

Aim

To determine the minimum inhibitory concentration of Streptomycin against given bacterial sample.

Principle

Antibiotics are chemical compound produced by one type of microorganism which at low concentration selectively inhibit or kill other organism. The sensitivity of microbes to antibiotics can be expediently tested in the laboratory by agar well diffusion method. In this method, the organism to be tested is heavily inoculated on the solidified medium on agar plate. The wells made in such a solid media are filled with varying concentration of antibiotics. The inoculated plates are observed after incubation for a definite period of time for the zone of inhibition. The diameters of zone of inhibition are measured. The inhibition is due to the diffusion of antibiotic from the wells an area of high concentration to an area of low concentration. The smallest amount of the antibiotic needed to inhibit the growth of microorganism is called as MIC.

Requirements

- Nutrient agar media
- Sterile Petri-plates
- Streptomycin
- Sterile test-tubes
- Gel puncher
- Bacteria culture

Procedure

1. Prepare serial dilution of a streptomycin antibiotic as fellow:

12.5mg + 25ml DDW -- 0.5mg/ml.....(A)

5ml (A)+ 5ml DDW -- 0.20mg/ml.....(B)

5ml (B)+ 5ml DDW -- 0.125mg/ml..... (C)

5ml (C)+ 5ml DDW -- 0.0625 (D)

5ml (D)+ 5ml DDW -- 0.03125.....(E)

5ml (E)+ 5ml DDW -- 0.015625.....(F)

5ml (F)+ 5ml DDW -- 0.0078125.....(G)

5ml (G)+ 5ml DDW -- 0.00390625..... (H)

5ml (H)+ 5ml DDW -- 0.001953125.....(I)

2. Arrange sterile tubes and no. Them (B) to (H).
3. Melt nutrient agar, pour them in Petri plates and allow them solidify.
4. Take 100ml bacterial sample and spread it properly an all the Petri plates.
Allow them culture to settle on agar.
5. Make 4 well on each plate and fill them with different concentration streptomycin solution.
6. Incubate the plate at 37°C for 24 hours and observe it.

Observation

Zone of inhibition were observed around the well containing varying concentration of streptomycin.

S.No.	Plate No.	Concentration of antibiotic mg/ml	Diameter of zone of inhibition in cm
1	A	0.5	
2	B	0.20	
3	C	0.125	
4	D	0.0625	
5	E	0.03125	
6	F	0.015625	
7	G	0.0078125	
8	H	0.00390625	
9	I	0.001953125	

Result

The minimum inhibitory concentration expressed as the lowest concentration of streptomycin dilution for given bacterial culture.