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Protocol status: Working
 We use this protocol and it's working

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Pseudomonas syringae seed infections

In 1 collection

William Rooney¹, Janet Laird (University of Glasgow)¹,
 Mechna Chowdhury (University of Glasgow)¹,
 Catriona MacIntosh (University of Glasgow)¹,
 Xiaoyi Deng (University of Glasgow)¹,
 Phoebe McBride (University of Glasgow)¹,
 Joel Milner (University of Glasgow)¹

¹University of Glasgow

William Rooney: will.rooney@glasgow.ac.uk
 Janet Laird (University of Glasgow): Janet.Laird@glasgow.ac.uk
 Joel Milner (University of Glasgow): Joel.Milner@glasgow.ac.uk

William Rooney
 University of Glasgow

DISCLAIMER

This protocol is adapted from:

Dutta B, Gitaitis R, Smith S, Langston D Jr (2014). Interactions of Seedborne Bacterial Pathogens with Host and Non-Host Plants in Relation to Seed Infestation and Seedling Transmission. PLOS ONE 9(6): e99215. <https://doi.org/10.1371/journal.pone.0099215>

ABSTRACT

Seed contamination represents a critical route for the transmission of bacterial plant diseases. Here we have created a step-by-step protocol for *in vitro* seed infections of *Pseudomonas syringae*. This protocol provides a robust method for *P. syringae* infection in cucumber. This synthetic seed infection assay is generally applicable for *P. syringae* as we have successfully adapted it for both tomato and soybean.

Keywords: bacterial infection, seed infection protocol, pseudomonas syringae, seed science, plant biology

GUIDELINES

This protocol is generally applicable and has been adapted for tomato and soybean. The variables we changed when optimizing for other seed varieties was the seed weight and the volume of inoculation buffer. Please note the characteristics of the seeds you are using - big or small, hard or soft etc.

MATERIALS

All materials are mentioned in the main methods. Having a vacuum pump and a chamber is critical to this success of these experiments

SAFETY WARNINGS

Eliminate biological waste using appropriate protocols. Always wear appropriate personal protective equipment when performing experiments.

BEFORE START INSTRUCTIONS

This protocol involves using sterile plastic, glassware and mortar and pestles. All components were either autoclaved and wiped down with ethanol before starting the protocol. Pipettes are wiped down with ethanol before use.

Overnight culture

- 1 Prepare a 5 mL Kings Broth liquid culture (20 gL⁻¹ peptone 10 gL⁻¹ glycerol, 1.5 gL⁻¹ K₂HPO₄, 1.23 gL⁻¹ MgSO₄ pH 7.2)
- 2 Inoculate a single colony of *Pseudomonas syringae* into a 5 mL liquid culture and incubate overnight in a shaking incubator for 16-18 hours, 28°C at 180-200 rpm.

Sterilising seeds

- 3 Transfer seeds to a sterile tube. For cucumber seeds used in this protocol, a 50 mL conical

centrifuge tube will suffice. This protocol can be adapted for bigger and smaller seeds.

4 Wash seeds in a 75% ethanol solution for 2 minutes and decant the ethanol.

5 Add the sterilisation solution (50% bleach (v/v water), 0.002% tween 20) and mix by inversion for 8-10 minutes. Decant the sterilisation solution and wash seeds in water to remove residual bleach.

5.1

Discolouration of blue roll indicates presence of residual bleach decreases with subsequent washes in water.

6 Once the bleach is no longer present transfer seeds into a sterile conical flask

Seed infection

7 Centrifuge the overnight culture using a bench top centrifuge 4,000 g for 15 mins

8 Re-suspend the pellet in sterile 10 mM MgCl_2 buffer and adjust the OD_{600} to 0.2. An OD_{600} is

approximately $\sim 1 \times 10^8$ colony-forming units per mL (cfu mL⁻¹)

- 9 Dilute the bacterial inoculate by 1/100 ($\sim 1 \times 10^6$ cfu mL⁻¹) and pour into the conical flask containing the seeds. Ensure the seeds are immersed in the inoculate.

9.1

Cucumber seeds in a conical flask containing the bacterial inoculate

- 10 Place the flask into a bell jar attached to a vacuum pump. Turn on the vacuum pump and allow the seeds to experience -1 atmospheres of pressure for 30 mins.

Equipment

Vacuum pump

NAME

BRAND

SKU

10.1

Set up

10.2

Vaccum pump

- 11 Switch off the pump, release pressure immediately and leave seeds for 5 minutes to settle.
- 12 Remove the flask and decant the inoculate, transfer seeds onto the sterile blue roll and remove excess moisture.
- 13 Wet some blue roll in a petri dish with sterile water and place the seeds on top of the blue roll.

13.1

- 14** At this point, you can take a sample for a 0-hour time point or incubate seeds in a growth chamber. For our purposes, the growth chamber conditions we used were: 16 h light/8 h dark (at 26 and 22 °C, respectively) and at a light level of 80 $\mu\text{mol}/\text{m}^2/\text{s}$.

Measuring bacterial load in seeds

- 15** Take note of the sample weight grind using a sterile mortar and pestle.
- 16** Dilute sample in 1000 μL of sterile 10 mM MgCl_2 and continue grinding.

17 Next transfer the "grindate" to a microcentrifuge tube.

Top tip: cutting the tip of the pipette tip will make it easier to transfer

18 Let the insoluble material float to the bottom and use the supernatant to measure bacterial load by spotting 10 µL onto Kings Broth agar plates.

Top tip: If you are recovering too few bacteria you can increase the volume of supernatant used on the plate. Conversely, if you have too much serially diluted the bacterial (1:10).

19 To calculate bacterial load:

bacterial load = [colony-forming units on plate] x [dilution factor] / [seed weight]

Example:

5 [colony forming units] x 1,000 [dilution factor] / 250 mg = 20 colony forming units/mg of tissue.