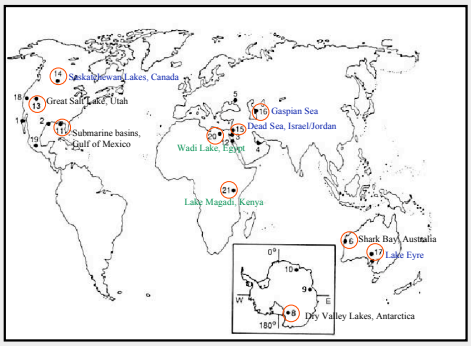
Halophiles: Environments and Diversity



Microbial Ecology
BSCI 464/MEES 698

Distribution of hypersaline environments worldwide



Salts composition of saline and hypersaline environments

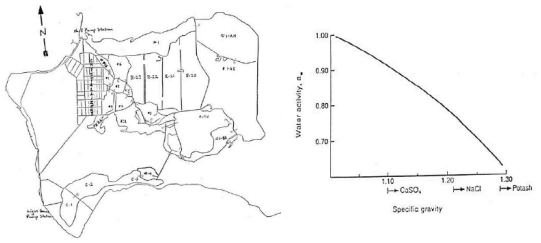
(g/l)

	Na^+	Mg^{2+}	Ca^{2+}	K^+	Cl^-	SO_4^{2-}	CO_3^+ HCO_3	Br^-	TDS,%	pH
Seawater	10.8	1.3	0.4	0.4	19.4	2.7			3.5	
@ gypsum saturation	49.5	6.8	1.7	2.0	91.5	12.5			16.4	
@ NaCl saturation	98.4	14.5	0.4	4.9	187	19.3			32.4	
@ potash saturation	61.4	39.3	0.2	12.8	189	51.2			35.4	
Great Salt Lake	105	11.1	0.3	6.7	181	27.0	0.72	0.2	27.5-33.3	7.7-8.2
Dead Sea	39.7	42.4	17.2	7.6	219	0.4		5.3	32.7	6.4
Lake Mushiki	62.2	29.0	low	low	114	237			34.2	
Solar Lake									6.8-18	6.8-8.8
Gavish Sabkha									12-36	ND
Deep Lake									28.0	7.4

(g/l)

	Na^+	Mg^{2+}	Ca^{2+}	K^+	Cl^-	SO_4^{2-}	CO_3^{2-} HCO_3^-	Br^-	TDS,%	pH
Lake Suribati	69.2	8.5	1.2	2.5	130	8.1			11.3-22.0	7-8
Lake Vanda	6.1	7.4	24.4	0.6	74.3	0.6			11.4	
Lake Bonney	56.9	21.7	1.2	2.3	162	2.9			24.8	
Don Juan Pond	11.5	1.2	114	0.2	212	0.01			33.9	
Mono Lake									9+	9.7
Big Soda Lake									2.6(top) 8.8(bot)	9.7
Lake Magadi									31.3	9.5-11.:
Hot Lake	16.8	53.6	0.7	1.5	1.9	243	3.1			
Wadi Natrun, Zugm	142	0	0	2.3	154.6	22.6	67.2		9.2-39.4	11.0
Wadi Natum, Gaar	137	0	0	1.4	173.7	48.0	6.6		9.2-39.4	10.9
Pink Lake									34.0	7.6-7.8
Seawater	10.8	1.3	0.4	0.4	19.4	2.7			3.5	

Solar saltern: precipitation of salts



Marine hypersaline basins



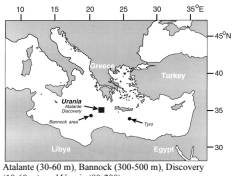
Distribution of hypersaline basins that are known to contain life.

- created by the evaporation and flooding of ancients seas
- brines enclosed in these basins are characterized by anoxic conditions, high pressure (around 35 MPa), and almost saturated salt concentrations
- high densities of the hypersaline brines limit mixing with overlaying oxic seawater
- physical separation and existence for thousands of years have resulted in the evolution of microbial communities specific to each hypersaline basin

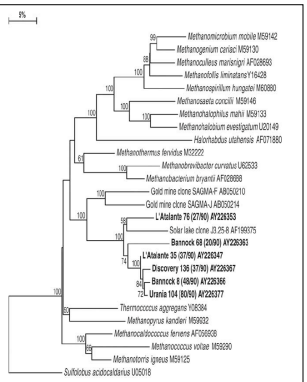
The Enigma of Prokaryotic Life in Deep Hypersaline Anoxic Basins

Paul W. J. J. van der Wielen,[1]† Henk Bolhuis,[1] Sara Borin,[2] Daniele Daffonchio,[2] Cesare Corselli,[3] Laura Giuliano,[4] Giuseppe D'Auria,[4] Gert J. de Lange,[5] Andreas Huebner,[6] Sotirios P. Varnavas,[6] John Thomson,[7] Christian Tamburini,[8] Danielle Marty,[8] Terry J. McGenity,[9] Kenneth N. Timmis,[9,10] BioDeep Scientific Party

Deep hypersaline anoxic basins in the Mediterranean Sea are a legacy of dissolution of ancient subterranean salt deposits from the Miocene period. Our study revealed that these hypersaline basins are not biogeochemical dead ends, but support in situ sulfate reduction, methanogenesis, and heterotrophic activity. A wide diversity of prokaryotes was observed, including a new, abundant, deeply branching order within the *Euryarchaeota*. Furthermore, we demonstrated the presence of a unique, metabolically active microbial community in the Discovery basin, which is one of the most extreme terrestrial saline environments known, as it is almost saturated with $MgCl_2$ (5 M).



Atalante (30-60 m), Bannock (300-500 m), Discovery (10-60 m), and Urania (80-200).



Phylogenetic tree showing the most dominant archaeal sequences

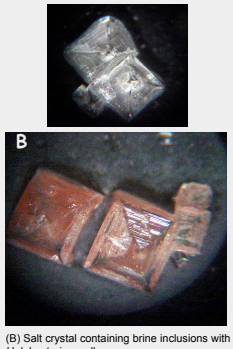
Vol 440|9 March 2006|doi:10.1038/nature04418 nature

LETTERS

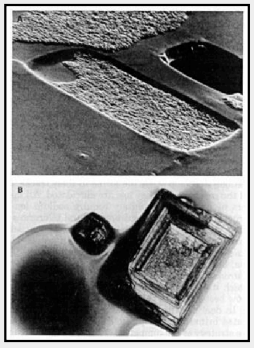
Stratified prokaryote network in the oxic–anoxic transition of a deep-sea halocline

Daniele Daffonchio[1], Sara Borin[1], Tullio Brusa[1], Lorenzo Brusetti[1], Paul W. J. J. van der Wielen[2], Henk Bolhuis[2], Michail M. Yakimov[3], Giuseppe D'Auria[3], Laura Giuliano[3], Danielle Marty[4], Christian Tamburini[4], Terry J. McGenity[5], John E. Hallsworth[5], Andrea M. Sass[5], Kenneth N. Timmis[5,6], Anastasios Tselepides[7], Gert J. de Lange[8], Andreas Hübner[9], John Thomson[9], Soterios P. Varnavas[10], Francesco Gasparoni[11], Hans W. Gerber[12], Elisa Malinverno[13], Cesare Corselli[13] & Biodeep Scientific Party*

Halophiles in salt crystals



(B) Salt crystal containing brine inclusions with *Halobacterium* cells



letters to nature

Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal

Russell H. Vreeland*, William D. Rosenzweig* & Dennis W. Powers†

- geological evidence support the 250-million year age of salt crystal
- radiation from ^{40}K (only radioactive material in salt crystal) is not sufficient to sterilize the salt (survival ~ 1.5 billion years)
- lipid profiling and molecular methods showed that Permian isolates differs significantly from their modern relatives

Data help rule out questions of contamination by modern strains during original isolation

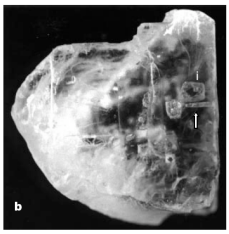
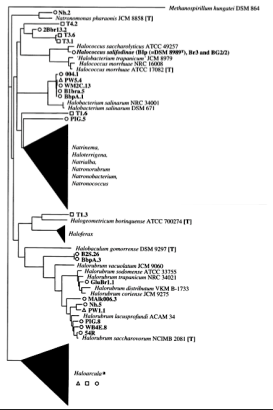


Figure 1. Halite crystals taken from the dissolution pipe at the 569m (1850 ft) level of the Salado Formation that yielded Bacillus strain 2-9-3. Crystal measured 3.5x3.5x2.5 cm. The inclusion that contained the bacterium (below i) measured approximately 3x3x1mm (9 cubic mm). The drill hole made during sampling of inclusion B is visible above the arrow.

NATURE (2000) 407:897



Phylogeny of haloarchaea isolated from salt deposits (bold) based on 16S rRNA sequences.

Isolated from salt deposits of period:
Permo-Triassic (235 - 260 Ma) ○
late Cretaceous (60-70 Ma) □
Miocene (c. 20Ma) △

The bar below represents 10 nucleotide substitutions per 100 bases

T type strain of the species
[T] type species of the genus
Ma, million years

McGenity et al., 2000

Geomicrobiology Journal, 24:275–282, 2007
Copyright © Taylor & Francis Group, LLC
ISSN: 0149-0451 print / 1521-0529 online
DOI: 10.1080/01490450701456917

Isolation of Live Cretaceous (121–112 Million Years Old) Halophilic *Archaea* from Primary Salt Crystals

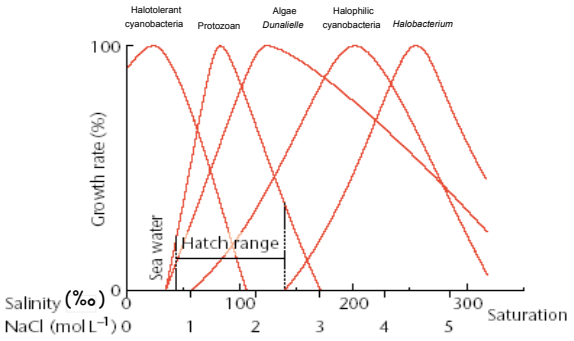
R. H. Vreeland
Ancient Biomaterials Institute, and Department of Biology, West Chester University, West Chester, PA 19383, USA

J. Jones, A. Monson, W. D. Rosenzweig
Department of Biology, West Chester University, West Chester, PA 19383, USA

T. K. Lowenstein, M. Timofeeff, C. Satterfield, B. C. Cho, and J. S. Park
Department of Geological Sciences, Binghamton University, Binghamton, New York 13902; School of Earth and Environmental Sciences, 302-19 Gwanak Campus, 151-752, Gwanak gu, Seoul National University, Seoul, South Korea

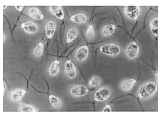
A. Wallace and W. D. Grant
Department of Infection, Immunity and Inflammation, Maurice Shock Medical Sciences Building University of Leicester, Leicester, United Kingdom LE1 9HN, UK

Growth characteristics of halophiles

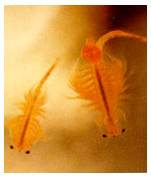


Diversity of halophiles: eukaryotes

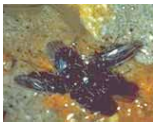
Organism	Type	Characteristics
Eukaryotes		
Artemia	brine shrimp	feeds on *Dunaliella*
Ephydra	brine fly	pupae look like grains of rice
Dunaliella	green algae	highly pigmented



Dunaliella



Artemia salina



Ephydra

and Bacteria

Aphanothece halophytica	unicellular cyanobacteria	commonly found on top of microbial mats
Oscillatoria sp.	filamentous cyanobacteria	grows under gypsum layer
Spirochaeta halophila	spirochaete	
Chlorobium limicola	green sulfur	external sulfur granules
Chloroflexus aurantiacus	green nonsulfur	filamentous
Rhodospirillum sp.	purple nonsulfur	
Beggiatoa alba	sulfur oxidizer	
Ectothiorhodospira sp.	purple sulfur	grows in alkaline brine pools
Desulfovibrio halophilus	sulfur reducer	
Haloanaerobium sp.	strict anaerobe	
Bacillus sp. 2-9-3	spore former	250 million year old salt

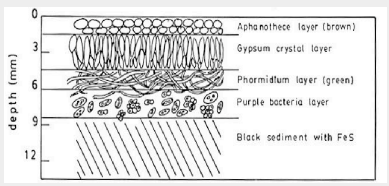
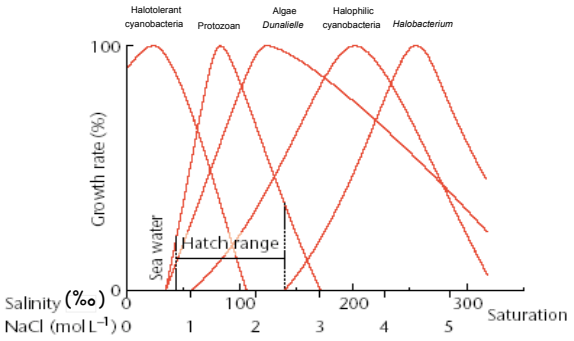
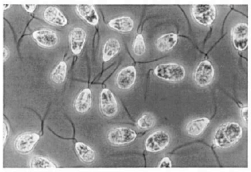


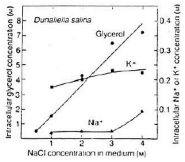
Diagram of microbial mat from marine saltern

Growth characteristics of halophiles

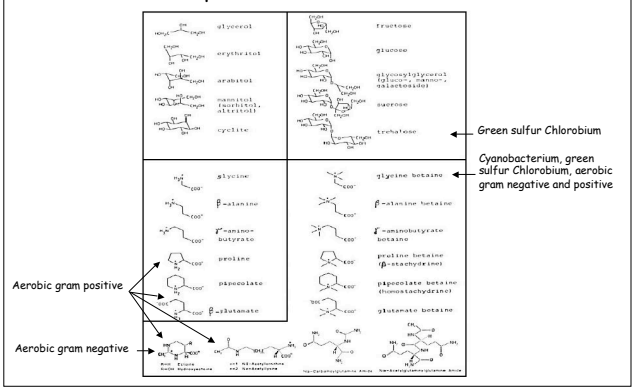


Dunaliella salina - intracellular Na^+, Ca^+, and glycerol concentrations

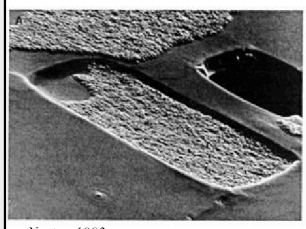




Compatible solutes in bacteria



Ionic composition of *Halobacterium* cells



Intracellular concentrations:

Na^+	1.37 +/- 0.21 M
K^+	4.57 +/- 0.12 M
Cl^-	3.61 +/- 0.07 M

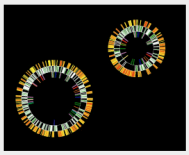
Ratios:

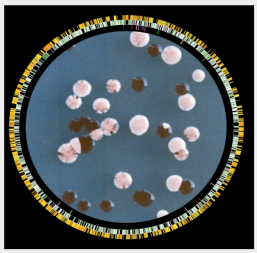
Cell Na^+ /medium Na^+	0.30
Cell K^+ /medium K^+	143
Cell Na^+ +K^+/medium Na^+	1.32
Cell Cl^- /medium Cl^-	0.80

Norton 1992

Topics

- *Halobacterium* physiology, genetics and biochemistry
- *Halobacterium* genomic
- *Natrosomonas* energetic
- *Haloquadratum walsbyi* or the "salt square of Walsby"
- Biotechnology applications

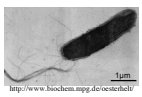




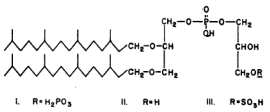
Adaptive features of *Halobacterium*

to hypersalinity, high solar radiation, microaerobic conditions, elevated temperature, limited nutrients

Growth	aerobic chemoorganotrophic	42°C - 3.5 to 5 M NaCl
	facultative anaerobic	
	phototrophic	
Motility	chemotaxis and phototaxis	
	gas vesicle-mediated flotation	
Cell envelope	retinal pigments (BR, SRI & II, HR)	
	S-layer sulfated glycoprotein	
	archaeol (branched ether) lipids	
	carotenoids	
Enzymes	salt requiring or tolerant	
Genetics	UV tolerant	
	genetic plasticity (IS elements)	

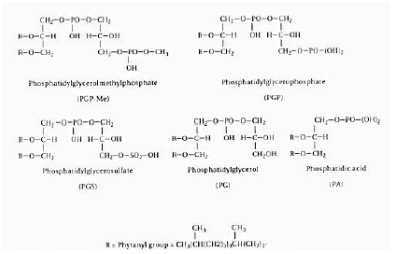


Halobacterium salinarum



I. R = H_2PO_3 II. R = H III. R = SO_3H

Halobacterium phospholipids



Cell envelope
S-layer
Sulfated glycoprotein



Left-handed
Z-DNA

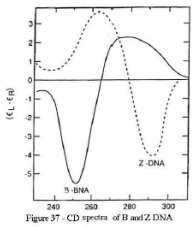


Figure 37 - CD spectra of B and Z DNA

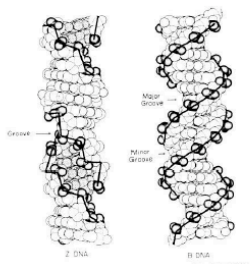
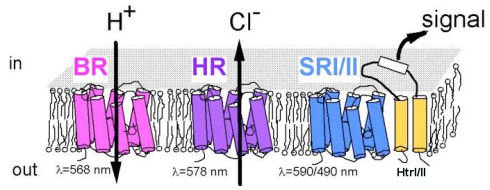


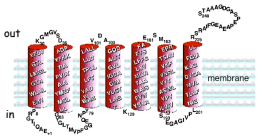
Figure 38 van der Waals models of Z-DNA an d B-DNA. The irregularity of the Z-DNA backbone is illustrated by the heavy lines that go from phosphate to phosphate residue along the chain. The Z-DNA diagram shows the molecules as they appear in the crystal. The groove in Z-DNA is quite deep, extending to the axis of the double helix. In contrast, B-DNA has a smooth line connecting the phosphate groups and two grooves, neither one of which extends to the helix axis of the molecule (D).

Retinal proteins in *Halobacterium*

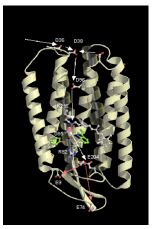


BR: bacteriorhodpsin
HR: halorhodopsin
SR: sensoryrhodopsin

Bacteriorhodopsin

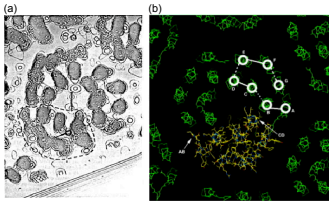


seven-transmembrane helix topology



3D structure of bacteriorhodopsin

(a) (b)



Purple membrane

Protein:lipid ratio is 75:25

2-dimensional crystal consisting of bacteriorhodopsin trimers

(a) Electron diffraction; (b) 2D-structure

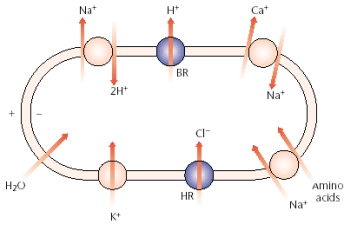


Figure 3 Flux of ions and molecules across the membrane of halobacteria. The light-driven proton and chloride pumps, bacteriorhodopsin (BR) and halorhodopsin (HR) (respectively), are shown in blue, and the sodium/proton antiporter, potassium uniporter, sodium/amino acid symporter and calcium/sodium antiporter are shown in pink. The directionality of ions, amino acids and water is indicated by arrows, and the electrical potential across the membrane is indicated by the + and − charges at the left (− inside).

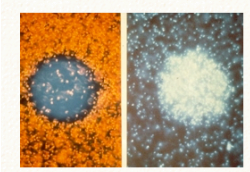
(DasSarma and Arora, 2001)

Phototaxis

Two transducers involved in phototaxis:

- sensory rhodopsin I - phototactic receptor SRI : mediates the photophilic response to orange and also the photophobic response to UV light

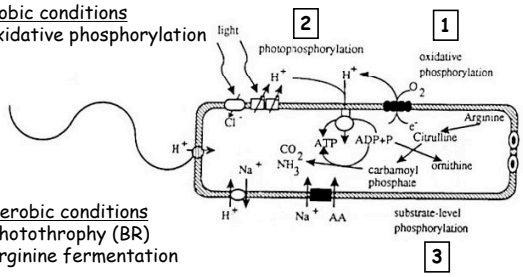
- sensory rhodopsin II - phototactic receptor SRII: mediates the photophobic response to blue light



Light trap experiment with Halobacterium: Left, cells escape from the central spot of blue light; right, cell accumulate in a central spot of orange light (false color representation) (Stoeckenius et al. 1988)

Energy metabolism of *Halobacterium*

Aerobic conditions
1. oxidative phosphorylation

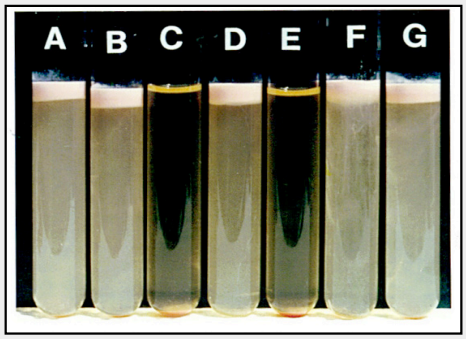


Anaerobic conditions
2. Photothrophy (BR)
3. arginine fermentation

The Hammer Experiment



Gas vesicle-mediated flotation of *Halobacterium*

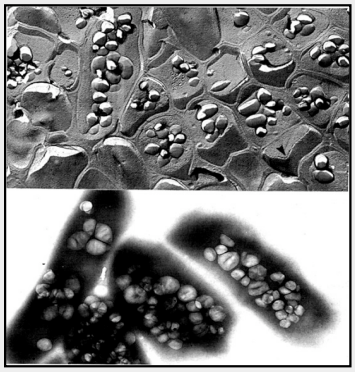


Halobacterium gas vesicles

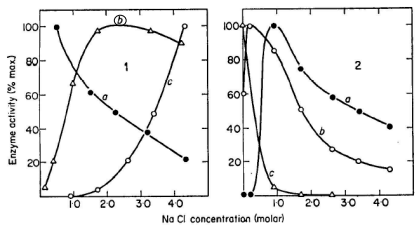
freeze-fracture

and

TEM

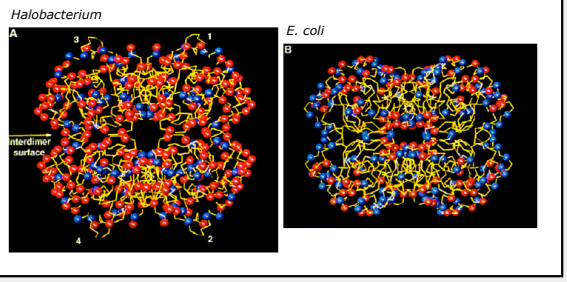


Salt sensitivity of *Halobacterium* enzymes

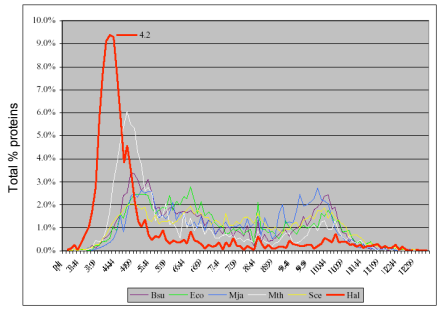


(1) Cysteine desulphydrase (a), cytochrome oxidase (b), NADH dehydrogenase (c) from *Halobacterium.*
(2) Malate dehydrogenase from 2 *Halobacterium* strains (a and b) and from pig liver (c).

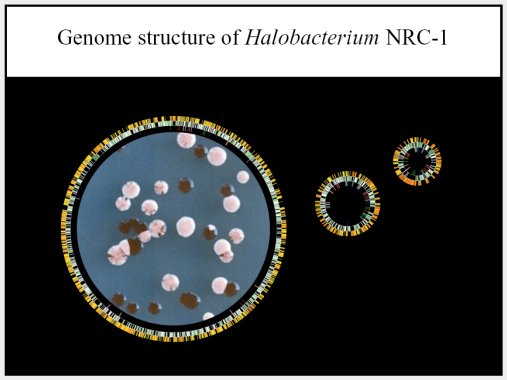
Structure of halophilic malate dehydrogenase



Distribution of isoelectric points



Genome structure of *Halobacterium* NRC-1



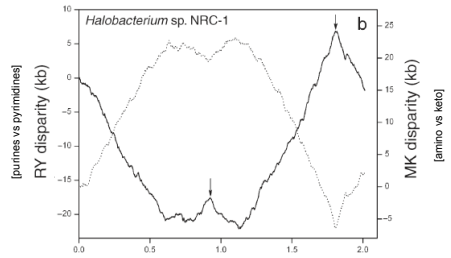
Essential and important genes on pNRC100 and pNRC200

Arginyl-tRNA synthetase
Cytochrome d oxidase
Cell division FtsZ protein
Potassium ion transporter
Sodium/proton antiporter
Orc1/cdc6 origin recognition proteins
DNA polymerase
TATA binding proteins
Transcription factor IIB
Thioredoxin
Thioredoxin reductase

Halobacterium NRC-1 genome statistics

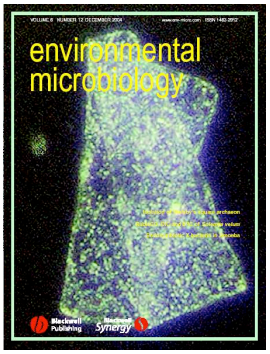
	Total	Chromosome	pNRC200	pNRC100
Size (bp)	2,571,010	2,014,239	365,425	191,346
G+C composition (%)	65.9	67.9	59.2	57.9
No. of IS elements	91	22	40	29
Coding (%)	84	87	76	71
No. of predicted genes	2,682	2,111	374	197
Named genes (%)	42	45	32	26
Conserved HPs (%)	22	24	13	16
Other HPs (%)	36	31	55	58

Halobacterium has two origins of replication



The Z-curves for the genome *Halobacterium* sp. NRC-1, Unbroken lines denote RY disparity curves, and broken lines denote MK disparity curves. Arrows indicate positions of cdc6 genes and predicted replication origins. Berquist and DasSarma (2003) identified a chromosomal autonomously replicating sequence element, which is at the location of the cdc6-3 (arrow at about 1.8 Mb).

Zhang and Zhang, 2005

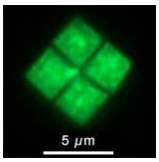


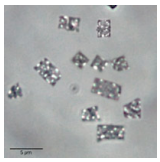
Haloquadratum walsbyi or the "salt square of Walsby"

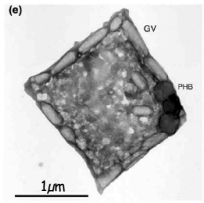
First description in 1980 by Anthony Walsby

Extremely halophilic square archaeon isolated in pure culture by 2 groups in 2004

Dark field microscopic image of *H. walsbyi*. Square sheet is 40X40 μm and filled with gas vesicles. Square cells is 5x5 μm (left)







Haloquadratum walsbyi

Thin square sheets with sharp corners
2 to 5 μm wide and 0.1 to 0.5 μm thick

GV: gas vesicles
PHB: poly-β-hydroxybutyrate granules

Require >3M sodium chloride
and up to 2M magnesium

Why are square cells so unusual?
Turgor pressure in freshwater cells
H. Walsbyi lack turgor pressure

What happens at the edges and corners?
Flattened cylinder with rounded edges

Advantages of thin sheets?
Large surface area for nutrient uptake
Light absorption highly efficient

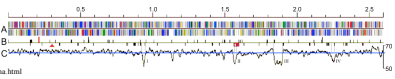
Living with two extremes: Conclusions from the genome sequence of *Natronomonas pharaonis*



Michaela Falb, Friedhelm Pfeiffer, Peter Palm, Karin Rodewald, Volker Hickmann, Jörg Tittor, and Dieter Oesterhelt[1]
Max-Planck-Institute of Biochemistry, Department of Membrane Biochemistry, D-82152 Martinsried, Germany
Genome Research (2005) 15:1336–1343

- *Natronomonas pharaonis* is an extremely haloalkaliphilic archaeon that was isolated from salt-saturated lakes of pH 11 (op. 3.5M NaCl, pH 8.5)
- 2.6-Mb GC-rich chromosome and two plasmids (131 and 23 kb)
- genome analysis suggests that it is adapted to cope with severe ammonia and heavy metal deficiencies that arise at high pH values
- respiration and oxidative phosphorylation were experimentally proven. These studies identified protons as coupling ion between respiratory chain and ATP synthase, in contrast to other alkaliphiles using sodium instead
- glycosylated cell surface proteins probably form a protective complex cell envelope

Representation of the chromosome of *Natronomonas pharaonis*



http://www.biochem.mpg.de/oesterhelt/genomics/intro_Napha.html

Biotechnological applications

- Large-scale production of biopolymers and proteins
- Stable enzymes for catalysis (organic solvents)
- Purple membrane films for holography
- Recombinant gas vesicles for separations and antigen presentation

Hypersaline environments and halophile diversity

1- Hypersaline environments are found all over the world, usually associated with modern marine pools or ancient evaporite basins, and can be neutral, basic or acidic.

2. Hypersaline environments are harsh with respect to salt, usually contain low oxygen concentrations, and are exposed to high solar radiation. They are extremely productive for microbial life, including formation of dense microbial mats similar to ancient stromatolites.

3. Moderate and extreme halophiles grow optimally in 5-10 X the concentration of salts in the sea, and include eukaryotes (algae, fungi, protozoa), bacteria (unicellular and filamentous cyanobacteria, sulfur/nonsulfur, purple photosynthetic bacteria, heterotrophic bacteria, sulfur oxidizing bacteria, sulfate reducing bacteria) and archaea (halobacteria and a few methanogens)

Halobacterium genetics, biochemistry, & physiology
Halobacterium genomics and post-genomic potential

Main points:

1. Extremely halophilic archaea, such as *Halobacterium* species, have adapted to the environmental challenges that they face, such as high salinity, low oxygen tension, limited nutrients availability, high solar radiation, etc.

2. *Halobacterium* proteins are highly acidic to resist salting-out by electrostatic repulsion and enhance solvation.

3. *Halobacterium* species have alternate physiological capabilities, aerobic respiration, substrate-level phosphorylation, and photophosphorylation.

4. *Halobacterium* species have evolved efficient photoprotective mechanisms to repair damage resulting from exposure to intense solar radiation.

5. Genomic sequencing has extended the understanding of the adaptation of *Halobacterium* to it's environment, including the discovery of an extremely acidic proteome, a complex stimulon responding to light, multigene families of transcription factors and regulators, acquisition of aerobic respiration by lateral gene transfer, etc.

6. The ease of culture and manipulation in the laboratory of *Halobacterium* species and other halophiles makes them valuable for future functional genomics studies and biotechnological applications.