Aim

Determine the sensitivity of Bacillus substillis against streptomycin using broth dilution method.

Principle

The Broth dilution method involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. Microdilution testing uses about 0.05 to 0.1 ml total broth volume and can be conveniently performed in a microtiter format. Macrodilution testing uses broth volumes at about 1.0 ml in standard test tubes. For both of these broth dilution methods, the lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the minimal inhibitory concentration or MIC. The MIC is thus the minumum concentration of the antibiotic that will inhibit this particular isolate. The test is only valid if the positive control shows growth and the negative control shows no growth.

A procedure similar to broth dilution is agar dilution. Agar dilution method follows the principle of establishing the lowest concentration of the serially diluted antibiotic concentration at which bacterial growth is still inhibited.

Requirements

- Nutrient agar media
- Sterile Petri-plates
- Streptomycin
- Sterile test-tubes
- Gel puncher
- Bacillus substillis 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 37ºC 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 zone sizes are looked up on a standardized chart to give a result of sensitivity, resistant, or intermediate.