# Lab.1: Isolation and selection of microorganisms used in biotechnology

The microorganisms used in biotechnology are; Bacteria, Actinomycetes, Fungi (Yeasts and Molds) and Algae. These microorganisms are isolated from different sources; soil is considered the most important source.

Soil sampling:

1- The sample (about 500 g) is taken deep in soil (about 5 to 10 cm) 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.

There are three methods used for isolation and selection of the bacterial isolates that have the ability to produce certain substances:

**A- Crowded plate technique**

For screening of antibiotic producing organisms, 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.

**Drawbacks:**

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 µl 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.

**B- Auxotrophic technique**

This technique is used to isolate the growth factor- producing bacteria, and the auxotrophic bacteria are used as indicator for such production of these factors.

Growth factor is an organic compound such as a vitamin or amino acid that must be provided in the diet to facilitate growth. Auxotrophy is the inability of an organism to synthesize a particular organic compound required for its growth. An auxotroph is an organism that displays this characteristic.

Auxotrophy is the opposite of prototrophy, which is characterized by the ability to synthesize all the compounds needed for growth.
Prototrophic Bacteria can grow on minimal medium, which only contains inorganic salts, a carbon source and water (wild type).
Auxotrophic Bacteria will not grow on minimal medium, but can grow on minimal medium plus one or more specific nutrients or supplements, or on rich media (mutant).

**Procedure**

1. A strip of sterilized filter paper is put on the bottom of a sterilized petridish in a way that the two ends of the strip are out of the petridish.
2. Another sterilized circular filter paper is put on the strip in a way that covers the petridish bottom and a nutrient agar is poured over the two filter papers.
3. When the agar is solidified, a sample of a diluted soil is spread over it in order to obtain well separated colonies that are recognized easily.
4. Another nutrient agar is prepared. By using pour plate method, the agar is cultivated with an auxotroph microorganism (which require a special growth factor like: vitamin or amino acid) or with a microbial species that we do not know its drug of choice (the antibiotic that is active on this species).
5. By using forceps, the first nutrient agar is transferred carefully and in aseptic technique to the medium surface in the indicator petridish (without put it upside down). The plate is incubated at 37$^{o}$C for 24 hrs.
6. The growth of auxotroph bacterial colonies is observed at the lower layer directly beneath colonies that grow at the upper layer (cultivated by the soil) because of the penetration of metabolites from the upper agar layer to the lower agar layer containing the indicator bacteria.
7. The colonies at the upper agar layer are selected for identification, reproduction and isolation of the produced growth factor.

### C- Enrichment Culture technique

This technique is used for detection of enzymes production especially alkaline proteases. In this procedure, Casein is used as a sole source of carbon and energy.

### Procedure

1. The diluted soil sample is exposed to 80$^{o}$C for 10 mins. in order to kill the vegetative cells without affecting the spores. Then this sample is cultivated on an agar-agar medium containing 0.5 % Casein.
2. The agar is incubated at 37$^{o}$C for 24-48 hrs.
3. The growth of protease producing colonies is noticed as a result of casein utilization.
4. The colonies are selected and sub cultured for their identification, as well as for extraction and purification of the enzyme.