

Isolation and selection of microorganisms used in Microbiology

The microorganisms that usually used in microbiology are; Bacteria, Actinomycetes, Fungi (Yeasts and Molds) and Algae. These microorganisms are isolated from different sources like water, air, clinical samples, etc. **but soil is considered the most important source.**

Bacterial isolation, purification and identification are the first steps to bacteriological studies

Soil sampling:

- 1- The sample (about 50 g) is taken deep in soil (about 5 to 10 cm deep) by clean spatula.
- 2- The sample is put in sterile sac and all necessary information recorded on it.
- 3- The sacs are transferred into the lab and maintain in the refrigerator at 4°C until used.

For screening of microorganisms, the simplest technique is “crowded plate” procedure. This technique is used where one is interested only in finding microorganisms that produces an antibiotic irrespective of its action against any specific organism. Hence, the sample is diluted only to such an extent that agar plates prepared from these dilutions will be crowd with individual colonies on agar surface, i.e. 300 to 400 colonies or more. Colonies producing antimicrobial activity are indicated by clear zone of growth inhibition surrounding the colony. Such colony is later on sub cultured, purified, and afterwards microbial inhibition spectrum is tested against selective microorganisms. Also, this technique is used for isolation of growth factors producing bacteria.

Advantages:

1. It's the simplest method to find antibiotic-producing microorganisms in soil samples.
2. It's also rapid, taking only a couple of days to produce results.
3. Introducing "test organisms" can help to determine whether a specific kind of microorganism (e.g., a disease-causing germ) is susceptible to the antibiotic compound. If it does indeed prove useful for this purpose, the compound can be isolated for further study.

Disadvantages:

1. It only detects microorganisms that produce compounds to kill bacteria found in their immediate environment.
2. These compounds could potentially be toxic to humans, and they may be lethal only to certain types of bacteria (e.g., soil bacteria), as opposed to the bacteria that actually cause disease in humans.
3. Moreover, they will only detect microorganisms that start to produce antibiotic compounds within a couple of days of being cultured and incubated, so they might well miss other compounds that could potentially be of interest.

Procedure

1. Suspend 1.0 gram of soil sample in about 9 ml of sterile normal saline.
2. Mix well and allow the soil particle to settle down.
3. Prepare serial ten-fold dilutions (10^{-1} – 10^{-6}) of the supernatant using sterile normal saline.
4. Spread 0.1 ml from each of the last three dilutions on sterile nutrient agar plates with glass spreader.
5. Incubate the plates at room temperature or 30°C for 24 to 48 hours.
6. Observe the plates at different intervals and look for the colony which shows the clear area surrounding the colony i.e. growth of inhibition.
7. Pick up such colony, perform gram staining and subculture it on similar media. Then after its microbial inhibition spectrum is determined as following:

- a- The colony suspend in 1 ml of sterile normal saline.
- b- Spread 0.1 ml of pathogenic bacterial culture on sterile nutrient agar plate with glass spreader, make 5 wells by sterile cork-borer and fill every well with 100 μ l of the culture suspension.
- c- Incubate the plate at 37°C for 24 hours.
- d- Inhibition is detected by a zone of clearing around the well.

How to do serial dilutions

The serial dilution method was first described in 1883 by German scientist and physician Robert Koch when he published his work on infectious disease-causing agents, and is now a standard technique in today's laboratories.

Serial dilutions are routinely used in microbiology to estimate the number of microorganisms in a sample with an unknown concentration. Imagine that you have a bacterial culture tube, and want to know how many bacterial cells are growing in it. As every milliliter of media could contain a billion bacteria, it is impossible to count them in such high concentrations. This is why you have to dilute a small portion of your bacterial culture until you get a more easily countable concentration of 30 to 300 bacterial cells per milliliter.

However, in order to get to such a low concentration from a sample with one billion bacteria per milliliter in one step, you would need to dilute one milliliter of the sample in 10,000 liters of diluent. An alternative and more practicable approach is to perform a serial dilution. After diluting one milliliter of the sample with one billion bacterial cells per milliliter in nine milliliters of diluent, you will get a concentration of one hundred million bacterial cells per milliliter. Diluting one milliliter of this dilution in nine milliliters of diluent will allow you to obtain a bacterial concentration of ten million cells per milliliter, and so on. This means that you will get to a

manageable concentration of 100 bacterial cells per milliliter in 7 dilution steps, using only 63 milliliters of diluent.

