Protocol Name: 0.75% Agar Chemotaxis Assay

Category: Chemotaxis

Date:

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Source(s):

Time Required: 2 day preparation + 2 hours + Incubation Period

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Additional Notes: While this assay did not provide evidence for chemotaxis in our scenario, this may not be true for other species or chemicals. Naringenin concentration was likely too high.

Materials

3x Standard Petri Dishes (per species)

0.75% (w/v) LB Agar

5ml 200µM Naringenin

5ml Sterile Distilled Water

1x 50ml Falcon Tube (per species)

15ml LB Broth (per species)

Sterile Pipette Tips

Sterile Inoculation Loops

Procedure

1) Prepare an overnight culture by inoculating 15ml LB in a 50ml falcon tube with a single colony from an agar preserve plate
    a. Incubate at the appropriate temperature for 12-16 hours
2) Microwave the 0.75% LB Agar and allow to cool to 40˚C before pouring
3) After pouring, allow to fully set
4) On the underside, draw the centre line and make two points 15mm either side of the middle of said centre line
5) Add 20µl of 200µM naringenin to the left side mark and 20µl of sterile water to the right.
6) Leave for 12-14 hours at RTP for the concentration gradient to form
7) Add 10µl of overnight culture to the middle of the centre line
8) Incubate for 24 hours at the appropriate temperature
9) Make qualitative observations of bacterial colonies
    a. Eg. Colony shape, whether there is distortion of growth towards one chemical