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METAGENOMIC-BASED FIELD MANAGEMENT OF WALNUT BACTERIAL BLIGHT DISEASE

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Objectives

Improve our understanding of walnut bacterial blight disease by discovering disease-specific biomarkers, improve treatments to mitigate disease development, and identify sources of resistance to this important disease of walnuts.

1. Field test new materials to combat copper resistance development and to protect walnut orchards against bacterial blight.
2. Conduct metagenomic and metaproteomic analyses of walnut bud tissues to improve the understanding of microbial community relationships in walnut bacterial blight disease and the development of biomarkers.

Background

Walnut blight (WB) is the most significant aboveground disease of *Juglans regia*, leading to loss of productivity and quality of harvested nuts, especially in wet years on early-leafing varieties. It is caused by *Xanthomonas arboricola pv. juglandis* (*Xaj*), a pervasive pathogen in California that is gaining resistance to copper-based pesticides used to control WB. In a previous study, we used a genomics-based approach to examine the extended period of host susceptibility to this pathogen that led to the identification of host and pathogen susceptibility factors. We also identified a natural compound, EPL, that kills copper-resistant strains of this pathogen, such as *Xaj*417. The genome of this reference strain was sequenced by us, and the strain was used to define a minimal inhibitory concentration (MIC value) of EPL needed to control the blight pathogen under lab conditions. We are performing comparative molecular studies to define biomarkers for host and pathogen susceptibility and virulence factors like copper resistance. We found that one of the sources of copper resistance is associated with a mobile genetic element acquired by other bacteria in the environment. Other virulence factors identified previously by us appear to be acquired from the orchard environment. Our data depicts the orchard as an “open laboratory” capable of supplying local *Xaj* strains with new genes that enhance bacterial fitness and disease susceptibility in the host plant. We have successfully used one of these genes to measure the amount of pathogen in walnut tissues by a new procedure – digital PCR.

Results & Discussion

Overuse of copper-based pesticides to control the disease has inevitably led to the emergence of copper resistance among pathogenic *Xaj* population in California. We compared 32 pathogenic and nonpathogenic *Xaj* genomes, revealing that bacterial virulence and copper resistance emerged by the acquisition of specific sets of pathogenesis-related genes commonly transferred among the members of the *Xanthomonas* genus. In addition, a hypothetical protein from the clade containing *Xaj*417 was selected as a potential molecular marker for a WB diagnostic. Using digital PCR, we could quantify *Xaj* in walnut hull inoculated with *Xaj*417.

The selective pressure generated in orchard systems by intensive spray application to control diseases led to selection of resistant bacteria and emergence of pathogens. We are testing new strategies to overcome resistance and understand the interaction between plant and its associated microbial community. The microbial community has an important role in this process as a source of genetic material that can be acquired, contributing to emergence of new pathogens. The microbial community of walnut buds is being investigated. A first level of analysis aims to identify the major microbial groups associated with the buds. Different DNA extraction methods were tested, using the wash solution of walnut buds, thus including microbes present on the surface of buds, while minimizing contaminating plant DNA. Our results provide novel insights into the emergence of virulence, adaptation, and tolerance to disease management strategies used in walnut orchard ecosystems.