



## Why to do this :

1. Check if the strain forms biofilms
2. Measure the strain adhesion

## 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  $\text{KH}_2\text{PO}_4$
- 2 g  $(\text{NH}_4)_2\text{SO}_4$
- 0,5 mg  $\text{FeSO}_4$
- 11 mL KOH 6,8 M
- 0,2 g  $\text{MgSO}_4$
- 0,5 mg B1 Vitamin
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### 2. Antibiotics concentrations

Chloramphenicol (Cm) : 2 mg/mL

Tetracycline (Tet) : 1 mg/mL

Kanamycin (Kann) : 5 mg/mL

Ampicillin (Amp) : 10 mg/mL

→ 50  $\mu\text{L}$  antibiotic / 5mL medium

3. Material : 24-well plates, P20, 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  $\mu$ L from the 24h-preculture ( $OD_{600} = 2$ ).

c) Incubate 24h at 30°C.

### 3. Adhesion Test

a) Aspirate the 2 mL of supernatant with a P1000 for each well by tilting the plate. Collect the supernatant in a test tube labeled "S".

b) Rinse gently each well by adding 1 mL of M63-Mannitol medium with a 5 mL pipette.

c) Collect the rinsing liquid with the P1000 and add it to the 2 mL of the previous supernatant.

d) Add 1 mL of M63-Mannitol medium in each well with a 5 mL pipette. Detach the biofilm by scraping the surface with the pipette tip and by successive suction/discharges.

e) Collect the bacterial suspension in a second test tube labeled "B".

f) Vortex 5 seconds to finish dispersing the bacterial clumps.

g) Measure  $OD_{600\text{ nm}}$  of each of the "S" and "B" tubes.

h)  $\% \text{ of adhesion} = \frac{OD_{600} \text{ "B"}}{\text{Total } OD_{600}} = \frac{OD_{600} \text{ "B"}}{OD_{600} \text{ B} + 3 \times OD_{600} \text{ "S"}}$