CRYSTAL VIOLET

Why to do this :

1. Visualize the property to form biofilm

What you need :

1. Culture media : LB and M63 supplemented with mannitol 0,2 g/L

LB medium (1L):

- 10 g bactotrypton
- 5 g yeast extract
- 5 g NaCl
- 0,5 mL NaOH 10N
- Qsp 1 L

M63 (1L):

- 13,6 g KH_2PO_4
- 2 g $(NH_4)_2SO_4$
- 0,5 mg $FeSO_4$
- 11 mL KOH 6,8 M
- 0,2 g $MgSO_4$
- 0,5 mg B1 Vitamin

2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL
Tetracycline (Tet) : 1 mg/mL
Kanamycin (Kann) : 5 mg/mL
Ampicillin (Amp) : 10 mg/mL

➔ 50 µL antibiotic / 5mL medium

3. Crystal Violet solution diluted 1/5 in water
4. Material : 24-well plates, P20, P200 P1000, 1 mL and 5 mL sterile pipettes.

How to do :

1. Bacteria culture

a) Prepare a preculture in LB (5 mL tube) during 24h at 30°C 250 rpm.

2. Biofilm culture

a) Dispense 2 mL of sterile M63 supplemented with mannitol 0,2 g/L per well.
b) Inoculate each well with 20 µL from the 24h-preculture (OD_{600} = 2).
c) Incubate 24h at 30°C.

3. Crystal Violet Test

a) Eliminate the 2 mL of supernatant with a P1000 for each well by tilting the plate.
b) Rinse gently each well by adding 1 mL of M63-Mannitol medium with a 5 mL pipette.
c) Eliminate the rinsing liquid with the P1000.
d) Fix the bacteria by heating 1h at 80°C.
e) Add 0,2 mL of diluted Crystal Violet in each well. Wait 2 minutes.
f) Eliminate the colorant with the P200. Rinse with water and let the plate dry on a paper towel.