Isolation of Soil Microorganisms

A Project for Elementary Grades

Title : Isolation of soil microorganisms
Objective : To isolate and count the microorganisms found in a sample of soil by the dilution method using aseptic techniques.

Materials:

- 1 Erlenmeyer flask containing 50ml of sterile agar (0.1%)
- 1 cup containing 0.5g of soil
- 4 small vials containing 4.5ml of sterile agar (0.1%)
- 5 sterile 1ml pipettes
- 1 sterile glass stirring rod
- 6 Petri plates containing about 10ml of PDA (potato dextrose agar)
- 6 strips of Parafilm
- paper towel
- disinfectant
- marking pens

Method:

1. Mark the dilutions on the vials (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) and Petri plates (10^{-4}, 10^{-5}, 10^{-6}).
2. Add the 0.5g soil to the Erlenmeyer flask containing 50ml of agar (this is the 10^{-2} dilution).
3. Shake well for at least one minute.
4. Using a sterile pipette, take 0.5ml and place it in a vial containing 4.5ml of 0.1% agar. (this is the 10^{-3} dilution).
5. Shake well for at least one minute.
6. Repeat steps 4 and 5 for the three other dilutions (10^{-4}, 10^{-5}, 10^{-6}).
7. Starting with the weakest dilution (10^{-6}), pipette 1ml onto each of 2 Petri plates containing PDA. Spread over the entire surface using the sterile glass stirring rod. (The 10^{-6} dilution first.) Follow the same steps for the 10^{-5} and 10^{-4} dilutions.
8. Seal the Petri plates with the Parafilm.
9. Incubate the Petri plates at room temperature.
10. Observe after 24, 48 et 72 hours.

Results:

- Record the number of colonies/Petri plate/dilution.
- Calculate the number of microorganisms per gram of soil using the following formula :# of colonies/plate × the dilution factor = # organisms / 1 gram of soil
- Record the characteristics of each colony.

Questions:

1. Why must you shake before pipetting?
2. On step 7, why begin with the weakest dilution?
3. Can you distinguish bacterial colonies from fungal colonies?
4. How can we have colonies that are only bacterial or fungal?
5. If you accidentally upend the vial containing the 10^{-6} dilution, what can be done to obtain this level of dilution on the Petri plate?

Instructions for supervisors:

- Take your time – you should have about an hour; the dilutions take around half an hour.
- Ask students to wash their hands and take a seat before beginning the lesson.
- Ask them to READ the instructions, and ask questions if anything is not clear.
- If the students would like to practice pipetting, there are NON-STERILE pipettes available with water.
- The vials and Petri plates are already labeled. Ask that the students write their initials on their Petri plates.
- The first thing to do is to clean up their workplace with disinfectant, and work on a damp towel.
- Remind them that once they have finished to collect their vials, etc., and place them in the sink. To clean their work place if messy, and to return to the classroom. There, they may watch the demonstration and ask any questions they have.
- As soon as everyone has left, return to the classroom to help answer questions.