



**CALIFORNIA STATE SCIENCE FAIR
2012 PROJECT SUMMARY**

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Project Title Saving Citrus Trees: Serological Detection of Bacteria Associated with Citrus Greening Disease, Year 2	
<p style="text-align: center;">Abstract</p> <p>Objectives/Goals Huanglongbing (HLB) is a devastating disease that has endangered the citrus industries around the world including Florida since 2005. It is associated with an unculturable bacterium, <i>Candidatus Liberibacter asiaticus</i> (LAS), and is spread by <i>Diaphorina citri</i>. The vector has been present in southern California since 2008 and HLB was detected in a backyard citrus tree in Hacienda Heights on March 30, 2012. Early detection is critical for protection of California citrus industry. This project is aimed at raising antibodies against select LAS surface proteins using recombinant DNA technology to aid in development of rapid high throughput serological detection methods.</p> <p>Methods/Materials Based on in-silico analysis two putative LAS surface proteins, lepB and yajC, were selected for the study. The two gene fragments were amplified by PCR of DNA of infected plants from Florida and cloned in a bacterial expression plasmid vector, pET101D/TOPO (Invitrogen), and used for transforming Top10 Escherichia coli cells. Plasmid DNA from positive clones confirmed by sequencing were used to transform the expression host, E. coli BL21 (DE3). The bacteria were grown to log phase and the expression was induced with IPTG. Total protein extracts were analyzed by Western blotting for detection of polyhistidine tag at the carboxy terminal of recombinant proteins. The expressed proteins were purified by affinity chromatography using Ni-NTA agarose, and dialyzed. The purified protein was used to raise antibodies in chickens. Test bleeds were tested for their specificity of reaction to the cognate antigens by Western blotting.</p> <p>Results Several clones with correct sequence of the entire gene, followed by V5 tag and polyhistidine tag, were identified and used for expression. Expressed protein from large scale expression and purification was tested for purity based on PAGE analysis, and the specificity was confirmed by Western blotting. The antibodies raised in chicken were able to detect the cognate antigen at up to 1:160,000 dilution.</p> <p>Conclusions/Discussion HLB has destroyed citrus industries around the world, and has not yet been effectively managed. Early detection is vital for management of the disease. The immunological method of detection is a useful alternate technique to complement the currently used PCR testing methods. The antibodies developed in this project need to be tested against live samples in the field in Florida.</p>	
Summary Statement Recombinant DNA technology was used to develop reagents required for development of early detection of huanglongbing, a devastating citrus disease currently threatening California's citrus industry.	
Help Received The research was conducted at the USDA Citrus Germplasm Repository under supervision of Dr. Manjunath Keremane. Dr. Richard Lee provided facilities. My parents helped for transportation and preparation of board.	