CONFOCAL MICROSCOPY : BIOFILM CULTURE

Why to do this :

1. Check if the strain forms biofilms
2. Visualize the biofilm with confocal microscopy
3. Mesure the biovolume and the biofilm thickness
4. Biofilm 3D reconstruction

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
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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 µL antibiotic / 5mL medium

3. Material : 96-well plates for confocal microcopy (black wall and thin bottom), P20, P200

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) Prepare your inoculation medium with X µL in M63 (supplemented with 0,2% in Mannitol and with an appropriate antibiotic) from your 24h preculture (OD_{600} = 2) in order to inoculate with an OD_{600} = 0,02.

b) Inoculate each well with 200 µL from the previous inoculation medium.

c) Incubate at 30°C.

d) After 1h of incubation, aspirate gently the 200 µL of supernatant with a P200.

e) Add 200 µL of fresh M63 medium (supplemented with 0,2% in Mannitol and with an appropriate antibiotic).

f) Incubate at 30°C for 15 hours.

3. Confocal microscopy observation and 3D reconstruction

a) Before confocal observation, change gently the M63 medium by replacing it with a fresh one.

b) Confocal observation: ZEISS LSM 510 META, 40x/1.3 OILDIC, laser : Argon 4 lines 30 mW (458 nm, 477 nm, 488 nm, 514 nm)

c) 3D reconstruction and biovolumes quantitation: IMARIS® and COMSTAT (ImageJ);instructions given → doi: 10.1007/978-1-4939-0467-9_18 *Contribution of confocal laser scanning microscopy in deciphering biofilm tridimensional structure and reactivity*. (Bridier A1, Briandet R.)