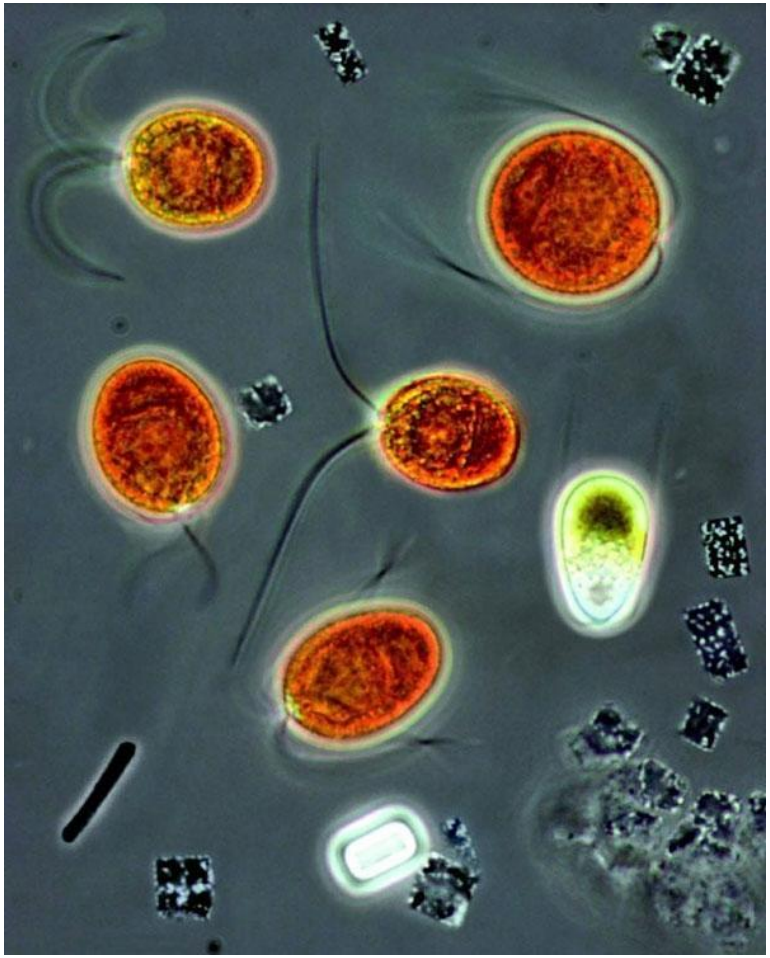
Halobacterium salinarum

by: Aigul Akimniyazova

- is not a bacterium, but is a model organism from the halophilic branch of Archaea
- It is classified as an extremophile due to its ability to survive in environments with very high salt concentrations.
- Due to their high salinity, these salterns become purple or reddish color with the presence of halophilic Archaea.

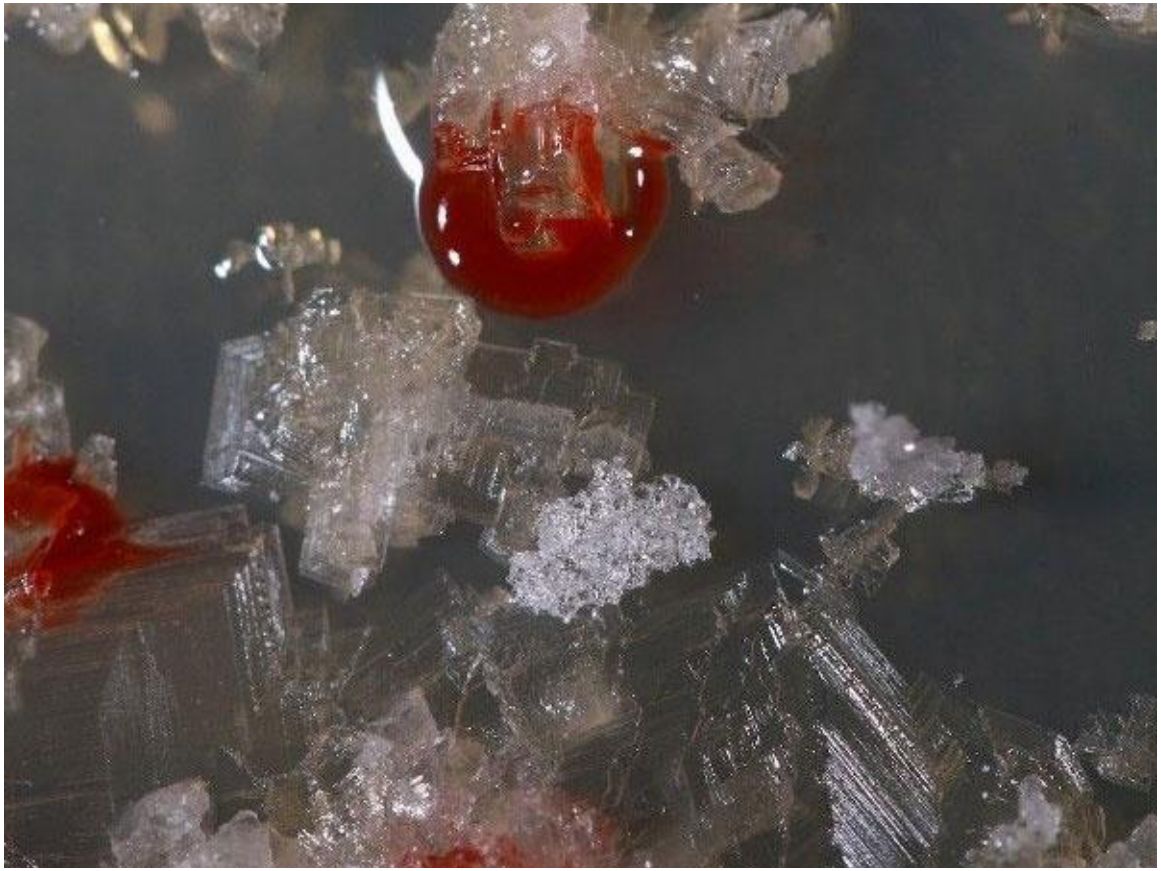
Halobacterium salinarum

- Domain: Archaea
- Kingdom: Euryarchaeota
- Phylum: Euryarchaeota
- Class: Halobacteria
- Order: Halobacteriales
- Family: Halobacteriaceae
- Genus: *Halobacterium*
- Species: ***H. salinarium***

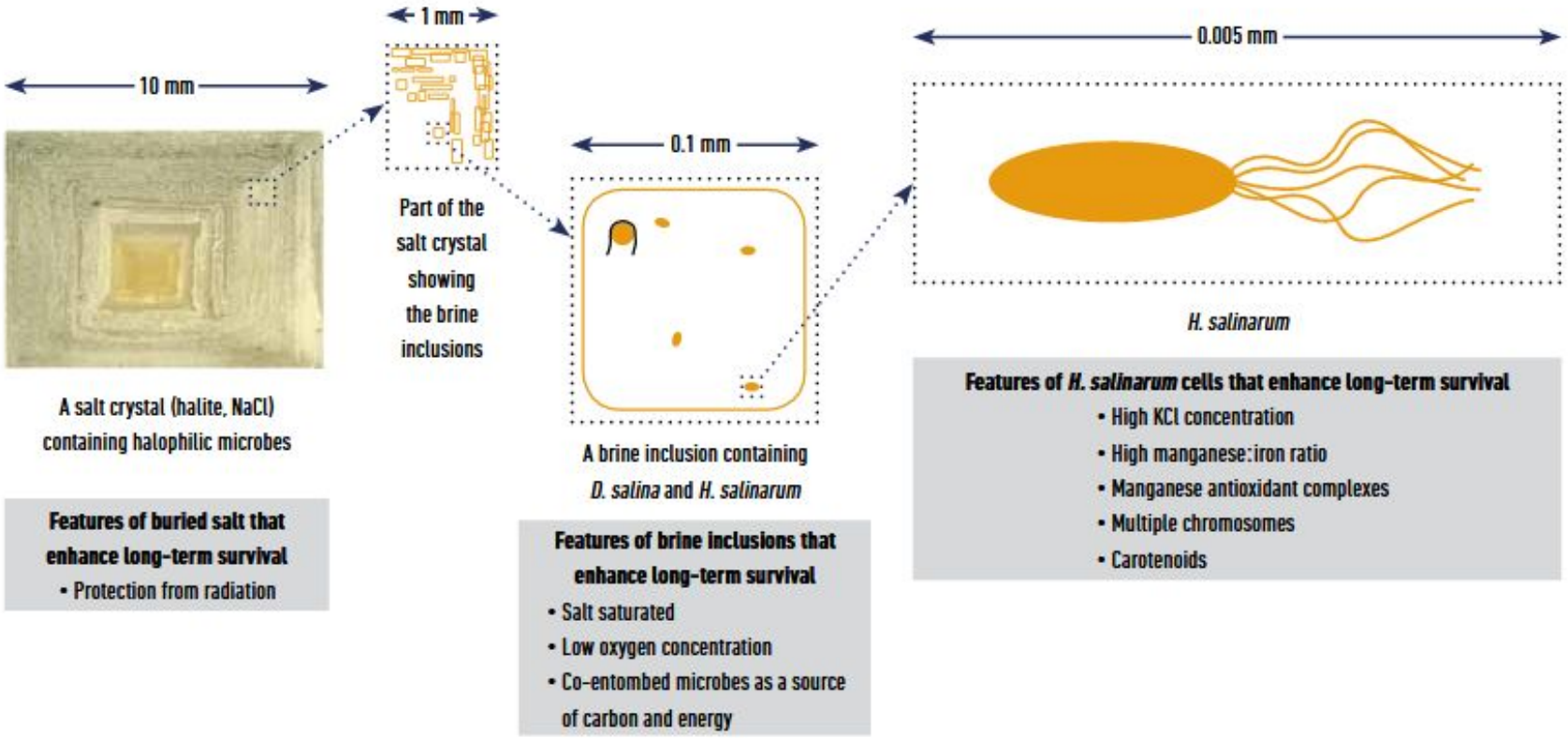


- For *H. salinarum* to grow in hypersaline environments, it contains a highly concentrated salt solution (mainly consisting of potassium chloride, KCl)
- This commitment to an extremely salty existence has its advantages; *H. salinarum* can grow with less interspecies competition than microbes living in more moderate conditions such as the ocean.

- Amino acids are the main source of chemical energy for *H. salinarum*, particularly arginine and aspartate, though they are able to metabolize other amino acids, as well.[2] *H. salinarum* have been reported to not be able to grow on sugars, and therefore need to encode enzymes capable of performing gluconeogenesis to create sugars. Although "H. salinarum" is unable to catabolize glucose, the transcription factor TrmB has been proven to regulate the gluconeogenic production of sugars found on the S-layer glycoprotein.



COLONIES OF *HALOBACTERIUM SALINARUM* GROWING ON SALT-SATURATED AGAR PLATE


10 mm
1 mm
0.1 mm
0.005 mm
A salt crystal (halite, NaCl) containing halophilic microbes
Part of the salt crystal showing the brine inclusions
A brine inclusion containing D. salina and H. salinarum
H. salinarum
Features of buried salt that enhance long-term survival
• Protection from radiation
Features of brine inclusions that enhance long-term survival
• Salt saturated
• Low oxygen concentration
• Co-entombed microbes as a source of carbon and energy
Features of H. salinarum cells that enhance long-term survival
• High KCl concentration
• High manganese:iron ratio
• Manganese antioxidant complexes
• Multiple chromosomes
• Carotenoids

For *Halobacterium salinarum* - **NOTE** the higher NaCl concentration.

2M NaCl, 27mM KCl, 15% (w/v) sucrose

For 1 Litre: Made up from sterile stock solutions or powders as follows:

1. Add the following components to a 1 or 2L beaker or graduated cylinder.

COMPOUND		**VOLUME** (Stock)	***OR***	**MASS**
	(final concn)			
NaCl	(2M)	400 ml (5M)		117 g
KCl	(27mM)	27 ml (1M)		2.01 g
Sucrose	(15%)	300 ml (50% w/v)		150 g

(MW NaCl= 58.5, MW KCl = 74.5)

Medium selection and its composition

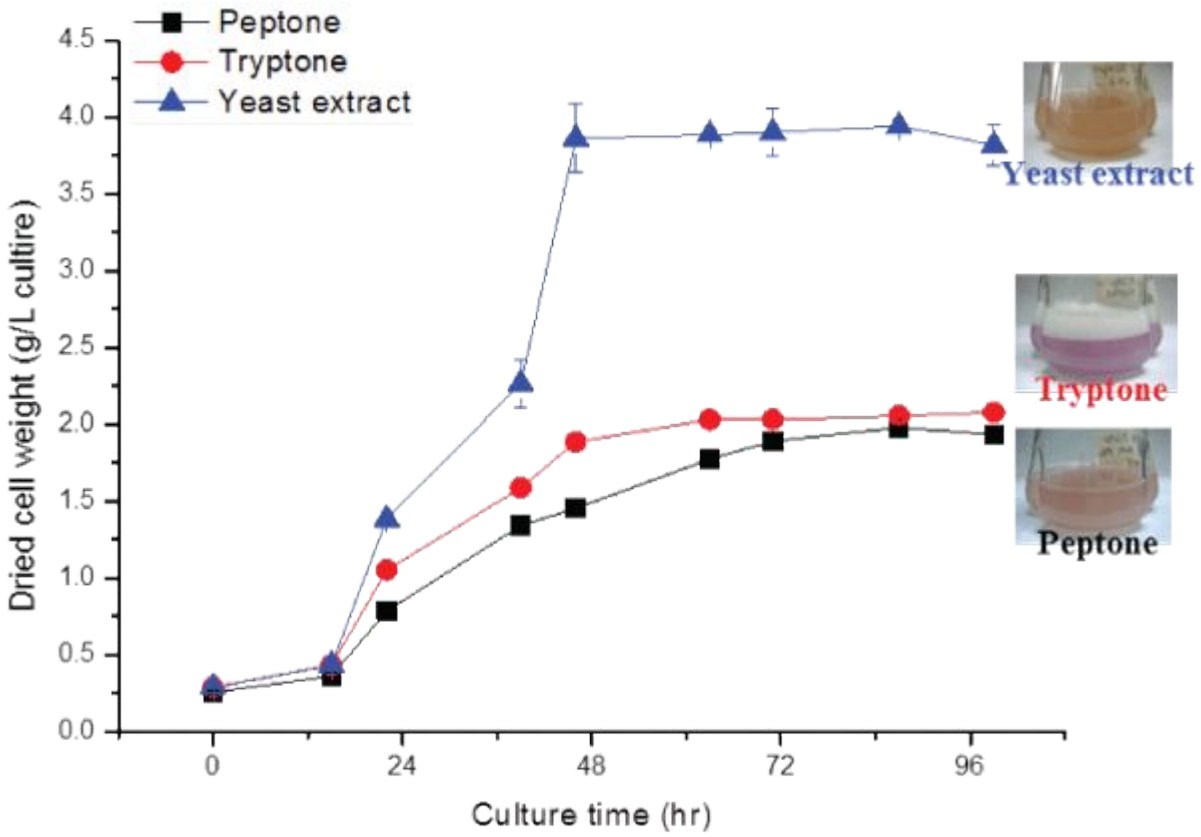
- can grow in a simple salts medium with lactate, pyruvate, glucose, or glycerol as sole carbon sources.

per Litre:

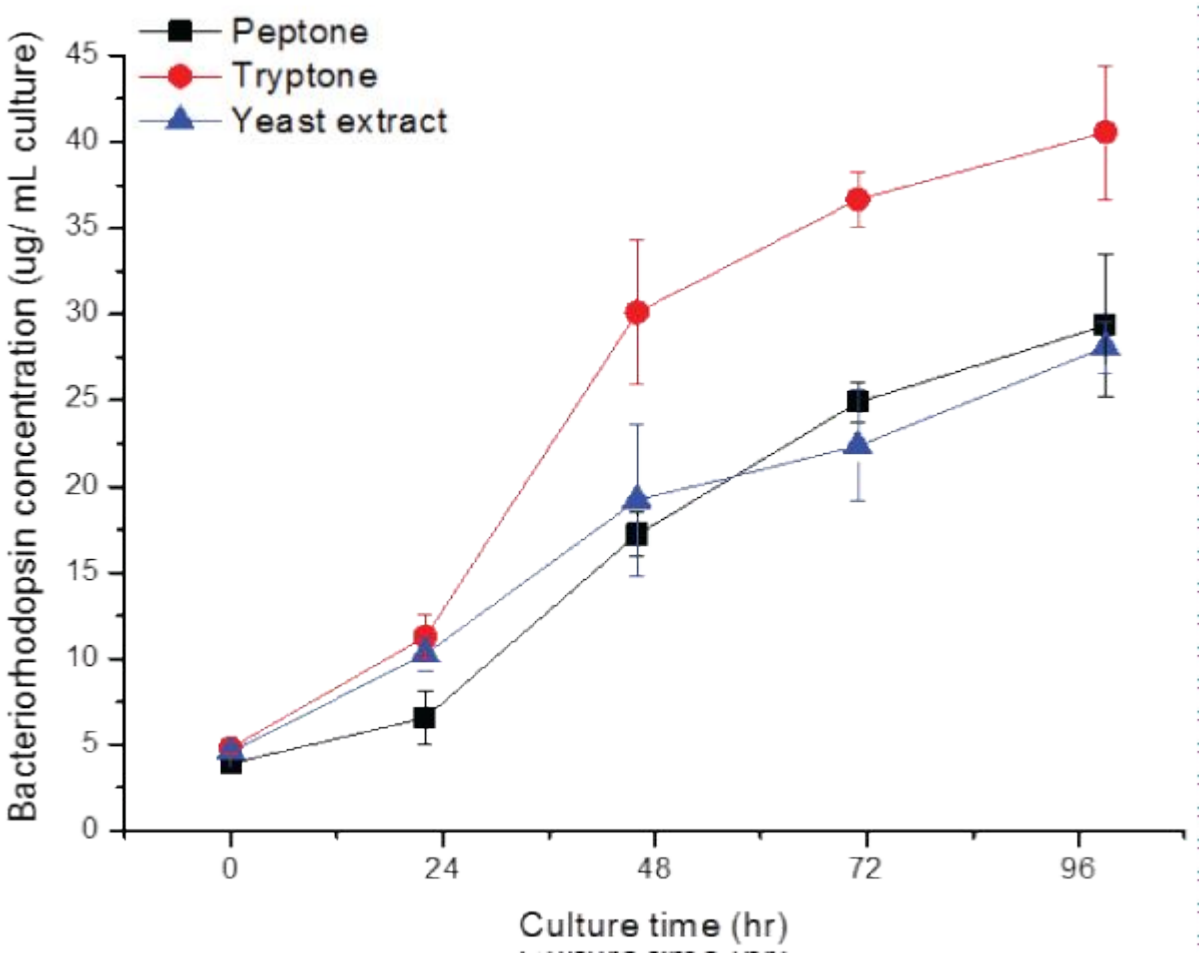
NaCl	250g
Tris.Cl (1M, pH7.4)	50ml
$MgSO_4.7H_2O$	20g
KCl	2g
$CaCl_2.2H_2O$	0.2 g
$Na_3Citrate.2H_2O$	3g
Bacto-Yeast Extract	3g
Bacto-Tryptone 5 g	

adjust to 1000ml and pH 7.4. Autoclave.

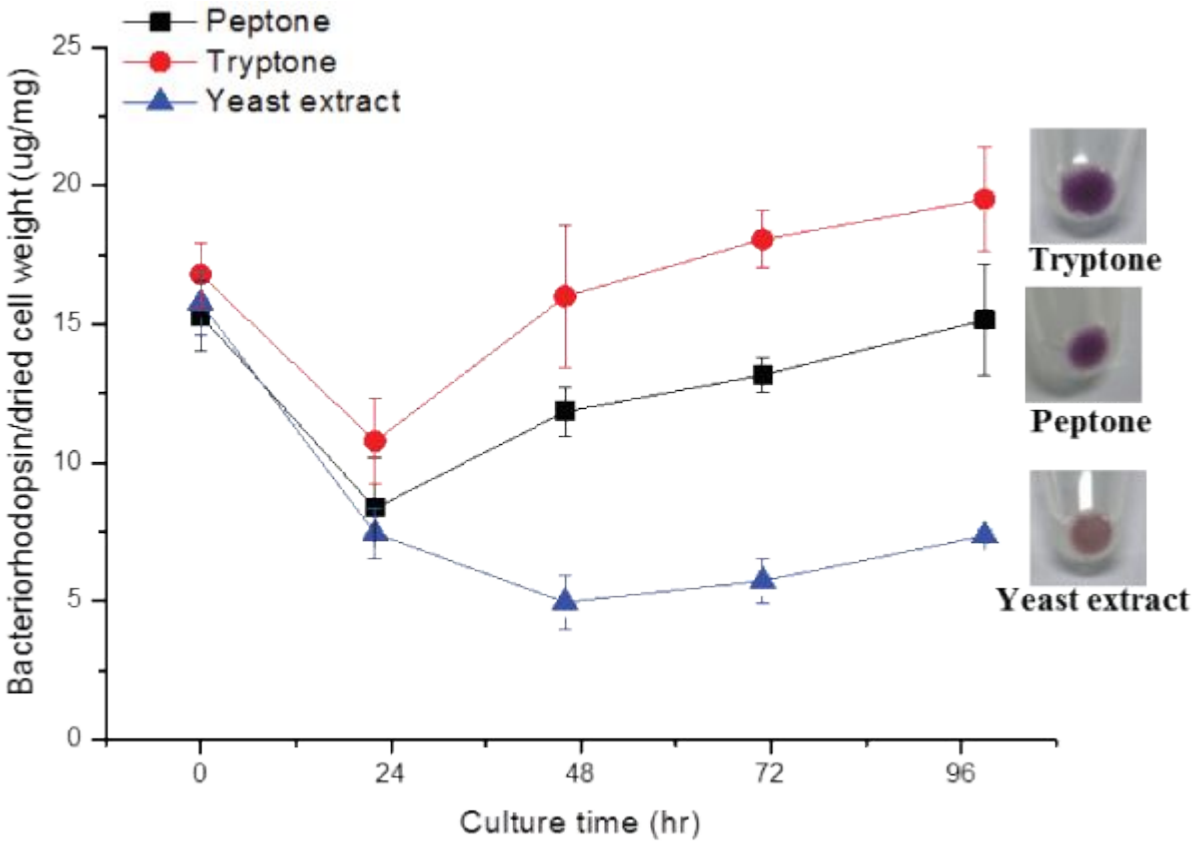
Growth studies



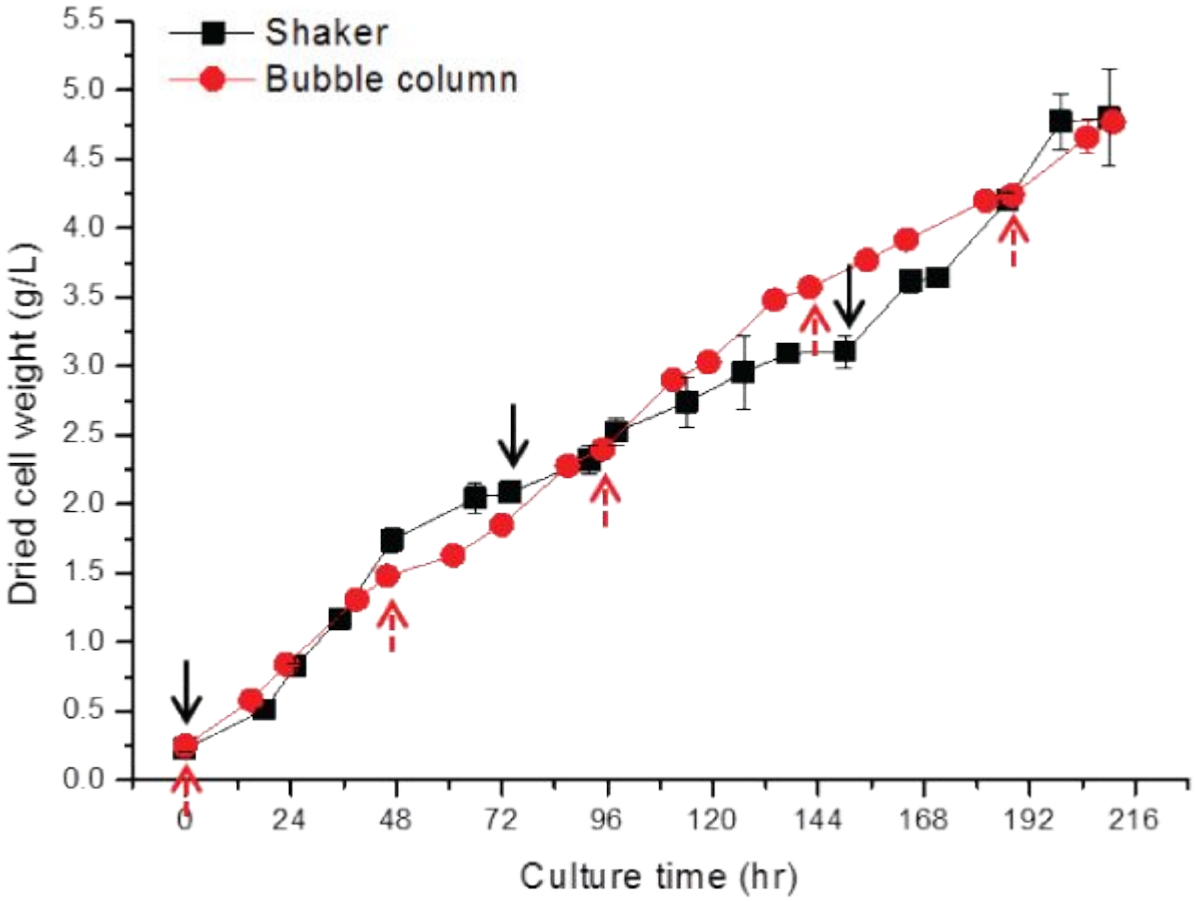
- **Figure 1:** Growth curves of *H. salinarum* cultivated in bacteriological peptone, tryptone and yeast extract medium.



- **Figure 2:** Bacteriorhodopsin produced by *H. salinarum* cultivated in bacteriological peptone, tryptone and yeast extract medium.

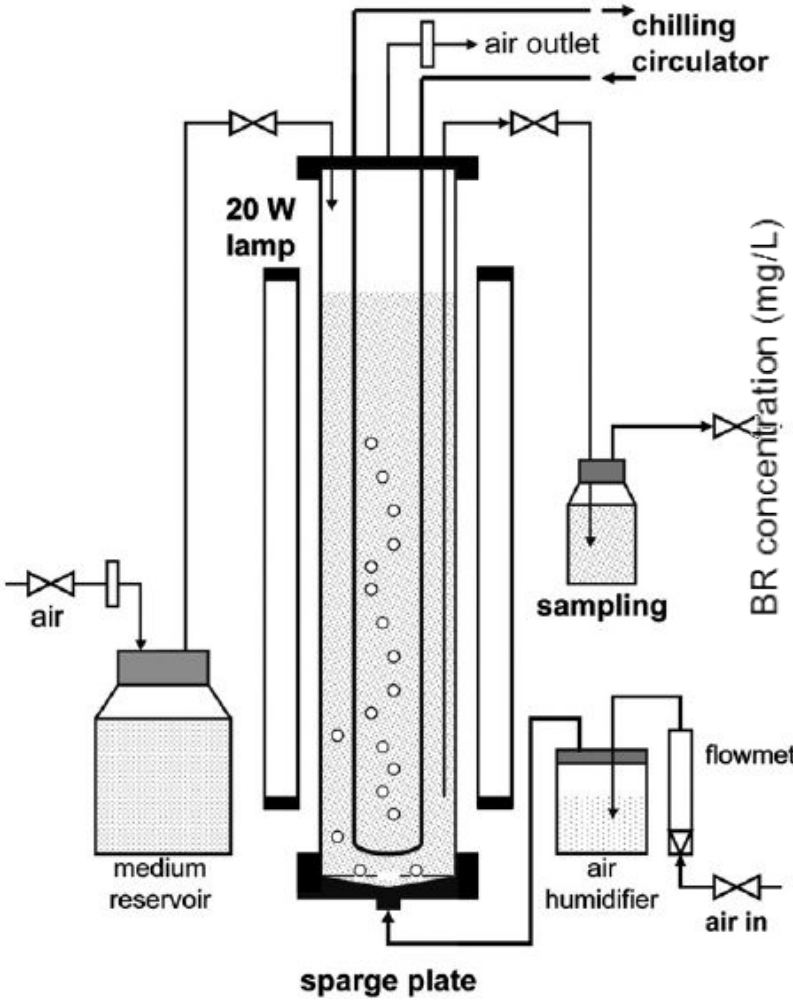


- **Figure 3:** Bacteriorhodopsin contents in *H. salinarum* cultivated in bacteriological peptone, tryptone and yeast extract medium.



- **Figure 4:** Repeated batch cultivation of *H. salinarum* in full-tryptone medium of a shaker flask and half-tryptone medium of a bubble column photobioreator, black-solid and red-broken arrow indicates full and half tryptone medium replacement.

- **Figure 5:** Images of *H. salinarum* cultivated with half-tryptone medium in a bubble column photobioreator under repeated batch operation. (pH 7,2)



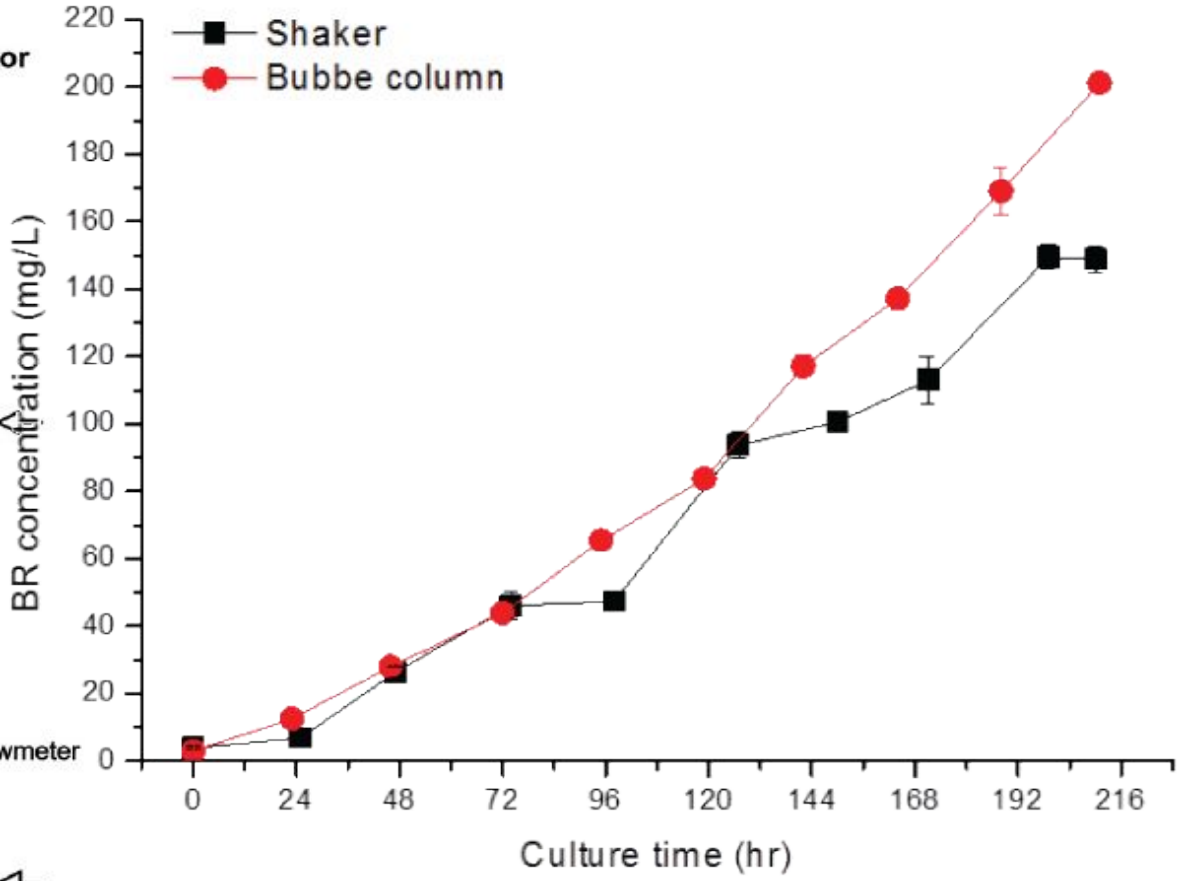


Figure 6: Bacteriorhodopsin produced by *H. salinarum* cultivated in fulltryptone medium of a shaker flask and half-tryptone medium in a bubble column photobioreator under repeated batch operation.

- **Protection against ionizing radiation and desiccation**
- *H. salinarum* is polyploid and highly resistant to ionizing radiation and desiccation, conditions that induce DNA double-strand breaks. Although chromosomes are initially shattered into many fragments, complete chromosomes are regenerated by making use of over-lapping fragments. Regeneration occurs by a process involving DNA single-stranded binding protein, and is likely a form of homologous recombinational repair.

Genome

- Whole genome sequences are available for two strains of *H. salinarum*, NRC-1[2] and R1.[20] The Halobacterium sp. NRC-1 genome consists of 2,571,010 base pairs on one large chromosome and two mini-chromosomes. The genome encodes 2,360 predicted proteins.[2] The large chromosome is very G-C rich (68%).[21] High GC-content of the genome increases stability in extreme environments. Whole proteome comparisons show the definite archaeal nature of this halophile with additional similarities to the Gram-positive *Bacillus subtilis* and other bacteria.

Genome sequence

- The genome was found to be 2,571,010 bp in size and composed of 3 circular replicons, a 2,014,239-bp-large chromosome and 2 smaller replicons, pNRC100 (191,346 bp) and pNRC200 (365,425 bp).

- This archaean has three chromosomes: a genomic chromosome of 2,015kb size, a 366kb replicon and a 191kb replicon. Its replicons have genes for DNA polymerase, transcription factors, mineral (K and PO_4) uptake, and cell division. The genomic chromosome has many transposon insertion sites. *Halobacterium salinarium* carries out aerobic respiration but in water up to 5M (25%!) NaCl (salt). It can be found in the Great Salt Lake in Utah and the Red Sea in Asia Minor.

1. *Hbt. salinarum* cells were grown at 40°C in liquid complex medium, shaken, until an OD_{550} of 0.9 – 1.0

2. Cells were spun down 3000× g at 4°C

3. Wash once with ice-cold FT buffer (4.3M NaCl, 27 mM KCl, 100 mM $CaCl_2$, 10 mM PIPES, pH 6.8)

4. Resuspend in 1 ml of ice-cold FT buffer.

If frozen stocks of competent cells are to be prepared, add glycerol to 10% (v/v) and store frozen at -70°C in 100 µl lots. Cells were then thawed when required and processed the same as fresh cells

5. A quantity of 1-2 µg plasmid DNA (in 2-5 µl FT buffer) was added to 100 µl of competent cells, and immediately mixed by tapping with a finger.

6. Leave on ice for 15 min, then store at –70°C for 30 min (to freeze).

7. Mixture thawed by incubation for 80 sec in 40°C waterbath, then kept on ice.

8. Add 900 µl of ice-cold complex medium, and incubate 60min at 40°C without shaking.

9. Plate 200 µl in 3ml of molten top agar (0.7% agarose, complex medium, kept at 60°C), and pour on top of support plates with selective drug (e.g. 10 µg/ml mevinolin/simvastatin)

10. Incubate 40°C until colonies arise.

Selectable markers and plasmid replicons

Plasmid	Marker/Replicon	Hosts (+/-)	Comment/Reference
pMDS20	NovR(Hfx.)/pHK2	*Hfx. volc., Hb. sal.* (+) *Har. hisp* (-), *Har.vall* (-)	Not sure why it doesn't work in *Haloarcula hispanica*. (Holmes et al., 1994)
pMDS30	as for pMDS20	as for pMDS20	Blue/White selection in *E.coli*. (Kamekura et al., 1996)
pMDS99	MevR(Har.)/pHV2	*Hfx. volcanii*	*Har. hispanica* derived MevR gene, does not recombine with *hmgA* in *Hfx. volcanii.* (Wendoloski et al., 2001).
pWL102	MevR(Hfx.)/pHV2	*Hfx.volc., Hfx. vall., Har. hisp. Hb. sal.* (all +)	Will not replicate in *radA* host. (Lam and Doolittle, 1992)
pUBP2	MevR(Hfx.)/pHH1	*Hfx. volc., vall., Har. hisp.* (all +)	(Cline and Doolittle, 1992) (Blaseio and Pfeifer, 1990)
TmR (dfr)	TmR(Hfx.)/pHV2	*Hfx.volc*. (+)	Only works in *Hfx. volcanii.* (Zusman et al., 1989)
pGRB1 pMPK series	MevR(Hfx.)/pGRB	*Hbt.sal*. (+)	(Krebs et al., 1991; Krebs et al., 1993)
pMLH32	NovR(Hfx.)/pHK2	*Hfx. volc* (+)	(Holmes and Dyall-Smith, 2000)
pUS-MEV[a]	MevR(Hfx) / non	*Hbt. sal*. (+)	Suicide vector. Positive clones only by integration in the genome; (Pfeiffer et al., 1999). See below

Lysis and RNA isolation

1. Inoculate 0.5 ~ 0.7 ml of haloarchaeal culture into fresh medium (e.g. 10 ml of 18% MGM, in a convenient bottle or tube), and shake at 190 rpm, 37°C, for 1 – 2 days, until mid-exponential phase (OD550 of around 0.5 – 0.8).
2. Take 0.5 – 1 ml sample into a clean 1.5ml microfuge tube and spin cells down (13,000 rpm, 1min, 4°C)

3. Put the tubes on ice and remove the supernatant as completely (get the last volume out with a micropipette), then add 80 µl of **lysis solution**. Pipette up and down to make sure the entire cell pellet is lysed and evenly mixed in the solution, but avoid making air bubbles.

Lysis Solution *(add reagents in order below)*

Reagent	**Stock Soln.**	**Volume** for 1ml
25mM NaOH	4 M	6.25 µl
5µM CDTA	(add powder)	1.7 mg
5mM EDTA	0.5 M	10 µl
8% Sucrose	30% w/v	270 µl
DEPC treated H_2O		664 µl
0.5% SDS	10% w/v	50 µl

**The hydroxide dissolves the CDTA powder easily, and the SDS is best added last*

CDTA = diaminocylcohexane tetraacetic acid (Sigma D8928)

- *The solution should go 'stringy', if it doesn't then the cells have not lysed properly.*
  4. Incubate the lysed cells at 37°C for 15 min, then place the tube on ice, leave for 2 min.
  5. Add 30 µl of ice-cold sodium acetate solution and vortex thoroughly. *(keep cold or on ice from now on)*
  6. Centrifuge the proteins down by spinning at 13,000 rpm, 30 min, 4°C.
  7. Remove the supernatant to a fresh tube, add 2 vol of ice-cold ethanol to precipitate the RNA, mix well.
  8. Centrifuge at 13,000 rpm, 15min, 4°C. Wash the pellets twice with ice-cold 70% ethanol.

- 9. Dry the pellets in a vacuum chamber for 30min at RT, dissolve in DEPC-treated water (e.g. 50-100 µl), and store at -70°C. You can also store at -20°C, but preparations last only a few weeks.
  Determine the yield of RNA by absorption at 260nm (in quartz cuvettes) using the formula 1A260 = 40 µg RNA

Thank you for attention!!!