

# Diagnosis and Therapy of Diseases Based Upon the Knowledge of Host Innate Immune Defense

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Innate immune system is the first line of defense against pathogenic organisms. However, pathogens have evolved multiple mechanisms to subvert host innate immune defense. The pathogenic mechanisms of subversion provide two important clues. One is for identifying the pre-symptomatic biomarkers, e.g., host innate immune genes, expression of which are significantly altered during the early (< 24 hours) stages of pathogenic infection. The other is for developing therapy strengthening the host innate defense, e.g., introducing protein chimeras that synergistically utilize pathogen recognition and lysis, which are two innate immune functions.

Here we will primarily illustrate our approach for Huanglongbing (HLB), the most devastating disease of citrus. Candidatus *Liberibacter* vectored by psyllid causes HLB. Typically HLB is diagnosed by visual symptom i.e., leaf mottling. However, the visual symptom appears several years after the initial exposure of *Liberibacter*, which is often too late for any disease management protocol because by then disease may have spread through many trees in the orchard. This warrants pre-symptomatic diagnosis long before the appearance of visual symptoms. Since innate immune response is induced early during infection, we performed transcriptome studies (i.e., RNA-seq and qPCR) to discover and validate the citrus genes that are induced early after *Liberibacter* infection and expressed throughout the tree. Thus, monitoring the expression of these citrus genes by taking samples from any part of the tree provided information if the tree is infected and if the infection is in the pre-symptomatic stage. The identified citrus biomarkers will facilitate pre-symptomatic diagnosis of HLB. We are also able to develop a therapy for HLB, which has no cure today. For this we designed a protein chimera that synergistically utilizes two arms of innate immunity: *Liberibacter* recognition and lysis. We constructed transgenic citrus that expresses an anti-*Liberibacter* chimera at the phloem (the site of *Liberibacter* colonization). Greenhouse studies showed that the transgenic citrus expressing the chimera is protected against HLB. Next-generation chimeras are currently undergoing testing. Thus, we demonstrated that diagnosis and therapy of HLB can be achieved based upon the knowledge of citrus innate immune system.

Finally, strengthening host innate immune defense offers novel therapy against diseases caused by antimicrobial resistant bacteria.

**Keywords:** host innate immunity; pre-symptomatic diagnosis; novel therapy

## References

- [1] Pathogen-specific Innate immune response. Ahmet Zeytun, Jennifer van Velkinburgh, JC, Paige Pardington, Robert Cary, and Goutam Gupta (2007). CURRENT TOPICS IN INNATE IMMUNITY, pp 342-357.
- [2] Analysis of Early Host Responses for Asymptomatic Disease Detection and Management of Specialty Crops. Abhaya M. Dandekar, Federico Martinelli, Cristina E. Davis, Abhinav Bhushan, Weixiang Zhao, Oliver Fiehn, Kirsten Skogerson, Gert Wohlgemuth, Raissa D'Souza, Soumen Roy, Russell L. Reagan, Dawei Lin, R. Bruce Cary, Paige Pardington, & Goutam Gupta (2010). *Critical Reviews™ in Immunology*, 30: 277-89.
- [3] Engineering of Novel Innate Immune Defense for Protection Against Pierce's Disease in Grape. Abhaya M. Dandekar, Ana Maria Ibanez, Hossein Gouran, Federico Martinelli, Russell L. Reagan, Charles Leslie, Gale McGranahan, George Bruening, Paige Pardington, Anu Chaudhary, and Goutam Gupta (2012). *Proc Natl Acad Sci U S A*. 109: 3721-3725.