

## Isolating *Bacillus* from Soil

**Introduction:** One of the key characteristics of bacteria that belong to the genus *Bacillus* is their ability to make a specialized cell type called a spore that is very resistant to heat, desiccation and ultraviolet light. The spore can survive for many years in the environment until there are favorable growth conditions. Then the spore germinates into a growing vegetative cell.

Many harmless species of *Bacillus* inhabit soil. Therefore, they are common contaminants in the microbiology laboratory. The heat resistance of *Bacillus* spores combined with the prevalence of harmless species of *Bacillus* in the soil provides for a fairly simple lab activity.

**Objective:** To isolate a strain of *Bacillus* from soil.

**Rationale:** Since spores are both heat resistant and activated to germinate by heat, boiling a sample of soil in nutrient broth will kill virtually all bacteria and will stimulate *Bacillus* spores in the soil to germinate. This is called heat shock. Incubating the heated broth allows the spores to germinate and produce vegetative cells. These cells will form *Bacillus* colonies on nutrient agar plates.

**Safety note:** The boiling water bath is a safety hazard and the teacher should insert and remove the tubes for the students using tongs or another safety device.

**Materials** (per experiment or group of students)

- soil
- clean test tube with loose top (should hold about 20 ml)
- 15 ml nutrient broth in a flask or tube
- 10 ml pipette
- boiling water bath suitable for holding test tubes (large beaker on a hot plate is good) - this will hold several tubes for heating
- nutrient agar plate
- inoculating loop
- marking pen

**Procedure**

1. Fill a large beaker half full of water and heat on hot plate or burner until water is simmering.
2. Have each group add about 1-2 grams of soil (about half a teaspoon) to an empty tube.
3. Fill the 10 ml pipette with nutrient broth and carefully add it to the tube with soil, washing any soil down the inside of the tube to the bottom of the tube.
4. Place the top on the tube and place the tube in the boiling water bath for 10-12 minutes. Make sure that the water in the beaker comes above the level of the nutrient broth so that all the broth gets thoroughly heated.
5. Remove the tube from the water bath and cool.
6. Incubate the tube at 30-37° C for 1-2 days.
7. Label the nutrient agar plate on the bottom with the student's initials and the date.
8. Using sterile technique, with the inoculating loop remove a loopful of the nutrient broth and streak it on a nutrient agar plate.
9. Incubate the plate 1-2 days at 30-37° C until colonies form.

**Results:** The nutrient agar plates should show colonies of *Bacillus* species. These are often characterized by a flat, chalky appearance, or a rhizoid (almost like a fungus) appearance. Many species of *Bacillus* are very motile; thus, you may find that the cells have migrated all over the plate completely covering it instead of producing colonies. Since the exact species of these strains is unknown, it might be best to seal the plates once they have grown and allow students to observe them only through the sealed lid.

**Related activity:** See the laboratory called "Simple staining of microbes" for an easy stain of *Bacillus megaterium*, a strain that is large enough to be seen with a 40X microscope objective. If the plate or slant of *B. megaterium* is old, many spores should be visible.

### **Teacher Instructions**

#### In advance:

1. Prepare nutrient broth according to instructions on package and sterilize about 15 ml in flasks or tubes. You will need one flask or tube per student group.
2. Prepare nutrient agar plates according to instructions on package. Pour plates of about 25 ml/plate. You will need 1 plate per student group.

#### The day before class:

Obtain soil samples for the class.

#### The day of class:

Set up the boiling water bath for the class and assemble the materials for each group.

### **Contributed by:**

Teresa Thiel, Ph.D.  
Department of Biology  
University of Missouri-St. Louis  
St. Louis, MO 63121  
USA