Growing Microbes in the Classroom

The field of microbial research is growing by leap and bounds right now. Everyday new discoveries are made about where microbes live, who or what they live on (or in) and what contributions they make to the health of the planet and every living thing on it. The nice thing about studying microbes is that they are all over the place, they don't take up a lot of space in a lab (or in a classroom) and they don't eat much. The problem with growing microbes is that some of them can be dangerous to human (or other living organism's) health, sometimes their nutrient or environmental needs are so specific that they won't grow in an artificial setting and individual microbes are too small to see with the unaided eye. Fortunately for us *Halobacterium*, a salt-loving microbe, meets the pros of growing microbes without having to deal with (most of) the cons – a single *Halobacterium* is too small to see without a microscope, but on the plus side, their colonies make bright pink colonies.

In nature *Halobacterium* species are found in bodies of water that have extremely high salt concentrations such as the Great Salt Lake or the Dead Sea. While the name *Halobacterium* suggests that these organisms belong in the domain of Bacteria, they actually belong in the domain of Archaea. (For more information about Archaea, please read the Encyclopedia of Life "Archaea" article included with this activity.) Because *Halobacterium* requires such high concentrations of salt to survive, the media used to grow *Halobacterium* in a lab setting is too salty for any potentially pathogenic (disease-causing) microbes or other bacteria to grow.

In this activity, you and your students will explore the nutrient needs of *Halobacterium* by trying to grow this microbe on media with and without nutrients. This activity is written for a class of up to 30 students who are working in pairs. At room temperatures, it takes about two weeks for *Halobacterium* cultures to show significant growth.

Materials

- 150 mL chicken stock (with at least 5 g protein/serving)
- 150 mL water
- 2 250-mL plastic beakers
- 82.5 g table salt, divided
- 6 g agar powder, divided
- 2 plastic spoons
- 30 2-oz mini-cups with lids
- 30 swizzle sticks with ball end
- Sharpie markers
- Microwave
- Digital scale
- Weigh boats
- 15 sandwich-sized re-sealable plastic bags
- *Halobacterium* culture
- Container with 10% bleach solution (20 mL bleach + 180 mL water)
- Out-of-the-way location where cultures can be stored for two weeks, but accessible for student observations

Set-up

Teacher Prep Area – *work area near microwave*

- 2 plastic beakers, 250 mL
- 1 box chicken stock (with at least 5 g protein/serving)
- 150 mL tap water
- microwave
- 1 canister table salt
- agar powder
- Digital scale
- weigh boats
- 2 plastic spoons
- Sharpie marker
- 1 swizzle stick for every student
- *Halobacterium* culture
- Container with 10% bleach solution (20 mL bleach + 180 mL water)

Student Work Area – *at student tables or work areas*

- 1 mini-cup with lid for every student
- 1 sandwich-sized re-sealable plastic bag for every pair of students
- Sharpie markers for labeling mini-cups

Microbe Growing Area – *out-of-the-way location where cultures can grow undisturbed but can be accessed easily for daily observations*

Procedure

1. **Student Task – Labeling Mini-Cups**
    a. Decide which student of the pair will try to grow *Halobacterium* on salty chicken stock agar and which will try to grow *Halobacterium* on salty plain agar.
    b. Each student should use a Sharpie marker to label the bottom of their mini-cup (NOT the lid!) with their initials, the date and either "chicken" or "plain."

2. **Teacher Task – Making Salty Chicken Stock Agar**
    a. Pour 150 mL chicken stock into one of the 250 mL beakers and use the microwave to heat up the chicken stock until it is just to the point of boiling.
    b. Add 41.25 g salt to the chicken stock and use one of the plastic spoons to stir the chicken stock until the salt dissolves.
    c. While the chicken stock is still hot, slowly sprinkle in 3 g of agar powder, stirring constantly.
    d. If the salty chicken stock agar starts to thicken, reheat to return it to a pourable consistency.
    e. Call up the students who will try to grow *Halobacterium* on the salty chicken stock agar.
    f. Into each mini-cup pour enough salty chicken stock agar to completely cover the bottom of the cup.
    g. Have the students cover their mini-cups with their lids and then return to their seats.
    h. Let the agar cool completely before moving on to Step 4. DO NOT touch the agar.

3. **Teacher Task – Making Salty Plain Agar**
    a. Pour 150 mL water into the second 250 mL beaker and use the microwave to heat up the water until it is just to the point of boiling.
    b. Add 41.25 g salt to the water and use the second plastic spoon to stir the water until the salt dissolves.
    c. While the salty water is still hot, slowly sprinkle in 3 g of agar powder, stirring constantly.
    d. If the salty plain agar starts to thicken, reheat to return it to a pourable consistency.
    e. Call up the students who will try to grow *Halobacterium* on the salty plain agar.
    f. Into each mini-cup pour enough salty plain agar to completely cover the bottom of the cup.
    g. Have the students cover their mini-cups with their lids and then return to their seats.
    h. Let the agar cool completely before moving on to Step 4. DO NOT touch the agar.

4. **Student Task – Inoculating Agar with *Halobacterium***
    a. When the agar in your mini-cup is cool (feel the bottom of the cup to determine this) you and your lab partner should take your mini-cups to the Teacher Prep Area and perform the following steps:
        i. Get a swizzle stick but be careful not to touch the ball-shaped end of the stick.
        ii. Open the lid of your mini-cup.
        iii. Open the lid of the *Halobacterium* culture and gently rub the ball-shaped end of the swizzle stick across the surface of the agar, picking up some of the pink *Halobacterium* colonies.
        iv. Replace the lid of the *Halobacterium* culture.
        v. Now gently rub the pink *Halobacterium* colonies that are on the end of your swizzle stick back and forth across the surface of the agar in your mini-cup.
        vi. Put the used swizzle stick ball-shaped end down into the 10% bleach container.
        vii. Replace the lid of your mini-cup.
    b. Put your mini-cup in a sandwich-sized re-sealable plastic bag with your lab partner's mini-cup.
    c. Seal the bag and store the mini-cups upside down in the area indicated by your teacher.

5. **Teacher Task – Cleaning Up**
    a. Allow the swizzle sticks to sit in the 10% bleach solution for at least 10 minutes. The sticks can then be rinsed and reused.
    b. Remove and discard any solidified agar from the 250 mL beakers. Wash the beakers with soap and water. They are now ready to be reused.

c. When you are ready to dispose of *Halobacterium* cultures (the initial culture used by the students to inoculate their mini-cups, or when the students are done making observations about their cultures) cover the surface of the agar with 10% bleach solution and let sit for at least 10 minutes. The cultures can then be placed in a plastic bag and thrown away.

6. **Student Task – Daily Observations**
   a. You and your partner should make observations during the school week about your two cultures. These observations can include pictures, drawings or written descriptions of what you notice about your *Halobacterium* cultures.

Date of Observation	***Halobacterium* on salty plain agar**	***Halobacterium* on salty chicken stock agar**

7. **Student Task – Report**
   a. You and your partner should write up a short report about your experiment. What do your observations tell you about the nutrient needs of *Halobacterium*? Did you make any unusual discoveries? What future experiment(s) would you like to try?