Practical-01: Primary Screening of Antibiotics Producing Microbes

Principle

“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 crowded 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.

Wilkins’s Method

Another technique mainly used is Wilkins’s method. A Wilking medium which contains a pH indicating dyes i.e. Bromo-thymol Blue which is green colour at neutral pH but colourless at acidic pH. This method differentiate antibiotic producer from the acid producer. Those colonies that produce antibiotics give zone of inhibition against sensitive organisms without changing colour surrounding it while in case of acid produce colony the we may find zone of inhibition due to fall in pH along with colourless area due high acid production which result in lower pH , ultimately change the colour of dye from green to colourless.

It has some additional advantage that the organism producing antibiotic against the microorganisms of choice can be secondary screened. Here dilutions of sample are applied to agar surface so as to get well isolated colonies. After the colonies have reached up to few millimetre in size the suspension of test organisms is spread over the surface of agar and incubated. The antibiotic activity is indicated by zone of inhibition around the antibiotic producing colony.

Requirements

- Soil sample
- Dilution blank
- Sterile pipette
- Glass spreader
- Sterile N-agar plate
- Sterile Wilkins’s broth
- Sterile Wilkins’s agar med
Procedure

1. Suspend 1.0 gram of soil sample in almost about 10 ml of sterile distilled water.
2. Mix well and allow the soil particle to settle down.
3. Prepare different dilution of the supernatant using dilution blank.

Crowded plate technique

1. Spread 0.1 ml of inoculum on sterile nutrient agar plate with glass spreader.
2. Incubate the plate at room temperature for 24 to 48 hours.
3. Observe the plate at different intervals and look for the colony which shows the clear area surrounding the colony i.e. growth of inhibition.
4. Pick up such colony, perform gram staining and subculture it on similar media. Then after its microbial inhibition spectrum is determined.

Wilkins’s agar plate

1. Spread 0.1 ml of inoculum from each dilution on sterile Wilkins’s agar plate.
2. Incubate at 37 degree for 24 hrs.
3. Next day observe the plate for growth of different organism.
4. Incubate about 2 ml of test culture into Wilkins’s broth/ sterile melted Wilkins’s top agar previously cooled at 45 degrees. Mix it well and pour it over the base Wilkins’s agar spread on previous day.
5. Allow it to solidify and incubate at 37 degrees for 24 hrs.
6. Next day observe the plates for colonies showing inhibition of growth of test organism surrounding its vicinity, i.e. antibiotic producing colony.
7. Mark the colony write colony characteristics, perform Gram’s staining, and subculture it for secondary screening.

References:

- Bacteriological Techniques By F.J. Baker
- Introduction to Microbial Techniques By Gunasekaran