Exp.4: Isolation of an antibiotic producer from soil

The constant search of soils throughout the world has yielded an abundance of antibiotics of great value for the treatment of many infectious diseases. Pharmaceutical companies are in constant search for new strains of bacteria, molds, and *Actinomyces* that can be used for antibiotic production. Although many organismsvin soils produce antibiotics, only a small portion of new antibiotics is suitable for medical use. In this experiment, an attempt will be made to isolate an antibiotic-producing *Actinomyces* from soil. Students will work in pairs.

FIRST PERIOD

(Primary Isolation)

Unless the organisms in a soil sample are thinned out sufficiently, the isolation of potential antibiotic producers is nearly impossible it will be necessary to use a series of six dilution tubes to produce a final soil dilution of 10^{-6}. Proceed as follows:

Materials:

per pair of students:

6 large test tubes

1 bottle of physiological saline solution

3 Petri plates of glycerol yeast extract agar

L-shaped glass rod

beaker of alcohol

6 1 ml pipettes

1 10 ml pipette

1. Label six test tubes 1 through 6, and with a 10 ml pipette, dispense 9 ml of saline into each tube.

2. Weigh out 1 g of soil and deposit it into tube 1.

3. Vortex mix tube 1 until all soil is well dispersed throughout the tube.

Make a tenfold dilution from tube 1 through tube 6 by transferring 1 ml from tube to tube. Use a fresh pipette for each transfer and be sure to pipette-mix thoroughly before each transfer.

5. Label three Petri plates with your initials and the dilutions to be deposited into them.

6. From each of the last three tubes transfer 1 ml to a plate of glycerol yeast extract agar.

7. Spread the organisms over the agar surfaces on each plate with an L-shaped glass rod that has been sterilized each time in alcohol and open flame. Be sure to cool rod before using.

Incubate the plates at 30° C for 7 days.

SECOND PERIOD

(Colony Selection and Inoculation)

The objective in this laboratory period will be to select *Actinomyces*-like colonies that may be antibiotic producers. The organisms will be streaked on nutrient agar plates that have been seeded with *Staphylococcus epidermidis.* After incubation we will look for evidence of antibiosis. Students will continue to work in pairs.

Materials:

per pair of students:

4 trypticase soy agar pours (liquefied)

4 sterile Petri plates TSB culture of *Staphylococcus epidermidis* 1 ml pipette

3 primary isolate plates from previous period water bath at student station (50° C)

1. Place four liquefied agar pours in water bath (50°C) to prevent solidification, and then inoculate each one with 1 ml of *S. epidermidis.*

2. Label the Petri plates with your initials and date.

3. Pour the contents of each inoculated tube into Petri plates. Allow agar to cool and solidify.

4. Examine the three primary isolation plates for the presence of *Actinomyces*-like colonies. They have a dusty appearance due to the presence of spores.

They may be white or colored. Your instructor will assist in the selection of colonies.

5. Using a sterile inoculating needle, scrape spores from *Actinomyces*-like colonies on the primary isolation plates to inoculate the seeded TSA plates. Use inoculum from a different colony for each of the four plates.

6. Incubate the plates at 30° C until the next laboratory period.

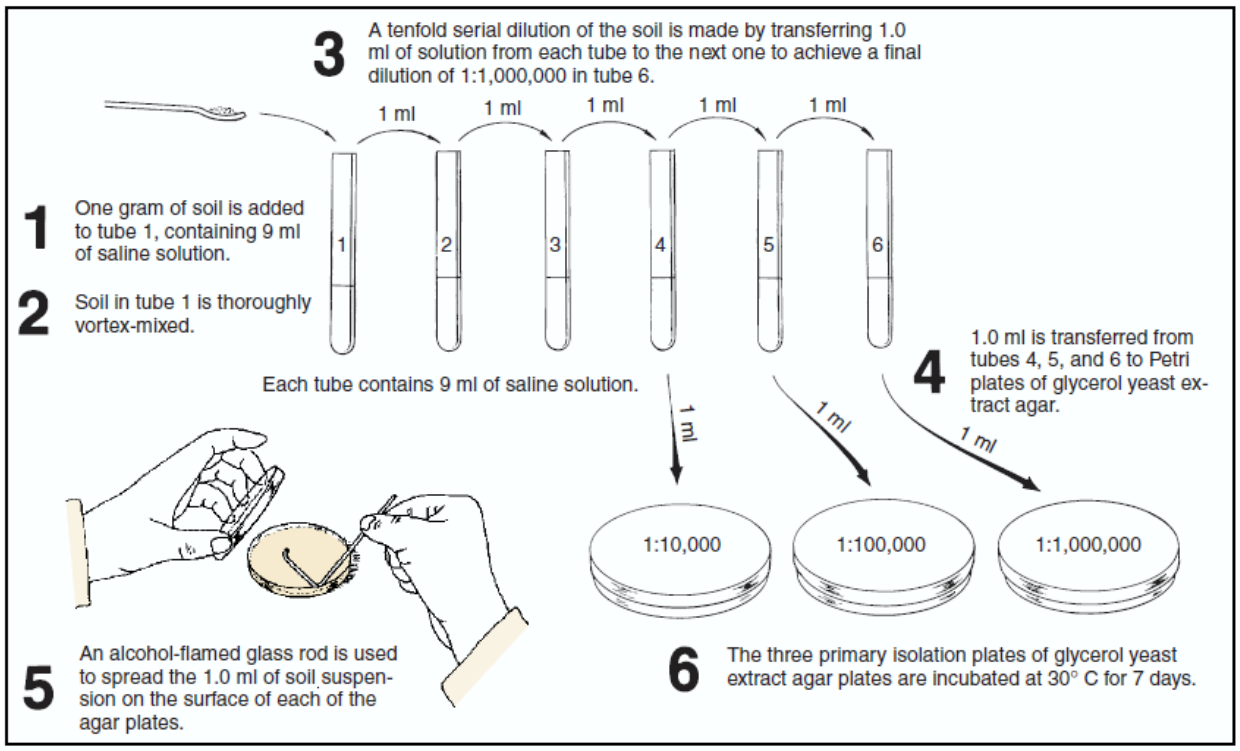
THIRD AND FOURTH PERIODS

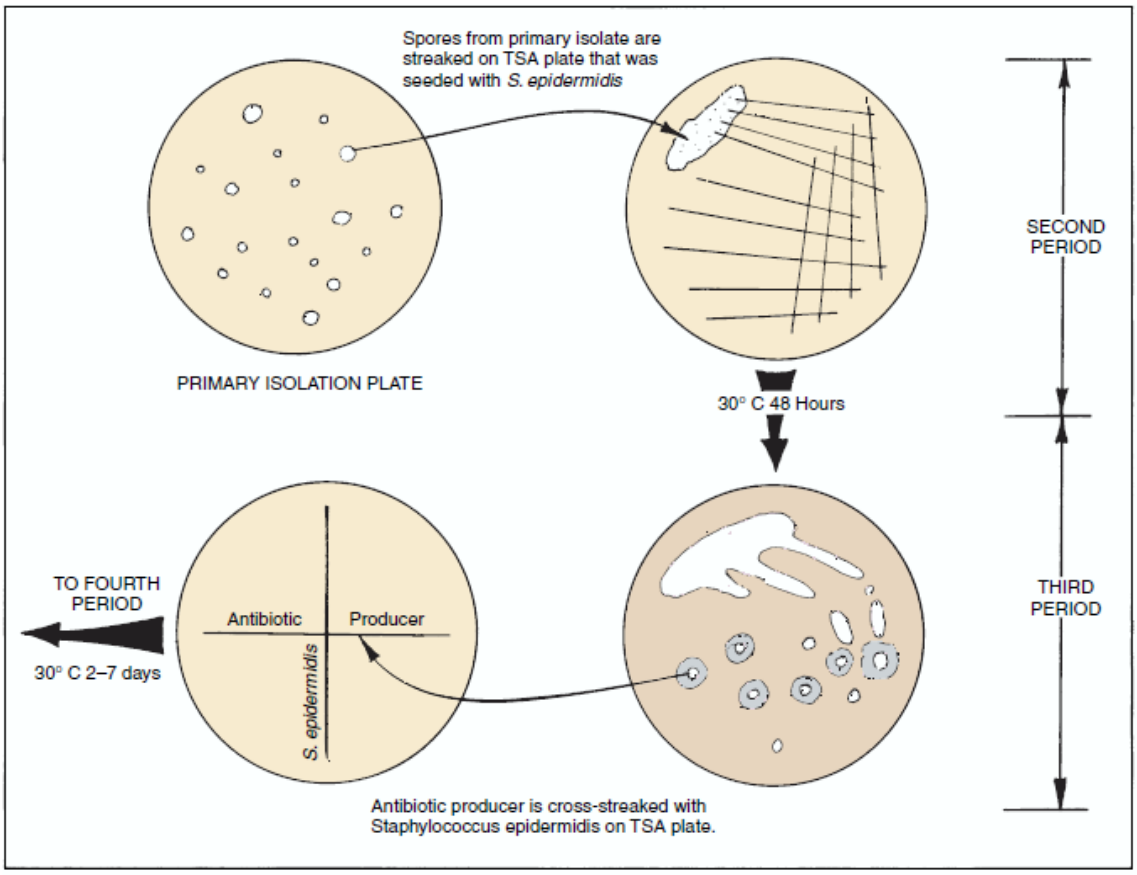
(Evidence of Antibiosis and Confirmation)

Examine the four plates you streaked during the last laboratory period. If you see evidence of antibiosis (inhibition of *S. epidermidis* growth), proceed as follows to confirm results.

Materials:

1 Petri plate of trypticase soy agar TSB culture of *S. epidermidis* If antibiosis is present, make two streaks on the TSA plate as shown in figure 57.2. Make a straight line streak first with spores from the *Actinomyces* colony, using a sterile inoculating needle. Cross-streak with organisms from a culture of *S. epidermidis.* Incubate at 30° C until the next period.




Spores from primary isolate are streaked on TSA plate that was seeded with S. epidermidis
PRIMARY ISOLATION PLATE
30° C 48 Hours
SECOND PERIOD
THIRD PERIOD
TO FOURTH PERIOD
30° C 2–7 days
Antibiotic
Producer
S. epidermidis
Antibiotic producer is cross-streaked with Staphylococcus epidermidis on TSA plate.