Pseudomonas Epiphytic Growth Overview

2024-07-12

Contents

Project Goals

Obtain data on epiphytic growth ability of different *Pseudomonas* strains to compare with their virulence in aphids.

Progress

Strains (from virulence assay)

Strain	Person	Reps
B728a	Sara	3
205	Sara	3
215	Sara	3
216	Sara	3
220	Sara	3
227	Sara	3
228	Sara	3
194	Havi	3
204	Havi	3
14	Havi	3
6	Havi	3
200	Havi	3
221	Havi	3

Protocol

Date Updated: 2023 November 24

Day -3: Plating

1. From -80°C/current plate, plate *Pseudomonas* strains/individual colonies on KB + NA (15 g/mL) + NFT (50 g/mL) plates.
2. Incubate for 24-72 hours at 28°C.

Day 0: Overnights of *Pseudomonas*

1. Pipette 10 mL of KB + NA (15 g/mL) + NFT (50 g/mL) media in 15 mL culture tube.

   - *Note: Make 1 tube/pot and don't forget a blank!*

2. Transfer a colony of *Pseudomonas* from plate to media.
3. Incubate in benchtop shaker at 28C overnight (~18 hrs).

Day 1: Spraying

1. Transfer overnight *Pseudomonas* culture from culture tube to falcon tube.
2. Pellet cells by centrifuging for 10 min on max speed
3. Discard supernatant.
4. Add 1 mL 10mM MgCl2 buffer and resuspend/vortex.
5. Pellet and discard supernatant again.
6. Add ~25 mL 10mM MgCl2 buffer and resuspend/vortex.
7. Transfer 100 ul of sample to a blank cuvette.
8. Measure OD of bacterial resuspension (include blank!).

   - Goal: OD = 0.2

9. Transfer 20 mL of bacterial resuspension into autoclaved spray bottle.

   - Clean tops using ethanol and sterile water.

10. Retrieve 14 day old plants from plant room and place in biosafety cabinet.
11. Spray ~20 mL bacterial solution onto all the leaves and all plant surfaces (stem, top, bottom) of pot until runoff (starting to drip).
12. Allow plant to dry in the biosafety cabinet.
13. Move plant to tent downstairs and note time.
14. Incubate for 72 hours in a tent at 70F and 85% humidity.

Day 4: Sampling

1. Retrieve plants from plant room and place in biosafety cabinet.
2. Sterilize cork borer and forceps using ethanol flame and place in a sterile petri dish in biosafety cabinet.
3. Take 10 samples from all over each plant in the pot.

   - Place all samples from a plant in a single falcon tube, 10 discs/tube.

     - Will end with end with 3 falcon tubes and 30 samples if sampling 3 plants (1 pot).

   - Add 10 mL of 10mM MgCl2 buffer to falcon tubes.

4. Sonicate falcon tubes for 10 min.
5. Vortex to dislodge epiphytic bacteria.
6. Make serial dilutions (Undiluted aka UD, 10^{-1}, and 10^{-2}) in 96 well plate using multichannel pipette.

- 450 uL buffer and 50 uL sample/dilution.

7. Plate 100 ul of each serial dilutions on KB + NA (15 ug/mL) + NFT (50 ug/mL) plates (2 technical replicates/dilution).

    - 6 plates per plant, 18 plates per pot

8. Allow plates to dry then flip upside and incubate at 28C for 24 hrs.

Day 5: Counting

1. Photograph plates.
2. Count colonies on each plate and note in spreadsheet.
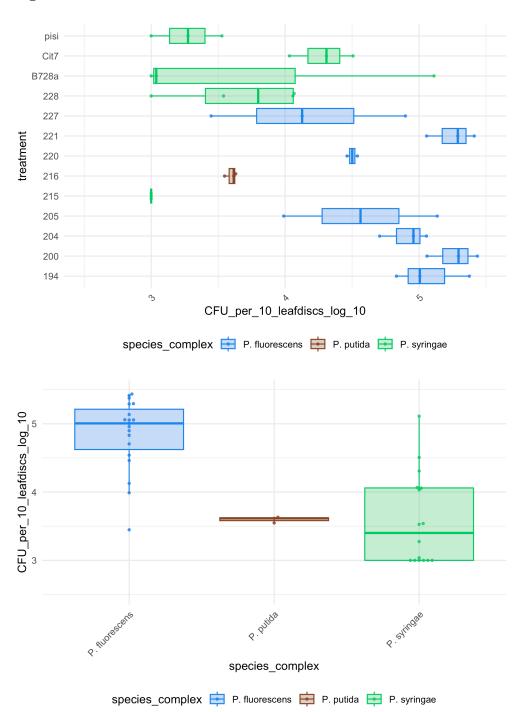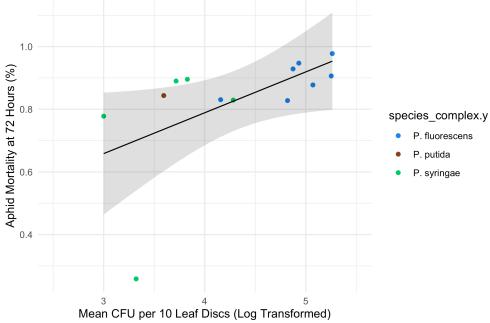3. Calculate CFU/10 mL bacterial resuspension (ie. per sample).

Results

Raw Spreadsheet

sample	person	block	treatment	plant_rep	CFU_per_10_leafdiscs	CFU_per_10_leafdiscs_log_10
194-A1	Havi	2-1	194	A1	4.00E+04	4.602059991
194-A2	Havi	2-1	194	A2	5.70E+04	4.755874856
194-A3	Havi	2-1	194	A3	1.37E+05	5.135132651
194-B1	Havi	2-1	194	B1	6.00E+04	4.77815125
194-B2	Havi	2-1	194	B2	1.46E+05	5.164352856
194-B3	Havi	2-1	194	B3	1.19E+05	5.074633618
194-C1	Havi	2-2	194	C1	2.30E+05	5.361727836
194-C2	Havi	2-2	194	C2	5.05E+05	5.703291378
194-C3	Havi	2-2	194	C3	1.15E+05	5.06069784
200-A1	Havi	2-6	200	A1	1.39E+05	5.142389466
200-A2	Havi	2-6	200	A2	1.39E+05	5.143951116
200-A3	Havi	2-6	200	A3	3.95E+05	5.596597096
200-B1	Havi	2-6	200	B1	3.00E+05	5.477121255
200-B2	Havi	2-6	200	B2	3.90E+05	5.591064607
200-B3	Havi	2-6	200	B3	1.28E+04	4.108057374
200-C1	Havi	2-6	200	C1	6.10E+05	5.785329835
200-C2	Havi	2-6	200	C2	1.95E+05	5.290034611
200-C3	Havi	2-6	200	C3	1.70E+05	5.230448921
204-A1	Havi	2-3	204	A1	8.90E+03	3.949390007
204-A2	Havi	2-3	204	A2	4.60E+05	5.662757832
204-A3	Havi	2-3	204	A3	1.83E+05	5.261262869
204-B1	Havi	2-2	204	B1	5.20E+03	3.716003344
204-B2	Havi	2-2	204	B2	NA	NA
204-B3	Havi	2-2	204	B3	4.95E+05	5.694605199
204-C1	Havi	2-2	204	C1	9.70E+04	4.986771734
204-C2	Havi	2-2	204	C2	1.14E+05	5.056904851
204-C3	Havi	2-2	204	C3	1.33E+05	5.122215878
205-A1	Sara	3	205	A1	1.10E+05	5.041392685
205-A2	Sara	3	205	A2	1.70E+05	5.230448921
205-A3	Sara	3	205	A3	NA	NA
205-B1	Sara	4	205	B1	<1.00E+03	<3
205-B2	Sara	4	205	B2	3.10E+04	4.491361694
205-B3	Sara	4	205	B3	3.00E+04	4.477121255
205-C1	Havi	2-8	205	C1	6.75E+05	5.829303773
205-C2	Havi	2-8	205	C2	1.80E+05	5.255272505
205-C3	Havi	2-8	205	C3	4.65E+05	5.667452953
215-A1	Sara	3	215	A1	<1.00E+03	<3
215-A2	Sara	3	215	A2	<1.00E+03	<3
215-A3	Sara	3	215	A3	<1.00E+03	<3
215-B1	Sara	4	215	B1	<1.00E+03	<3
215-B2	Sara	4	215	B2	<1.00E+03	<3
215-B3	Sara	4	215	B3	<1.00E+03	<3
215-C1	Havi	2-9	215	C1	<1.00E+03	<3
215-C2	Havi	2-9	215	C2	<1.00E+03	<3
215-C3	Havi	2-9	215	C3	<1.00E+03	<3
216-A1	Sara	3	216	A1	2.20E+04	4.342422681
216-A2	Sara	3	216	A2	2.00E+03	3.301029996
216-A3	Sara	3	216	A3	<1.00E+03	<3
216-B1	Sara	4	216	B1	NA	NA
216-B2	Sara	4	216	B2	4.70E+03	3.672097858
216-B3	Sara	4	216	B3	3.90E+03	3.591064607
216-C1	Sara	7	216	C1	4.70E+03	3.672097858
216-C2	Sara	7	216	C2	1.50E+04	4.176091259
216-C3	Sara	7	216	C3	<1.00E+03	<3
220-A1	Sara	5	220	A1	4.00E+04	4.602059991
220-A2	Sara	5	220	A2	3.60E+04	4.556302501
220-A3	Sara	5	220	A3	2.90E+04	4.462397998
220-B1	Sara	6	220	B1	3.00E+05	5.477121255
220-B2	Sara	6	220	B2	NA	NA
220-B3	Sara	6	220	B3	2.80E+03	3.447158031
220-C1	Sara	7	220	C1	3.30E+05	5.51851394
220-C2	Sara	7	220	C2	4.70E+05	5.672097858
220-C3	Sara	7	220	C3	NA	NA
221-A1	Havi	2-7	221	A1	3.45E+05	5.537819095
221-A2	Havi	2-7	221	A2	3.50E+05	5.544068044
221-A3	Havi	2-7	221	A3	6.10E+04	4.785329835
221-B1	Havi	2-7	221	B1	2.10E+05	5.322219295
221-B2	Havi	2-7	221	B2	3.60E+05	5.556302501
221-B3	Havi	2-7	221	B3	1.95E+04	4.290034611

221-C1	Havi	2-7	221	C1	1.21E+05	5.080987047
221-C2	Havi	2-7	221	C2	2.20E+05	5.342422681
221-C3	Havi	2-7	221	C3	6.50E+05	5.812913357
227-A1	Sara	5	227	A1	2.20E+04	4.342422681
227-A2	Sara	5	227	A2	<1.00E+03	<3
227-A3	Sara	5	227	A3	<1.00E+03	<3
227-B1	Sara	8	227	B1	8.70E+04	4.939519253
227-B2	Sara	8	227	B2	2.50E+03	3.397940009
227-B3	Sara	8	227	B3	1.11E+04	4.043362278
227-C1	Sara	8	227	C1	1.44E+05	5.158362492
227-C2	Sara	8	227	C2	4.70E+05	5.672097858
227-C3	Sara	8	227	C3	7.30E+03	3.86332286
228-A1	Sara	5	228	A1	4.20E+04	4.62324929
228-A2	Sara	5	228	A2	<1.00E+03	<3
228-A3	Sara	5	228	A3	3.80E+04	4.579783597
228-B1	Sara	6	228	B1	NA	<3
228-B2	Sara	6	228	B2	2.30E+04	4.361727836
228-B3	Sara	6	228	B3	6.50E+04	4.812913357
228-C1	Sara	7	228	C1	NA	#VALUE!
228-C2	Sara	7	228	C2	1.20E+04	4.079181246
228-C3	Sara	7	228	C3	<1.00E+03	<3
228-D1	Sara	8	228	D1	NA	NA
228-D2	Sara	8	228	D2	NA	NA
228-D3	Sara	8	228	D3	<1.00E+03	<3
B728a-A1	Sara	4	B728a	A1	3.60E+05	5.556302501
B728a-A2	Sara	4	B728a	A2	2.40E+04	4.380211242
B728a-A3	Sara	4	B728a	A3	2.50E+05	5.397940009
B728a-B1	Sara	8	B728a	B1	<1.00E+03	<3
B728a-B2	Sara	8	B728a	B2	<1.00E+03	<3
B728a-B3	Sara	8	B728a	B3	<1.00E+03	<3
B728a-C1	Sara	8	B728a	C1	<1.00E+03	<3
B728a-C2	Sara	8	B728a	C2	1.30E+03	3.113943352
B728a-C3	Sara	8	B728a	C3	1.00E+03	3
Cit7-A1	Havi	2-3	Cit7	A1	1.30E+04	4.113943352
Cit7-A2	Havi	2-3	Cit7	A2	2.70E+04	4.431363764
Cit7-A3	Havi	2-3	Cit7	A3	9.45E+04	4.975431809
Cit7-B1	Havi	2-3	Cit7	B1	1.65E+03	3.217483944
Cit7-B2	Havi	2-3	Cit7	B2	3.65E+05	5.562292864
Cit7-B3	Havi	2-3	Cit7	B3	1.40E+04	4.146128036
Cit7-C1	Havi	2-4	Cit7	C1	4.00E+03	3.602059991
Cit7-C2	Havi	2-4	Cit7	C2	1.20E+04	4.079181246
Cit7-C3	Havi	2-4	Cit7	C3	2.60E+04	4.414973348
pisi-A1	Havi	2-4	pisi	A1	1.75E+04	4.243038049
pisi-A2	Havi	2-4	pisi	A2	<1.00E+03	<3
pisi-A3	Havi	2-4	pisi	A3	2.55E+03	3.40654018
pisi-C1	Havi	2-5	pisi	C1	1.38E+05	5.138302698
pisi-C2	Havi	2-5	pisi	C2	<1.00E+03	<3
pisi-C3	Havi	2-5	pisi	C3	<1.00E+03	<3
pisi-A1	Havi	2-10	pisi	A1	<1.00E+03	<3
pisi-A2	Havi	2-10	pisi	A2	<1.00E+03	<3
pisi-A3	Havi	2-10	pisi	A3	<1.00E+03	<3
pisi-B1	Havi	2-10	pisi	B1	<1.00E+03	<3
pisi-B2	Havi	2-10	pisi	B2	<1.00E+03	<3
pisi-B3	Havi	2-10	pisi	B3	<1.00E+03	<3
pisi-C1	Havi	2-10	pisi	C1	<1.00E+03	<3
pisi-C2	Havi	2-10	pisi	C2	3.25E+03	3.511883361
pisi-C3	Havi	2-10	pisi	C3	3.30E+03	3.51851394

Figures

Pearson's Correlation Coefficient: 0.56