```
Termine Ökophysiologie Hoersaal II
Freitags 10:30 – 12 Uhr

5.3.     10:30-11:15
12.3.    entfaellt.
19.3.    10:30- 12 Uhr
26.3.    10:30-11 Uhr

2.4. und 9.4. Osterferien
16.4.    entfällt

23.4.    10:30-12:00

29.4.    10.30-12:00
7.5.     entfällt

14.5.    10:30-12:00

21.5.    10:30-12:00
28.5.    10:30 – 12:00

4.6.     Prüfung
```

Osmotic Effects on Microbial Growth

Halophiles

Microbes aim for:

Positive water balance

Cell has a higher solute concentration than the environment

-->water diffuses into the cell through osmosis

water availability depends on:

•water content of habitat (moist/dry)

•concentration of solutes dissolved in water (salts, sugars, etc)

--> ‘water activity’

Wasseraktivität: Maß für frei verfügbares Wasser

A_w- Wert = $\underline{p/p_o}$

Wasserdampfdruck über einem Material/
Wasserdampfdruck über reinem Wasser
(bei best. Temperatur)

Optimal für Bakterien: 0,98 - 1

Xerophile Mikroorganismen wachsen bei sinkendem Wassergehalt besser:
Halophile Bakterien (a_w: 0,6)
Osmophile Hefen (zuckerliebende)

Table 6.2 Water activity of several substances

Water activity (a_w)	Material	Example organisms[a]
1.000	Pure water	*Caulobacter, Spirillum*
0.995	Human blood	*Streptococcus, Escherichia*
0.980	Seawater	*Pseudomonas, Vibrio*
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as *Staphylococcus*
0.850	Salami	*Saccharomyces rouxii* (yeast)
0.800	Fruit cake, jams	*Saccharomyces bailii, Penicillium* (fungus)
0.750	Salt lakes, salted fish	*Halobacterium, Halococcus*
0.700	Cereals, candy, dried fruit	*Xeromyces bisporus* and other xerophilic fungi

[a] Selected examples of prokaryotes or fungi capable of growth in culture media adjusted to the stated water activity.

Table 6-2 Brock Biology of Microorganisms 11/e

• Some microorganisms (**halophiles**) have evolved to grow best at reduced water potential, and some (**extreme halophiles**) even require high levels of salts for growth.

• **Halotolerant** organisms can tolerate some reduction in the water activity of their environment but generally grow best in the absence of the added solute

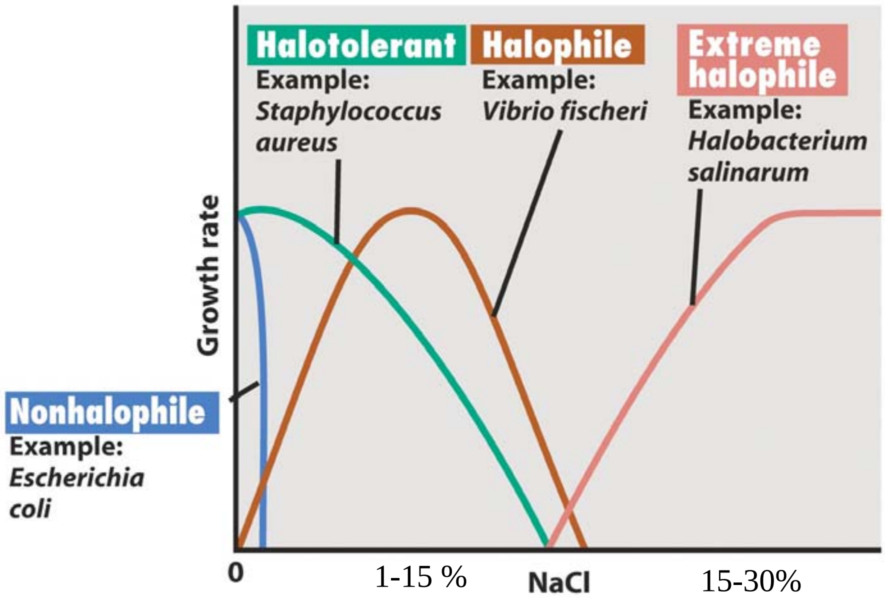
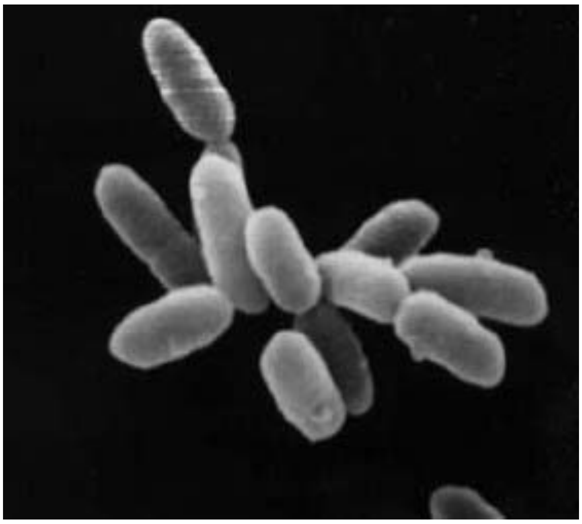


Figure 6-23 Brock Biology of Microorganisms 11/e

Halobacterium salinarum



Red color: growth of halobacteria

Adaptations of halophiles:

High internal ionic strength

Acidic proteins

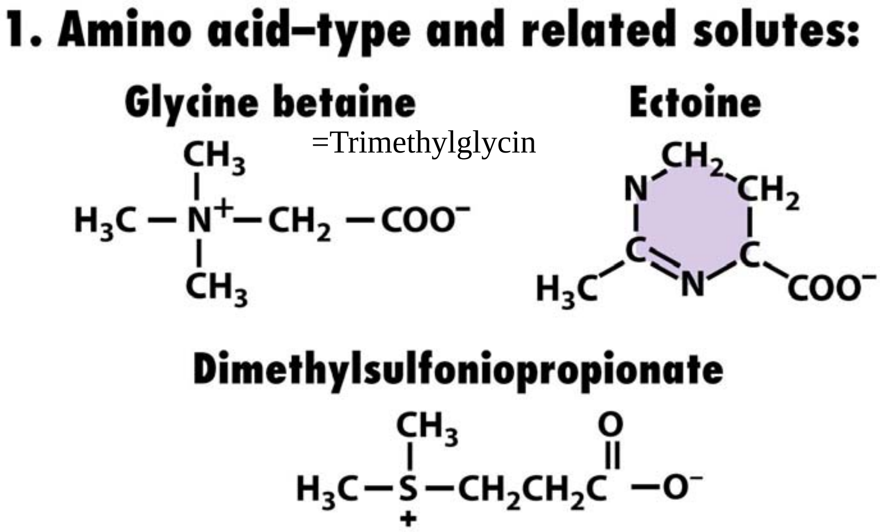
• Water activity becomes limiting to an organism when the dissolved solute concentration in its environment increases.

• To counteract this situation, organisms produce or accumulate intracellular **compatible solutes** (dt. 'verträglicher gelöster Stoff') that maintain the cell in positive water balance.

Kompatible Solute:
Osmolyte, die auch bei hohen cytoplasmatischen Konzentrationen
nicht mit dem Zellstoffwechsel interferieren,

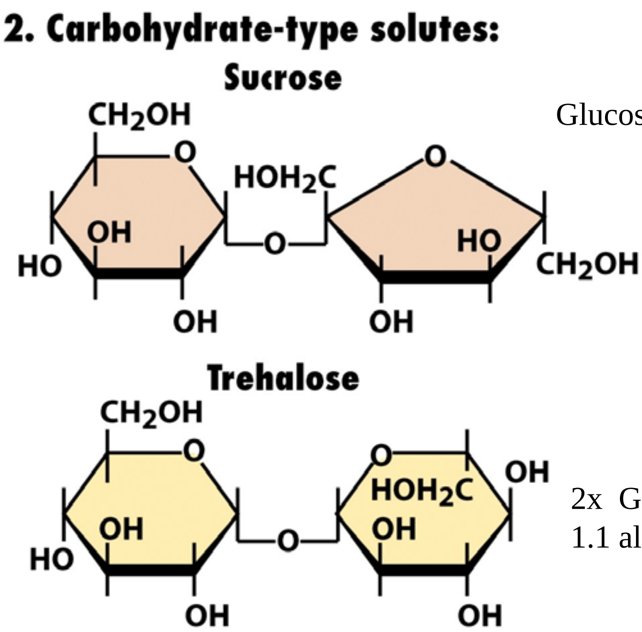
organische Verbindungen,
geringe molare Masse
bei physiologischem pH Wert elektrisch neutral,
polar: hohe Löslichkeit in Wasser

Compatible solutes



=Trimethylglycin

Figure 6-24 part 1 Brock Biology of Microorganisms 11/e



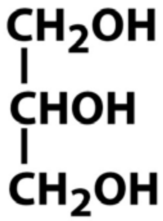
Glucose+Fructose

2x Glucose
1.1 alpha/alpha

Figure 6-24 part 2 Brock Biology of Microorganisms 11/e

3. Alcohol-type solutes:

Glycerol



Mannitol

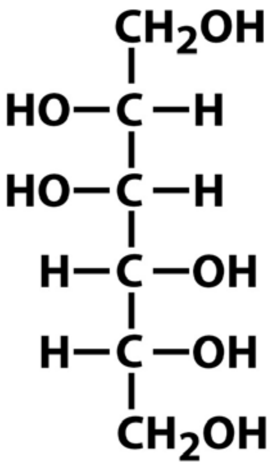


Figure 6-24 part 3 Brock Biology of Microorganisms 11/e

Table 6.3 Compatible solutes of microorganisms

Organism	Major solute(s) accumulated	Minimum a_w for growth
Bacteria, nonphototrophic	Glycine betaine, proline (mainly gram-positive), glutamate (mainly gram-negative)	0.97–0.90
Freshwater cyanobacteria	Sucrose, trehalose	0.98
Marine cyanobacteria	α-Glucosylglycerol	0.92
Marine algae	Mannitol, various glycosides, proline, dimethylsulfoniopropionate	0.92
Salt lake cyanobacteria	Glycine betaine	0.90–0.75
Halophilic anoxygenic phototrophic *Bacteria* (*Ectothiorhodospira*/*Halorhodospira* and *Rhodovibrio* species)	Glycine betaine, ectoine, trehalose	0.90–0.75
Extremely halophilic *Archaea* (for example, *Halobacterium*) and some *Bacteria* (for example, *Haloanaerobium*)	KCl	0.75
Dunaliella (halophilic green alga)	Glycerol	0.75
Xerophilic yeasts	Glycerol	0.83–0.62
Xerophilic filamentous fungi	Glycerol	0.72–0.61

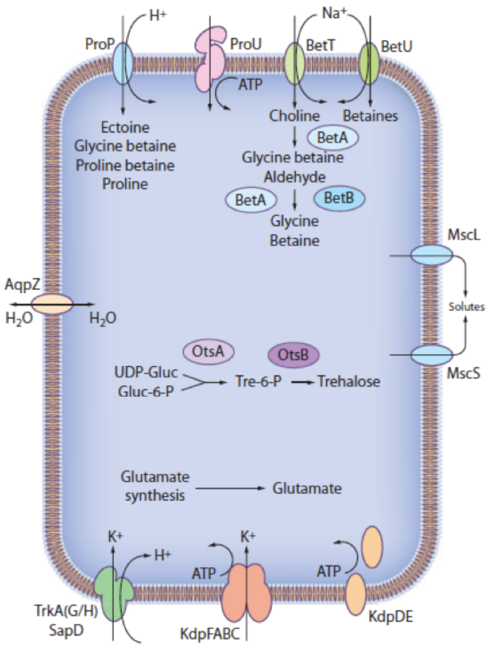
Table 6-3 Brock Biology of Microorganisms 11/e

How to react to Osmostress?

Increase internal osmolarity!

1. Pump K^+ inside
2. Pump other compatible solutes inside
3. Synthesize compatible solutes

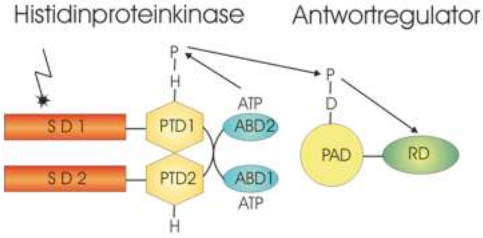
Osmoprotection in *E. coli*:



1. Aquaporin: waterflux
2. K+ transport increases
3. KdpD/E: 2-component system
4. Suppression of glutamate metab. (counterion to K+)
5. accumulation/intransport of compatible solutes
6. De novo synthesis of compatible solutes

Fig. 1. Osmoregulatory systems of *E. coli.* Aquaporin AqpZ mediates transmembrane water flux. K^+ transporters TrkA(G/H)/SapD and KdpFABC mediate K^+ accumulation in response to high osmotic pressure. KdpD (a membrane-integral sensor kinase) and KdpE (a cytoplasmic response regulator) constitute a two-component regulatory system that controls *kdpFABC* transcription in response to K^+ supply and osmotic stress. Suppression of glutamate catabolism leads to its accumulation as K^+ counterion. Transporters ProP, ProU, BetT, and BetU mediate organic osmolyte accumulation at high osmotic pressure. ProU is an ortholog of OpuA from *L. lactis*. BetT and BetU are orthologs of BetP from *C. glutamicum*. Enzymes BetA and BetB mediate glycine betaine synthesis from choline, and enzymes OtsA and OtsB mediate trehalose synthesis from glucose at high osmotic pressure. Mechanosensitive channels MscL and MscS mediate solute efflux in response to decreasing osmotic pressure.

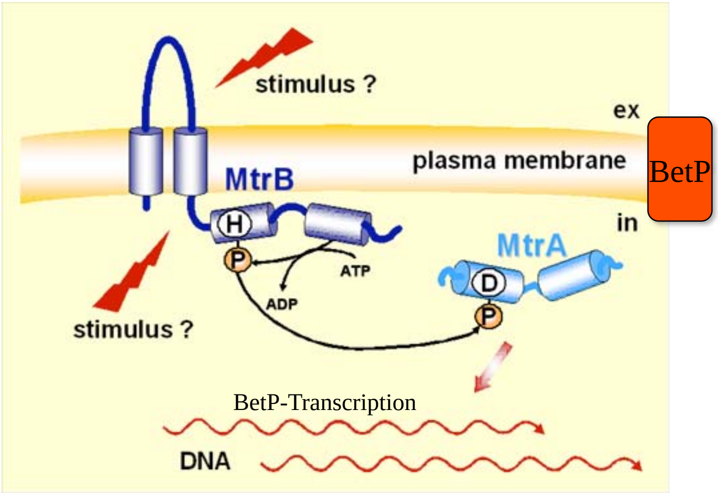
Zweikomponentensystem:
Die einfachste Signaltransduktionskette in Bakterien



SD: Sensor Domäne, PTD: Phosphotransferdomäne,
ABD: ATP-Bindedomäne, H: Histidin
PAD: Phosphoakzeptordomäne; RD:
Reaktionsdomäne, D: Aspartat

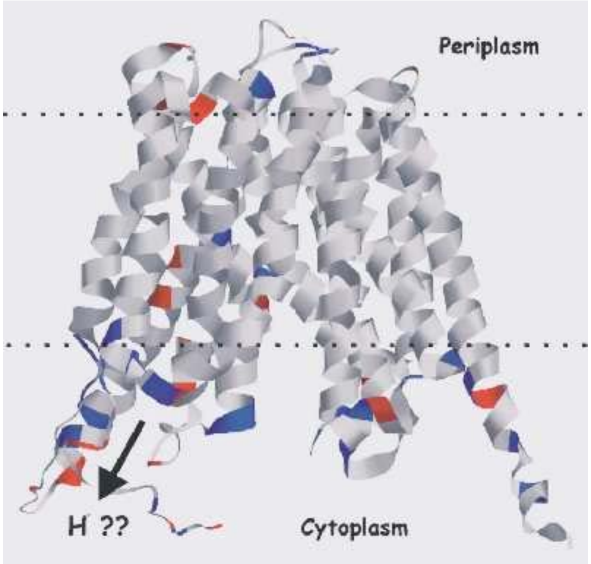
Histidinkinase – Response Regulator

Osmo sensoring:Signal transduction via two-component-systems:
also in other bacteria (Corynebacterium glutamicum)

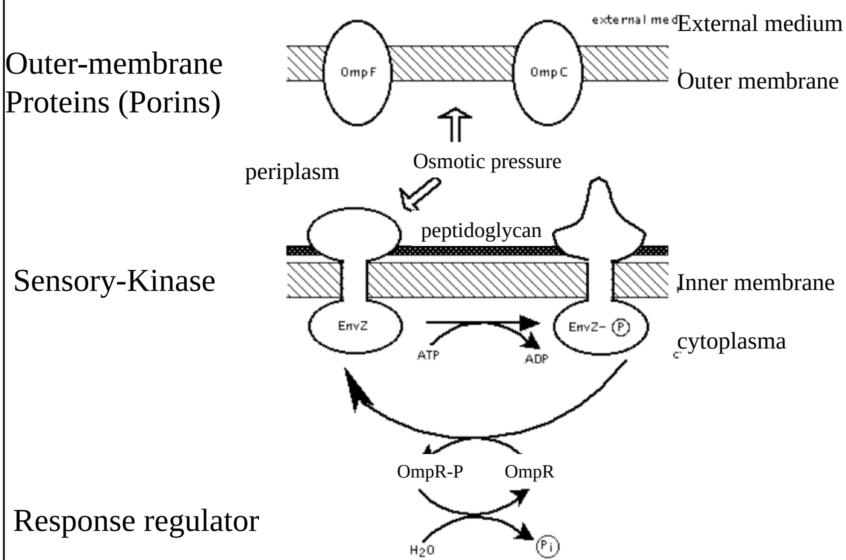


Two systems respond to hyperosmotic stress in C. glutamicum,
the two component system MtrAB and the glycine-betaine uptake system BetP

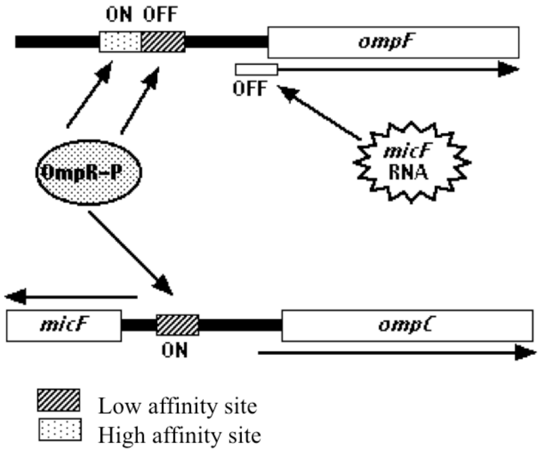
Crystal structure of the sensor protein



Sensoring of osmotic pressure via EnvZ (membrane protein)



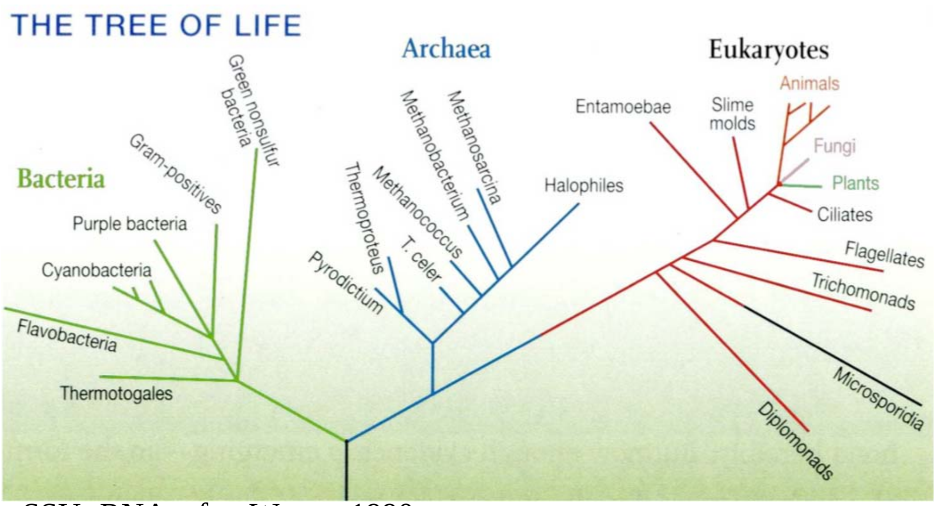
A switch from ompF transcription to ompC transcription at increasing osmotic pressure



micF: small non-coding RNA, blocks ompF translation

At low medium osmolarity a reduced level of OmpR-P, due to the decreased kinase/phosphatase ratio of EnvZ, favors the transcription of ompF. At high medium osmolarity an elevated OmpR-P level, resulting from the increased kinase/ phosphatase ratio of EnvZ, allows the activation of ompC transcription. On the other hand, more OmpR-P molecules bind to the ompF promoter upstream region causing repression of ompF expression.

Die halophilen Archaea



SSU rRNA, after Woese, 1990

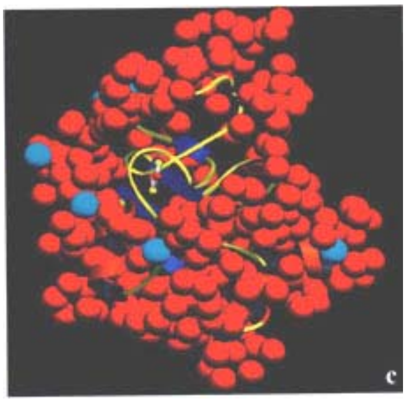
Adaptations of halophiles:

High internal ionic strength: K+

Acidic proteins

Proteins of halophiles

Highly acidic
Negatively charged surface
Makes it more soluble in 1-4M range
More flexible at high salt concentration



Habitate: hypersaline Seen, Salzgewinnungsanlagen

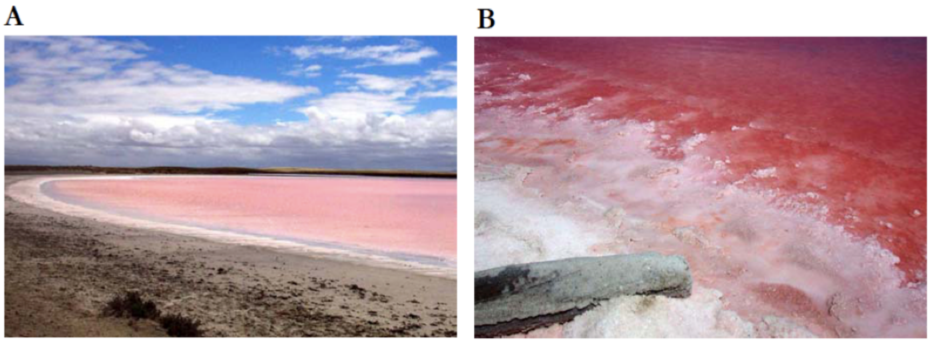


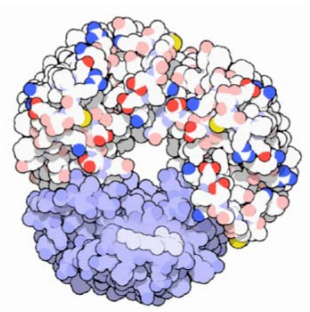
Abb. 2. Hypersaline Habitate. A: Pink Lake Reserve, Südaustralien B: Salzsee auf Kangaroo Island. Die rötliche bzw. rosa Färbung ist auf die Pigmentierung der halophilen Organismen zurückzuführen (s. Text). Fotos: M. Dyall-Smith.

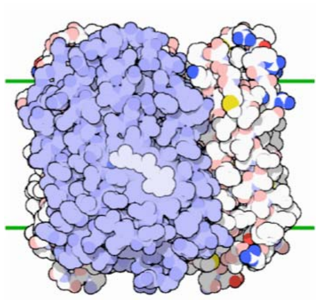
Zellen voller Pigmente: UV-Schutz und Energiegewinnung

Bakteriorhodopsin:

'Photosynthese', Licht-getrieben Protonenpumpe zur ATP-Gewinnung

Protein + Retinal (analog dem Opsin)

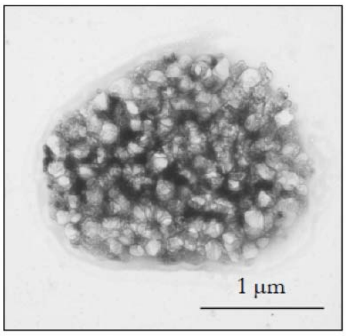




'Notstrom-aggregat'

Haloarchaea sind meist obligat aerob

Bildung von Gasvesikeln als ‘Schwimmkörperchen’ um in der Sauerstoff- und Licht-reichen oberen Wasserzone zu bleiben



Das Gasvesikel: eine einzigartige mikrobielle Struktur

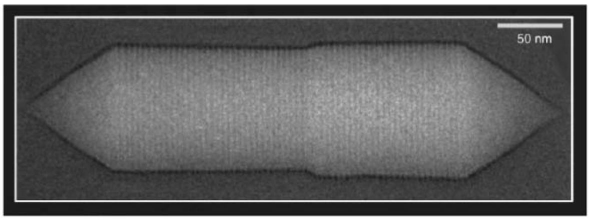
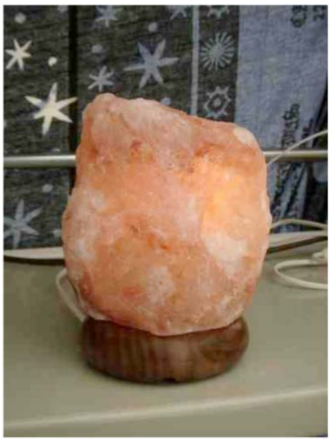
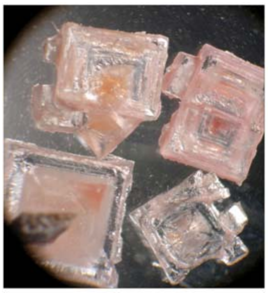


Abb. 4. Elektronenmikroskopische Aufnahme eines Gasvesikels von *Hbt. salinarum* (Offner *et al.*, 1998, verändert). Ketten von GvpA bilden die senkrecht zur Zylinderlängsachse angeordneten 5 nm breiten Rippen.

‘Wiederbelebung’ von Haloarchaea aus Salzkristallen





culture storage crystals

- Kultivierungsberichte aus Millionen Jahre altem Salzstein
- Kandidaten für Mars-Lebensformen