Studies towards Development of POC Devices for Detecting Citrus Fungicides and HLB using LFA and LAMP Platforms

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Impact on California Agriculture: California's citrus industry is a major part of the state's agricultural economy. It generates about $3.4 billion annually. Huanglongbing (HLB), also known as citrus greening disease, is one of the biggest threats to the citrus industry and California is no exception. It is caused by a bacterium (Candidatus Liberibacter asiaticus, aka CLas), spread by the Asian citrus psyllid (ACP). Once a tree is infected with HLB, there is no cure. Thus it is critical for citrus growers to have a portable and inexpensive device to detect HLB early, and subsequently eliminate or contain the infected plants. Fungicides are widely used in citrus industry, there are growing concerns about food safety, worker exposure, and environmental impact on their uses. It is important to have inexpensive and rapid tests for these fungicides.

Rationale/Introduction: we used emerging POC technologies to study portable devices for rapid, on-site testing of important analytes in food safety and agriculture such as allergen, pesticides and pathogens such as CLas for HLB. The technologies lateral flow immunoassay (LFIA), and loop-mediated isothermal amplification (LAMP) of nucleic acids from pathogens.

Experimental Approach: For LAMP experiment, the 177 bp DNA fragment of CLas encompassing a phage related genomic region was the "target gene". All the publicly available Las sequences for the 177 bp target region were aligned and confirmed to be highly conserved in Las strains from different geographical regions. The primers F3, B3, F1P and B1P required for LAMP were designed and synthesized. For LFIA, three types of gold nanoparticles have been studied. We have determined that using 40-nm diameter DCNovation (DCN) colloidal gold nanoparticles with an optical density (OD) of 0.560 at 520 nm, at a pH of 9, in conjunction with a detector concentration of 6.25 ug/mL, yields the best results. In analyzing the fungicide imazalil using LFIA strips, we used custom-synthesized antibody for test line. We have successfully detected imazalil at a detector concentration of 3 μg/mL. We also developed LFA testing strip for the peanut allergen protein Ara h1 with the concentration of 0.05 μg /mL.

Major Conclusion: LAMP technology could be used for the rapid and cost-effective detection of citrus huanglongbing (HLB). Positive samples with different titers (Ct22-Ct28) were detected and differentiated. The technique of lateral flow immunoassay (LFIA) can be used to detect fungicide such as imazalil, good sensitivity has been achieved. Further studies are ongoing

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