

Arom Jantasorn 2007: Investigation and Detection on Causal Agent of Citrus Greening  
(*Candidatus Liberibacter asiaticus*) in Thailand. Master of Science (Agriculture), Major Field: Plant  
Pathology, Department of Plant Pathology. Thesis Advisor: Associate Professor Niphone  
Thaveechai, Ph.D. 117 pages

Citrus greening disease is an important disease in citrus cultivation worldwide including Thailand since it cause severe destruction and yield losses. The causal agent is known as *Candidatus Liberibacter asiaticus* (CLA) and uncultured on synthetic medium, phloem – restricted bacterium. Symptomatological study for diagnosis of the greening disease indicated two typical symptoms in which the first type shows Zn-deficiency like symptom, yellowing of new leaves, and molting with blotchy of fully expanded leaves whereas the other shows reduced, narrow and thick leaves along with green midrib surrounding by yellow area and pointed upright. These symptoms were frequently observed in mandarin, pomelo and sweet orange. Development of GB and GE primers and *rpIJ*-DIG probes for detection of citrus greening with polymerase chain reaction (PCR) and DNA dot blot hybridization (DBH) demonstrated that they were specifically reacted with CLA but negative reacted with healthy citrus and bacteria; *Xanthomonas citri* subsp. *citri*, *Ralstonia solanacearum* and *Escherichia coli*. Designed primers could amplify 513 bp fragment of *rpIJ* gene of CLA by PCR. The *rpIJ* gene was cloned and sequenced and further deposited to GenBank as accession number DQ852665. Detection of citrus showing typical symptoms as above from different growing provinces for 65 samples found that there were 100% positive for both methods. Synthesis of recombinant protein GST-RPLJ of 45 KDa which consisted of GST protein from plasmid vector and RPLJ protein of CLA at the size of 27 and 18 KDa, respectively was done in *E. coli*. Purification of GST-RPLJ from *E. coli* was carried out for antiserum production in New Zealand White rabbit. Antiserum titer was 1:10,000 which was sensitive to detect as low as 10 ng RPLJ protein of CLA and was highly specific to CLA but negative reaction with bacteria and virus pathogen of citrus by Enzyme-Linked Immunosorbent Assay (ELISA). It is the first report for antiserum production from protein antigen of gene from citrus greening pathogen which provided highly specific and detection by ELISA gave equally the same results from developed primers and probes for PCR and DBH techniques. Results from this study demonstrated that all three developed detection methods could be used for diagnosis of citrus greening and citrus grown in Thailand was infected by CLA in all investigated growing areas.

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