

Semi-solid agar motility assay

Aim

To access the motility of bacterial species on semi-solid agar

Day 1

Semi-solid agar plate preparation

1. Prepare tryptone broth containing 0.25% agar, selective antibiotic and theophylline at the desire concentration(s) [0mM, 0.5mM, 1.0mM, 1.5mM]
2. Pour the agar into 85mm petri-dishes and allow to cool and set

Inoculation of cultures

Inoculate 4ml selective LB broth with transformed bacteria containing:

- Pr-t1-Ch-V-dt (BBa_K537011)
- Pr-t2-Ch-V-dt (BBa_K537012)
- Pr-t1-V-dt (BBa_K537009)
- Pr-t2-V-dt (BBa_K537010)

Day 2

1. Inoculate 2ml of each of the above cultures into 50ml selective LB broth and grow until mid-log phase ($OD_{600}=0.55$)
2. Plate 1.5 μ l of the transformant into the centre of the agar plate
3. Repeat for all transformants at all concentrations of theophylline
4. Three plates of each theophylline concentration should be inoculated for each transformant
5. Carefully transfer the plates into the 37°C incubator for 24 hour

Day 3

1. Photograph the plates, preferably using a gel dock set to the upper white light. The appearance of a halo around the site of inoculation indicates migration of bacteria from the start position. This distance can be measured in mm and recorded