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7.13 Experimental Microbial Genetics

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BIOFILM ASSAY

Materials

- o/n liquid culture
- medium + antibiotic
- 1% crystal violet solution
- 95% ethanol

Method

- 1) Grow overnight cultures of strains to be assayed.
- 2) Add 150ul of medium (+ antibiotic if necessary) to a 96-well U-bottom Falcon plate (Falcon #3911). Inoculate 3ul o/n culture into each well.
NOTE: Leave several wells of medium un-inoculated. These will serve as controls and should be treated exactly as the inoculated wells in the steps below.
- 3) Incubate on a rotating platform for 6-8 hours.
- 4) Discard supernatant and wash with water. This can be done in one the following ways:
 - a. Submerge plate in tub of water. This will simultaneously discard the medium and wash the wells.
 - b. With a pipette, aspirate supernatant from each well, gently wash with 200ul water and aspirate once again. A multichannel pipette can be used if needed.
- 5) Stain the cells that remain attached to the plate with 200ul of a 1% crystal violet solution.
- 6) Incubate on a rotating platform for 15 minutes.
- 7) Pipette off crystal violet and discard. Wash wells once with water. Subsequent washes can be performed one of two ways as above.
NOTE: Washing at this step may be easier with the tub submersion method. Multiple tubs of water are required for each plate due to the strength of the crystal violet dye. Wash plates in successive tubs of fresh water until minimal traces of dye are released after plate submersion.
- 8) Add 200ul 95% ethanol to each well to dissolve the crystal violet. Incubate covered (to avoid ethanol evaporation) for 5 minutes.
- 9) Transfer 100ul to a flat-bottomed 96-well plate and read OD570 (including control wells)

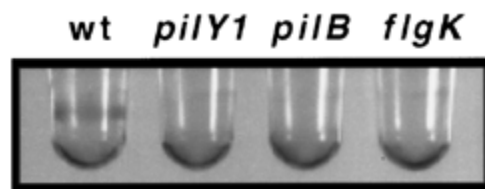


Fig. 1 . Shown is the biofilm formation phenotype of the wild-type *Pseudomonas aeruginosa* PA14 strain and three representative *sad* mutants (*pilY1*, *pilB* and *flgK*).

Figure adapted from: George A. O'Toole & Roberto Kolter. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Molecular Microbiology* Vol. 30, 2 Pages: 295-304