STUDIES OF BACTERIAL PROBLEMS OF ONION IN NEW YORK – 2010

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Bacterial problems of onions have become more important lately for reasons that are not completely clear. Although there are few anti-bacterial tools currently available for use on onions, understanding the disease cycle and the factors affecting disease incidence and severity should aid the development of disease management strategies. That is the thrust of our recent and planned research.

During the 2010 growing season, we encountered serious incidences of Sour Skin, caused by *Burkholderia cepacia,* and Center Rot, a recently described disease in New York caused by *Pantoea ananatis.* In addition, we identified two pathogens causing internal bulb decay that are new to New York onions. *Pantoea agglomerans* and *Enterobacter cloacae* had been described as problematic for onion growers in other regions.

The first step in identifying a bacterial pathogen generally involves its isolation in pure culture. Determining the microbiological and biochemical capabilities of the bacterium and its molecular biological properties then can be pursued to properly identify the bacterium and to determine its pathogenic capability.

To isolate bacterial pathogens from diseased plants, portions or extracts of the infected tissues generally are spread in Petri dishes containing a semi-selective medium suitable for culturing the suspected bacterium. Bacteria grown on media commonly-used for isolation of onion-pathogenic bacteria generally require 5 or 6 days of incubation before recognizable colonies develop. In an effort to speed up the identification of bacteria responsible for internally decayed bulbs, we are developing a specific onion-extract medium (OEM) that requires less time for sufficient growth of bacteria in pure culture. This medium, which contains ingredients that inhibit the growth of many organisms not pathogenic to onions, is promising. Thus far, it appears that most important bacterial pathogens of onion grow to recognizable colonies that are distinctive from each other within 24 hours of spreading on OEM (Figure 1). These include *P. ananatis* (Center Rot), *B. cepacia* (Sour Skin), *E. cloacae* (Enterobacter Bulb Decay), *Pectobacterium carotovorum* subsp. *carotovorum* (Bacterial Soft Rot), and *Xanthomonas axonopodis* pv. *allii* (Bacterial Leaf Blight). Thus, a tentative visual identification is possible, but more importantly, isolated bacterial colonies are available for further testing within 24 hours of examining affected plants or bulbs. The development of OEM and a rapid means of testing pathogenicity have greatly facilitated our ability to assess and diagnose onions submitted to our lab by growers, field consultants and Extension Educators. These procedures are applicable to onions at all stages of growth, as well as those with internal decay problems following storage after harvest.

Bacterial diseases encountered in onions in 2010

In cooperation with Cornell Cooperative Extension Educators, we visited onion fields in July and August and an onion storage facility a few months after harvest in three important onion-growing regions of New York: Orange County, Oswego County, and the Elba muck land. Onions in each had symptoms suggestive of bacterial disease. In addition, we examined several lots of symptomatic onions from Extension Educators and identified the bacteria that caused the problems. The plants or bulbs were processed for isolation of bacterial pathogens using OEM. The isolated bacteria first were tested with simple microbiological tests indicative of *B. cepacia, P. ananatis* and *E. cloacae.* Colonies of suspected onion pathogens were then tested for pathogenicity by inoculation of onion sets or bulbs, and sample colonies were tested in polymerase chain reactions (PCR) using appropriate primers (Figure 2). In some cases, the amplicon produced was sequenced by the Cornell University Biotechnology Resource Center. Based on these tests, isolated bacterial colonies were tentatively identified as *P. ananatis* or *B. cepacia*, as suspected, or as *P. agglomerans* and *E. cloacae* (Figure 3). Inoculation of onion sets with some of these bacteria resulted in symptoms similar to those inoculated with *P. ananatis.* Apparently, *P. ananatis* and *P. agglomerans* can cause similar disease symptoms. In addition, some strains of *Enterobacter cloacae* were identified from growing symptomatic onions and from onions that had been stored for 2 to 3 months after harvest.

Where do bacterial pathogens come from each season, especially *Pantoea ananatis*?

What is the source of the pathogen (a/k/a "inoculum") for New York-grown onions? Does inoculum overwinter in soil so that it is always present in onion-growing areas, or is it introduced anew into onion-growing areas each season? Is inoculum brought into onion fields with planting material, seed, sets or transplants or by equipment, people or insect vectors, particularly onion thrips? Knowledge of the source of inoculum is critical to development of control recommendations. Studies during the early spring emphasized possible sources of inoculum of Center Rot. Working closely with Extension Educators, we sampled and analyzed soil, seed and transplants for the presence of *P. ananatis*.

P. ananatis was isolated only from one of 16 soil samples taken from onion-growing fields; none of the other isolated strains had the microbiological characteristics of the Center Rot pathogen. (Sampling of a muck soil analyzed in 2009 for *B. cepacia*, yielded strains of *P. ananatis*; that finding prompted the more extensive screening in 2010.) However, when the bacteria recovered from one Genesee County field were tested for pathogenicity in onion sets, several strains were pathogenic; some of these were identified as *P. ananatis.* Several others were identified subsequently, using PCR (Figure 2) and techniques of gene sequencing, as *Enterobacter cloacae*, a pathogen of onion that had been described in the Columbia Basin of Washington. Thus, muck soil sampled from two important onion-growing areas in New York was not a consistent source of *P. ananatis* for the 2010 growing season. In contrast, the Sour Skin pathogen (*B. cepacia*) was isolated consistently from all soils analyzed. In addition, other bacterial pathogens of onion were isolated from several of the soil samples.

Five samples of transplants received from growers were tested for the presence of bacterial pathogens. None of approximately 40 strains isolated from more than 250 transplants proved pathogenic to onion sets in tests that we had developed for onion pathogenic bacteria. Similarly, tests of 10 lots of seed obtained from onion growers also proved negative for the presence of bacteria pathogenic to onions. Although some bacteria were isolated from seed, none

of the strains recovered had characteristics of *P. ananatis*, and none of the strains caused symptoms reminiscent of Center Rot in inoculated onion sets or bulbs.

Overall, the question of the source of inoculum of the Center Rot pathogen, *P. ananatis*, for New York onion fields in 2010 remains open. Although the pathogen was recovered from one of 16 muck soils sampled, muck soil seemed not to be a significant source of inoculum. Interestingly onion-pathogenic strains of *E. cloacae* and strains of *P. agglomerans* were isolated from several soil samples. No onion-pathogenic strains of bacteria were isolated and identified as *P. ananatis* from onion transplants or onion seed.

Although the four diseases mentioned are likely to originate in the field during the later stages of onion growing, the problems often are not obvious until after harvest and following storage for some time. However, for all the diseases, the specific conditions that are critical to disease development are not clear. We anticipate addressing possible sources of inoculum and the conditions under which the diseases develop in future studies.

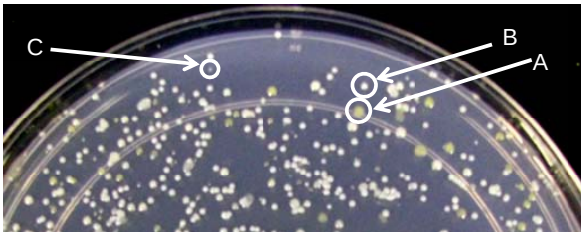

Figure 1. Appearance of onion pathogenic bacteria after growing on OEM for 24 hr at 26° C. A: Yellow mucoid colony of *Pantoea* species; B: Gray-white colony of *Enterobacter cloacae*; C: Small pale-white colony of *Burkholderia cepacia*.

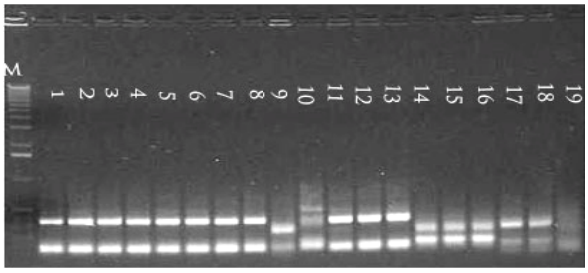

Figure 2. Agarose gel electrophoresis for PCR products of bacterial strains isolated from harvested onions during 2010. M: 1 kb reference ladder; Lanes 1 – 8: Strains isolated from onion bulbs with symptoms of Enterobacter decay; Lane 9: Strain isolated from a bulb with symptoms of Sour Skin; Lanes 10 and 11: Strains isolated from soil taken from an onion field in NY; Lanes 12 and 13: Reference strains of *E. cloacae* CU6882 and CU 6881; Lanes 14 and 15: *P. ananatis* isolated from stored onion bulbs (NY); Lane 16: *P. agglomerans* CU2019; Lanes 17 and 18: *B. cepacia*; Lane 19: No bacteria control.

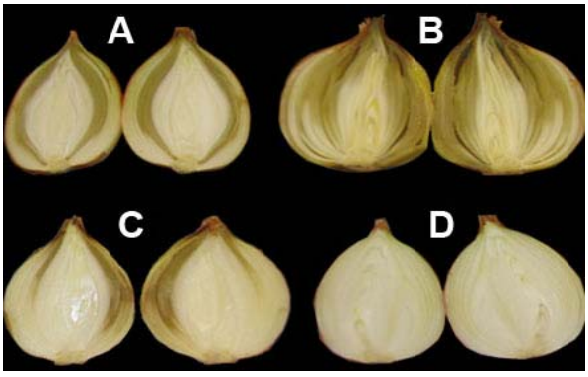

Figure 3. Symptoms of Enterobacter bulb decay in halved bulbs following artificial inoculation with three strains of *Enterobacter cloacae*. A: Strain CU0295; B: Strain ECWSU1 from Washington state; C: Strain AZ-22 isolated from a NY onion; D: Bulb inoculated with sterile water as a negative control.