Enrichment & Isolation Lab

OBJECTIVE

We will learn and practice both streak and spread plate techniques for isolating bacteria. These techniques, once mastered can be used in end endless variety of exercises and experiments. We will use broth cultures of *Serratia marcesens* and luminescent bacteria to practice streak plate technique. To practice spread plate technique we will perform a serial dilution of soil. When combined with serial dilution the spread plate technique makes it possible to both enumerate and isolate cultivatable bacteria from any sample.

STREAK PLATE EXERCISE: SERRATIA AND LUMENESCENT BACTERIA

1) Find and review the 'Streak plate' instructions attached to this exercise (from page 11 of the booklet "Basic Practical Microbiology – A manual").

2) Obtain a tube of *S. marcesens* and a tube of luminescent bacteria from the front of the room (there will be one tube of each culture for every 4 people so you will have to share). Each group of 2 people will also need an inoculating loop, a burner, 2 nutrient agar plates and 2 seawater complete agar plates.

3) Each person should follow the Streak Plate instructions and streak one plate of nutrient agar with *S. marcesens* and one plate of seawater complete agar with luminescent bacteria.

SPREAD PLATE EXERCSIE: COUNTING AND ISOLATING BACTERIA FROM SOIL

1) Find and review the 'Spread Plate' and 'Using a Spreader' instructions attached to this exercise (from pages 13-15 of the booklet "Basic Practical Microbiology – A manual").

2) Each group should obtain a spreader, a burner, a 1 ml pipette and tips, 6 tubes of sterile 1x PBS (9 ml), and 3 dilute nutrient agar plates. A glass Petri plate containing 70% ethanol should also be available.

3) Label your tubes Tube 1 through Tube 6.

4) Weigh out 1 g of soil and add it to Tube 1. Cover the top with parafilm and shake aggressively for about a minute, being sure to keep your finger over parafilm to prevent it from spilling (the parafilm doesn't have to be sterile at this point since there will be many millions of bacteria in the soil). Allow the soil to settle for about a minute.

5) Transfer 1 ml of Tube 1 to Tube 2, and mix well by swirling.

6) Transfer 1 ml of Tube 2 to Tube 3, and mix well by swirling. Continue sequentially in this manner until you have carried the dilution through all 6 tubes.

7) Label your dilute nutrient agar plates 10^{-5}, 10^{-6}, and 10^{-7}.

8) Spread 0.1 ml of tube 4 onto the plate marked 10^{-5}, then spread 0.1 ml of tube 5 on plate 10^{-6}, and 0.1 ml of tube 6 on plate 10^{-7}.

9) Take the plates to the instructor so that they can be incubated at 30ºC.

10) Following incubation for 3-5 days count the number of colonies on each plate. Divide the number of colonies you find by the dilution factor (ie: 10^{-6}) to obtain the number of cultivatable bacteria per g of soil. You should only count plates that have distinct colonies (generally <300 per plate).

Notes on Counting and Isolating Bacteria from Soil

A wide variety of growth media and incubation conditions can be used to isolate bacteria from soil. In general bacteria will grow faster on rich media (nutrient agar, TSA, etc.) then on dilute media (dilute nutrient agar) and will grow faster at 30ºC than at room temperature. You should be aware that on rich media you will frequently encounter organisms that will grow rapidly and spread to cover the entire plate, which can make counting problematic. You will also find that the total count of bacteria that you obtain will vary with choice of media, incubation conditions, and even incubation time. The vast majority of bacteria in soil (90-99%) will not grow on your plates at all. This technique only counts the 'cultivatable' bacteria and since the growth requirements of bacteria will vary the growth conditions you choose will have a strong impact on how many and what types of bacteria you isolate. The spread plate technique is used widely and a few of the uses for this techniques can be found in hospital laboratories, in the food industry, and to test water quality at beaches and water parks, and waste treatment facilities.

You could vary this protocol by comparing soils from different sources or using different types of environmental samples (water samples, leaf surfaces, insect guts. . .). You can also compare different incubation conditions or different types of media to see whether this has an effect on the number or types of colonies you observe.

Media Recipies

(Most ingredients can be purchased from Carolina Biological but otherwise can be found at Difco Media (www.vgdusa.com/DIFCO.htm), VWR (http://www.vwrsp.com/), or Sigma-Aldrich (www.sigmaaldrich.com)).

Nutrient agar:
Can be purchased as a powder from Carolina Biological, Difco Media or other distributors

Seawater complete agar:
15 g/l NaCl, 2.25 g/l $MgCl_2$ hexahydrate, 0.15 g/l $CaCl_2$ dihydrate, 0.15 g/l KH_2PO_4, 0.38 g/l KCl, 5 g/l peptone, 3 g/l yeast extract, 15 g/l agar

Dilute nutrient agar:
0.08 g/l nutrient broth (purchased as a dehydrated powder), 15g/l agar

1x PBS:
8 g/l NaCl, 0.2 g/l KCl, 1.43 g/l Na_2HPO_4, 0.24 g/l KH_2PO_4