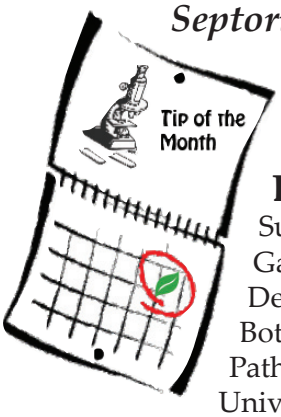

Septoria lycopersici Isolation from Tomato Leaves

Submitted by Gail Ruhl for J.C; Department of Botany and Plant Pathology, Purdue University

Have you ever tried to isolate *Septoria* from tomato leaves at the request of a colleague for a clean culture; not as easy as it sounds. ☺ The following tips may be a helpful resource to reduce contamination when isolating from foliar tissue.

1. Collect tomato leaves infected with *Septoria lycopersici*. Lyophilize for 2–3 days to fully dry the leaves. (This reduced contamination of fast growing fungi in my experience.)
2. Cut section of leaf containing *Septoria* lesion and soak in 10% bleach for 45 seconds. (I use 10 mL in a 14 mL tube.) Transfer leaf to 10 mL sterile water in a 14 mL tube and invert 5–6 times to rinse. Gently blot dry between paper towels or kimwipes.
3. In a regular petri plate, put a piece of Whatman #1 filter paper circle in the bottom and add a little sterile water, just until wet. Lay a sterile rubber band on top and then a circle of sterile screen on top of that to provide a raised platform for your leaf sample. When the lid is placed on the petri plate, this will create a humid chamber without your leaf sample sitting in water (figure 1).
4. After you've blotted your surface sterilized leaf sample dry, place on top of the screen, lesion side up. Put the lid on the petri plate and put all your plates in a closed bag. Incubate at 24°C overnight.
5. Under the dissecting microscope, look for spores oozing out of the lesions. Touch spores with a sterile needle then transfer spores to clarified V-8 agar plates containing kanamycin or other antibiotic (see recipe below). Incubate these plates at 24°C for several days. *Septoria lycopersici* will be dark in color with spores appearing later as small pinkish spots (figure 2).

Clarified V-8 preparation: http://wiki.bugwood.org/Clarified_V8_agar

1. Add 5 g $CaCO_3$ to 340 mL V-8 juice and stir for at least 15 minutes. Aliquot into 50 mL tubes and centrifuge balanced tubes for 15 minutes at 4,000 rpm. Carefully pour 40 mL of the clarified V-8 into 50 mL tubes for storage at -20°C. Label the tubes "5X clarified V-8" along with the volume.
2. When ready to prepare agar plates, thaw an aliquot(s) of the clarified V-8 and mix:

   40 mL 5X clarified V-8 juice
   160 mL dH2O
   3 g agar
   0.2 g sucrose
   0.04 g yeast extract
   200 mL total volume
3. After sterilization, add 400 µL Kanamycin (25 mg/mL, filter sterilized) to 200 mL cooled media and pour plates.

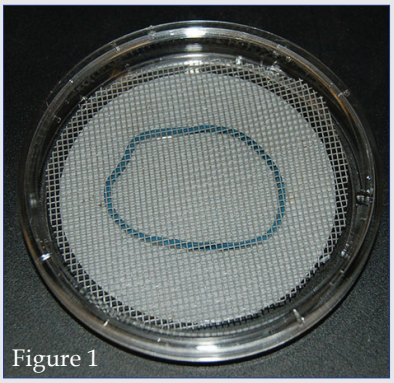
Figure 1

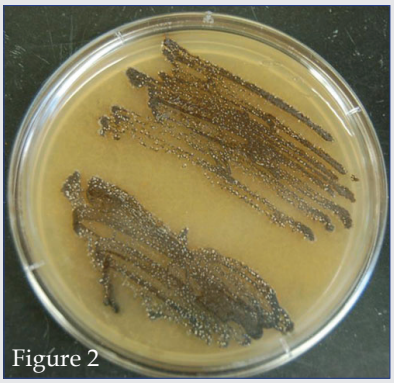
Figure 2

Photos courtesy of J. C., Purdue University.