

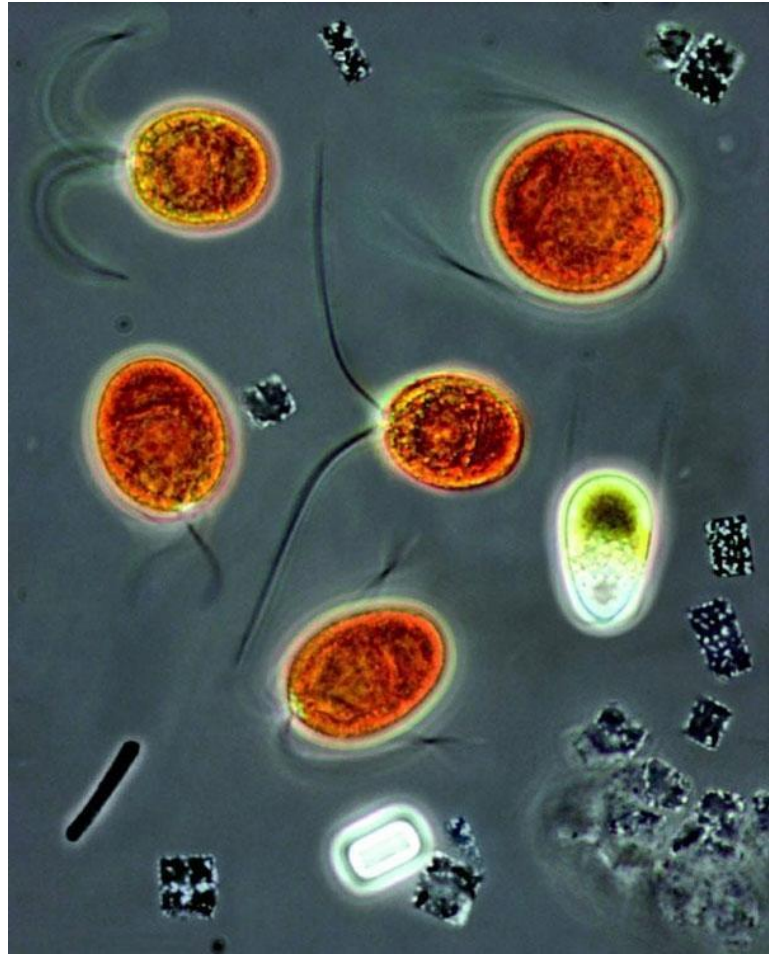
# Halobacterium salinarum

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. salinarum***

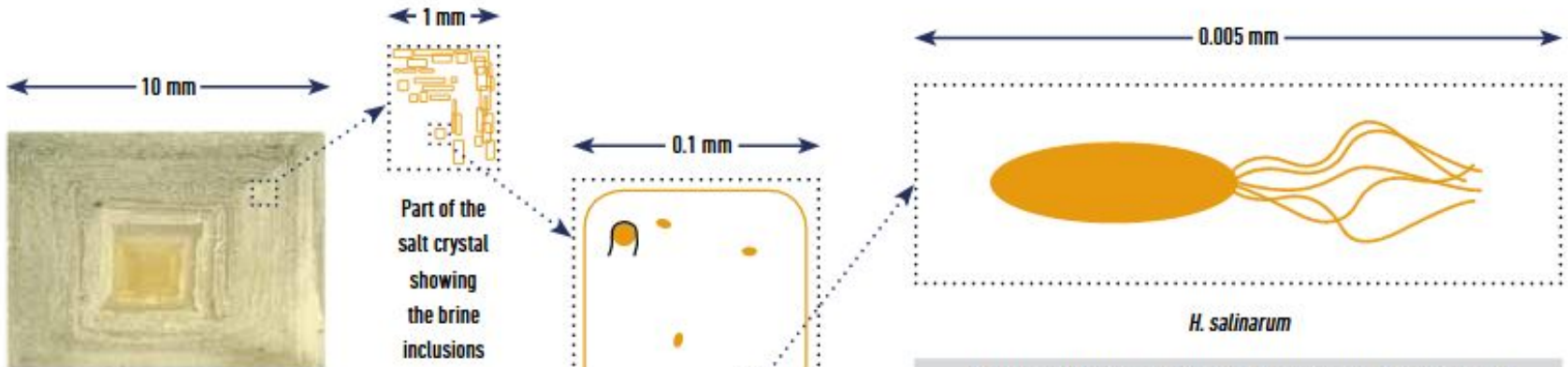


- For *H. salinarum* to grow in hypersaline environments, it contains a highly concentrated salt solution (mainly consisting of potassium chloride, KCl)
- This commitment to an extremely salty existence has its advantages; *H. salinarum* can grow with less interspecies competition than microbes living 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. <sup>[2]</sup> *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**



A salt crystal (halite, NaCl) containing halophilic microbes

**Features of buried salt that enhance long-term survival**

- Protection from radiation

A brine inclusion containing *D. salina* and *H. salinarum*

**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.

<b>COMPOUND</b>	<b>VOLUME (Stock)</b>	<b>OR</b>	<b>MASS</b>
(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

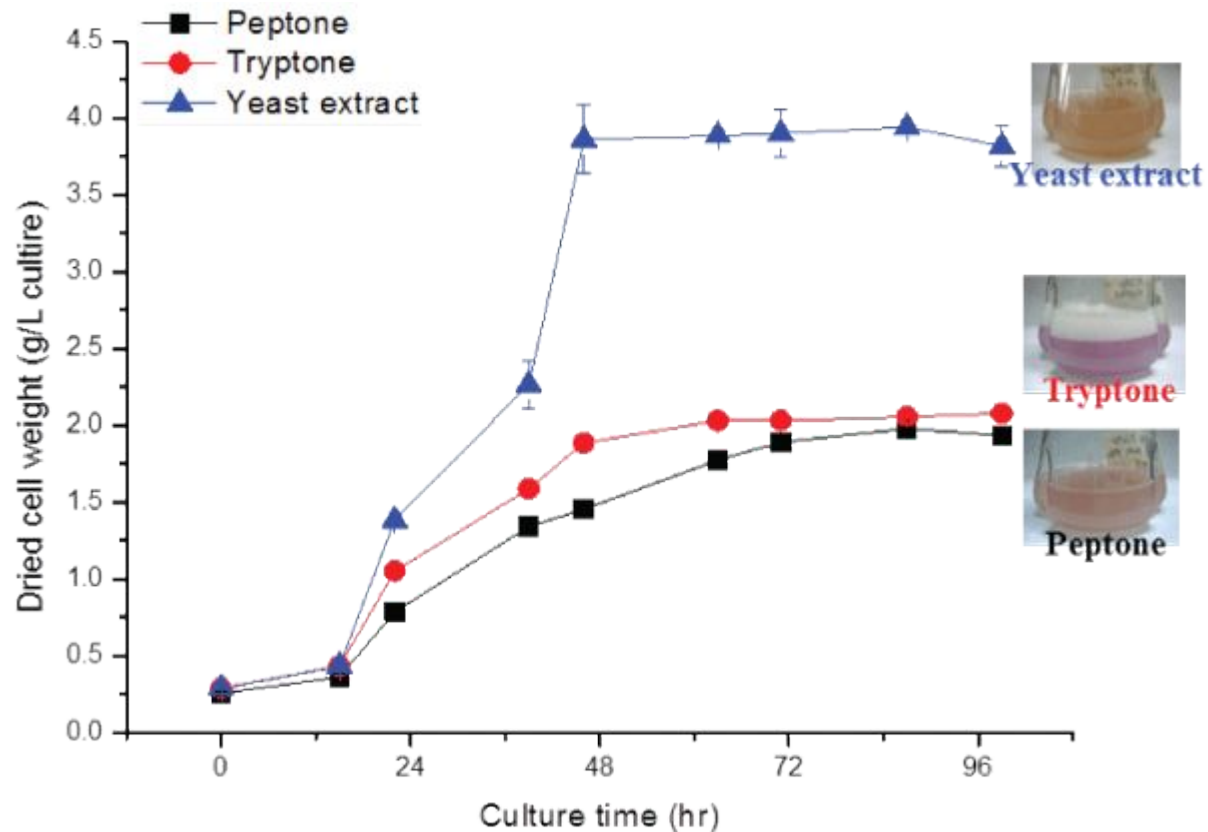
- can grow in a simple salts medium with lactate, pyruvate, glucose, or glycerol as sole carbon sources.

per Litre:

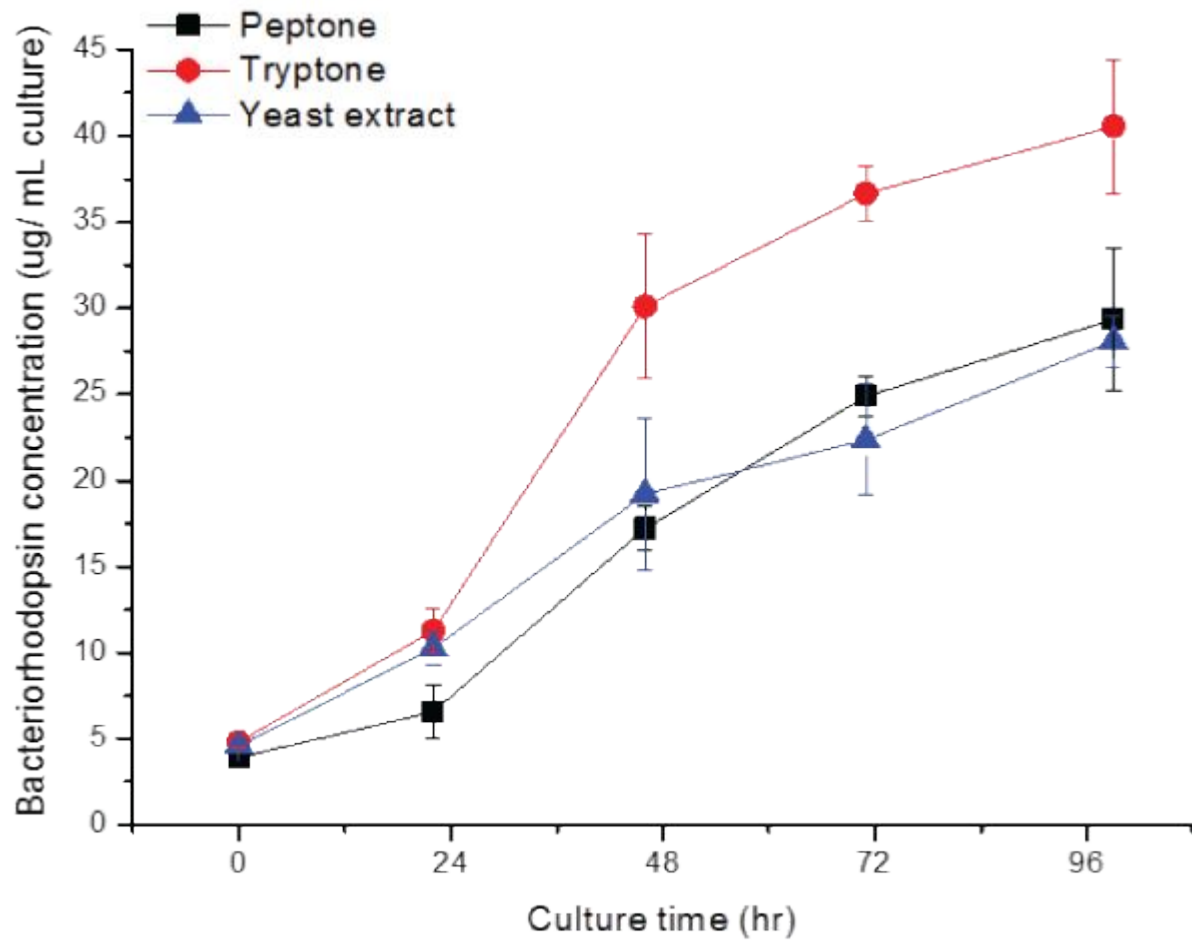
NaCl	250g
Tris.Cl (1M, pH7.4)	50ml
MgSO <sub>4</sub> .7H <sub>2</sub> O	20g
KCl	2g
CaCl <sub>2</sub> .2H <sub>2</sub> O	0.2 g
Na <sub>3</sub> Citrate.2H <sub>2</sub> O	3g
Bacto-Yeast Extract	3g
Bacto-Tryptone	5 g

*adjust to 1000ml and pH 7.4. Autoclave.*

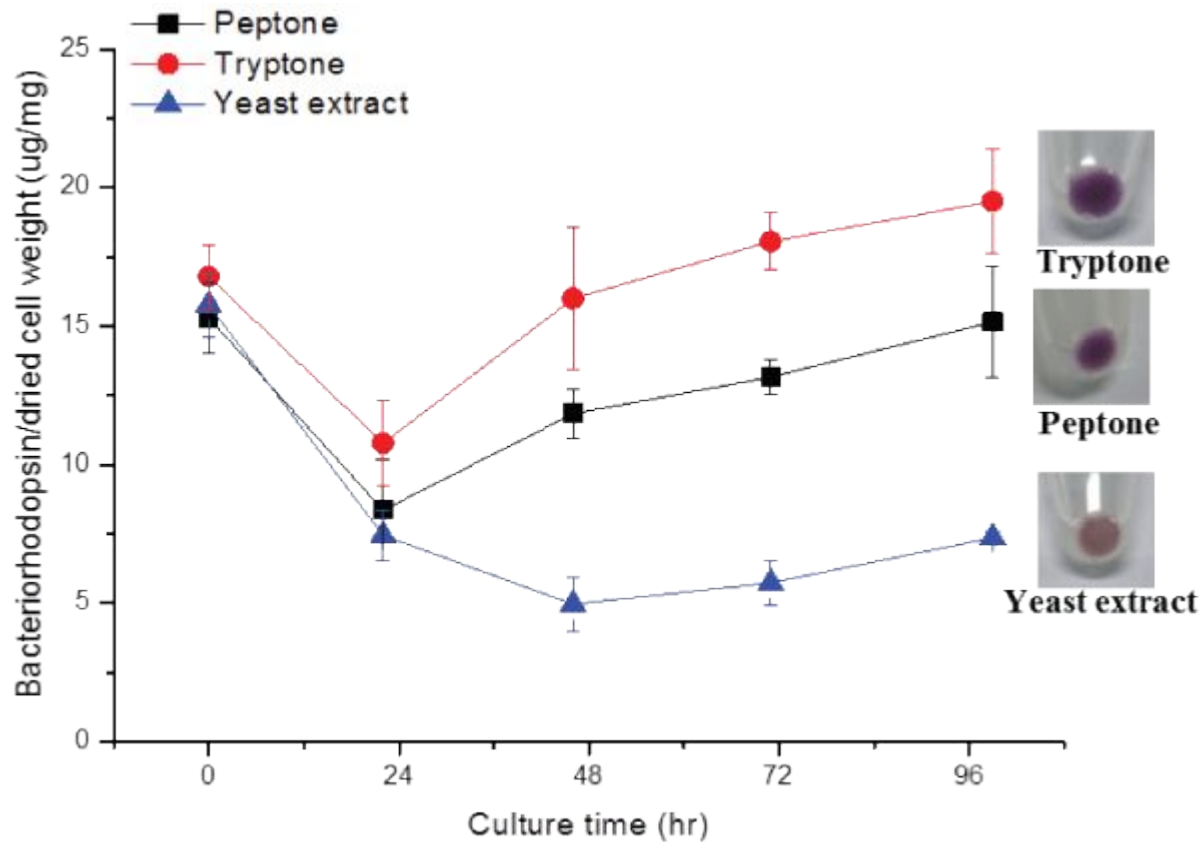
# Growth studies



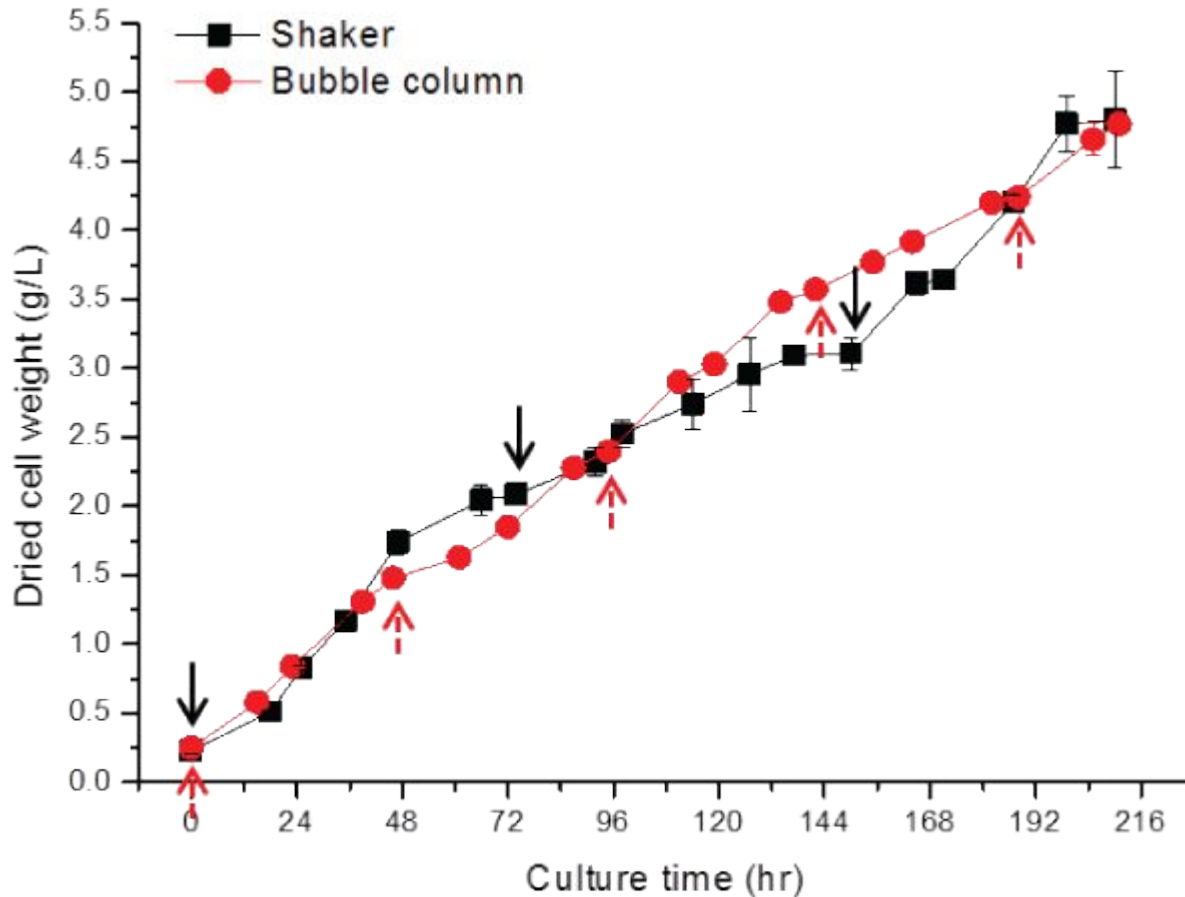
- **Figure 1:** Growth curves of *H. salinarum* cultivated in bacteriological peptone, tryptone and yeast extract medium.



- **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.



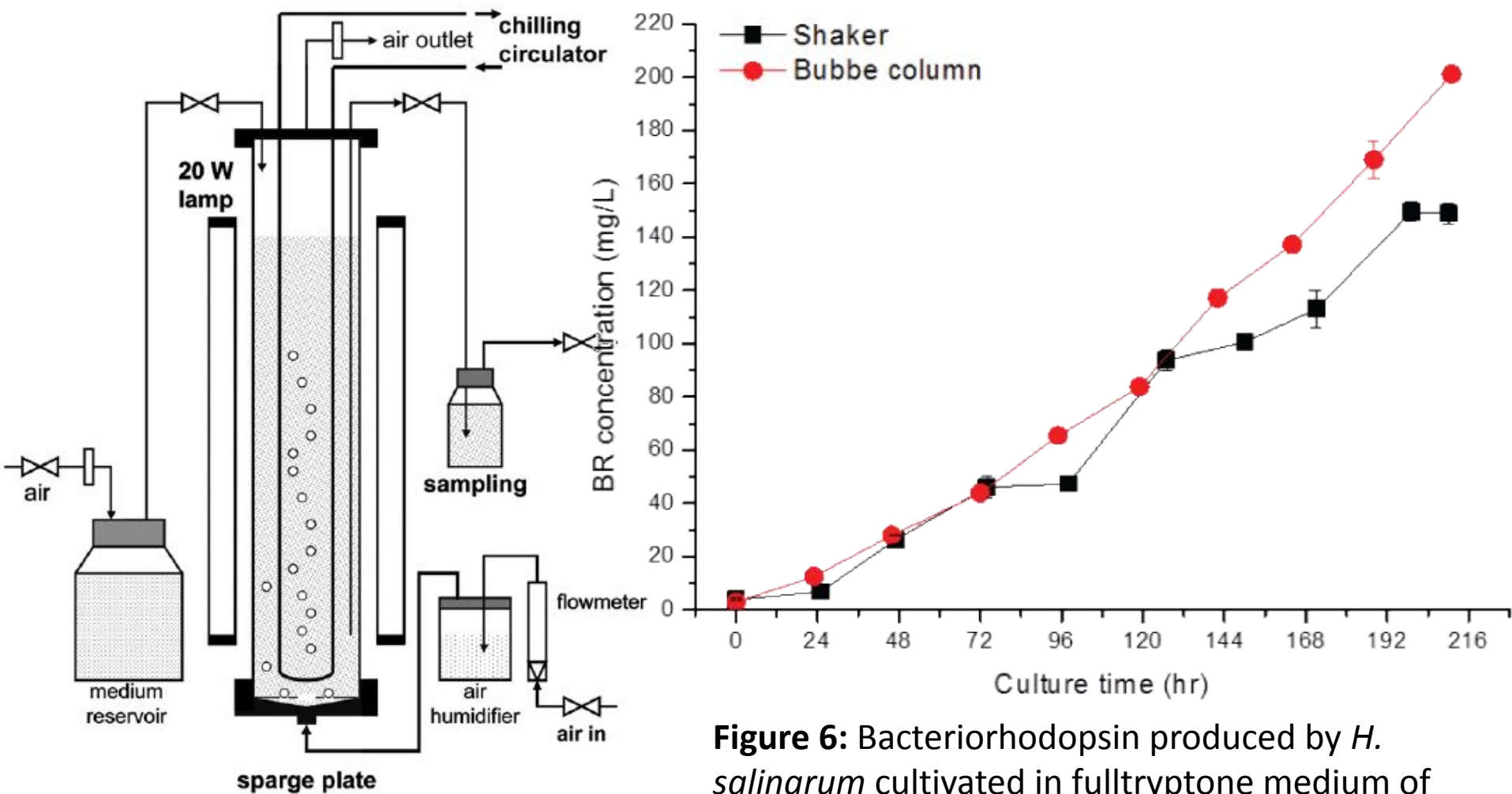
0 hr

72 hr

142 hr

211 hr

- **Figure 5:** Images of *H. salinarum* cultivated with half-tryptone medium in a bubble column photobioreactor 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<sup>[2]</sup> and R1.<sup>[20]</sup> 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.<sup>[2]</sup> The large chromosome is very G-C rich (68%).<sup>[21]</sup> 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  $\text{PO}_4$ ) 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<sub>550</sub> 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<sub>2</sub>, 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	<i>Hfx. volc.</i> , <i>Hb. sal.</i> (+) <i>Har. hisp</i> (-), <i>Har.vall</i> (-)	Not sure why it doesn't work in <i>Haloarcula hispanica</i> . (Holmes et al., 1994)
pMDS30	as for pMDS20	as for pMDS20	Blue/White selection in <i>E.coli</i> . (Kamekura et al., 1996)
pMDS99	MevR(Har.)/pHV2	<i>Hfx. volcanii</i>	<i>Har. hispanica</i> derived MevR gene, does not recombine with <i>hmgA</i> in <i>Hfx. volcanii</i> . (Wendoloski et al., 2001).
pWL102	MevR(Hfx.)/pHV2	<i>Hfx.volc.</i> , <i>Hfx. vall.</i> , <i>Har. hisp.</i> <i>Hb. sal.</i> (all +)	Will not replicate in <i>radA</i> host. (Lam and Doolittle, 1992)
pUBP2	MevR(Hfx.)/pHH1	<i>Hfx. volc.</i> , <i>vall.</i> , <i>Har. hisp.</i> (all +)	(Cline and Doolittle, 1992) (Blaseio and Pfeifer, 1990)
TmR (dfr)	TmR(Hfx.)/pHV2	<i>Hfx.volc.</i> (+)	Only works in <i>Hfx. volcanii</i> . (Zusman et al., 1989)
pGRB1 pMPK series	MevR(Hfx.)/pGRB	<i>Hbt.sal.</i> (+)	(Krebs et al., 1991; Krebs et al., 1993)
pMLH32	NovR(Hfx.)/pHK2	<i>Hfx. volc</i> (+)	(Holmes and Dyall-Smith, 2000)
pUS-MEV <sup>a</sup>	MevR(Hfx) / non	<i>Hbt. sal.</i> (+)	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  $\mu$ l 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)***

<b>Reagent</b>	<b>Stock Soln.</b>	<b>Volume for 1ml</b>
25mM NaOH	4 M	6.25 $\mu$ l
5 $\mu$ M CDTA	(add powder)	1.7 mg
5mM EDTA	0.5 M	10 $\mu$ l
8% Sucrose	30% w/v	270 $\mu$ l
DEPC treated H <sub>2</sub> O		664 $\mu$ l
0.5% SDS	10% w/v	50 $\mu$ l

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*\*The hydroxide dissolves the CDTA powder easily, and the SDS is best added last  
CDTA = diaminocyclohexane 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  $\mu$ l 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  $\mu$ l), and store at  $-70^{\circ}\text{C}$ . You can also store at  $-20^{\circ}\text{C}$ , but preparations last only a few weeks.

Determine the yield of RNA by absorption at 260nm (in quartz cuvettes) using the formula  
 $1A_{260} = 40 \mu\text{g RNA}$

**Thank you for  
attention!!!**