Bacterial Streaming Test for Rapid Diagnosis of Vegetable Bacterial Diseases

Successful plant disease management depends first on identifying the type of pest that is causing the disease. Several types of pests including fungi, bacteria, viruses, and nematodes can cause disease on plants. A rapid test that can be used to confirm that bacteria are the cause of disease is bacterial streaming. Bacterial streaming is a phenomenon that describes the leaking, movement, or exit of bacteria out of plant tissue when the diseased area of the plant tissue is cut in water. Once a plant is infected, bacteria multiply quickly inside the plant tissue and the plant develops disease symptoms. Common bacterial disease symptoms (**Figure 1**) are:

- Water soaked spots, which are typically seen during the early stages of infection (**Figure 1A**).
- Brown (necrotic) spots that may or may not have yellow (chlorotic) halos (**Figure 1B**).
- Spots that initiate on the edges or margins of leaves and progress inward forming a "V" shape (**Figure 1C**).
- Holes in the leaf tissue (shot holes) that are surrounded by brown necrotic or chlorotic tissue (**Figure 1D**).
- Severe wilting of the entire plant (**Figure 1E**).

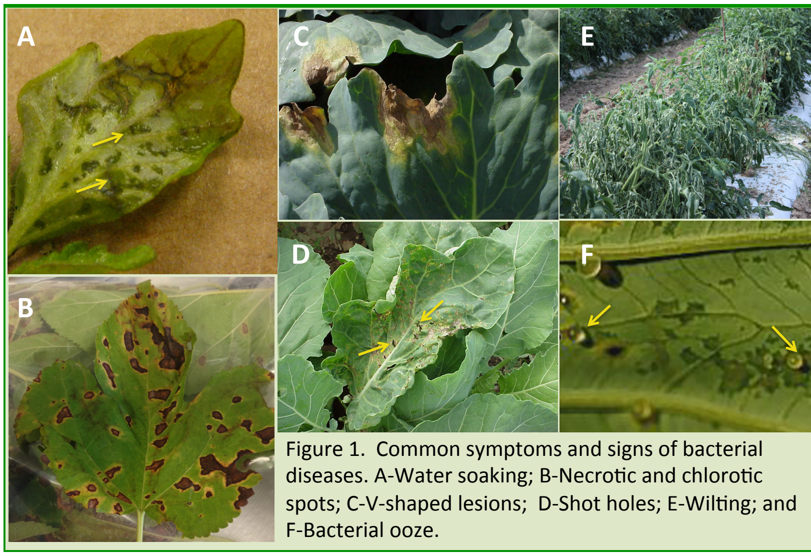


Figure 1. Common symptoms and signs of bacterial diseases. A-Water soaking; B-Necrotic and chlorotic spots; C-V-shaped lesions; D-Shot holes; E-Wilting; and F-Bacterial ooze.

Signs of the disease, such as bacterial ooze (**Figure 1F**), can also be seen on heavily infected plant tissues.

Bacterial Streaming Test

The bacterial streaming test is simple, inexpensive, and environmentally friendly and can be used in the field or laboratory. The **field test** is most commonly used to confirm the presence of bacteria in stem (**Figure 2A and B**), rhizome, and fruit (**Figure 2C**) tissues. The field test does not require any type of magnification; it only requires a clear drinking glass or small jar, clean water, and a sharp blade. A cross section through the symptomatic tissue is made, followed by suspending the tissue in water (**Figure 2A**) for 30 seconds to 1 minute. Alternatively, two cut pieces can be pressed together and slowly separated to reveal bacterial streaming (stringing) (**Figure 2B and C**).

The **laboratory test** can be used to confirm the presence of bacteria in nearly all types of plant tissue. The test takes less than 10 minutes, but requires the use of a light microscope. Other aids include a sharp blade, clean water, and a microscope slide with coverslip.

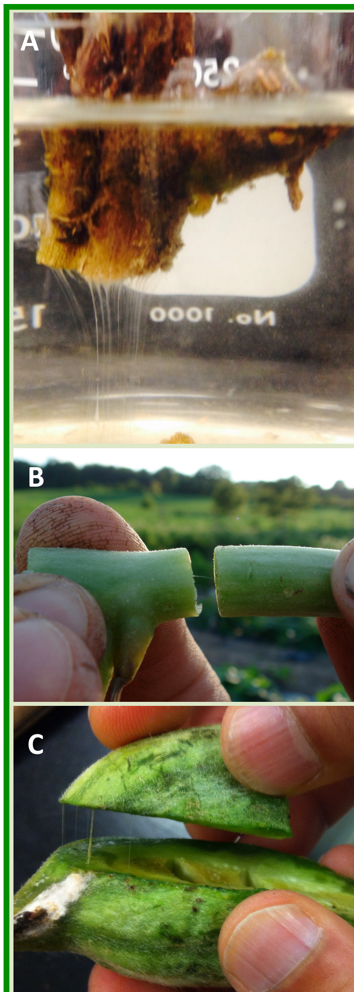


Figure 2. Bacterial streaming from: A-tomato stem; B-cucumber stem; and C-immature cantaloupe fruit.

Laboratory Test Steps (Box 1)

1. Collect plant tissue with visible disease symptoms. Dead or dried out plant tissue will not stream bacteria.
2. Make small sections of tissue by cutting directly through the spot or lesion. For stem tissue, very thin cross- or longitudinal-sections should be made. The final tissue section should include diseased and healthy tissue.
3. Place a drop of clean water onto a microscope slide.
4. Place the cut section of tissue in the water and gently lay a coverslip over the edge of the tissue. Place the microscope slide under a light (compound) microscope. Do not set the light source too high or you will not see streaming.
5. Under low (10X objective) or medium (40X objective) power, focus on the cut edge of the tissue at the margin of the spot or lesion.
6. If bacteria are present, a cloud of bacteria can be seen exiting (streaming) from the cut edge of the tissue.

Important Facts to Know about the Bacterial Streaming Test

While the bacterial streaming test is an easy and quick method to confirm that bacteria are present, mistakes in disease confirmation can occur. To avoid coming to the wrong conclusion it is important to be aware of the following facts about the bacterial streaming test.

- Not every spot or lesion will stream. Therefore, it is important to check several spots before coming to a conclusion.
- Cellular debris and plant sap will also stream or ooze from freshly cut tissue. Generally cellular debris and plant sap streams more quickly and the particles are larger than bacterial cells. Distinguishing between cellular debris and bacterial streaming becomes easier with experience.
- Bacterial streaming is easier to detect with a phase contrast microscope. However, by adjusting the condenser and diaphragm of a compound microscope bacterial streaming can easily be seen (Figure 3).
- The appearance of bacterial streaming can vary depending on the amount of bacteria present in the tissue, the condition of the sample and the type of bacteria causing disease. For example, streaming from tissues infected with Xanthomonads (i.e. bacterial leaf spot of pepper and tomato, black rot of crucifers, and bacterial spot of pumpkin), *Clavibacter* spp. (i.e. bacterial canker of tomato), and *Ralstonia solanacearum* (bacterial wilt of tomato) tends to be very cloudy, thick and slow to stream. Streaming from tissues infected with *Pseudomonas* spp. (bacterial speck of tomato, Syringae seedling blight of pepper, and halo blight of beans) is rapid and diffuse, and often sparkles.

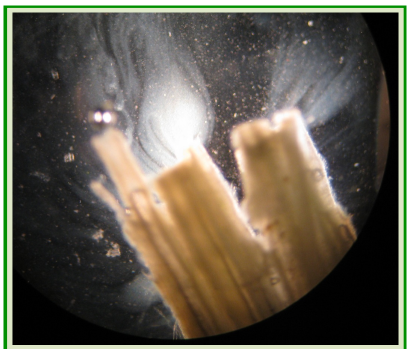
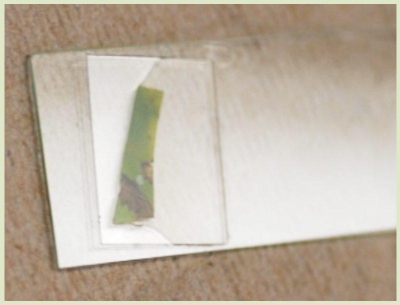


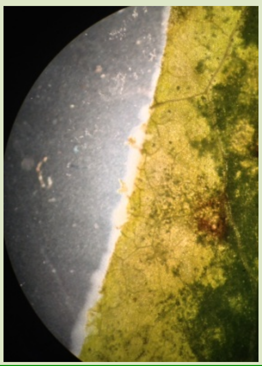
Figure 3. Cloudy secretion of bacterial streaming from a tomato stem as viewed under a compound microscope.

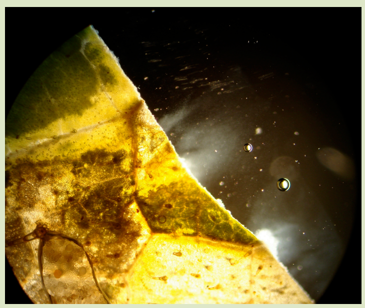
Box. 1

Steps 1-2. Using a sharp blade a section of tissue is cut from a diseased piece of tissue. The final tissue section should include healthy and diseased tissue.



Steps 3-4. A drop of clean water is placed on a microscope slide, the cut section of tissue is then placed in the water and a coverslip is gently placed over the tissue. Additional drops of water can be added to the slide to ensure that there are no air pockets under the coverslip.





Steps 5-6. Using a light microscope, focus on the cut edge of the tissue. Bacteria will stream from tissue at the margin of the spot or lesion. Streaming can be cloudy, diffuse and/or sparkling.

Dr. Melanie L. Lewis Ivey
Allysson Lunos
LSU AgCenter
Department of Plant Pathology & Crop Physiology
mivey@agcenter.lsu.edu

Images courtesy of A) Richard Raid, University of Florida (Figure 1F) and B) Allysson Lunos and Lacy Brooks, LSU AgCenter (Bacterial streaming test steps).
Peer reviewed by Dr. Clayton Hollier, LSU AgCenter, Department of Plant Pathology & Crop Physiology.

PPCP-VEG-005 **August 2014**

The LSU AgCenter is a statewide campus of the LSU system and provides equal opportunities in programs and employment. Louisiana State University is an equal opportunity/access university.