

C1. (Hunter) HLB–psyllid cell culture. The goal of this project is to determine if the disease bacterium can be cultured with a culture of insect cells taken from psyllids. (W. Hunter) **Progress as of September 30, 2008 –**

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Culturing HLB Pathogens

A postdoctoral researcher was hired on this funding to examine the- Development of psyllid cell cultures for use in the propagation of *Can. Liberibacter asiaticus*. We have evaluated Nine different commercially available media for their suitability to culture psyllid cells. Different and variable combinations of these media continue to be evaluated. The most efficient medium determined so far is the SF900-III-supplemented. We can currently support psyllid cells in a combination of this media, plus 10% Fetal bovine serum for over three months. We have also screened and continue to screen the efficiency of using different starting tissues from psyllids: early eggs, red-eye stage eggs, nymphs, dissected adult tissues- midgut, ovaries, and fatbodies. So far there has only been limited success and cells although viable grow extremely slowly, which demonstrates that one or more components may still be missing or in the wrong concentrations. Thus we have started a multivariate mixing array to address the effects of different concentrations to produce the best medium. This involves producing and screening of 100+ different media combinations and concentrations made in 10 mL volumes, using the best media, FBS, and a salt solution (Hanks Salts) at varying concentrations from 0% to 100%, in 10% increments. These are currently being evaluated. Another approach- was to evaluate *Liberibacter* growth/survival in a commercially available insect cell line, SF9, which is from Lepidoptera, *Spodoptera frugiperda*. These trials resulted in no survival of *Liberibacter* which was undetectable 1 day after inoculated (PCR analysis using primer sets, Las). Evaluation of other bacteria within Asian citrus psyllid. In this study, we used two PCR primer sets (16S and a pair of 10F, 489R) which were chosen to detect novel endosymbiont bacterial 16s rDNA sequences (Munson et al, 1991, Hansen et al., 2007). Almost the entire length of 16s rDNA was successfully amplified from the total psyllid DNA. A total of 47 cloned 1.5 kb DNA fragments were sequenced and were subjected to homology search in DNA databases. Sequence homology results found 40 of the 47 were 100% homologous to a *Syncytium* endosymbiont of *D. citri* (accession number EF433792) and seven were 100% homologous to *Wolbachia* of *D. citri* (accession number EF433793). The 2.5 kb of PCR product with 10F, 489R primer pairs contained six major types of sequences. These methods identified the presence of eight different bacteria. Biological functions of these endosymbionts and gut fauna bacteria were not investigated. Here we provide a first report of functional bacterial homologs isolated from *D. citri*. These homologs included: Carbazole degrading bacterium, *Janthinobacterium* sp. IC161, an ammonia-oxidizing bacterium, *Nitrospira multiformis* ATCC25196, known as a biodegradation bacterium, *Acinetobacter* sp. and an alkane-degrading bacterium, *Alkanindiges*. These data provide evidence that Asian citrus psyllids are supported by a rich bacterial fauna of many endosymbiotic, and gut fauna bacteria of various types all of which have important interactions between each other and may interact with *Can. Liberibacter asiaticus* when it occurs in psyllids. The psyllid cell cultures in their current state are being used to evaluate *Liberibacter* survival post inoculation. We also detected eubacterium 16S rDNA sequences in psyllid cell cultures 92 days post cultured. These bacteria persist at low concentrations, grow slowly, and did not overwhelm the cultures, providing evidence of success in culturing at least one of the psyllid's endosymbionts.