Protocol Name: Quantitative Capillary Assay

Category: Chemotaxis

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Source(s):

Swiecicki J-M, Sliusarenko O, & Weibel DB (2013) From swimming to swarming: Escherichia coli cell motility in two-dimensions(). Integrative biology : quantitative biosciences from nano to macro 5(12):1490-1494.

Reyes-Darias JA, García V, Rico-Jiménez M, Corral-Lugo A, Krell Tino (2016) Identification and Characterization of Bacterial Chemoreceptors Using Quantitative Capillary and Gradient Plate Chemotaxis Assays. Bio-protocol 6(8): e1789

Time Required: 3 days

Additional Notes: While this assay did not provide evidence for chemotaxis in our scenario, this may not be true for other species or chemicals

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Materials

LB 50mL

Falcon Tubes

Sterile Pipette Tips

Chemotaxis Buffer (10 mM potassium phosphate, 0.1 mM EDTA, 10 mM glucose)

100µM Naringenin

Sterile Distilled Water

Sterile 96-well plate

Parafilm

1µL Capillary Tubes

1.5ml Eppendorf Tubes

0.9% NaCl Solution

1% LB agar Plates

Procedure

1) Prepare an overnight culture by inoculating 15ml LB in a 50ml falcon tube with a single colony from an agar plate.
    a. Incubate at the appropriate temperature for the species for 12-16 hours
2) Innoculate 15ml of fresh LB in a 50ml falcon tube with 1.0-2.0mL of overnight culture.
3) Incubate at the appropriate temperature until the culture reaches an early stationary phase (approximately OD_{600}= 0.25-0.35)

4) Centrifuge the bacteria for 5 mines at 1,667xg (4°c) to allow a pellet to form. Once formed, decant the supernatant
5) Resuspend the pellet with 4ml of Chemotaxis Buffer
6) Repeat steps 4 and 5
7) Aliquot 250µl of bacterial solution into 6 wells of a 96-well plate
    a. Repeat as necessary for other species
8) Submerge the end of the sterile 1µl capillary tube in 100µM naringenin and Chemotaxis Buffer for 10 minutes
9) Seal the end of the capillary with parafilm (or heat) and place one capillary in each well, with the open end submerged
    a. There should be an equal number of controls and test wells
10) Incubate for 45 minutes at RTP
11) After incubation, remove the capillary from well and wash the end submerged in the bacteria solution with sterile water
12) Dispense the contents of the capillary tube into a Eppendorf tube containing 1ml of 0.9% NaCl
13) Centrifuge the Eppendorf tube for 5-10 seconds to mix the bacteria
14) Spread 50µl of bacterial 0.9% NaCl solution onto one third of a 1% LB agar plate
15) Repeat steps 11-14 with all capillaries, keeping the control and naringenin capillaries on separate agar plates
16) Incubate at appropriate temperature for 24 hours
17) Count $CFU.\mu l^{-1}$ by counting the number of colonies per third
18) Perform statistical analysis as required