June 23rd, 2017

Title: The Effect of Fluoride on CHOP on E. coli growth at different concentrations of Chloramphenicol

Expected outcome: If there is the presence of fluoride, then CHOP will grow on Chloramphenicol.

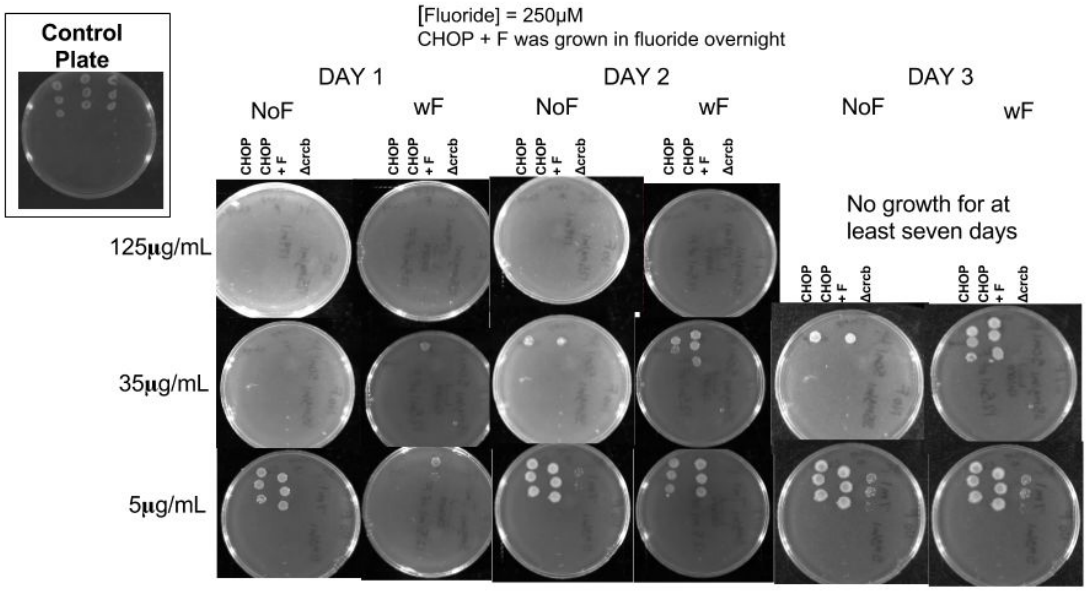
Procedure:

1. Measure growth of bacteria:
    a. Spectrophotometer used to detect bacteria levels
        i. Set the wavelength to 600 nm
        ii. Make a 10-fold dilution of the bacteria (i.e. 1.35mL water 150µL of cells)

        Results: .45 CHOP + F, .48 FLOP, .39 CHOP, .49 CRCB

2. Make serial dilutions:
    a. Add 45µL LB each well
    b. Add 5µL of E. coli to the first well in the 96-well plate
        i. Order of bacteria: CHOP; CHOP + F; ΔCrcB – ΔCrcB; FLOP
    c. Mix 5µL of the top well with the next well down, pipette up and down 5 to 10 times, discard tips, and repeat for each well.
    d. Add 5µL of each well to the agar plates.
    e. Allow plates to grow O/N at 37 C

Results:



Conclusion:

For 5ug/mL of chloramphenicol, both CHOP and delta-crcB grew, while for 125 ug/mL, none of them grew for all three days. However, on 35ug/mL, there was distinct growth between CHOP, CHOP with fluoride, and delta-crcB (which did not grow as it does not have the chloramphenicol resistance gene). This allowed us to determine **35ug/mL** as a reasonable concentration of chloramphenicol for future experiments. This means that our CHOP system is able to provide qualitative data of the difference in growth between the presence of fluoride and the absence of fluoride.

Note: There was an error in the serial dilutions.