California Leafy Greens Research Program
Project Report for 2017-2018

Title: Development of a molecular assay for *Fusarium oxysporum* f. sp. *lactucae*

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Abstract
Having the ability to rapidly and accurately diagnose *Fusarium oxysporum* f. sp. *lactucae*, the cause of Fusarium wilt of lettuce, would significantly aid efforts to control this pathogen. Due to similarities in morphology when grown in culture it is very difficult to differentiate strains of the pathogen from other strains in the soil that cannot infect lettuce or saprophytically colonize lettuce tissue. The objective of this project is to develop a molecular diagnostic assay using comparative genomics to identify unique regions of the genome that will be highly specific for the pathogen and provide a rapid means of detection. The approach is to sequence the genomes of a number of pathogenic strains and then compare these DNA sequences with library of genome sequences that represent a wide range of *Fusarium oxysporum* strains that do not infect lettuce to identify unique regions to use as targets for the molecular assays. The genomes of 10 isolates of race 1 of the pathogen collected from California and Arizona have been sequenced; DNA from other race 1 isolates as well as other races of the pathogen not currently present in the USA (race 2, 3, and 4) has been obtained from collaborators in Europe and Japan and been sequenced as well. These sequences are currently being used in a comparative genomics study to select unique regions of the lettuce wilt pathogen to target for development of a diagnostic assay.

Objectives

The main objective is to develop a molecular diagnostic assay for all four races of *Fusarium oxysporum* f. sp. *lactucae* that will be useful for soil quantification of pathogen inoculum in the field as well as rapid in-field diagnostics. The technology will be transferred to

the UC Cooperative Extension, commercial diagnostic labs and university researchers so it will be available to the industry.

Procedures

Given the large number of *formae specialis* in *F. oxysporum* and the fact that some isolates of the same *formae specialis* are not phylogenetically related it can be difficult to develop molecular diagnostic assays for these pathogens. The approach we used for development of specific molecular assays for *F. oxysporum* f. sp. *fragariae* was to sequence the genomes of 5 isolates representing the different evolutionary groupings by Illumina and do a preliminary assembly of the genomes. We also sequenced the genomes of five isolates recovered from strawberry roots that were not pathogenic and used genomic sequence data that had been collected for 13 other *formae specialis* and several saprophytic isolates to identify unique sequences in f. sp. *fragariae.* These loci were then evaluated for specificity for only the pathogen by real time TaqMan PCR amplification. To ensure specificity the diagnostic assays were tested against purified DNA from over 45 other closely related *formae specialis* that was provided by collaborators. Thus far the marker system has been highly specific for only f. sp. *fragariae* and is currently being evaluated to determine the relationship between results of the real time PCR assay and inoculum level of the pathogen in the soil. This approach for soil quantification has been effective for estimating pathogen inoculum densities for *Verticillium dahliae* (Bilodeau et al. 2012) and *Macrophomina phaseolina* (Burkhardt et al. 2018). In addition to the TaqMan real time PCR assay, an isothermal assay that can be done directly in the field in as little as 10 minutes using a technology called recombinant polymerase amplification (RPA) was developed and provided to the UCCE lab for their use (they also have also been using our RPA assays for *Phytophthora*, *Macrophomina phaseolina* and *Verticillium dahliae*; see Burkhardt et al. 2018, Miles et al. 2015 and Rojas et al. 2017).

Standard techniques will be used for culturing the isolates and extracting DNA. Any additional genomic sequencing has been done at the UC Davis Genome Center and the data downloaded over the web onto workstations in the PIs office. The necessary computer hardware and software is available on these workstations for processing the data, assembling the genomes and identifying unique regions by comparative genomics for developing specific molecular markers for *formae specialis lactucae.*

Since the work with the Fusarium wilt pathogen of strawberry was started our genomic sequence database for *F. oxysporum* has been expanded to include over 150 isolates representing 50 different f. sp. of *F. oxysporum* (and 24 nonpathogenic isolates). This will significantly improve our ability to bioinformatically identify unique sequences to target development of molecular assays.

Results and discussion

Fusarium oxysporum is an important lethal vascular wilt pathogen of a wide range of specialty crops in CA with limited options aside from host resistance for their control. Morphological variation among isolates is not observed but host range differences are, with host specific isolates classified as "*formae specialis*" (or f. sp.) according to the host they infect. For example, isolates that infect only strawberry are *F. oxysporum* f. sp. *fragariae,* those that infect

only lettuce are *F. oxysporum* f. sp. *lactucae*. Approximately 100 *formae speciales* of host specific *F. oxysporum* have been described, with 30-40 regularly encountered in agricultural production systems.

There is limited ability to identify & quantify particular *formae specialis* in soil plating assays, making it difficult to assess the potential for disease to occur in a field. In addition, plating assays can take days to complete, and do not always provide an accurate soil quantification due to the presence of isolates that are nonpathogenic on the host under study. The development of a molecular diagnostic assay would address potential problems with plating assays.

Fusarium wilt of lettuce had been reported only from Japan until 1993 when it was observed from several fields in the area of Huron, CA. The pathogen has since spread to Arizona in 2001 and is becoming an increasing production problem in the California and Arizona production areas. Currently only race 1 isolates have been identified in the US production areas but isolates representing race 2, 3 and 4 have been reported in Japan and Europe; Gilardi et al. 2017). Having the ability to rapidly and accurately determine if the pathogen is not just found in a field, but also the amount of it that is present will help growers evaluate the risk of disease based on quantitative knowledge of the pathogen. The availability of rapid detection techniques (the isothermal RPA assay) will also help growers and the research community rapidly diagnose diseased plants and evaluate how cropping practices influence pathogen populations in the soil, thereby contributing to development of a more integrated control program.

The genomes of ten isolates of *formae speciales lactucae* race 1 recovered from California and Arizona (isolates were provided by Tom Gordon) have been sequenced with Illumina technology. Additional race 1 isolates from Italy (3) and Japan (1) as well as isolates representing race 2 (Japan, 1 isolate), race 3 (Japan, 1 isolate) and race 4 (Italy, 2 isolates) have been sequenced as well. Comparative genomics is in progress, once unique regions present in all races of the pathogen have been identified they will be tested for specificity using TaqMan real time PCR against a broad array of *formae speciales* we currently have DNA for. Once the TaqMan assay has been fully validated the marker will be transferred to the isothermal RPA platform so a rapid, in field detection assay will be available to diagnosticians.

While only race 1 is currently present in the USA our intention is to develop a marker that will identify all four races so the tools will be available for detecting the other races in the event they are introduced into our production area. Preliminary phylogenetic analysis using the genomic data indicates race 1 and 4 are closely related with race 2 and 3 a little less so. We are in the process of expanding this analysis to include a broader array of *formae speciales*. In the event a single assay is not able to detect all races, assays for individual races will be developed.

References

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