Lab Log

Daniel Padfield

18/10/2017

- Autoclaved the soil again
- Autoclaved 1 L of DI millicule water
- Autoclaved 1 L of 0.6% soft agar
- Autoclaved a spoon to weigh out soil
- Got some $SBW25\phi2$ from Floh. Around 1 mL at 7.26 x 10^8.
  - Grow up overnight
  - Transfer 60 µl of bacteria and 10 µl of phage (around 7 x 10 ^{6} pfus) into 6 mL of KB agar
  - Should give a concentration of around 10^8 phage/mL
  - Done this in triplicate
- Grow up *lacZ* and *WT* strains overnight. Should give concentration of around ~10^8 cells in 60 µl.
- Do these in triplicate
- Added 60 µL of frozen overnight culture from first experiment (18/08/2017 *lacZ* and WT)

Retrospectively work out density of the overnight stocks and phage

19/10/2017

- Put 80g of soil into each 10cm x 10cm microcosm
  - Used autoclaved spoon
  - Placed scale in laminar flow hood (cleaned with ethanol before and after)
- Placed 5 mL (~ 200 µl per microcosm) of *lacZ* and WT into separate 12 mL centrifuge tubes
  - Centrifuged for 15 minutes at max speed (~4500 r.p.m) on big centrifuge
  - Want to get to 5 mL per microcosm for inoculating (~ 125 mL in total)
  - Resuspended pellet into 2250 µl, vortexed and placed 620 µl, 620 µl and 810 µl into three different falcon tubes
  - Filled these three falcon tubes up to 40 mL, 40 mL and 45 mL respectively
    - This guaranteed the same concentration of sample in each falcon tube
  - Placed 5 mL of *lacZ* or WT strain into each microcosm
  - Froze (-80 ºC) 900 µl of inoculate in 900 µl of glycerol (25% final concentration)
- In the no phage treatments, we added 5 mL of M9
- Added 5 mL of phage to phage treatments
  - Place 900 µl of bacteria + phage into three centrifuge tubes
  - Add 100 µl (10%) chloroform into each tube (under fume hood)
  - Vortex rigorously
  - Centrifuge for 2-3 minutes at full speed (minifuge)
  - Take out supernatant and placed in a single tube (took out 800 µl of each tube)
  - Put 40 mL of M9 into 6 tubes
  - Added 400 µl into each tube (~100 fold dilution from the initial stock)
  - Shake each tube and add 5 mL into each microcosm
- Place microcosms into the 26 ºC incubator (Level 1 incubator room)

22/10/2017

- setup 2 WT microcosms up for spot assays of phage
- used a crystal from T_0
- autoclaved 2 L of KB agar. Put in small autoclave

23/10/2017

- phage spot test 10_{-1} to 10_{-8}
  - Floh's phage (7.26 x 10^8)
  - Phage we inoculated with
  - Extra phage tube (should have lower concentration)
  - blank plate
- plated T_0 of WT and *lacZ P. fluorescens*

24/10/2017

- phage spot test was no good (streaky)
- likely that we did not wait long enough for the spot to dry
- set up more T_0 bacteria overnight

25/10/2017

- phage spot assay again
- plating only 4 spots per plate instead of 8
- counted T_0 counts (plated 30 µl)
  - *lacZ* @ 10^{-4}: 18
  - WT @ 10^{-4}: 64

26/10/2017

- check phage spot, 10^{-6} looks to be the correct dilution
- further counts are done by putting the 10 µl phage into the 1% bacteria soft agar
  - vortex and plate
  - leave to dry
  - incubate overnight

30/10/2017

- grow up some T_0 bacteria overnight

31/10/2017

- serial dilution of phage to 10^{-6}
- 50 µl bacteria + 10 µl phage + 5 ml soft agar
  - vortex
  - pour
- set samples up for inoculated phage, floh's phage stock and our phage dregs (each in triplicate)
- in 28 ºC incubator overnight