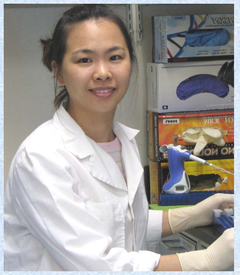

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Asian Citrus Psyllid (Diaphorina citri) Cell Cultures for Liberibacter Propagation

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Abstract: We successfully cultured the bacterium *Can.* Liberibacter asiaticus in cell lines produced from Asian citrus psyllid (AsCP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), which is a highly competent vector of the phloem-inhabiting bacterium *Candidatus* Liberibacter asiaticus, *CLa,* that is associated with the citrus disease huanglongbing (HLB). Commonly referred to as citrus greening disease in the USA, HLB causes reduced fruit yields and quality leading to tree death and is considered the most serious citrus disease. HLB has become a major limiting factor to the production of citrus world-wide. Studies of HLB have been impeded by the fact that *CLa* has not yet been cultured on artificial nutrient media. After being acquired by a psyllid, *CLa* was reported to replicate within the psyllid and was retained by the psyllid throughout its life span. Therefore, we hypothesized that *CLa* could be cultured *in-vitro* using psyllid cell cultures as the medium and investigated the establishment of a pure culture for AsCP cells. Commercially available insect cell culture media was screened and a new media developed to culture cells from AsCP embryos. Successful cell lines from psyllid tissues adhered to the plate and migration was observed within 24 hrs. Cells were maintained at ~21°C. We successfully established several psyllid cell lines, consecutively referred to as DcHH-1, 2, …, for *Diaphorina citri* Hert-Hunter-, and the newly defined media as Hert-Hunter-70, HH70.

Material and Methods

Media and supplements

Hert-Hunter-70 (Hert-Marutani, 2009) and L-glutamine solution 200mM

Cells from psyllid eggs

Embryos at the blastokinetic stage of development were used as the source for psyllid cell cultures. Eggs were collected using an insect pin (size 2) under a microscope. Approximately 100 eggs were collected in 1.5 ml micro centrifuge tube and were disinfected by submersion in 70% ethanol for ten min. After rinsing five times with 1 X Hank's Salt™ sol (Sigma, St. Louis, MO), eggs were crushed with a glass rod. One ml of culture medium containing the antibiotics penicillin (10,000 U/mL) and streptomycin (10 mg/ml) (Sigma) was added to the crushed eggs, and the eggs were then incubated in 24 well plates (Costar®, Corning, NY) at 20°C. The media was changed at intervals of 7 to 10 days

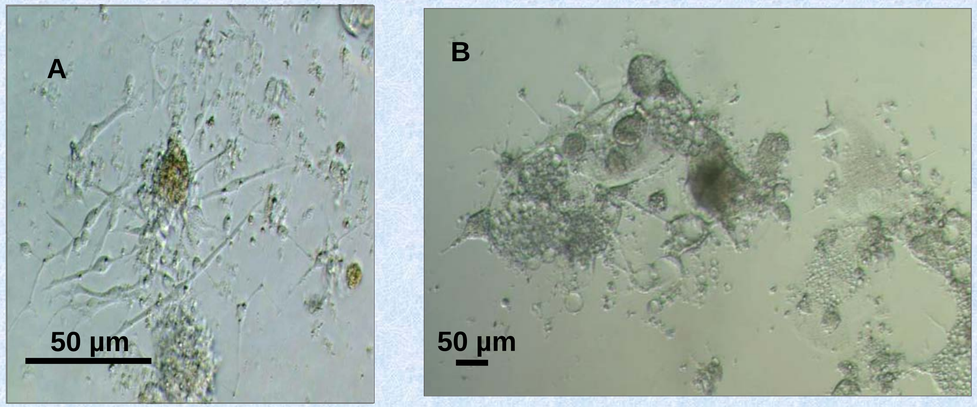

Figure *Diaphorina citri*, primary cell growth in Hert-Hunter-70 Medium at 8 days (A) and 80 days (B) post processing.

Liberibacter asiaticus was detected in Psyllid midgut cell cultures over 40 days,

The Psyllid-*Wolbachia* was detected in psyllid cell cultures for over 60 days.

We successfully cultured psyllid cells cultures using the HH-70 medium.

Direct cell counts demonstrated ~10 day double time, at 21°C.

Applications of Psyllid cell lines as a needed research tool are now being applied to development pure *Candidatus* Liberibacter cultures.

These Psyllid cell cultures increases the opportunity for *in vitro* research, on many aspects Liberibacter biology including its role in HLB pathology.

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Reference: Marutani-Hert, M, Hunter, WB., Hall, DG. 2009. Establishment of Asian citrus psyllid (*Diaphorina citri*) cell lines. In Vitro Cellular & Developmental Biology-Animal (DOI 10.1007/s11626-00909188-3..