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

- looked at phage spots (10^{-6} dilution)
- calculated how many phage are there

$$Phage\ concentration = \frac{number\ of\ plaques}{dilution\ factor\ \times\ volume added}$$

Volume added is almost always 10 µl. I wrote a mini function to calculate this

```
d <- data.frame(dregs = c(3,4,6), phage = c(6,2,4))

# back calculate number in there
# PFU/ml = plaque number / (dil fac * volume added)
plaq_num <- function(x, dilfac){return(x/(dilfac*0.01))}

d <- dplyr::mutate_at(d, c('dregs', 'phage'), plaq_num, dilfac = 10^-6)

knitr::kable(d)
```

dregs	phage
3e+08	6e+08
4e+08	2e+08
6e+08	4e+08

```
# concentration of phage added
mean(d$phage)
```

```
## [1] 4e+08
```

```
# concentration of other phage
mean(d$dregs)
```

```
## [1] 433333333
```

- 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_g
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
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)

03/11/2017

- all no phage treatments had no phage and all phage treatments had phage
- plated all the bacteria + phage treatments at 10^{-4} and 10^{-5} (30 µl)
  - left on bench over the weekend

06/11/2017

- counted all the plates
  - all still had bacteria in and were not obviously contaminated (Yays)
- picked 20 colonies from each sample and placed in KB media in 48 well plates
  - 750 µl of KB media
  - used matchsticks
- grown statically for 2 days at 26 ºC

08/11/2017

- put 500 µl of 50% glycerol into each well (final concentration 20%)
- froze in the -80 ºC
- autoclaved
  - 2x 800 mL hard agar
  - 2x 800 mL soft agar

13/11/2017

- prep for sampling soil microcosms tomorrow. Labelled samples. Booked downstairs hood with power access.

14/11/2017

- sampled all 48 microcosms
- froze 900 µl of bacteria + phage in 900 µl of 50% glycerol (final concentration equals 25%)
- phage sample in fridge
  - 1-12: 750 µl
  - 13-48: 600 µl
- setup soft agar plates of ancestral bacteria and did phage spot assays

sample	weight_g
1	2.2
2	2.2
3	2.3
4	2.1

sample	weight_g
5	2.2
6	2.1
7	2.1
8	2.0
9	2.0
10	2.0
11	2.2
12	2.1
13	2.0
14	2.2
15	2.2
16	2.0
17	2.1
18	2.3
19	2.0
20	2.0
21	2.1
22	2.0
23	2.1
24	2.2
25	2.0
26	2.0
27	2.2
28	2.0
29	2.0
30	2.0
31	2.3
32	2.1
33	2.2
34	2.2
35	2.1
36	2.1
37	2.1
38	2.3
39	2.0
40	2.0
41	2.3
42	2.0
43	2.2
44	2.2
45	2.0
46	2.2
47	2.0
48	2.0

15/11/2017

- all phageless samples are still phageless, all phage samples still contain phage

16/11/2017

- plated all T_2 samples
  - 10^{-3} and 10^{-4}
  - 30 µl of on plate
- **used undiluted M9 x10 on Tuesday!!!**
- made up 400 mL of M9 salts x10 for autoclave

20/11/2017

- counted plates.
  - Most are ok at 10^{-3}. Counts were much lower. Want to be certain that the reduction in abundance is not due to using concentrated M9.
- full sample again, T_3.
  - Took 700 µL samples of phage
- setup overnight stock of ancestral bacteria
- autoclaved 2400 mL of KB agar

sample	weight_g
1	2.0
2	2.0
3	1.9
4	2.3
5	2.0
6	2.2
7	2.0
8	2.3
9	2.0
10	2.1
11	2.2
12	2.1
13	2.0
14	2.1
15	2.0
16	2.3
17	2.3
18	2.1
19	2.2
20	2.0
21	2.1
22	2.1
23	2.1
24	2.2
25	2.1
26	2.0
27	2.2
28	2.1
29	2.1
30	2.0
31	2.2
32	2.0
33	2.1
34	2.1

sample	weight_g
35	2.2
36	2.2
37	2.0
38	2.1
39	2.1
40	2.0
41	2.1
42	2.2
43	2.0
44	2.3
45	2.2
46	2.1
47	2.1
48	2.0

21/11/2017

- plated all 48 T_3 samples at 10^{-2} and 10^{-3}
- re-plated all T_2 replicates that did not contain > 20 colonies at 10^{-2}
- poured ~150 Xgal plates. Stored in the cold room
- autoclaved two boxes of ependorfs
- put beads in Virkon to clean
- phage spot assay on phage extractions from T_3. Used ancestral bacteria as the bacterial lawn

23/11/2017

- all T_3 samples were ok (> 30 colonies at 10^{-3}) apart from 12 and 37
  - 12 an 37 were re-plated at 10^0 and 10^{-1}
- picked 20 colonies from each sample and placed in KB media in 48 well plates
  - 750 µl of KB media
  - used matchsticks
- grown statically for 2 days at 26 ºC
- Checked phage spot assays

25/11/2017

- picked colonies from 12 (10^0) and 37 (10^{-1})
  - incubate for two days
- put 500 µl of 50% glycerol into each well of the other replciates that had been growing for two days (final concentration 20%)
- froze in the -80 ºC

27/11/2017

- autoclaved 4L of KB hard agar

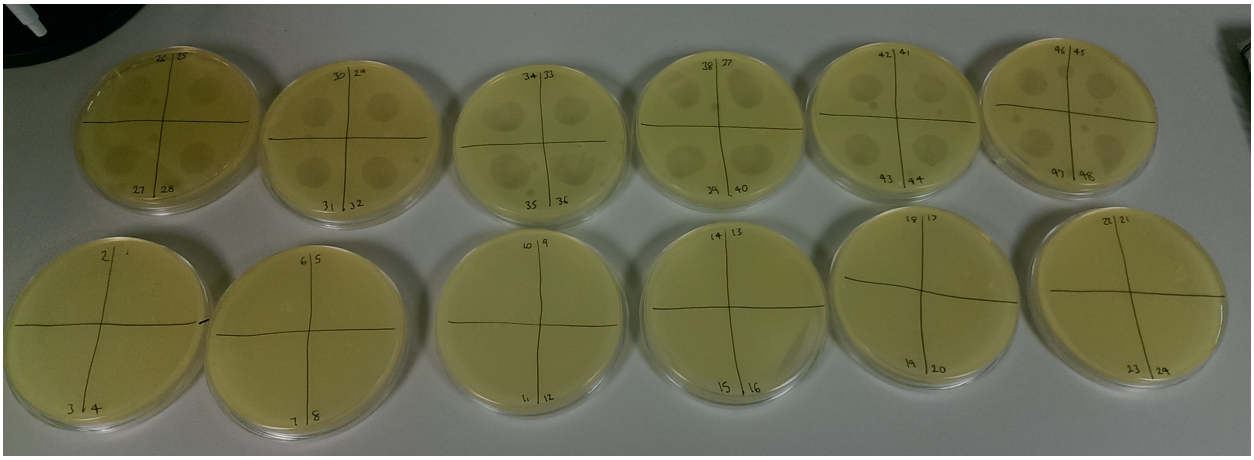

Figure 1: **Figure 1. phage spot assays are on point**

28/11/2017

- poured 2L of KB agar into square plates (Xgal)
- put 180 µl of KB media into 96 well plates
- grew T_F clones up overnight from frozen isolates (phage present microcosms only, 25-48)
  - placed in 28 ^oC incubator

29/11/2017

- look at coevolution of phage and host using streak plates
- streak each clone against contemporary phage, ancestral phage, a paired WT phage and a paired *lacZ* phage
- spot 30 µl of each phage onto a square plate, let run down the plate then let it dry
- 1 plate per microcosm
- streak clones across the line of phage using matchsticks and the template

30/11/2017

- looked like it didnt work
- everything grew everywhere, even the controls
- set up an ancestral overnight

01/12/2017

- set up some phage spot assays of random phage against the ancestral phage
- did some streak assays

02/12/2017

- phage streak worked
- all phage spots worked
- **current hypothesis of problem is that we did not mix the phage properly**

05/12/2017

- grew up all clones overnight
- poured lots of square plates

06/12/2017

- did another phage streak day

08/12/2017

- counted the phage streaks. Processed these but they do not look as expected.
- autoclaved 4 L of hard KB agar
- autoclaved 1 L of KB media

10/12/2017

- setup isolated clones overnight in 180 µl of KB media
- poured many square plates
- amplifying T_F phage in ancestral WT overnight
  - 60 µl of ancestor and 10 µl phage
- setup some phage spot assays (P.27, P.38, P.41)
  - spotted 10 µl from 10^{-1} to 10^{-8}

11/12/2017

- Unfortunately some of the lids of the phage amplifications had come off
- Reset these today
- Plated all the phage with ancestral bacteria at 10^{-3}

12/12/2017

- Counted phage

13/12/2017

- Did phage streaks using comb

14/12/2017

- Marked phage streaks

03/01/2018

State of play - It is worrying that there is not fluctuating selection. That there is no variation in resistance across phages - Resistance to ancestral phage by communities without phage has never been observed

Options - Do phage spot tests on some replicates to see if same results hold (whether it is the test) - Re-isolate some replicates - Test resistance of ancestral clones - Test presence of phage in no phage treatments with larger phage volume - Test resistance after T_1

- Am going to use 4 communities for the tests here.
  - 1, 10 (controls that had resistance)
  - 37, 42 communities that had resistance
- Grow each clone up overnight in 200 µl of KB media
- Grow Ancestral clone up overnight in KB media

04/01/2017

- Phage spot tests on community 1, 10, 37 and 42
- 1 and 10 are controls that showed resistance. Phage these are against are ancestral and their own phage
- 37 went against ancestral phage, contemporary phage and phage 38 and phage 25
- 42 went against ancestral phage, contemporary phage and phage 41 and phage 30
- 7.5 µl spots were used
- put in all ~200 µl of culture into 5 mL of soft agar
- placed at 28 ºC overnight
- looked at whether there is phage in the controls just at low concentration
  - put 100 µl of bacteria (WT) and 100 µl of "phage" (1 and 10) in 5 mL of soft agar
- plated WT (10^{-4} and 10^{-5})
- plated 1, 10, 37 and 42 to re-isolate clones (from T_2)
- plated 30 µl as always