Aim 49 Penicillin Production and Testing of Antimicrobial Activity

Introduction

Antibiotics are chemical compounds produced by one type of microorganisms that selectively inhibit the growth or kill the other microorganisms even at low concentration.

The antimicrobial activity of the penicillin can be tested by the disc diffusion method in the lab. In this method, the organism which is tested is inoculated on agar medium. Different concentrations of penicillin are loaded on filter paper discs of uniform diameter. These discs are placed onto the surface of agar medium. After incubation for a definite period of time clear zones (zone of inhibition of growth) are appeared around the disc. With increase in the concentration of penicillin the diameter of zone of inhibition is also increases. The antibiotic will diffuse from the disc into the agar and this result in the inhibition of bacterial growth.

Requirements

1. Penicillin producing strains of *Penicillium chrysogenum* or *Penicillium notatum.*
2. **Czapek-Dox medium (pH 7.3)**

Magnesium sulphate	0.5 g
Dipotassium hydrogen phosphate	1 g
Sucrose	30 g
Sodium nitrate	2 g
KCI	0.5 g
Ferrous sulphate	0.01 g
Distilled water	1.0 litre

3. 18 hour nutrient broth culture of *Staphylococcus aureus*.
4. Sterile standard filter paper discs.
5. Inoculating loop.
6. Sterile nutrient agar plates.
7. Solution of penicillin (50 units concentration).

Procedure

1. Prepare Czapek-Dox medium.
2. By autoclaving sterilize it at 121°C for 20 minutes.
3. Aseptically, culture of *Penicillium chrysogenum* is inoculate on the Czapek-Dox medium (in 100 ml two flasks).
4. At 25-28°C incubate these flasks for 7-14 days.
5. The production of penicillin by the mold is indicates by development of golden yellow colour in the medium.
6. Centrifuge the above mixture and discard the debris.
7. For testing, the antimicrobial activity of penicillin against *Staphylococcus aureus*, supernatant/filtrate is taken.
8. Inoculate 1 ml culture of *Staphylococcus aureus* on nutrient agar plates and spread it using a spreader.
9. Allow the agar surface to dry for 10 minutes.
10. Use sterile forcep to pick up the sterile filter paper and dip it into the penicillium culture filtrate.
11. Place the paper disc on the surface of inoculated plate.
12. Similarly, disc impregnated with standard penicillin solution (50 units) is put on the surface of inoculated plate.
13. Third disc is kept as a control.
14. Repeat the same in another nutrient agar plate.

15. At 37°C, incubate these plates and a plate as control for 24-48 hours in an inverted position.

Results

The antimicrobial activity of the penicillin is indicated by no growth around the disc.

Precautions

1. The disc should be pressed softly with the help of sterile forcep to ensure firm contact with the agar surface.
2. The experiment should be done in aseptic environment.