Practical Biotechnology

Lab 3

Searching for antagonistic properties of soil microbes

By Dr. Shaymaa H. Ali
Assistant Professor of Molecular biology
Mrs. Najat Taher
MSc. In molecular Biology

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Soil Microbes

Most soil microbes can be classified as fungi, bacteria, archaea, protozoa, or viruses.

soil organic matter include plant litter, living or dead soil organisms .and larger soil fauna like worms and insects, as well as their waste.

soil microbes significantly affect soil and crop health. Some of its activities include:

1. nitrogen-fixation.
2. phosphorus solubilization.
3. suppression of pests and pathogens.
4. decomposition that leads to soil aggregation.

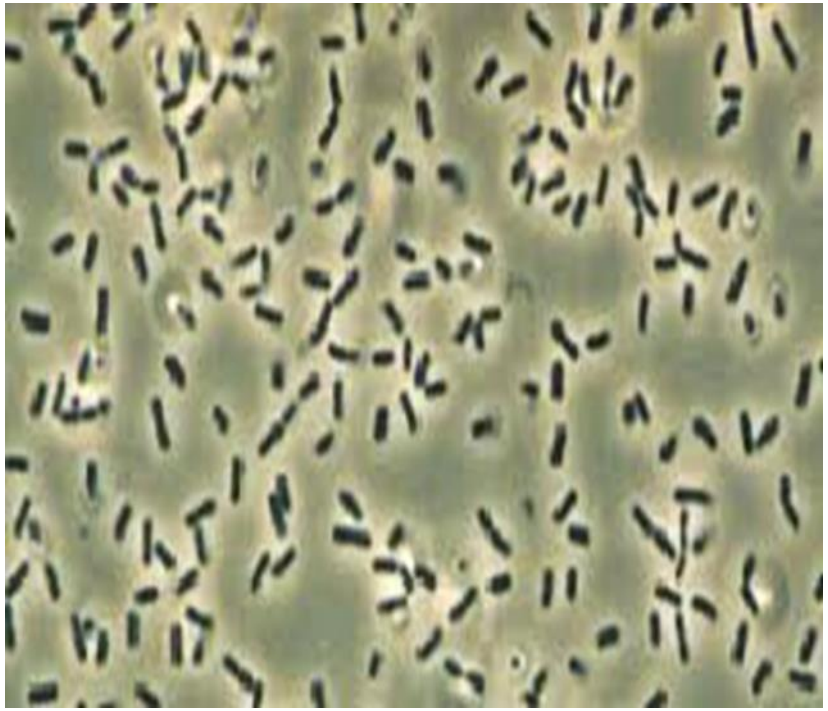
However, soil microbes can also be harmful to crops if they cause disease or compete for nutrients.

A Few Important Bacteria in Soil

- Nitrogen-fixing bacteria, change ammonium to nitrate, its the preferred form of nitrogen for grasses and most row crops.
- Actinomycetes are a large group of bacteria that grow as hyphae. They are responsible for the “earthy” smell.

- Actinomycetes important in degrading recalcitrant (hard-to-decompose) compounds, such as chitin and cellulose.

- A number of antibiotics are produced by actinomycetes such as Streptomyces.



Isolation of Bacteria from Soil

Methods of Isolation :

Bacteria present in soil can be isolated by several methods:

1. Dilution Plate Method
2. Streak Plate Method
3. Pour Plate Method
4. Membrane Filter Technique

Dilution Plate Method

Principle:

This basic technique used for bacteria especially from soil, helps in obtaining isolated colonies where dilutions prevent crowding of colonies.

Requirements:

1. Soil samples and sieve (2 mm pore).
2. Incubator, oven, balance ,Burrell's wrist action shaker.
3. Agar plates—Nutrient agar.
4. Bunsen flame.

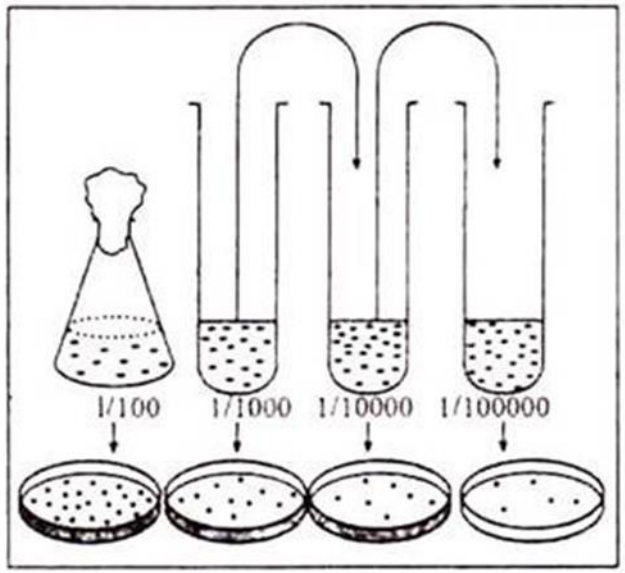


Fig. 2.1: Soil dilution and inoculation

Procedure:

1. Sift soil through a 2 mm sieve and weigh one gram each (2 samples) in sterile previously weighed containers.

2. Keep 1 g in oven at 105°-110°C overnight, reweigh the dried sample.

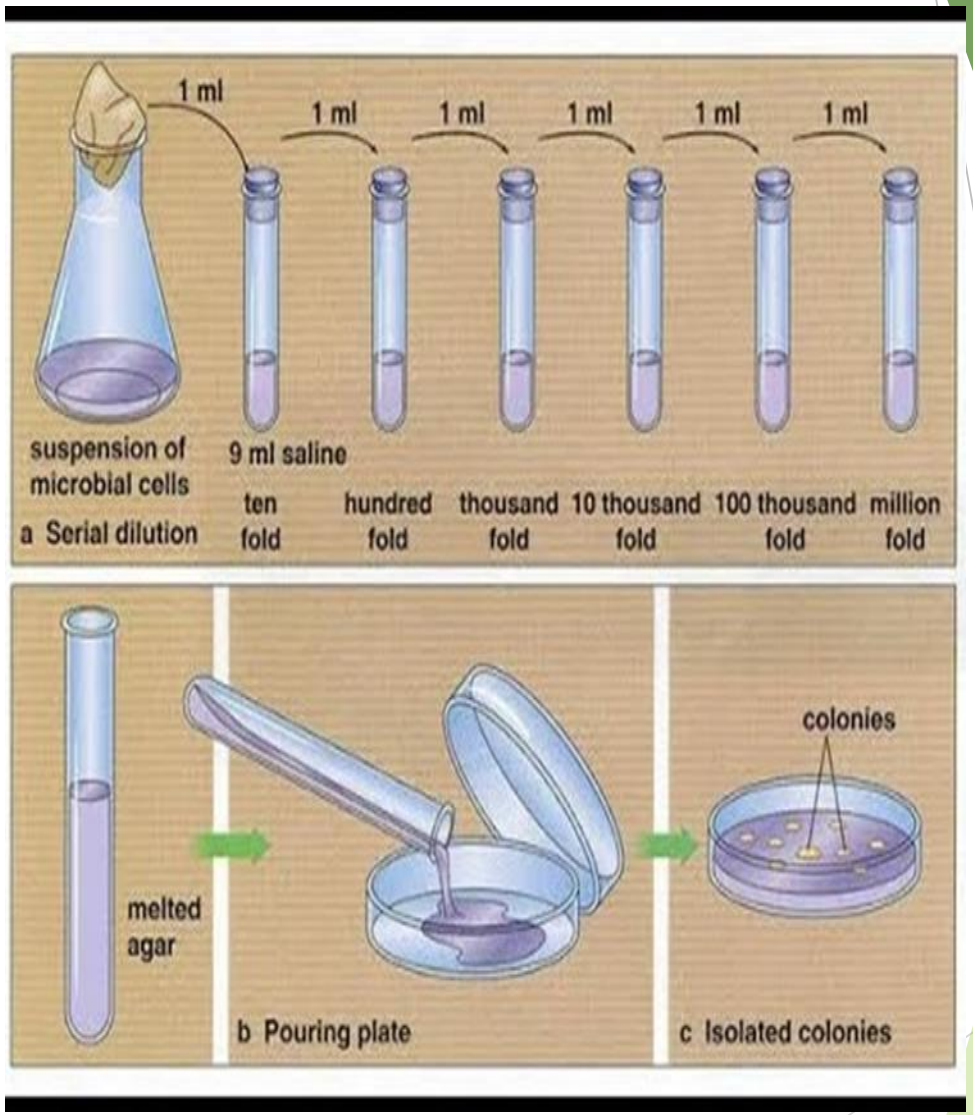
3. Add the other one gram sample to a 99 ml water in a 250 ml flask.

4. Keep the flask on a Burrell's wrist action shaker for 30 minutes and label, this as 1/100 dilution.

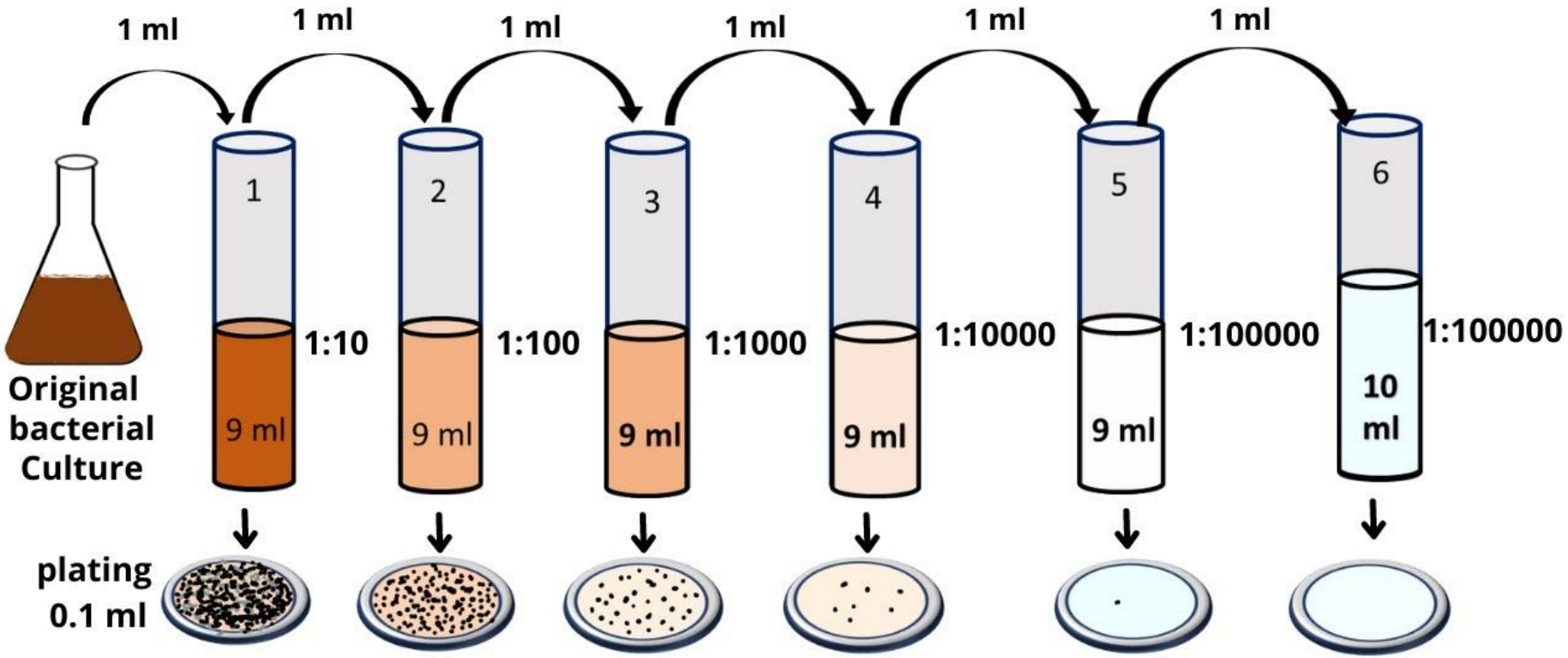
5. While the flask is in motion, pipette out one ml of 1/100 dilution to a 9 ml water 100 blank, label this as 1/1000 dilution.

6. Transfer 1ml dilution through successive 9 ml water blanks until a final dilution of 1/1000,000 is obtained.

7. Transfer aseptically 0.1 ml/(1.0 ml for fungi and actinomycetes) of each dilution, after shaking vigorously, to each of the several Petri dishes with 12-15 ml of appropriate agar medium (Nutrient agar for bacteria) temperature (45°C).



Serial Dilution



8. Spread the suspension in case of solidified medium by rotating the plate or spread by means of a sterile glass (rod) spreader and in case of semi-solid medium, rotate the plate and allow it to solidify.

9. Incubate at 37°C for 24-48 hours for bacteria (5-10 days for fungi and actinomycetes).

10. After incubation, count the colonies on a colony counter (visually for fungi) for bacteria and actinomycetes.

Calculations:

Number of bacteria / per gram of soil = Number of colonies X Dilution factor on a dry wt. basis, e.g. if there are 20 colonies in a plate with1/1000 dilution.

The number of viable cells/ per gram of soil = 20 X 1000 = 20,000.

Calculate on a dry weight basis.

11. Note down colony characters and transfer them to the respective agar slants, incubate and preserve them for further studies.

Thank you