

Arom Jantasorn 2012: *In Vitro* Cultivation of ‘*Candidatus Liberibacter asiaticus*’, the Causal Agent of Citrus Huanglongbing and Determination of Its Presence in Alternate Host and Vector in Thailand. Doctor of Philosophy (Tropical Agriculture), Major Field: Tropical Agriculture, Faculty of Agriculture. Thesis Advisor: Associate Professor Niphone Thaveechai, Ph.D. 100 pages.

‘*Candidatus Liberibacter asiaticus* (Las).’ is a phloem inhabiting bacterium that causes huanglongbing disease (HLB) and also known as citrus greening in which is presently associated with three species of α -Proteobacteria in the genus ‘*Candidatus Liberibacter* sp’. This research was to develop a medium to cultivate Las. A medium contains periwinkle extract and growth factor for Las bacteria to grow in the medium. Cultures obtained positive with Real time PCR at 24 hours after growth. The highest populations of ‘*Candidatus Liberibacter asiaticus*’ bacterium culture were obtained from infected periwinkle, citrus and psyllids at 24 hours of cultivations in the developed medium. The medium composition and culture condition were determined. Fastest growth of Las obtained from cultivation at 25°C and pH 5.8, containing ATP at 2,000 ppm and glucose at 2% as an energy and a carbon source, respectively. Scanning electron microscope, LIVE/DEAD cell, DAPI and FISH techniques were used to examine the pathogen in the culture. The morphology of ‘*Candidatus Liberibacter asiaticus*’ in culture is a clump of cells and after treated with proteinase K the cells were separated to show individual rod shape cell. The cell cultures were confirmed by using 16s rDNA primer real time PCR. This is the first report on successful cultivation of Las with the high titer of HLB bacterium in culture. However, the Ar medium can support growth of Las for 3 times subcultures at 48 hours as an interval. A first report on assessment of the infection frequency of HLB on psyllid vector from *M. paniculata* in Thailand was demonstrated as a potential source of inoculum. Murraya plant samples and psyllids on the Murraya plants from ten diverse geographical regions of Thailand were collected and DNA was extracted to evaluate the presence and titers of Las by real time PCR using two different methods. The data showed variation of Las levels both in Murraya and psyllids in Thailand. Different titers among individual psyllid and Murraya plant sample were observed in each province of Thailand which indicate potential insect vector and source of inoculum of HLB in Thailand.

Student’s signature

Thesis Advisor’s signature