Tissue culture assay to quantify bacterial adhesion to HeLa cells

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1) Grow Hela cells to confluence in T-75 (approximately $8.0X10^6$ cells)

2) Remove media, wash cell monolayer 3X with PBS

3) Remove PBS, add 1ml Trypsin-EDTA

4) After cells have detached, add 2ml DMEM+ 10% FBS w/o antibiotics (This should give approximately $2.67X10^6$ cells/ml).

5) Pipette 9ml DMEM+ 10%FBS, w/o antibiotics into a 15ml centrifuge tube.

6) Add 1ml of cell suspension. (This should yield a total of 10ml at $2.67X10^5$ cell/ml).

7) Plate 5 wells of a 6 well plate (with 4 sterile 12mm glass cover slips in each well) at 2ml per well. At this cell density, the plate should be ready to use the next day.

8) Pipette 19ml DMEM+ 10%FBS, w/o antibiotics into a 50ml centrifuge tube.

9) Add 1ml of cell suspension from step 4. (This should yield a total of 20ml at $1.3X10^5$ cells/ml).

10) Plate 20 wells of a 24 well plate (with 1 sterile 12mm glass cover slip in each well) with 1ml per well. At this cell density, the plate should be ready to use the next day.

11) Grow overnight cultures of *E. coli* and *Erwinia* (or other bacterial species of interest).

12) Pipette 250µl of turbid broth (overnight culture) into 1.5ml centrifuge tubes.

13) Centrifuge at 13,000rpm for 5 minutes.

14) Remove supernatant, and resuspend pellet in 250µl of PBS+Mg.

15) Inoculate each well of both the 6 and 24 well plates with 50µl (for 6 well plate) and 10µl (for 24 well plate), respectively, of cell suspension.

16) Serially dilute cell suspension in PBS to 10^{-6}, and plate 100 µl of the 10^{-5} and 10^{-6} dilutions on BHI agar plates. (i.e., a 10^{-6} and 10^{-7} final dilution).

17) Put the BHI agar plates in a 37^0C incubator overnight.

18) Place the 6 and 24 well plates in a 37^0C incubator with CO_2 for two hours.

19) After 2 hours, remove plates from incubator, remove media from each well, and wash each well containing cover slips 3X with PBS+Mg. After the third wash, add PBS+Mg to each well.

20) Remove cover slips and place in 15ml centrifuge tubes containing 5ml of .1% Triton X100. For the 6 well plates, all 4 coverslips from each well in the 6 well plate are added to one 15 ml tube. For the 24 well plate, all 4 coverslips from the 4 wells inoculated with the same bacterial strains are added to a single 15 ml tube. Thus, each 15 ml tube contains 4 coverslips.

21) Vortex each tube for 30 seconds.

22) Serially dilute the cell lysate to 10^{-3}, then plate 100µl of each dilution on BHI agar plates. Plate the 10^{0} dilution once, plate the other dilutions in duplicate.

23) Put plates in a 37^{0}C incubator overnight.

24) Count colonies and tabulate results.