DIP GROWTH CURVE PROTOCOL

Kunkel Lab

1. Inoculate plants by dipping the plants into bacterial suspensions containing 0.02% Silwet-L77 (a wetting agent). The bacterial solution should contain ~5 x 10^8 cells/ml (an OD_{600} of approximately 0.5). The inoculated plants should then be kept under a dome overnight. You will be able to see and record disease symptoms 3-5 days after inoculation.

2. Obtain 1 micro centrifuge tube for each sample you wish to take, and weigh them on an analytical balance. Record the weight on the tube or in your notebook.

3. Remove 1 medium sized leaf from each infected plant you wish to sample (minimize the amount of petiole tissue) and place in the pre-weighed microfuge tube. For best results you should take at least four replicate samples for each interaction you wish to analyze. Weigh the tube to determine the mass of the tissue sample. These measurements must be taken on an analytical balance because the sample will be only 10-30 mg.

4. This step is optional, but recommended for day 0 samples: Put on a pair of gloves and safety goggles. Surface sterilize the leaves by adding 1 ml 15% H_2O_2. Mix by vortexing and allow to stand for 5 minutes. Make sure that the leaves stay immersed in the H_2O_2. Carefully wash away the H_2O_2 by removing as much liquid as possible with a P-1000 (including liquid between the leaf and the side of the tube, and from the cap of the tube). Tap the tube on the bench and remove as much fluid as possible with a pipet. Wash away the remaining H_2O_2 by adding 1 ml of sterile water, mixing and removing the liquid. Repeat the water wash two more times removing as much liquid as possible from the tube.

5. Add 200 ml of 10mM $MgCl_2$ and thoroughly grind the sample in the microfuge tube with a blue pestle. **NOTE**: if you see frothing in the tube upon grinding of the tissue, you probably did not remove all of the H_2O_2. It is unlikely that any bacterial will survive in this sample. Add 800 ml of 10mM $MgCl_2$, and make several serial dilutions. Below are examples of suitable dilutions for Day 2

Day	Susceptible Interaction	Resistant Interaction	Unknown:
2	10^{-4}, 10^{-5}, 10^{-6}	10^{-2}, 10^{-3}, 10^{-4}	10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}

6. Plate the appropriate dilutions onto NYGA plates containing the appropriate antibiotic. Use a sterile spreader, dipped in ethanol, flamed and cooled, to spread the bacteria evenly over the plates.

   Note: If you are interested in saving plates, you can divide each plate into quadrants, and plate 20 ul of each dilution onto a separate quadrant of the plate. Make sure the plates are very dry, prior to spreading the bacteria, to avoid having the different dilutions run into each other.