## PEACH BACTERIAL SPOT - DETACHED LEAF ASSAY PROTOCOL (*XANTHOMONAS ARBORICOLA*)

Sterilize the following items using autoclave:

- Paper filter
- Distilled water
- Making media (to keep leaves with humidity during experiment days)
- Bacto™ agar (15g/L) and sterilize by autoclaving. After cool down add propiconazole 2.0 ppm (0.4 ml of 5000 ppm propimax to 1 L media)

### INOCULUM PREPARATION:

The strains *X.a.*88 (used in this test) and *X.a.*42 were isolated from peach. Strains were isolated in glycerol 25% under -80°C conditions in an ultra-low freezer.

### X.A. STOCK PREPARATION:

1. Prepare liquid media Difco™ LB Broth, Miller (Luria-Bertani) 25 g in 1000 ml (1L) and sterilize by autoclaving.
2. Use 15 ml tube and add around 8 ml LB media and take a loopful of bacterial growth suspended in sterile LB media and leave for 48 hours to growth in shaker agitation with temperature around 30°C.
3. Spin down 400 µl liquid culture (grown in LB) in a sterile 1.5 ml Eppendorf tube
4. Decant supernatant
5. Add 400 µl 25% glycerol (sterilize by autoclaving) to the pellet
6. Mix and store at -80°C

### INOCULUM SUSPENSION:

Inoculum suspensions were prepared from 48-hr-old cultures on Difco nutrient agar. A loopful of bacterial growth was suspended in sterile phosphate buffered saline (1X PBS) containing 0.05% Tween 20 and adjusted to 3 different concentrations: Low ($A_{600nm}$ = 0.1), Medium ($A_{600nm}$ = 0.2) and High ($A_{600nm}$ = 0.4). Control treatment = 1X PBS + 0.05% Tween 20.

### MAKE 1 LITER 10X PBS (PHOSPHATE BUFFERED SALINE):

1. 80 g NaCl
2. 2 g KCl
3. 14 g Na2HPO4
4. 2.4 g $KH_2PO_4$
5. 0.05% Tween 20 (add 0.5 ml/1L)
6. Adjust pH to 7.4
7. Sterilize by autoclaving

### PLANT MATERIAL:

Collect shoots from (20 -25 cm long) placed inside sealable plastic bags on ice to transport to the lab. Expanded leaves (3rd to 5th leaf from tip) were removed, cut in half across leaf center and used the distal part of the leaf, sterilized by soaking for 5 min in 10% bleach, and then rinsed with sterile distilled water. Ten (10) leaves were assayed per treatment.

## INFILTRATION:

Disinfested leaves were placed on sterile filter paper. Using a sterile, 3 ml syringe (needle removed), bacterial suspension was loaded and placed firmly against the abaxial surface. Leaves were infiltrated by applying gentle pressure until a 2-3 mm water soaked spot appeared on the leaf. The soaked spot eventually disappears. Several spots (8 each leaf) were applied per leaf half, approximately I cm apart. Some inoculum spilled around the syringe tip at the infiltration site. The reference says that spilled inoculum will not cause infection, and this seemed to be true.

***Freshly Infiltrated Leaf***

Inoculated leaves were placed in Petri dishes on the surface of water agar (1.5%) with the abaxial side up. And plates left at room temperature on lab bench.

Development of symptoms on leaves was evaluated periodically up to 21 dpi. Lesion counts were performed at 14 dpi.

## REFERENCES:


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