community



Between-pond differences in Archaeal communities;

No significant change in Archaeal communities over time



Weeks Entombed

Haloarchaea are ~ uniformly entombed & survive equally inside halite over 21 weeks

Huby, Clark, McKew, McGenity subm.

How long can life exist in salt? – Salar Grande, Chile

•45 x 5 km inter-montane basin•Hydrologically inactive



Gramain A et al. (2011) Environ Microbiol 13: 2105–2121.

25 metre core from G. Chong Diaz



Chong Díaz et al. (1999) Palaeogeog Palaeoclim Palaeoecol 151: 39-54

Salar Grande core samples investigated by PCR of enrichment cultures & DNA extracted directly from the cores









Halobacterium noricense & relatives isolated (i) or detected (d) in ancient halite

Country	Age of deposit (million years)
Chile	> 1.8 (i & d)
Poland	11-15.8 (d)
China	33.9-41.3 (i)
Brazil	121 (i & d)
China	123 (i)
Austria	250 (i & d)
USA	419 (d)

Are they as old as the salt?

- Or, laboratory contamination?
- Or, recent contamination of the environment?
- Or, derived from populations trapped millions of years ago?
- Or, yes?



Gruber C et al. (2011) Extremophiles 8:431-439

Features of living in halite that support long-term survival



Features of buried salt that enhance long-term survival

 Protection from radiation



Schubert et al. (2009) 34,000 year halite



Features of brine inclusions that enhance long-term survival

- Salt saturated
- Low oxygen concentration
- •Co-entombed microbes as a source of carbon and energy

Carbon & Energy Requirements

20°C over 100 million years a cell would need 50,000 times its own mass in C to avoid DNA depurination (Price and Sowers 2004)

A100 μm cube inclusion with a1:20 cell:brine ratio would have with 50,000 cells (McGenity et al. 2008)

One *Dunaliella salina* cell would provide enough C & energy for a single miniaturized cell to survive for 12 million years (Oren 2016)

Features of *Halobacterium* spp. and living in halite that allow for long-term survival



Features of buried salt that enhance long-term survival

 Protection from radiation



Schubert et al. (2009) 34,000 year halite



Features of brine inclusions that enhance long-term survival

Salt saturated

< 1 mm -

- Low oxygen concentration
- •Co-entombed microbes as a source of carbon and energy



Features of *Halobacterium* that enhances long-term survival

Features of *Halobacterium* spp. that support long-term survival



Protective Cellular Environment -Damage Avoidance



- •High K⁺ and halide concentrations
- •High Manganese : Iron
- Manganese antioxidant complexes
- Carotenoids
- •DNA with high G&C content
- ROS-protecting enzymes

Metabolism

- •Anaerobic metabolism
- Diverse C & energy sources
- •Storage compounds (e.g. PHA; DNA)
- •Cell miniaturisation (energy limitation)
- •Archaeol (non-leaky) lipids

Efficient Repair Mechanisms

- Photo-reactivation
- Nucleotide excision repair
- Base excision repair
- •Homologous recombination (polyploidy)



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A good general read





WP2 Deep Life

ESR 9

Long-term survival of microbes in halite

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