Halobacterium:

One Stop Shopping for Student Labs

KABT Fall Meeting

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***Feel free to contact me with questions, comments, etc.!***

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**Overview to implement *Halobacterium* into Biology Course**

K. Tuel’s plan is to use periodically throughout first semester with Biology I (compiled with help from “lesson plans” on HaloEd Project website). Depth and breadth depends on students and actual class. May or may not be in this order:

1. **Classification**

Domains: Overview of Halobacteria including their biotechnological applications

2. **The Cell**

* + - Morphology, motility, etc.
    - Exponential growth and growth curves
    - Dilutions, Plating, Liquid culture
      * ***Plate cultures now and save for next section!***
    - Antibiotics
    - Osmosis (lyse the cells on microscope slide with just water!! Watch it happen!)
    - Colony morphology
      * ***Observations using plates they made previously.***
    - Complementation (Transformation from Vac- to Vac+)
  1. **DNA** 
     + Genotype and Phenotype
       - ***Observations using plates they made previously.***
     + DNA extraction
       - ***Using the plates they made previously…protocol below.***

1. **Bioinformatics**
   * + Genomics
     + Biotechnology. Patents and practical uses of *Halobacterium.*
     + PCR
       - ***Using own primers & DNA extracted from Halobacterium colony***
     + Gel Electrophoresis
       - ***Preparing and running a gel***
2. **Evolution**

Revisit domains. Conditions of early earth that may aided Archaean Extremophiles survival.

**Possible Open-Ended Activities**

**(check out HaloEd Project for springboard to get you thinking of even more!)**

**Middle School**

* Gas vesicle/pressure relationship.

Increase in pressure causes gas vesicles to pop. Culture appears translucent.

* Wavelengths of light & Halobacterium preference (UV or blue light vs. green light).
* Grow Artemia (Sea Monkeys). They share the same extreme salt environment with *Halobacterium.* Try feeding some *Halobacterium* to the Sea Monkeys! Research why pink flamingos are PINK.
* Chemotaxis? Phototaxis?
* Osmosis experiments

Observe under microscope on slide with cover slip. Wick dH20 across and watch lysis

**High School**

* Bioinformatics applications

Ex: research bacteriorhodopsin and metabolic role. SEE the 3D structure of this protein; examine the structure for a structure-function exploration. View protein-specific phylogenic tree.

* Use the DNA from “Extremely Easy DNA Extraction” for further study (protocol on next pg)!!
* Abiotics experiments (light/radiation, pressure, temperature, water, etc.)
* Methods of microbiology with this safe model microbe! Use this organism to learn sterile technique without worry of contamination or sick students!
* See if you can get it to grow phototrophically by removing Carbon source (without Sodium Citrate) in the media

* *Halobacterium* is supposedly UV-tolerant. Can you expose it to enough UV light to wipe out the repair (photolyase enzyme repairs)?

**DNA Extraction Options**

**DNA Storage/ Protocol after “Extremely Easy DNA Extraction\***

1. After following “Extremely Easy DNA Extraction”, remove the precipitated DNA by suction with sterile plastic pipette or micropipette tip. Try to remove as little alcohol as possible but getting all the DNA.
2. Expel the DNA clump into a new, sterile microcentrifuge tube.
3. Spin at maximum speed for 5 minutes.
4. Extract the supernatant off as completely as possible, then wick the rest of the supernatant off using a twisted corner of Kim Wipe.
5. Allow the DNA to dry completely in the microcentrifuge tube by leaving the cap open on the lab bench for 15 or more minutes.
6. Resuspend the pellet in 100 uL (or more)TE buffer (pH 7.5) OR dH2O by pipetting up and down several times to mix. Vortex.
7. Using the spectrophotometer, determine amount of DNA you have. Dilute with TE buffer OR dH2O until DNA measures between 12-30 ng/mL on the spectrophotometer. My protocol with PCR worked fine reliably even if I had as little as 0.8 ng/mL

\*This DNA can be used for further experimentation. IF the original Petri dish contained ONLY pure culture, this DNA could be used for PCR amplification, etc.

**OR**

**Extraction from 1 colony/ Cell Lysis technique**

1. Label tubes

Label 2 microcentrifuge tubes, “#1” and “#2 extract”. Set the tube “#2 extract” aside for later use.

2. Add d water

Add 500 μL of sterile distilled water to tube “#1”.

3. Swipe a colony

Chose a Halobacterium colony that you wish to extract DNA from. Using a new micropipette with a sterile

tip, pick up your colony from the surface of the agar using a swiping motion (be careful not to stab the agar

with your sterile tip). Make sure not to touch your pipette tip to any other colony or surface.

4. Transfer into tube

Put the pipette tip (containing the colony) into your tube labeled #1. Pipette up and down at least 10 times in

the distilled water to release the cells from the tip. Securely close the cap of your microcentrifuge tube.

5. Sit for 5 minutes

Allow your microcentrifuge tube to sit undisturbed on the lab bench for 5 minutes. You now have “cell

lysate” in your tube. If you let the cell lysate stand too long, your DNA may be degraded by cellular

enzymes called DNases.

6. Mix

Vortex (or tip side to side) until mixed.

7. Microcentrifuge 1 minute

Spin your microcentrifuge tube containing the cell lysate in a microcentrifuge at maximum speed for 1

minute to pellet the cellular materials (other than the water soluble materials like nucleic acids). A pellet may not be visible.

8. Transfer supernatant

Transfer 100 μL of the supernatant into your other, unused tube labeled “#2 extract”. The extracted nucleic

acid in microcentrifuge tube #2 can now be used for PCR. Freeze it until ready to use!

**PCR Protocols**

**PCR prep of *Halobacterium* DNA (using “Ready to go” PCR Beads)**

**(note: Primer concentrations are not included here. Contact me if you are interested in this)**

1. Extract on ice

Place your microcentrifuge tube (#2 extract) containing the cell lysate onto ice. It should remain on ice while you are working.

2. Obtain PCR tube

Notice that the PCR microcentrifuge tube contains a white bead. The waxy bead contains DNA polymerase, dNTPs and buffer which will be released from the interior of the bead when placed in the warm thermal cycler.

3. Add 20 μL Primer Mix

Pipette 20 μL of the Primer Mix into your PCR tube, trying not to disturb the bead when doing so. The Primer Mix contains a forward primer and reverse primer, DMSO and gel electrophoresis loading dye.

4. Add 4 μL extract

With a new sterile tip (or new disposable pipette), add 4 μL lysate from the microcentrifuge tube (#2 extract) from the extraction activity to the liquid in the bottom of the PCR tube, again trying not to disturb the PCR bead.

5. Mix

After securely closing the PCR tube, mix the contents by tipping side to side. DO NOT try to mix with your pipette tip as the contents of the bead may have been released and will stick to your tip.

6. Place in thermal cycler

**Thermal cycler times**

Program the thermal cycler with the following times and temperatures:

Step I. 3 minutes at 94°C

Step II. 15 seconds at 94°C

Step III. 15 seconds at 58°C

Step IV. 1 minute at 72°C

Step V. Return to Step II – Step IV, repeating 30 times

Step VI. 5 minutes at 72°C

Step VI. Infinitely held at 4°C (Note: some machines do not have this capacity and you may have to be present at the end of the cycle to transfer the samples to a refrigerator or freezer.)

The PCR reaction will take approximately 53 minutes or more hours to run. The time will vary with the machine.

**BSYC Medium (makes 1 Liter)**

Compiled with permission from Carolina Biological

***Materials***

1 L beaker or flask

1 L graduated cylinder

2 of 1 liter bottles

Autoclave tape

Deionized water

Stir bar

Stir/Heat plate

Weigh boats

250 g sodium chloride (NaCl)

20 g magnesium sulfate heptahydrate (MgSO4 \* 7 H2O)

3 g (Tri) sodium citrate dihydrate (Na3C6H5O7 \* 2 H2O)

2 g potassium chloride (KCl)

5 g yeast extract

5 g casamino acids

5 M sodium hydroxide (NaOH) for adjusting pH

Concentrated HCl for adjusting pH

***Instructions:***

1. Place a beaker with stir bar and 700 mL of deionized water onto a stir plate and set the speed to low.
2. Add NaCl and turn the heat to low-medium. Warm to 50 °C in order to help dissolve the salt (this may take up to 15 minutes). Do not heat too high.
3. Once the salt has fully dissolved, add the MgSO4 \* 7 H2O, Na3C6H5O7, KCl, yeast extract, and casamino acids.
4. Once all the ingredients have dissolved, adjust the pH to 7.2 using 5 M NaOH or concentrated HCl.
5. Bring the volume to 1 L with deionized water. Mix.
6. Split the 1 L into two 1 L bottles to prevent overflow during autoclaving.
7. Autoclave at 15 lbs/in2 and 121° C for 35 minutes.

***Instructions for solid BSYC media*** (listed in Carolina Biological “Special Media” booklet):

1. Follow protocol for liquid BSYC media as written.
2. After adjusting the pH, add 20 grams agar to 1 liter BSYC liquid media.
3. Boil until clear (approx. 45 minutes for 500 mL BSYC with 10 g agar)
4. With distilled water, adjust volume back to starting volume after boiling (since some of your water boiled off while dissolving the agar).

***Additional Notes from K. Tuel:***

* Because of the extreme salinity, I found autoclaving unnecessary
* If stored in bottles for later use, autoclaving to melt the solid agar does speed up melting time (I found it could take approx. an hour in a boiling water bath). **HOWEVER, when pouring in bottles for later use, do not fill bottles more than 1/3 full**.

**Time Involved & Trouble Shooting**

**Time Estimations**

(Just estimate! Always allow longer when preparing cultures so they’ll be ready when you want them!)

* Making BSYC media – 45 minutes
* Using BSYC media to make agar – allow about an hour for the agar to completely dissolved (will be CLEAR)
* Melting the pre-made agar (~135 mL) – 45 min to 1 hour in water bath. DO NOT MICROWAVE!!!
* Growth
  + ON AGAR
    - 42°C - plating **should be done at least** 7-10 days prior to the intended date of activity
    - 37°C - allow 10-14 days for adequate growth
    - Room temperature - plates may be incubated at on the lab bench, but it may take longer than 2 weeks before they are ready for use.
  + IN BROTH
* In tube, 3-5 days for turbid growth. Longer for “pink froth”
* In flask, 7-10 days for turbid growth. Several weeks for “pink froth”

**Trouble Shooting**

* Crystals form!
  + Probably air got to them when incubating.
    - Liquid culture
      * Make sure tubes/flasks sealed well with several layers of parafilm or caps!
    - Solid culture
      * Squeeze out all air and double bag with FREEZER bags or 1 freezer bag into plastic resealable container
* Nothing is growing!!
  + ARE YOU SURE IT WAS ALIVE WHEN YOU INNOCULATED? Did you subculture enough?
    - Liquid culture
      * Needs oxygen to really grow! Grow on shaker (recommendation: 37°C with 150-200 shakes per minute)
    - Solid culture
      * Did you flame the loop/spreader (which technically isn’t necessary with this organism, but good practice)?? If the loop/spreader was too hot, it could’ve killed your culture.
* Something funky is growing in my culture!
  + Table salt is discouraged from use when making BSYC media because it may contain other halophiles
  + Were they incubating a long period of time? May be a mold.
* The “melt and pour” agar blew up in my microwave
  + Because of the extreme salt content of this media, DO NOT MICROWAVE. You’ll have to do the old fashioned water bath on hot plate melting method.
* I’d like to use this next year. How do I store it?
  + Research brine inclusions (from liquid culture)
  + Store on BSYC agar slant at 4°C. May keep up to a year.

**Resources & Presentation References**

**4 great online resources:**

1. **HaloWeb** [**http://halo4.umbi.umd.edu/**](http://halo4.umbi.umd.edu/)

Contains links to bioinformatics through NCBI with all the parameters already set specific to *Halobacterium* sp. NRC-1. Click on Halobacterium sp. NRC-1. Look at gene map, get info on individual proteins (example, gvpA), link to BLAST proteins against the database of *Halobacterium* sp. NRC-1, view the gene sequence and more!

1. **Carolina Biological Supply carolina.com**

Free teacher manuals for the kits that are currently published. These will be available for the next week or so, until they change their website! Wild type (NRC-1) and mutant (KBT-1) cultures can be purchased here.

1. **HaloEd** [**http://halo4.umbi.umd.edu/~haloed/**](http://halo4.umbi.umd.edu/~haloed/)

Contains education related ideas, resources, lessons, etc. related to *Halobacterium.*

1. **MolGenT (Molecular Genetics Tutor) Access by link on left side of HaloEd website**

Contains animations for understanding various molecular biology concepts including DNA, inheritance, gene expression and technology.

**References from K. Tuel’s KABT PowerPoint**

1Sean P. Kennedy, et. al. Understanding the Adaptation of *Halobacterium* Species NRC-1 to Its Extreme Environment through Computational Analysis of Its Genome Sequence. *Genome Research*, 11:1641-1650.

2HaloEd Project. Accessed 4/9/11. <http://halo4.umbi.umd.edu/~haloed/>

3 Frank T. Robb. Archaea: A Laboratory Manual Halophiles. Cold Spring Harbor Laboratory Press, 1995.

4S. DasSarma, et. al. 1987. A Plasmid-encoded gas vesicle gene in a halophilic archaebacterium. Molecular Microbiology 1(3):365-370.

5 M.J. Danson, et. al. The Archaebacteria: Biochemistry and Biotechnology. University Press. Biochemical Society Symposium 58.

6Haloweb. Accessed 4/11/11. <http://halo4.umbi.umd.edu/>

7K. Tuel, et. al. The Genotype-Phenotype Connection: Molecular Genetics and Bioinformatics Skills. Laboratory Activity through Carolina Biological. *In Press.* Expected to be available November 2012.

8Halobacterium salinarum – overview. Max Planck Institute of Biochemistry. Available online. Accessed 4/12/2011. <http://biochem.mpg.de/en/eg/oesterhelt/web_page_list/Org_Hasal/index.html>

9C. Gruber, et. al. 2004. “Halobacterium noricense sp. Nov., an archaeal isolate from a bore core of an alpine Permian salt deposit, classification of Halobacterium sp. NRC-1 as a strain of H. salinarum and emended description of H. salinarum.” Extremophiles (6): 431-9.

**Kits available to order**

**Halobacterium sp. NRC-1 kits**

**Item # Name Cost (as of 4/11)**

154771 Basic Microbiology Skills Kit, Part 1 $63.55

154772 Basic Microbiology Skills Kit Part 2 $70.20

154776 Antibiotics in Action Kit $94.90

154773 Extremely Easy DNA Extraction Kit $81.70

154770 Introduction to Life in an Extreme Environment Kit $48.00

XXXX The Genotype:Phenotype Connection Kit approx. $195.00

**Kit Descriptions**

* [Basic Microbiology Skills Part 1](http://www.carolina.com/product/basic+microbiology+skills+kit,+part+1.do?keyword=halobacterium&sortby=bestMatches)  
  Teach your students how to grow microbes on an agar plate using the safe, visually stimulating microbe, *Halobacterium* sp. NRC-1. Students learn the sterile and plate-streaking techniques used in biotechnology and the food and medical industries.
* [Basic Microbiology Skills Part 2](http://www.carolina.com/product/basic+microbiology+skills+kit+part+2.do?keyword=halobacterium&sortby=bestMatches)  
  This kit is a great way to introduce your students to this novel model microbe. *Halobacterium* is an earthly model of organisms that could be found on Mars.
* [Antibiotics in Action](http://www.carolina.com/product/antibiotics+in+action,+per+kit+.do?keyword=antibiotics+in+action&sortby=bestMatches)  
  Introduce your students to antibiotics and a technique that tests microbes for antibiotic resistance. Students create a lawn of *Halobacterium* sp. NRC-1 and determine which antibiotics the microbe is susceptible to by looking for a lack of microbial growth around antibiotic-impregnated disks.
* [Extremely Easy DNA Extraction Kit](http://www.carolina.com/product/extremely+easy+dna+extraction+kit.do?keyword=halobacterium&sortby=bestMatches)  
  This kit helps students visualize DNA and its properties and meets the National Science Content Standards in Life Science. Using kit materials, students extract and spool out DNA from living microbes using techniques similar to those in top research laboratories.
* [Introduction to Life in an Extreme Environment](http://www.carolina.com/product/introduction+to+life+in+an+extreme+environment+kit.do?keyword=halobacterium&sortby=bestMatches)  
  This inquiry-based introduction to the colorful life of the Archaea helps students learn about microbes and their natural habitat—in this case, the extremely salty areas of the world, such as the Great Salt Lake and Dead Sea. It can also be used to illustrate how organisms adapt to a changing environment. Students culture the extreme halophile (salt-loving) microbe, *Halobacterium* sp. NRC-1, from a safe grain of salt.
* The Genotype:Phenotype Connection (Basic Bioinformatics and Molecular Biology Skills) Kit

Students choose a phenotypically colony to experiment with. They will explore colony morphology, DNA extraction (cell lysis), PCR and gel electrophoresis. The final gel image of their selected colony will allow a comparison between genotype to phenotype because of the potential Insertion Sequence that is present in many of the mutant (red) colonies.

**A la carte items available for order**

**Item # Name Cost (as of 4/11)**

775950 Halobacterium Agar, 135 mL prepared media bottle (~7 plates) $6.70

821449 Halobacterium Broth, 5 mL tubes, Pack of 10 $17.00

154777 Culture Packet of Brine Salt Inclusions $12.60

154800 Culture Halobacterium sp. NRC-1, Tube $9.75

154801 Culture Halobacterium sp. NRC-1., Plate $11.60

**Make the BSYC media yourself**

Priced from Sigma-Aldrich, <http://www.sigmaaldrich.com>

**Verify that these items/numbers are the correct chemical!!! Do not just order from my list in case I made a mistake!!!**

Check your chemical supply closet – you probably already have a lot of these!

**Item # Name Cost (as of 4/11)**

S7653-1KG sodium chloride (NaCl) $60.10

63138-250G magnesium sulfate (MgSO4 \* 7 H2O) $56.80

S1804-500G (Tri) sodium citrate (Na3C6H5O7) $69.20

P9541-500G potassium chloride (KCl) $31.90

Y1625-250G yeast extract $81.40

22090-100G casamino acids $14.80

S8045-500G sodium hydroxide (NaOH) pellets; make 5 M solution $70.10

H1758-100ML Concentrated HCl for adjusting pH $46.50