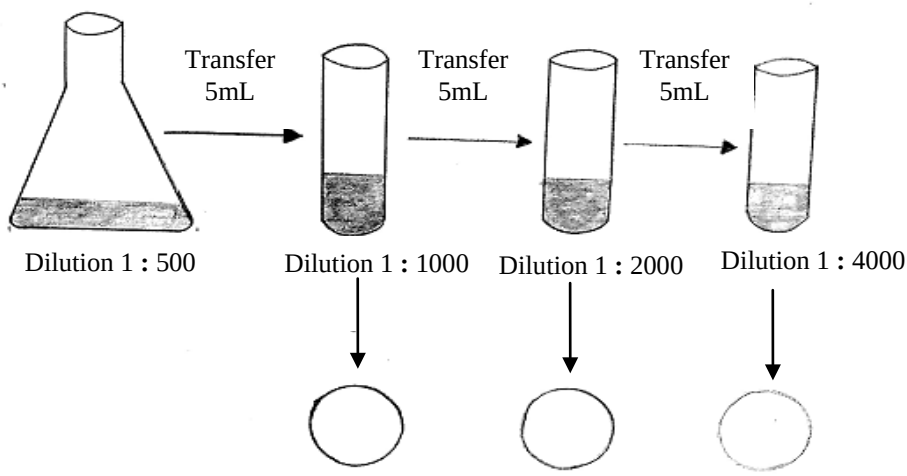
Aim 22

To Isolation Antibiotic Producing Microorganisms from the Soil

Introduction

Antibiotics (Anti-against, bios-life) are those compounds which are produced by one kind of micro organisms and kill or destroy the other micro organisms. Billions of micro organisms are present in soil. Microbes have been isolated from the soil that produces antibiotics. They are *Micromonospora, Bacillus, Penicillium streptomyces*, and *Cephalosporium.*

For the isolation of antibiotic producing microbes from the soil, Crowded plate technique can be used.



Scheme for the isolation of Antibiotic- producing Micro organisms from soil by crowded- plate Technique

Requirments

1. Soil sample (freshly collected from grassland)
2. Trypticase soy agar medium (pH 7.3)

Trypticase (animal peptone)	15 g
Soy peptone	5 g
Agar	15 g
Distilled water	1.0 liter

3. Sterile pipettes
4. Mechanical pipetting device
5. Sterile test tubes
6. Sterile flask
7. Sterile petri plates
8. Sterile water

Procedure

1. Take three sterile petri plates, mark them with dilutions (1:1000, 1: 2000, 1:4000). Take three tubes, label them as 1, 2, 3. Add 5 ml sterile water in each tube.
2. Prepare serial dilutions of the soil sample by taking 0.1 g of soil and dissolve it in 50 ml of sterile water (1:500 dilutions). Then, mix them.
3. By using 5 ml sterile pipette, transfer 5 ml of this mixture to tube no. 1 and mix well (dilution 1: 1000).
4. With the help of sterile pipette, transfer 5 ml of suspension from tube no. 1 to tube 2 and mix well (dilution 1: 2000).
5. Similarly, transfer 5 ml of suspension from tube no. 2 to tube 3 (dilution 1: 4000).
6. By using separate sterile pipettes, pipette 1 ml of these three dilutions to labeled petri plates.
7. Into each petri plates, pour the autoclaved trypticase soy agar medium that maintained at 45°C, then mix gently.

8. Allow the plates to solidify.
9. At 25°C, incubate the plates for 3-4 days.
10. Examine the colonies showing zone of growth inhibition.

Results

Due to the action of antimicrobial substances produced by the micro organisms present in the soil, the colonies show zone of inhibition. The antibiotic producing microorganism can be isolated and their anti-microbial activity can be checked against different test microorganisms.