Biofilm assay

Aim

To evaluate the biofilm inhibition or biofilm dispersal capacity of substances in 96 well plate.

Procedure

Plating

1. Grow bacteria overnight on Agar plates (with appropriate antibiotics) at 37 °C. (BHI media for *S.aureus* and TSB media for *P.aeruginosa*)
2. Inoculate bacteria from overnight plate into 10 ml of LB broth in 50 ml Falcon tube and incubate overnight.
3. Measure the OD600 and adjust to 0.01 in culture medium.
4. Take 200 μl of adjusted cell suspension in to wells of a 96 plate. Prepare 16 technical replicates.
5. Incubate at 37 °C for 24 or 48 hrs in a moist chamber (normally a plastic box with wet tissue in it).
6. Biofilm takes one or two days to develop. For inhibition of biofilm, the treatment should be added after plating. For dispersal, treatment should be given after the static biofilm has developed.

Staining

7. Carefully remove the culture with a multichannel pipette.
8. Wash the wells two times with 250 μl PBS or DW.
9. Add 250 μl 0.4% crystal violet and incubate for 10 minutes at RT.
10. Take picture to the adherence to the well wall or the bottom if observed.
11. Carefully wash the wells with distilled water 2-3 times and dry the plate at RT.

Quantification

12. Dissolve the biofilm with 200 μl 30% acetic acid (alternative can be DMSO, however note that the biofilm has to be completely removed from the wall of well for quantitative assessment).
13. Measure OD at 595 nm. (Biofilm 550 nm program at 5th floor, NUNC 96 wells plate). If required, transfer 100 or 200 μl to a new plate for measurement). Measure the raw data (the entire well include the control).

Sources

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182663/
Revised by Shady Kamal and Annika Cimdins in Ute Romling'r research group.