Life at the Extreme

Working with haloarchaea

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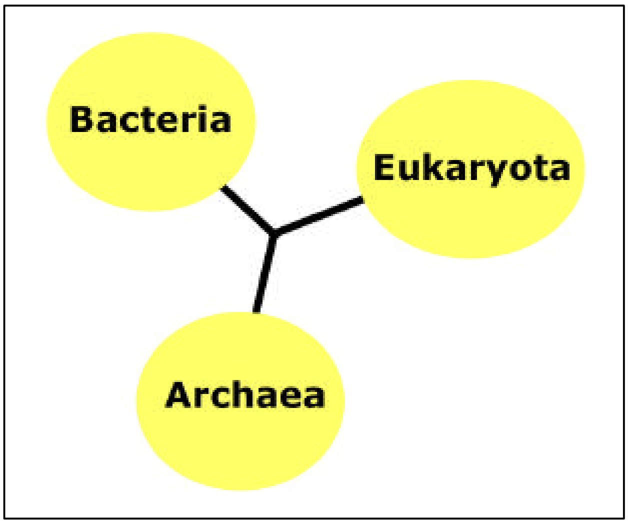
Introduction

It is common to imagine that life can only exist under conditions we are familiar with. The prospect of finding organisms not only surviving, but thriving in hostile environments seems unlikely when considered through our human paradigm. If our environment becomes too hot or cold, too dry or wet, or too contaminated with chemicals such as acids, alkalis, hydrocarbons or salts, we soon become uncomfortable and will eventually succumb unless "normal" conditions can be restored. However, environments that would be fatal to humans provide a haven for many microorganisms that have become known as "extremophiles".

Types of Extremophiles

Extremophiles are found amongst the eukaryotes and bacteria, but the largest membership is represented by the archaea.



Archaea ("ancient ones") were once classified with bacteria, but are now considered to be a separate and distinct domain, although, like bacteria, they are regarded as prokaryotes.

Perhaps because of the early lack of distinction in nomenclature, many microbial extremophiles that are described as bacteria are, in fact, archaea.

Since the first microbial extremophiles were discovered in the 1970s, many new examples have been found in all kinds of environments that had not been contemplated in earlier times.

It seems no extreme is severe enough to exclude the possibility of finding microbial life:

- Low pH (acidic)
- High pH (alkaline)
- Low temperatures
- High temperatures
- Dry conditions
- Deep underground
- Frozen regions
- Saturated salt environments
- High levels of radiation
- High pressures

In many cases, extremophiles can live in combinations of extreme conditions. These are called “polyextremophiles”.

Origins of Life

Many extremophiles can trace their origins to earth’s earliest life forms. The first inhabitants on the earth *had* to be able to survive in extreme conditions. The high temperatures and lack of oxygen could never have supported life as we know it today.

The discovery of extremophiles has led to renewed interest in the field of astrobiology. The harsh environments of earth’s planetary neighbours could harbour extremophiles. There is even speculation that life on earth could have arisen from extremophiles that traveled on an asteroid from elsewhere in the universe.

A Closer Look at Halophiles

Sea water (approximately 3.5% w/v salt) sustains an abundance of microbial life forms, the vast majority of which remains unknown. However, at higher salt concentrations, only specialised halophiles are able to survive. Halophiles live at salt concentrations of 12% to saturation (approximately 35%), and most prefer the range 20-25%.

Many halophilic archaea are polyextremophiles that can also tolerate exposure to solar UV radiation, high diurnal temperature fluctuations, and in some cases, low or high pH environments. Haloarchaea have also been isolated from antarctic lakes that remain liquid below 0^{o}C due to hypersalinity. Even ancient subterranean salt deposits have yielded evidence of viable haloarchaea.

Taxonomy

Class:	Halomebacteria
Order:	Halobacteriales
Family:	Halobacteriaceae

Members of this class are often described in general terms as “haloarchaea”, and sometimes (but less correctly) as “halobacteria”.

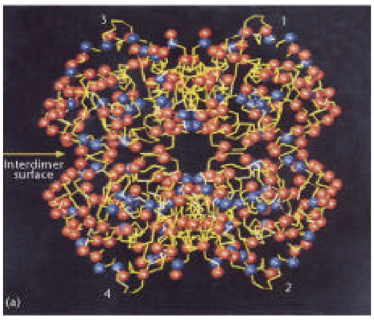
Unique Features of Halophiles

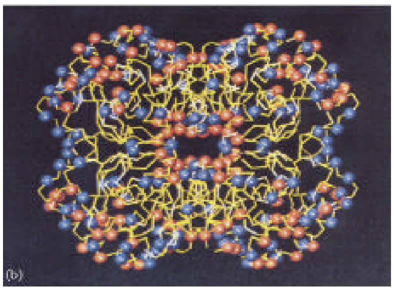
Osmotic Balance

Most cells cannot survive in concentrated salt solutions, but halophiles are able to accumulate high solute concentrations in the cell cytoplasm. When an osmotic balance with their surroundings is achieved, cell volume is maintained. Some halophiles accumulate amino acids, sugars and polyols as balancing solutes, but most haloarchaea use KCl.

Protein Structure

The proteins of haloarchaea tend to contain an excess of acidic amino acids. This is thought to be important for their solvation and activity at high salt concentrations. The image to the right depicts the structure of an enzyme (malate dehydrogenase) from a halophile. Red spheres represent acidic side chains, and blue spheres represent basic side chains.





The large number of acidic residues on the exterior of the halophilic protein is readily apparent when compared to the image on the left which shows a similar non-halophilic enzyme, lactate dehydrogenase.

(images from reference 3)

Pigmentation

The hot dry regions where haloarchaea abound are exposed to high levels of solar radiation, so it is not surprising that they contain pigments that are useful in photochemical processes.

Purple Membrane

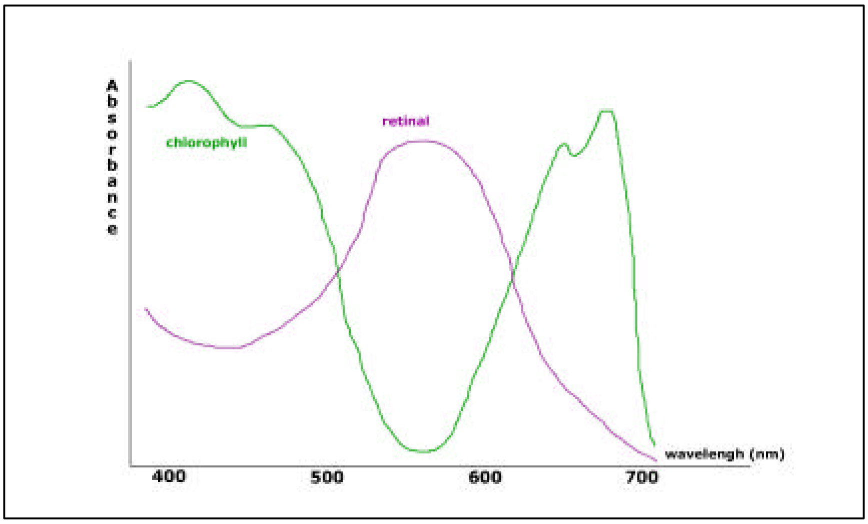
In low oxygen and high light conditions, some haloarchaea express bacteriorhodopsin, a 1:1 complex of bacterioopsin (a protein) and retinal (a light absorbing chromophore), which builds up on the cell surface to form a “purple membrane”. This acts as a solar powered pump to transport protons from the interior to the exterior of the cell. The resulting electrical potential across the cell wall drives ATP synthesis that supplements the normal heterotrophic pathways.

Other Protein-Retinal Complexes

Halorhodopsin is another protein-retinal complex found in some haloarchaea. Its function is to act as a solar powered chloride pump to regulate electrolyte concentration in the cell. There are also sensory rhodopsins that control phototaxis so as to optimize the photochemical processes. This involves moving towards beneficial (green) light, and away from harmful shorter wavelength blue and UV light.

Retinal Pigments and Evolution

Rhodopsins are also present in many “true” bacteria and also in eukaryotes (consider human vision), so these pigments may have evolved from some common ancestor that predates the divergence of archaea, bacteria and eukaryotes. Another possibility is that lateral gene transfers might have occurred after divergence. Either way, an interesting hypothesis suggests that chlorophyll-based photochemical processes might have evolved to take advantage of light that was not being absorbed by the established retinal-based processes.



Chlorophyll-based metabolic pathways went on to supplant the reliance on retinal for growth, leading to the earth's atmosphere becoming oxygenated and allowing the development of life as we know it today.

Carotenoids

The characteristic red colour of many haloarchaea is mainly due to the presence of carotenoid pigments. Their function is to act as a sunscreen and to assist in the repair of cell damage caused by UV exposure. One particular carotenoid pigment, ß-carotene, also acts as a source of retinal when it is cleaved by oxidation.

Motility

Living in concentrated salt solutions means experiencing gradients of all kinds. For example:

- Salt concentration can vary with depth due to surface evaporation, or an in-flow of fresh water, or as a result of gravity.
- Temperature can change with depth due to insulation and light reflection at the surface.
- Oxygen concentration is reduced as salt concentration rises.

To cope with these environmental gradients, many haloarchaea use flagella to move as conditions change. Some also have gas filled vesicles that confer buoyancy. Floating to the surface increases access to oxygen, or, in low oxygen situations, provides light exposure to power the purple membrane ATP synthesis mechanism.

Haloarchaea and Biotechnology

Since their relatively recent discovery, there has been huge growth in the number of haloarchaea that have been isolated and characterized. There is also a lot of interest in the haloviruses, which are thought to outnumber the haloarchaea by a factor of 10.

Besides shedding light on genetic and evolutionary questions, there are many potential applications for haloarchaea in industry. Unlike other extremophiles that can be difficult to isolate and culture, halophiles grow readily in manageable conditions.

(Image from web site reference 1)

Research is aimed at extracting enzymes that are capable of functioning in conditions that would denature similar enzymes from conventional sources. Halophiles have also been suggested for environmental remediation of polluted land and waters.

Why Study Haloarchaea?

Knowledge of extremophiles helps students understand many fundamental biological concepts. However, most of these organisms cannot be studied in a practical way. The haloarchaea are an exception because they are not difficult to culture and are safe to work with. They can also provide an introduction to microbiological techniques with none of the risks that come with conventional microorganisms.

Safety Aspects

We recommend using standard microbiological methods when working with haloarchaea, but they are completely harmless and pose no hazard whatsoever to humans. They can be cleaned up by washing with tap water, and high-salt culture media are incapable of growing non-halophilic microorganisms. This makes them ideal for students to learn the basic techniques of microbiology.

Details of Haloarchaea Available from Southern Biological

Through the courtesy of Dr M Dyall-Smith, formerly of the Department of Microbiology at the University of Melbourne, we will shortly be able to offer four haloarchaea for study by Australian students.

Halorubrum coriense (_Hrr. coriense_)

Haloferax volcanii (_Hfx. volcanii_)

Haloarcula hispanica (_Har. hispanica_)

Halobacterium sp. strain NRC-1 (NRC-1)

These will be available as broths that can be streaked or spread as a lawn on high-salt agar plates.

DNA Extraction

Haloarchaeal cells can be lysed (ruptured) by immersion in water, thus allowing DNA to be extracted and collected as follows:

1. Grow a lawn of a suitable haloarchaea on a high salt agar plate.
2. Add 5mL of distilled water to the lawn and gently swirl. Be careful not to spill the contents.
3. Allow to stand (with occasional swirls) for 10-15 minutes.
4. Add 3.5mL of chilled 95% ethanol to a small test tube.
5. Carefully pour the lysate from the agar plate through a small plastic funnel into the test tube containing the ethanol. Pour at an angle to minimise agitation.
6. Allow to stand for a few minutes then use a spooling stick to wind the white precipitate that forms at the interface of the liquids in the tube.

References

An internet search will turn up hundreds of references to extremophiles and haloarchaea. The following were used as source material for this workshop:

1. The Halohandbook, Protocols for Haloarchaeal Genetics. Compiled and edited by Dr M Dyall-Smith, University of Melbourne. Version 6.01, July 2006. Available from www.haloarchaea.com.
2. S DasSarma, Extreme halophiles are models for astrobiology, *Microbe*, vol 1, no 3, pp120-126, 2006.
3. S DasSarma and P Arora, Halophiles, in *Encyclopedia of Life Sciences*, Nature Publishing Group, 2001.
4. S DasSarma, Extreme Microbes, *American Scientist*, vol 95, May-June, pp224-231, 2007.
5. S McCready, J A Müller, I Boubriak, B R Berquist, W L Ng, S DasSarma, UV irradiation induces homologous recombination genes in the model archaeon, *Halobacterium sp. NRC-1*, *Saline Systems*, vol 1, no 3, 2005. Available from www.salinesystems.org/content/1/1/3.

Useful Web Sites

1. www.haloarchaea.com. The home of the Haloarchaeal Genetics Laboratory in the Department Microbiology and Immunology at the University of Melbourne.
2. http://halo.umbi.umd.edu/~haloed/Introduction.htm. The home of the HaloEd Project, an education initiative coordinated by Priya DasSarma of the University of Maryland.
3. www.cheethamsalt.com.au. Useful information about salt production in Australia.
4. www.microbeworld.org. Site of the American Society for Microbiology with lots of information suitable for students, including a comprehensive list of podcasts and vodcasts.