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Pseudomonas syringae seed infections

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bhwhj7b6

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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.

DOI

dx.doi.org/10.17504/protocols.io.bhwhj7b6

PROTOCOL CITATION

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KEYWORDS

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

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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 TEXT

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

SAFETY WARNINGS

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

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

BEFORE STARTING

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} K_2HPO_4, 1.23 gL^{-1} $MgSO_4$ 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.

Sterlising 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.

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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 ~ 1 x 10^8 colony-forming units per mL (cfu mL^{-1})

9 Dilute the bacterial inoculate by 1/100 (~1 x 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.

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Vacuum pump

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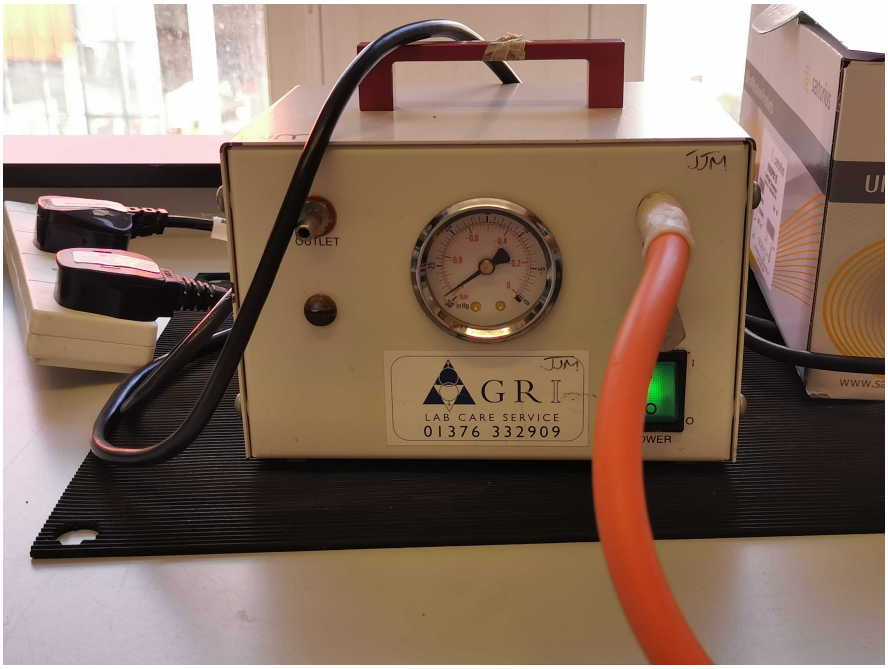
10.1

Set up

10.2



Vaccum pump

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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 µmol/m^2/s.

Measuring bacterial load in seeds

15 Take note of the sample weight grind using a sterile mortar and pestle.

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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.

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