4.13 Efficient Enrichment of the Pathogen DNA from HLB Infected Host

Chen C., Yu Q., Gmitter Jr F.,

University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850, USA

The presumed causal agent of citrus HLB, Candidatus Liberibacter spp., is a phloem-restricted alpha-proteobacterium and has not yet been cultured. Consequently, little progress has been made to determine its genome sequence and size, or to characterize the pathogen and disease, all routine tasks for most other bacteria. Very few HLB sequences have been cloned by conserved probes, degenerate primers, or genomic walking methods; these slow, inefficient methods have only claimed shorter than 30 kb of the pathogen genome cloned over the years. These sequences have been widely used to design primers for PCR detection. On the other hand, the inability to culture HLB in vitro continues to preclude simple isolation of the pure bacterial DNA. Direct separation of the intact bacterial genome in pulsed field gel or indirect enrichment of the bacterial genome by removal of overabundant host genome is essential for subsequent cloning and sequencing. The basics of the indirect enrichment has been characterized as genomic difference cloning – to isolate sequences present in one genomic DNA population that are absent in another. The low-titer Liberibacter genome present in infected host tissues, but absent in the healthy host genome, is such a case.

In addition to direct separation and size determination of the intact bacterial genome in pulsed field gels, three genomic subtractive approaches were compared to remove the overabundant host genome and enrich the low-titer Liberibacter genome. We monitored the DNA quantity during the processes using spectrophotometry and PCR with host and Liberibacter primers. Among them, the newly developed neoschizomer- and adaptor-mediated approach could remove a majority of host DNA, and resulted in a small amount of enriched DNA that was believed likely to contain a substantial portion of Liberibacter DNA. The neoschizomer cleavage could completely prevent re-associated healthy host genomic DNA from ligating in the subsequent cloning process. Over ten thousand clones were picked, more are under way and subject to further identification and sequencing. The details on the approaches and their efficiency, and library screening and available sequence data, as well as PFGE separation and size determination of the genome, will be presented. These genomic sequences may benefit many aspects of understanding the pathogen and managing the disease, such as diagnosis, transmission, infection, pathogenicity, culture, vector control, and resistance breeding.