

6.11 Development of a Potato Psyllid (*Bactericera cockerelli*) cell culture

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Zebra Complex (ZC), was first documented in potato fields around Saltillo, Mexico in 1994, and first identified in the USA in 2000 in commercial potato fields in Pearsall, TX. Over the past eight years, ZC has spread to a number of other states, including NE, CO, KS, NM and CA. This disease was sporadically important economically until the 2004 and 2005 growing seasons, when it caused millions of dollars in losses to both potato producers and processors in numerous locations. This disease has recently been associated with a new, fourth, pathogenic *Candidatus* Liberibacter species (called “psyllaurous”) (Hansen et al., in press). *Candidatus* Liberibacter psyllaurous has also been associated with a similar disease in tomatoes and peppers. In all three cropping systems, the association of this pathogen with disease was originally missed because the traditional diagnostic method, PCR and QRT-PCR, did not amplify DNA from this fourth species. The bacterium is transmitted by the potato psyllid (*Bactericera cockerelli*), an insect that is prevalent in most growing areas of the US.

The causal agent of ZC was unknown until just a few months ago; however, the potato psyllid’s involvement with the disease was determined a few years ago by our lab and others (Goolsby 2007, Munyaneza 2007). It is now suspected that this disease is caused by the presence of *Candidatus* Liberibacter psyllaurous, a pathogen that thus far remained elusive in the development of pure cultures. Following the successful development of an Asian citrus psyllid cell culture by Dr. Hunter’s lab, we have worked toward developing a potato psyllid cell culture (figure 1). By developing an insect cell line, we can attempt to isolate and culture *Candidatus* Liberibacter psyllaurous. Several commercially available insect cell culture media were screened for viability to culture cells/tissues from potato psyllid embryos and midgut tissues without success. Following the protocols of the Hunter lab, we have determined an insect culture medium, labeled Hert-Hunter-70, which permitted psyllid cell lines to be established.

Materials and Methods

Cells from psyllid egg. Psyllid eggs were isolated then disinfected by submersion in 70% ethanol, 10 min, then rinsed 3 to 5 times by submersions in 0.05% sodium hypochlorite (= 1% chlorine bleach). Eggs were rinsed six times with sterile distilled water and transferred to a tube where they were crushed with a glass rod. Culture medium (Hert-Hunter-70) containing antibiotics (Pen-Strep) was added and the culture was incubated at 25 °C.

Cells from adult Psyllid alimentary tract. Adult psyllids were surface sterilized by immersion in 70% ethanol for 30 min. Then, they were rinsed five times with sterile distilled water. Sterilized insects were fixed on a glass slide and circled with a PAP pen and the circle was filled with PBS buffer (pH6.5). A dissection was performed in the buffer under a dissecting microscope where a forceps and needles were used to remove the gut. Next, the excised gut was placed into a tube and crushed with a glass rod. Culture medium containing antibiotics was added and the cells were cultured at 25 °C.

Results and Discussion

The psyllid cell line being developed will be screened for the presence of *Liberibacter*, and other bacteria using quantitative real-time PCR.

References

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Figure 1. Development of a psyllid cell culture, early stage.

