Biofilm quantification

This protocol aims at quantifying the adherence of any given strain in a 24 well plate. All solutions used need to be sterile. EDTA and CoCl2 solutions were sterilized by filtration on a 0.2µm filter.

Procedure

1. Prepare 10 mL of the desired media in 10mL falcon tubes. For LB/2, dilute 25mL sterile LB with 25mL sterile water and aliquot this mother solution. For M63G, prepare 100mL M63 + 1mL glucose 20%+ 1mL LB (all sterile) and aliquot 10mL of this mother solution. Each 10mL tube will be used to fill one column of 4 wells.

2. Grow 5mL cultures of the desired strains overnight with the appropriate antibiotic if the strain has a resistance (50µL) in the same media as the ones that are going to be used for the test.

3. Add any other required solution to the 10mL tubes (100 µL antibiotic, CoCl2 to obtain the desired concentration...).

4. Measure the OD600 of the 5mL bacterial cultures and add an appropriate volume of bacteria to the 10 mL tubes to obtain a concentration of 10^7 bacteria/mL (0.6 OD units is roughly equivalent to 2.10^8 bacteria/mL)

5. Wash the 24 well plate with sterile EDTA 4mM, empty the plate, wash with sterile water, empty the plate. This step allows the capture of metals that are on the plate and which could interfere with the test.

6. Fill the wells with 2mL each from the 10mL tubes.

7. Incubate at 30°C for 24 to 48 hours, depending on the medium (M63 takes longer than LB)

8. OD600 method Transfer the 2mL supernatant into small test

tubes (S) Gently wash the biofilm with 1mL medium (the same as the one used for the culture) and add this to the S tubes. Add 1mL medium and scratch the biofilm. Pour the biofilm with the medium into a test tube (B) and vortex for 20s. Measure the OD600 of each tube. The % of adherence is 100*B/(B+3S)

8. Methyl violet method Eliminate the supernatant Gently wash the biofilm with 1mL medium and eliminate the wash liquid Heat the plate at 80°C for 1h. Add 0.2mL of a methyl violet solution and wait for 2mn. Methyl violet is toxic and should be manipulated under a hood with gloves. Eliminate the dye solution and wash with water. Dry the plate.

General culture conditions

Antibiotics were used at the following concentrations :
Ampicilin (Amp) : 100µg/mL
Chloramphenicol (Cm) : 20µg/mL
Spectinomycin (Spc) : 100µg/mL
Kanamycin (Kan) : 30µg/mL
The solvent is a 70/30 (v/v) water/ethanol mix for chloramphenicol, other antibiotics were dissolved in water. All volumes mentionned are meant for a 100X mother solution.

The composition of the different media we have used is the following :
LB : 10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl for 1L of liquid LB.
M63G : 100/1/1 (v/v) of M63, glucose 20% and LB
LB/2 : 50/50 (v/v) mix of LB and water