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 ^{o}C incubator overnight

01/11/2017

- sampled all soil microcosms (after 13 days)
  - ~2g of soil into a 12 mL centrifuge tube (with ~ 7 glass beads)
    - sample using the big end of a 5 mL pipette and weigh on scales under hood
  - add 10 mL of M9

  - vortex for ~ 1 minute
  - add 900 µl of sample to 900 µl of 50% glycerol and freeze at -80 ºC (bacteria + phage)
  - make phage suspension for each tube
    * add 900 µl of sample to centrifuge tube
    * add 100 µl of chloroform under fume hood
    * vortex
    * centrifuge at full speed for 4 minutes
    * put supernatant into separate tube
      · samples 1-17 800 µl, samples 18-48 750 µl
- names of samples
- 1-12: WT no phage
- 13-24: *lacZ* no phage
- 25-36: WT + phage
- 37-48: *lacZ* + phage
- Autoclaved things
  - 2 empty 500 mL bottles
  - M9 salts x10 (500 mL)
    * converted the weight of Na_2HPO_4 to the amount of $Na_2HPO_4.7H_2O$ needed.
    * MW of $Na_2HPO_4.7H_2O$ is 268. MW of Na_2HPO_4 is 142.
    * to convert from weight of Na_2HPO_4 to $Na_2HPO_4.7H_2O$ is to multiply by $\frac{268}{142}$
  - 1 L of DI water
  - 2 L hard agar
  - centrifuge tubes.

sample	weight
1	2.1
2	2.0
3	1.9
4	2.0
5	2.0
6	2.0
7	2.0
8	2.0
9	2.1
10	1.9
11	2.2
12	2.1
13	2.1
14	1.9
15	2.2
16	2.2
17	1.9
18	2.2
19	2.0
20	2.3
21	2.3
22	2.2
23	1.9
24	2.1
25	2.1
26	2.2
27	1.9
28	2.0
29	2.2

sample	weight
30	2.3
31	2.0
32	2.2
33	2.2
34	2.2
35	2.0
36	2.0
37	2.3
38	1.9
39	2.2
40	1.9
41	2.2
42	2.0
43	2.0
44	2.2
45	2.1
46	2.2
47	2.2
48	2.1

02/11/2017

- set up phage spot tests of all soil phage suspensions against ancestral WT on soft agar.
  - just checking for presence/absence of phage
  - 5 mL per plate, 4 spots per plate, 12 spots in total
  - important to let the spot dry before moving
- poured plates (all Xgal)