

Lab Log

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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* from Floh. Around 1 mL at 7.26×10^8 .
 - Grow up overnight
 - Transfer 60 μ L of bacteria and 10 μ L of phage (around 7×10^6 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 $\sim 10^8$ cells in 60 μ L.
- Do these in triplicate
- Added 60 μ L 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 μ L 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 μ L, vortexed and placed 620 μ L, 620 μ L and 810 μ L 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 $^{\circ}$ C) 900 μ L of inoculate in 900 μ L 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 μ L of bacteria + phage into three centrifuge tubes
 - Add 100 μ L (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 μ L of each tube)
 - Put 40 mL of M9 into 6 tubes
 - Added 400 μ L 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 $^{\circ}$ C 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 (7.26×10^8)
 - Phage we inoculated with
 - Extra phage tube (should have lower concentration)
 - blank plate
- plated T₀ 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 T₀ bacteria overnight

25/10/2017

- phage spot assay again
- plating only 4 spots per plate instead of 8
- counted T₀ counts (plated 30 µl)
 - *lacZ* @ 10⁻⁴: 18
 - WT @ 10⁻⁴: 64