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Introduction to Life in an Extreme Environment

TEACHER'S MANUAL WITH STUDENT GUIDE

by Priya and Shiladitya DasSarma

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Introduction to Life in an Extreme Environment

Teacher's Manual

Photocopy Masters

About the authors

Priya and Shil DasSarma have studied *Halobacterium* sp. NRC-1 for over 20 years. They are enthusiastic about making these safe, convenient microbes accessible to educators and students at all levels. Visit their website at http://www.carolina.com/life_science/halobacteria for more information, ideas, and contact information.

On the cover: Solar salt-production facility showing colorful halophile blooms arising at various salinities. Pastel and watercolor by Satyajit DasSarma, age 7.

Introduction to Life in an Extreme Environment

Overview

In this activity, students grow microbial cultures of the extreme halophile *Halobacterium* sp. NRC-1 using liquid media. Salt crystals containing live, entrapped *Halobacterium* sp. NRC-1 cells are used for inoculation. When the cultures grow, students can observe the microbes under a microscope. The activity introduces students to an extremophile and an archaeon, and is an ideal starting point for independent, inquiry-based projects.

This Teacher's Manual contains an overview of extremophiles, Archaea, and *Halobacterium* sp. NRC-1, preparation information, step-by-step procedures, lists of additional sources of information, a glossary, a reproducible blackline master of background information for students, and a set of student worksheets. Materials in this kit are sufficient for 32 students working individually.

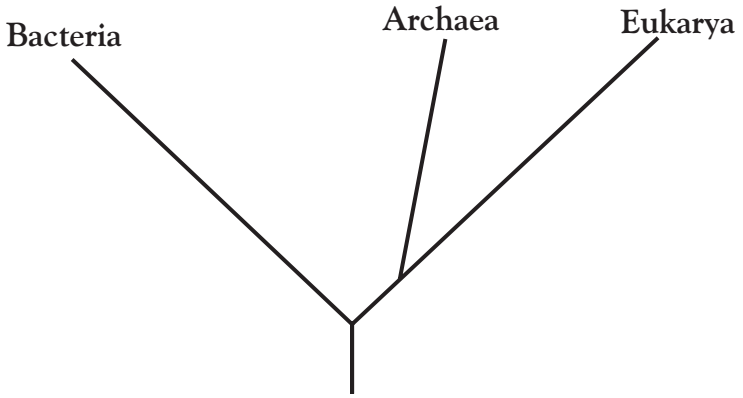
Background

"Extreme" is a relative term based on the human perspective. A variety of life-forms on earth tolerate or even require environments which we would consider extreme. Collectively, these organisms are known as **extremophiles** ("lovers of extremes"). Environmental extremes and their inhabitants include the following:

1. Extremes of temperature. Heat-loving **thermophiles** thrive at temperatures above 80°C, in places such as geothermal springs in Yellowstone National Park in Wyoming. Cold-tolerant **psychrophiles** grow at temperatures of –15°C or below (and up to 20°C) and are commonly found in Antarctica, for example.
2. Extremes of pressure. **Barophiles** thrive at high hydrostatic pressures in the deep ocean, in places such as the Mariana Trench north of New Guinea.
3. Extremes of pH. **Acidophiles** thrive at pH below 3.0, in mineral sulfide mine waters among other places. **Alkaliphiles**, on the other hand, prefer environments at pH greater than 10.0, such as soda lakes.
4. Environments containing toxins at a level that normally destroys life. **Toxitolerant** organisms do well in environments with high levels of materials that are harmful to most other organisms, e.g., areas contaminated with nuclear materials or toxic chemicals.
5. Extremely dry environments. **Xerotolerant** organisms can survive desiccation.
6. Environments lacking oxygen. **Anaerobes** require oxygen-free environments to survive.
7. Extremes of saltiness. Moderate **halophiles** grow optimally at salt concentrations of 0.85 M to 3.4 M (5%–20%, weight/volume) NaCl. Extreme halophiles grow optimally at 3.4 M to 5.1 M (20%–30%) NaCl, such as found in solar salterns for sea salt production. In contrast, nonhalophiles grow optimally at less than 0.2 M NaCl.

Many extremophiles are members of a recently identified third branch or "domain" of life, the **Archaea** ("ancient" in Greek) and are distinct from the other two domains, **Eukarya** (or Eukaryota) and **Bacteria**. **Eukaryotes**, members of the domain Eukarya, have cells with nuclear envelopes. Organisms in the other two domains, Bacteria and Archaea, are **prokaryotes**, and are partially characterized as having cells without nuclear envelopes. Eukaryotic cells may be 10–100 times larger than prokaryotic cells (including both Bacteria and Archaea), which typically range from 1–3 μm in length and 0.1–1.5 μm in diameter. The archaea were identified as being evolutionarily distinct from bacteria in 1977 by Carl Woese, et al. (reviewed in Woese 1987; Woese 1981), based on their unique 16S rRNA, a ribosomal molecule important for building protein in all cells. *Halobacterium* was named before the discovery that Archaea is phylogenetically distinct from Bacteria, at a time when the term "bacterium" was used for most microscopic organisms.

Research indicates that archaea may have been among the original organisms on earth. Despite morphological similarities with members of Bacteria, some of their molecular characteristics (such as their information transfer systems—DNA replication, transcription, and translation systems) more closely resemble Eukarya than Bacteria. Eukaryotes and bacteria have cell membranes composed mainly of glycerol-ester lipids, while the cells of organisms in the domain Archaea contain glycerol-ether lipids. Unlike many bacteria, members of the domain Archaea lack a peptidoglycan cell wall, but instead contain an S-layer also present in some bacteria. The S-layer is a crystalline protein coating on the microbe's surface (S = surface) that acts as a cell wall in place of the peptidoglycan cell wall used by most bacteria. This makes them more stable. Although some organisms in the domain Archaea are thermophiles, many, especially halophilic archaea, are **mesophiles**, preferring temperature conditions similar to what humans find comfortable. All known archaea are believed to be harmless to higher organisms and none has been known to cause disease in humans. In fact, one type is frequently present in human intestines.



Members of the domain Archaea are divided into three types on the basis of their environmental requirements:

1. **Halophiles** thrive in highly saline environments.
2. **Methanogens** produce methane and thrive in anaerobic conditions.
3. **Thermophiles** grow at high temperatures.

Halophiles

The extreme halophiles, such as the rod-shaped *Halobacterium* sp. NRC-1, are common in extremely salty environments such as the Great Salt Lake in Utah (DasSarma and Arora 2002; Ng, et al. 2000; DasSarma 2004). These environments are harsh in a number of ways. The microbes are frequently exposed to elevated temperature, solar radiation, desiccation and, most characteristically, extremes of salinity. Their growth requirement typically is for at least 3 M NaCl. This compares with concentrations of about 0.6 M NaCl for seawater and 5.3 M NaCl in a saturated solution. At such high salinity, most organisms are osmotically stressed—cells lose water and membrane potential, their proteins aggregate and denature, and they perish.

Halophiles can also tolerate great fluctuation in their environment. For example, in the Great Salt Lake, where *Halobacterium* is the dominant microorganism, the temperature of the surface varies, ranging from –6°C to about 27°C. Many halophiles, e.g., *Halobacterium* sp. NRC-1, also tolerate the fluctuations in salinity that occur when rainfall and river runoff dilute the saline levels in the environment and when evaporation creates a higher concentration of salt in the environment, often to the point of saturation.

Remarkably, extreme halophiles can even survive lengthy periods of time inside salt crystals in brine inclusions. Unlike fungal and bacterial cells that form endospores to survive extremes of desiccation and salinity (Echigo et al. 2005), *Halobacterium* forms no spores. The mechanism of salt tolerance, which is likely related to its desiccation and radiation tolerance, is the subject of much current research (see e.g., Kennedy, et al. 2001; McCready, et al. 2005).

Another question of current interest is why, in the absence of its ability to form spores, *Halobacterium* has been found dispersed all over the world. Many other microorganisms are dispersed throughout the world via their spores.

Solar salt, produced by the evaporation of seawater, and salt from salt mines, both were used in food preservation and leather production. Early microbiologists discovered halophiles growing in such products. When high salinity levels combined with enough moisture, *Halobacterium* species emerged from dissolved salt crystals and thrived on the organic matter, causing deterioration of the food or leather quality. The *Halobacterium* was visible as pink blotches on pickled herring, and as red "burns" on animal hides. Such spoilage could have been avoided if the salt were heat sterilized. However, heat sterilization is not generally used in the production of table salt.

Recently, additional interest in halophiles has come from the search for extraterrestrial life. Do life-forms exist, or did they ever exist, on other planets? If so, what would they look like? Where would they live and how would they survive? Halophiles seem to be well adapted to conditions found on Mars (Landis 2001; DasSarma, 2006). Halophiles can withstand ultraviolet and even gamma radiation (a very high energy form of radiation in space), as well as long periods of desiccation. In addition, brine is postulated to have existed on the Martian surface or subsurface and on Jupiter's moon, Europa.

Halobacterium in the Classroom

In this exercise, students grow liquid cultures using salt crystals with entrapped brine inclusions that contain live *Halobacterium* sp. NRC-1 cells. Once the cultures are grown, students observe the flagellated, motile cells under a microscope. If they have access to a phase-contrast microscope, they will be able to observe the phase-bright gas vesicle inclusions. These microbial organelles enable the *Halobacterium* cells to float near the surface of brine where the light and oxygen are more abundant. These gas vesicles also affect the appearance of the cultures. Cultures without gas vesicles are translucent and red. When gas vesicles are present they refract light, making the red coloration appear pink and imparting opacity to the culture. The coloration is due to the presence of two pigments in cells, carotenoids and bacteriorhodopsin. Carotenoids are light-absorbing molecules that protect the cells against damaging radiation. Bacteriorhodopsin is a pigment in the cell membrane used by the cell to obtain energy from light in an alternative fashion to that of photosynthesis. This pigment is related to the human visual pigment, rhodopsin.

Halobacterium sp. NRC-1 is ideal for use in the science classroom. These halophiles grow at 20°C–45°C, with an optimum of 42°C, and require neither anaerobic hoods nor high temperatures. The American Type Culture Collection (ATCC) classifies the microbe at Biosafety Level 1 (BSL-1). Materials classified at BSL-1 "are not known to cause disease in healthy adult humans." Additionally, and perhaps even more critically, the media in which students will grow the microbes is so **hypersaline** (highly salty) that virtually no other organisms can survive in it. This allows students to manipulate *Halobacterium* sp. NRC-1 without the fear of contaminating the media, as most microbes cannot grow in the media. Nevertheless, it is prudent to always follow good microbiological safety procedures when handling any microbe, including *Halobacterium* sp. NRC-1.

Another advantage of working with *Halobacterium* sp. NRC-1 is that the entire genome of this microbe has been sequenced. It was the very first genome completed with funding from the U.S. National Science Foundation, and was among the first genomes ever to be sequenced (Ng, et al. 2000; DasSarma 2004). Students can explore the genome sequence and current research being done on *Halobacterium* sp. NRC-1 at the HaloEd Project's Web site: http://halo.umbi.umd.edu/~haloed/.

Since the sequencing of its genome, *Halobacterium* sp. NRC-1 (Ng, et al. 2000; DasSarma 2004) has gained popularity among researchers. Today, laboratories all over the world are exploring the many facets of this fascinating, versatile microbe. For advanced research and laboratory work, you can use the laboratory manual, *Archaea: A Laboratory Manual—Halophiles* (DasSarma, et al. 1995). Students can develop their own research projects to investigate growth requirements of *Halobacterium* sp. NRC-1. They can try growing the cells at various salinities, various temperatures, or both. Please note that as students decrease the salinity they start to run the risk of growing other microorganisms. Good microbiology technique must be used. Students can also, with adequate protection, expose the cultures to sunlight or ultraviolet light from a germicidal lamp. Additionally, students can dry their cultures down to crystals and, by inoculating crystals obtained over several intervals of

time, determine how long the cells remain viable. The DasSarma Team would be happy to discuss projects with students and educators and can be reached by email: dassarma@comcast.net.

Materials

The materials in this kit are sufficient for 32 liquid cultures. The materials are supplied for use with the educational exercises in this kit only. Carolina Biological Supply Company disclaims all responsibility for other uses of these materials.

Included in the kit

96 salt crystals containing entrapped *Halobacterium* sp. NRC-1 cells
1 bottle liquid media, 200 mL
5-mL bulb pipet or graduated cylinder
32 sterile test tubes with caps
35 dropping pipets
microscope slides
coverslips
Teacher's Manual with photocopy masters

Needed, but not supplied

wax markers or lab pens
test tube racks
sterile forceps
microscopes
*shaker incubator, 37°C or 42°C
hand lenses (optional)

*If an incubator is not available, the tubes can be placed in a test tube rack and shaken manually each day. However, this will significantly lengthen the time required to grow a visually stimulating culture.

Teacher Preparation

You will need to

- read the entire booklet to familiarize yourself with the information and instructions given to the students.
- photocopy sets of student instructions (Student Background and Student Worksheets) for individuals or teams.
- decide how and when to share the Student Background information with the students.

Procedure

At an appropriate point before the activity, have students complete worksheet sections I and II. During the activity, make sure that your students always follow good microbiological safety procedures when handling the cultures. *Halobacterium* sp. NRC-1 is safe to use in the classroom, however it is important that your students learn good technique.

1. Distribute one capped test tube to each student or group of students.
2. Use the bulb pipet or graduated cylinder to add 5 mL of liquid media to all of the students' test tubes.
3. Give students two or three salt crystals to inoculate each tube of media. Have students hypothesize whether they will be able to grow cells from the raw salt crystals. Have them complete worksheet Section III, recording their observations and hypotheses. You may allow them to use microscopes or hand lenses to observe the crystals in greater detail.
4. Have students use sterile forceps to drop the salt crystals into the test tubes. Note that the worksheet for Section III includes step-by-step instructions for this procedure, which your students should follow.
5. As a negative control, reserve one test tube of media without salt crystals. **Note:** If you have fewer than 32 students, use one of the extra test tubes; otherwise, select one student or group to serve as the negative control. This offers a learning opportunity as students compare their media and cultures to the negative control tube.
6. Place the test tubes in an incubator. If you do not have access to a shaking incubator, shake the cultures daily to aerate them. If the tubes are incubated at 42°C, a pink culture should appear in approximately 7 days. At 37°C, the culture may take 1–2 weeks to become visible. If the tubes are incubated at room temperature, the culture may take two weeks or longer to become visible.

Once these highly motile, flagellated cells are visible as a culture in the students' test tubes, they can be observed under a microscope. When the cultures are ready, proceed with the next step.

7. Have students complete worksheet Section IV, recording their observations and analysis.
8. Distribute dropping pipets, microscope slides, and coverslips. Have students use a dropping pipet to place a drop of their culture onto a slide and then gently lay a coverslip over the drop. Have them observe the slides under a microscope. No staining is required. If students use phase-contrast microscopes, gas vesicles will be visible as refractive bodies in the cell. **Note:** Caution students to place the coverslips gently—if pressure is exerted on the microbes, the gas vesicles within will collapse. However, note that even if the vesicles collapse the microbes themselves will remain viable and still motile (using their flagella).
9. On a separate sheet of paper, have students draw the halophilic microbes that they observe under the microscope.

Disposal of Cultures

Because these microbes are so extremely halophilic, they require 3 M salt concentrations or greater in order to maintain cell membrane integrity, and they lyse at salt concentrations below 2.5 M. However, we suggest that you follow standard good microbiology laboratory practice and autoclave the labware or soak it in a 10% bleach solution before disposal.

Answers to Selected Worksheet Questions

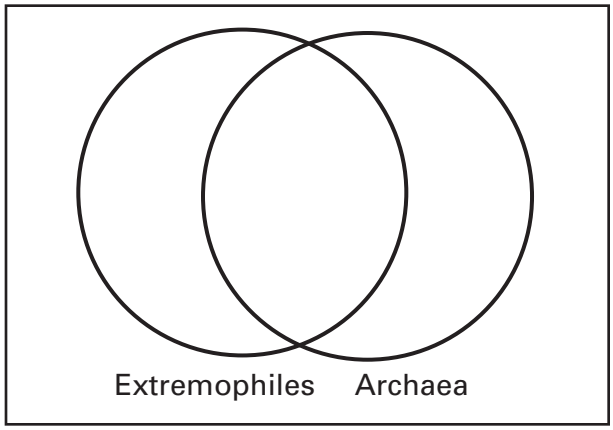
Section I

The letters from the word bank should appear in the paragraph in the following order:

extremophiles, thermophiles, psychrophiles, barophiles, acidophiles, alkaliphiles, toxitolerant, xerotolerant, anaerobes, halophiles.

Section II

In the space below, draw a Venn diagram that shows the relationship between extremophiles and Archaea.



Section III

1. Answers will vary depending upon the exact nature of the crystals and the students' own perceptions. The crystals will vary in shape and will range in color from yellow to pink.
2. At this point the media should be clear and amber.
3. Answers will vary.
4. A negative control is an experimental variation set up to demonstrate that the effect that you see in response to the variable you are testing is due to the variable and not some other unknown factor.

Section IV

2. At this point the media should appear opaque and pink.
3. Answers will vary depending upon what students focus on.
6. Allowing the culture to dry out to form crystals is the simplest way to preserve the organisms. Some students may suggest that the culture could be placed out in the sun to speed the process.
7. The fact that halophiles are able to tolerate desiccation and a range of high salt concentrations suggests this approach. The fact that they are UV tolerant suggests that the sun may be used to dry the cultures to create the crystals. The characteristics of being able to tolerate desiccation, UV light, and varying salt concentrations also allows *Halobacterium* to thrive in nature where they might be exposed to sun as well as to varying degrees of moisture and salt.

Glossary

acidophiles organisms which thrive in relatively acidic environments.

alkaliphiles organisms which thrive in alkaline environments.

anaerobes organisms which do not require oxygen to live.

Archaea prokaryotic domain of life composed of extremophilic microorganisms including halophiles, organisms that grow in high-salt environments; methanogens, anaerobes that produce methane; and thermophiles, which thrive in high-temperature environments.

Bacteria most common prokaryotic microorganisms; distinct from Archaea.

barophiles organisms which thrive at high hydrostatic pressures.

Eukarya domain of life whose members possess a nucleus.

eukaryotes organisms possessing a nuclear envelope.

extremophiles organisms which thrive in environmental extremes (e.g., extreme heat, cold, pressure, pH, salinity, or toxicity) or under very dry or anaerobic conditions.

halophiles organisms which thrive in salty environments, at concentrations of 0.85 M NaCl to 3.4 M NaCl. Extreme halophiles thrive at 3.4 M NaCl to 5.1 M NaCl.

hypersaline highly salty.

mesophiles organisms which grow best at 20°C–40°C.

methanogens anaerobic, methane-generating members of the domain Archaea, including some found in the intestines of many mammals including some humans

prokaryotes organisms lacking a nuclear envelope.

psychrophiles cold-loving organisms.

thermophiles organisms which thrive at high temperatures in places such as hot springs and deep-sea vents.

toxitolerant able to withstand environments and/or substances that are deadly to most other living creatures.

xerotolerant able to withstand a dry environment and dehydration.

Further Reading

At the time of this printing, the following Web sites are active. You may wish to perform an independent search for similar sites.

Anonymous. 2000. *First salt-loving bug sequenced*. BBC News Online. 2 October, 2000. http://news.bbc.co.uk/1/hi/sci/tech/953356.stm

Buchanan, Rachael. 2004. *Utah microbes point to Mars*. BBC News Online. 18 May, 2004. http://news.bbc.co.uk/2/hi/science/nature/3725973.stm

DasSarma, S. 2006. Extreme halophiles are models for astrobiology. *Microbe* 1(3):120–26.

DasSarma, S. 2004. Genome sequence of an extremely halophilic archaeon, in *Microbial Genomes*, pp. 383–99. C.M. Fraser, T. Read, and K.E. Nelson (eds.). Humana Press, Inc., Totowa, NJ.

DasSarma, S., et al. (eds). 1995. *Archaea: A Laboratory Manual—Halophiles*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

DasSarma, S., and P. Arora. 2002. "Halophiles," *Encyclopedia of Life Sciences*, Vol. 8, p. 458–66. London: Nature Publishing Group. Also: http://els.wiley.com/els/public/home/default.asp?sessionid=public

Echigo, A., M. Hino, T. Fukushima, T. Mizuki, M. Kamekura, and R. Usami. 2005. Endospores of halophilic bacteria of the family Bacillaceae isolated from non-saline Japanese soil may be transported by Kosa event (Asian dust storm). *Saline Systems* 1:8.

Kennedy, S.P., W.V. Ng, S.L. Salzberg, L. Hood, and S. DasSarma. 2001. Understanding the adaptation of *Halobacterium* species NRC-1 to its extreme environment through computational analysis of its genome sequence. *Genome Res*. 11:1641–50.

Landis, G.A. 2001. Martian Water: Are There Extant Halobacteria on Mars? *Astrobiology* Vol. 1, No. 2, pp. 161–4.

McCready, S., J.A. Müller, I. Boubriak, B.R. Berquist, W. Ng, and S. DasSarma. 2005. UV irradiation induces homologous recombination genes in the model archaeon, *Halobacterium* sp. NRC-1. *Saline Systems* 1:3.

Mullen, Leslie. 2002. Salt of the Early Earth. *Astrobiology Magazine* [Online]. June 11, 2002. http://www.astrobio.net/news/article223.html

Ng, W.V., S.P. Kennedy, G.G. Mahairas, B. Berquist, M. Pan, H.D. Shukla, S.R. Lasky, N.S. Baliga, V. Thorsson, J. Sbrogna, S. Swartzell, D. Weir, J. Hall, T.A. Dahl, R. Welti, Y.A. Goo, B. Leithauser, K. Keller, R. Cruz, M.J. Danson, D.W. Hough, D.G. Maddocks, P.E. Jablonski, M.P. Krebs, C.M. Angevine, H. Dale, T.A. Isenbarger, R.F. Peck, M. Pohlschroder, J.L. Spudich, K.H. Jung, M. Alam, T. Freitas, S. Hou, C.J. Daniels, P.P. Dennis, A.D. Omer, H. Ebhardt, T.M. Lowe, P. Liang, M. Riley, L. Hood, S. DasSarma. 2000. Genome sequence of *Halobacterium* species NRC-1. *Proc. Natl. Acad. Sci.* USA 97:12176–81.

Rothschild, L.J., and R.L. Mancinelli. 2001. Life in extreme environments. *Nature* 409:1092–101.

Wayne's Word. California's Pink Salt Lakes: A Strange Phenomenon Caused by Red Halobacteria. http://waynesword.palomar.edu/plsept98.htm

Woese, C.R. 1981. Archaebacteria. *Scientific American* (Jun):98–122.

Woese, C.R. 1987. Bacterial evolution. *Microbiol Rev*. 51:221–71.

Additional Resources

The HaloEd Project: A Web site dedicated to biotechnology education. http://zdna2.umbi.umd.edu/~haloed/

Carolina Biological Supply Company [Online]. *Halobacterium* sp. NRC-1: The NEW Model Microbe. http://www.carolina.com/life_science/halobacteria/index.asp

Name _____

Date _____

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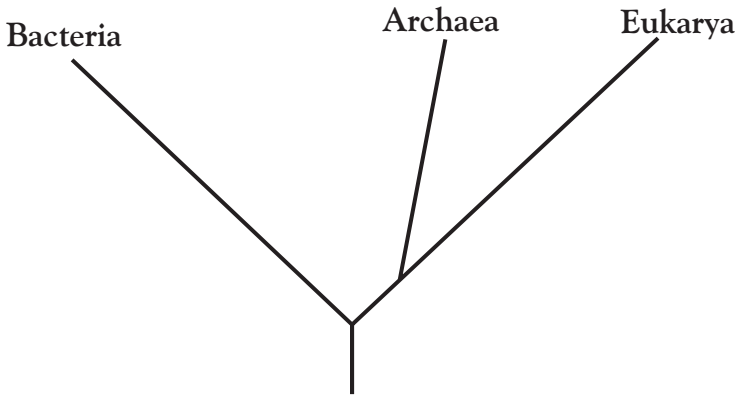

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Halophiles

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Recently, additional interest in halophiles has come from the search for extraterrestrial life. Do life-forms exist, or did they ever exist, on other planets? If so, what would they look like? Where would they live and how would they survive? Halophiles seem to be well adapted to conditions found on Mars (Landis 2001; DasSarma, 2006). Halophiles can withstand ultraviolet and even gamma radiation (a very high energy form of radiation in space), as well as long periods of desiccation. In addition, brine is postulated to have existed on the Martian surface or subsurface and on Jupiter's moon, Europa.

Halobacterium in the Classroom

In this exercise, you will grow liquid cultures using salt crystals with entrapped brine inclusions that contain live *Halobacterium* sp. NRC-1 cells. Once the cultures are grown, you will observe the cells in motion under a microscope. The microbes contain flagella which allow them to "swim," and gas vesicles which allow them to float. If you have access to a phase-contrast microscope, you should be able to see the gas vesicles. However, the flagella may only be seen with an electron microscope. Gas vesicles, which are uncommon microbial organelles, enable the *Halobacterium* cells to float near the surface of brine where light and oxygen are more abundant. Gas vesicles also affect the appearance of cultures. Cultures without gas vesicles are translucent and red. When gas vesicles are present, they refract (bend) light, making the red coloration appear pink and imparting opacity (a milky appearance) to the culture. The coloration is due to the presence of two pigments in cells, carotenoids and bacteriorhodopsin. Carotenoids are light-absorbing molecules that protect the cells against damaging radiation. Bacteriorhodopsin is a pigment in the cell membrane and is used by the cell to obtain energy from light in an alternative fashion to that of photosynthesis. This pigment is related to the human visual pigment, rhodopsin.

Name _____

Date _____

Life in an Extreme Environment

Section I

Complete the sentences below by filling in each blank with the correct word from the word bank.

Word Bank

acidophiles	alkaliphiles	anaerobes	barophiles	extremophiles
halophiles	psychrophiles	thermophiles	toxitolerant	xerotolerant

A variety of life forms on earth thrive in or even require extreme environments. Collectively, these organisms are known as _____. Heat-loving _____ thrive at temperatures above 80°C. Cold-tolerant _____ grow best at temperatures of –15°C or lower and up to 20°C. _____ thrive at high hydrostatic pressures in the deep ocean. _____ thrive at pH below 3.0. _____, on the other hand, prefer environments at pH greater than 10.0. _____ organisms survive in environments, such as nuclear waste dumps, that are deadly to most other organisms. _____ organisms can survive dehydration. _____ do not need oxygen to survive. _____ are salt-loving microbes that thrive at salt concentrations many times that of sea water.

Section II

In the space below, draw a Venn diagram that shows the relationship between extremophiles and Archaea.

Name _____

Date _____

Life in an Extreme Environment

Section III

1. Record today's date. Observe the crystals that you will put into the media in the test tube. Describe the size, shape, color, and texture of the crystals, as well as any other interesting features you observe. You may use a microscope or magnifying glass to look at them.

2. Observe the media in the test tube. Describe the color and consistency of the media, as well as any other interesting characteristics you can see.

3. On the basis of your own ideas and the information you have, write a hypothesis describing what you think will happen to the salt crystals and the media that you place the crystals in after you place the crystals in the media and incubate.

4. What is a negative control and what purpose does it serve in laboratory research?

Procedure

1. On the upper portion of the test tube containing your media, clearly write the date and your initials or group name. The writing should not obscure the media or appear on or under the cap. If you are doing a negative control, label it as such.

2. Obtain two or three salt crystals from your teacher. Handle them as sterilely as possible.

3. Using good sterile technique, remove the cap from your test tube. Use sterile forceps to drop the crystals into the media in your test tube. Place the cap back on the test tube.

4. Incubate the test tube containing the crystals under the conditions prescribed by your teacher.

Name _____

Date _____

Life in an Extreme Environment

Section IV

1. Record today's date. How many days have passed since you placed the salt crystals into the media in the test tube?

2. Observe the media in the test tube again. Describe the color and consistency of the media, and note any changes that you can see. What caused these changes?

3. Compare your test tube results with the results of your classmates.

   a. In what ways are your results similar to theirs?

   b. Are there any differences in your results? If so, describe what is different and give a reason why this might be so.

4. Observe your cells under a microscope. Use a dropping pipet to place a drop of culture onto a slide and **gently** lay a coverslip over the drop. Observe the slides under a microscope. No staining is required. **Note:** Place the coverslip gently—if pressure is exerted on the microbes, the gas vesicles within will collapse.
5. On a separate sheet of paper, draw what you observe under the microscope.
6. If you wanted to preserve the extreme halophiles for use in future experiments, how do you think you could preserve them?

7. What characteristics of halophiles support the preservation approach that you described above? How are these characteristics essential to the survival of extreme halophiles in nature?

Carolina Biological Supply Company
2700 York Road, Burlington, North Carolina 27215
Phone: 800.334.5551 • Fax: 800.222.7112
Technical Support: 800.227.1150 • www.carolina.com
CB163140605