

# The Isolation of *Bacillus* from soil

Name : \_\_\_\_\_ Date : \_\_\_\_\_

"I have neither given nor received unauthorized aid on this test or assignment."

## Purpose

Gram-positive Bacteria as a group are common soil organisms. *Bacillus* species are very common Gram-positive mesophilic, aerobic heterotrophs that produce heat-resistant endospores. The enrichment and isolation of *Bacillus* is straightforward - a sample of rich soil (which is typically rich in *Bacillus*) is heated to kill non-spore-forming mesophiles, and then plated on rich media and incubated aerobically at 30C. Thermophiles will not grow at this temperature, and anaerobic spore-formers (e.g. *Clostridium*) will not grow aerobically. Other mesophilic aerobic endospore-formers (e.g. *Heliospirillum*) are rare and phototrophic, requiring lots of light for growth.

## Caution



The organisms we'll be working with are common soil bacteria. On one hand, they are generally not particularly dangerous, and rarely infect humans. On the other hand, these are undomesticated organisms of unknown identity or pathogenicity. *Bacillus anthracis* and *Clostridium tetani* are also soil bacteria (although not common)! Handle all cultures with respect and using standard microbiological procedure.

The heating block used to treat the samples is *very hot*. Do not touch the heating block, and allow your sample to cool before handling.

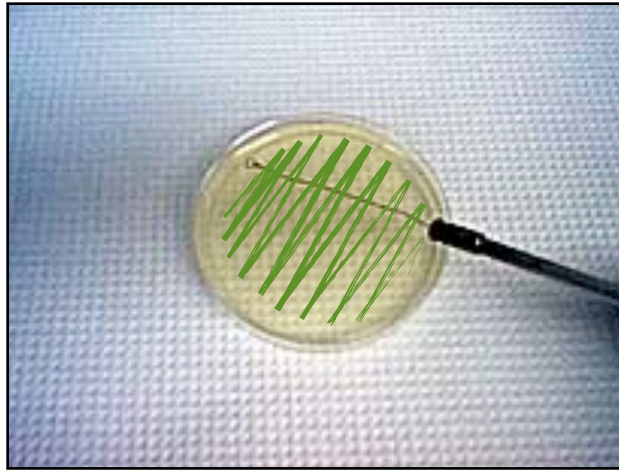
## Materials

- Inoculum : A sample of soil
- Media : LB, PYD or NA agar plates (1 per student)
- Supplies : Inoculating loop & burner
  - Sterile distilled water
  - Sterile 1.5ml microcentrifuge tubes
  - Heating block at 80C
  - Bleach discard beakers and biohazard disposal bag
  - Sterile cotton swabs

## Procedure

### Part 1 - Day 1

1. In a microcentrifuge tube, suspend a small amount (about the size of a BB) of soil in 1-2 drops of sterile water. Mix well and incubate at 80C for 10 minutes.
2. Use a sterile cotton swab or loop (flamed) to swab a sample of the heated soil suspension across the entire surface of the plate, from one side to the other.



3. Write your name and the date in Sharpie on the bottom of the plate (the half with the agar), and incubate agar-side up at room temperature for 1-2 days. (The instructor will transfer your plates to the fridge after this amount of time.)

### Part 2 - Day 2

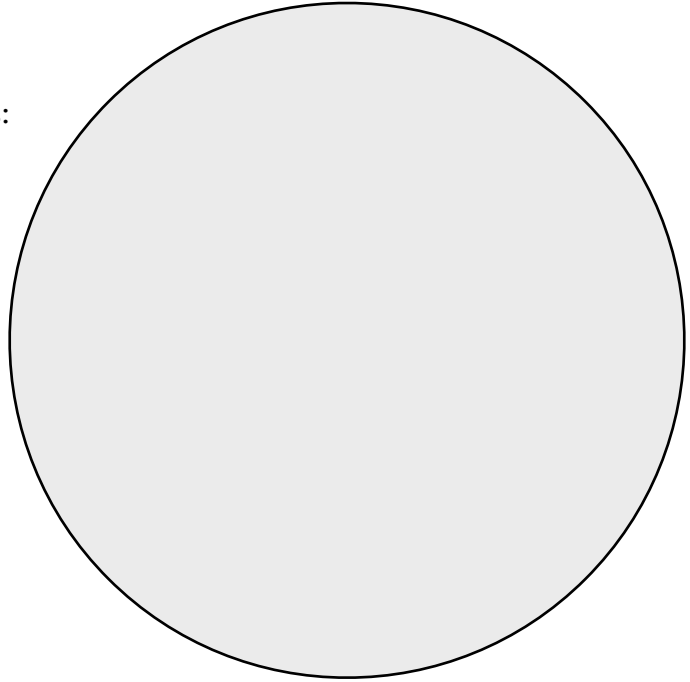
1. Examine samples of various colonies by eye. The most common *Bacillus* colonies are white, opaque or translucent (not transparent), and rough, granular, or wispy.
2. Starting with likely-looking isolated colonies, make notes about colony morphology, then smear a tiny bit onto a slide with a flamed loop. Add a small drop of water and top off with a coverslip, and view under high-power, phase contrast. Look for large rod-shaped organisms, often with a distinctly mottled appearance, non-motile with some pairs or longer chains, and especially the presence of endospores (some example micrographs are shown below).

Example: *Bacillus megaterium*



**Notes**

1. Make a drawing of your plate with colonies:



2. Make a close-up drawing of your *Bacillus* colony:

3. Make a drawing of your *Bacillus* under the microscope:

### Questions

1. Not every colony on your and your neighbors plates is likely to be a member of the Family *Bacillus*. Given the enrichment and isolation procedure used, if you came up with something other than *Bacillus*, how might this have happened?
2. How would you modify this procedure if you wanted to isolate a member of the Family *Clostridium*?
3. Some endospore-formers are photosynthetic - the *Heliobacteria*. How would you modify this procedure if you wanted to isolate one of these species?
4. The numbers of *Bacillus* in you soil sample were surely very, very high, and yet you only got a relatively small number of colonies. Give at least one reason why this might be.
5. What is the difference between a *selection* and a *screen*?