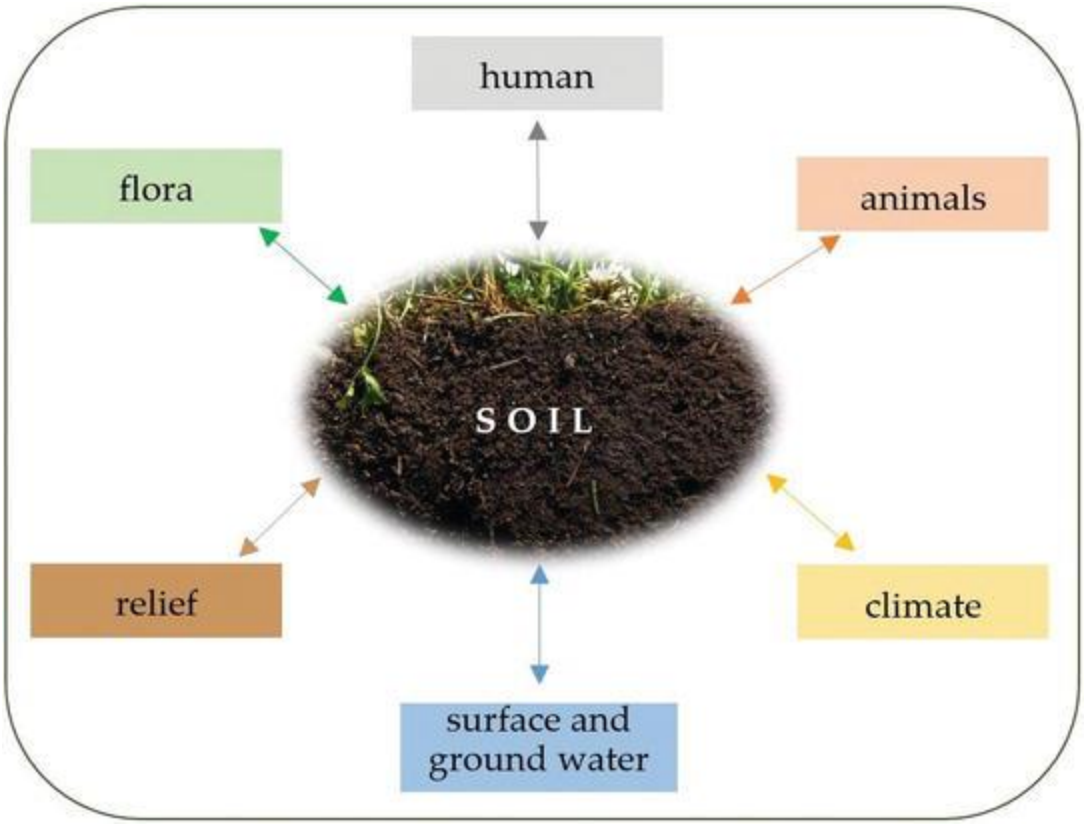
Soil Microbiology second stage /Environmental science

Prepared by: Dr.sarween O. Taha



2021-2022

Salahaddin university
Dr. Sarween O. Taha
PHD. Molecular microbiology

Lab 1 and 2
soil microbiology practical
second stage/Environmental science

Introduction of soil microbiology

Soil microbiology is the study of microorganisms in soil, their functions, and how they affect soil properties. It is believed that between two and four billion years ago, the first ancient bacteria and microorganisms came about on Earth's oceans. These bacteria could fix nitrogen, in time multiplied, and as a result released oxygen into the atmosphere. This led to more advanced microorganisms, which are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa.

Surface soils are a heterogeneous mixture of inorganic and organic particles that combine together to form secondary aggregates. Within and between the aggregates are voids or pores that visually contain both air and water. These conditions create an ideal ecosystem for bacteria, so all soils contain vast populations of bacteria, usually over 1 million per gram of soil.

Bacteria are the simplest of microorganisms, known as prokaryotes. Within this prokaryotic group, there are the filamentous microbes known as actinomycetes. Actinomycetes are actually bacteria, but they are frequently considered to be a unique group within the classification of bacteria because of their filamentous structure, which consists of multiple cells strung together to form hyphae.

Bacteria and Archaea, the smallest organisms in soil apart from viruses, are prokaryotic. They are the most abundant microorganisms in the soil, and serve many important purposes, including nitrogen fixation.

Some bacteria can colonize minerals in the soil and help influence weathering and the breaking down of these minerals. The overall composition of the soil can determine the amount of bacteria growing in the soil. The more minerals that are found in area can result in a higher abundance of bacteria. These bacteria will also form aggregates which increases the overall health of the soil.

Culturing and Enumerating Bacteria from Soil Samples:

Principles

One way to enumerate the number of bacteria present in a soil sample is to utilize dilution and plating methodology. This methodology utilizes agar as a medium for bacterial growth, a process termed, "culturable technology." Because of the vast numbers of bacteria found within soils, a small sample of soil is serially diluted in water, prior to being plated on agar within a Petri plate. Typically, a small amount of soil contained within 0.1 to 1 mL of the diluted soil suspension is "spread" over the surface of the agar plate.

Dilution and plating Method:

This experiment demonstrates the dilution and spread plating methodology used to enumerate the number of bacteria within a soil sample.

Procedure:

1. Preparation of Soil Dilutions

To begin the procedure, weigh out 10 g of soil sample and add to 95 mL of deionized water. Shake the suspension well, and label as "A".

Before the soil settles, remove 1 mL of the suspension with a sterile pipette and transfer it to a 9-mL deionized water blank. Vortex thoroughly, and label as "B".

Repeat this dilution step three times, each time with 1 mL of the previous suspension and a 9-mL deionized water blank. Label these sequentially as tubes C, D, and E. This results in serial dilutions of 10-1 through 10-5 grams of soil per Ml.

2. Making Spread Plates for Bacterial Culture

To grow bacterial colonies, take three pre-prepared peptone-yeast agar plates and label them as C, D, and E. Vortex samples C, D, and E, and pipette 0.1 mL onto each plate. This increases the dilution value further, by a factor of ten (C = 10-3, D = 10-4, E = 10-5).

Incubate the bacteria plates at room temperature for 1 week. Make sure the plates are inverted during the incubation to prevent drops of moisture from condensation from falling onto the agar surface.

3. Making Spread Plates for Actinomycetes

To grow actinomycetes, take three pre-prepared glycerol-casein plates and label them as B, C, and D. Using the techniques shown previously, spread plate 0.1 mL from the suspensions B, C, and D. The lower dilutions are used because actinomycetes are typically present as 1/10th of the bacterial population (B = 10-2, C = 10-3, D = 10-4).

Incubate the actinomycete plates (inverted) at room temperature for 2 weeks.

4. Bacterial and Actinomycete Counts

After incubation, examine all of the bacteria plates carefully, and note differences in colony size and shape. When grown on agar, bacteria produce slimy colonies ranging from colorless to bright orange, yellow, or pink. In contrast, actinomycete colonies

are chalky, firm, leathery, and will break under pressure, where other bacterial colonies will smear. This allows colonies to be distinguished by touch with a sterile loop.

Count and record the number of bacterial colonies, including any actinomycetes. Only count plates with 30-200 colonies per plate.

5. Isolation of Pure Cultures

Select individual bacterial colonies from any of the plates. More colonies can be selected if there is particular interest in the soil. Use a high dilution plate, as it tends to have pure colonies that are separated well. Choose only colonies that are well-separated from neighboring colonies and look morphologically distinct from each other.

Sterilize the loop by dipping it in alcohol and flaming it. Quickly open the Petri dish of interest, and touch the loop to a bare spot in the agar to cool it. Then, remove a small amount of a colony of interest onto the loop.

Taking a fresh peptone-yeast plate, make a streak a few centimeters long on one side. Sterilize and cool again, then make a streak that crosses the initial streak only on the first pass. Repeat this process twice more in the same manner. This streaking “dilution” results in cells on the loop being separated from one another. Place the plate in a dark area to incubate at room temperature for two weeks.

Results:

A 10-g sample of soil with a moisture content of 20% on a dry weight basis is analyzed for viable culturable bacteria via dilution and plating techniques.

Number of CFU per g moist soil = 1/dilution factor X number of colonies