Development and Management of a Genomics Database for Microarray-based Detection Systems for Citrus Pathogens

Edwin Civerolo
USDA-ARS, Parlier

The specific objective is to develop and manage a database of genomics information related to citrus pathogens and infection and to citrus pathogen-host interactions. The main targeted citrus pathogens for this project are *Xylella fastidiosa* (Xf), *citrus tristeza virus* (CTV) and *huanglongbing* (HLB).

Value and benefits to the California citrus industry: to provide industry and regulatory agencies with the basis for developing rapid, reliable, and cost effective methods (e.g., microarray-based) for detection of citrus pathogens. In addition, this information will be useful for developing novel therapeutic approaches to citrus disease management.

Specific Progress: A database of Xf genomics and bioinformatics was constructed. The website for this database is http://fresno.ars.usda.gov/citrusdisease/CVC_index.htm. In this database, sequences of each of the four available Xf genomes are compared with each other. All of the known genome sequences (Xf-CVC strain9a5c, Xf-OLSD strainAnn1, Xf-PD strain Temecula and Xf-ALSD strain Dixon), including nucleotide sequences, amino acid sequences and noncoding sequences were collected. These differences can be used as a basis for the design new of and specific primers for PCR-based applications (e.g., detection, identification, and functional genomics). Another feature of this database is that unique sequences are listed. A unique sequence is a region in the genome of a strain that is not been found in other strains based on the currently available genomics information. This database provides a convenient tool for researchers. The users can easily download these sequences for analysis or download unique genes and sequences for primer design.

We have begun to demonstrate the utility of this database for specific PCR primer design. Specific primers are being designed for each gene and intergenic region as well as for every 1.0kb, 0.5kb and 0.25kb fragment of each of the genomes. Thus, the primer database will provide additional tools for pathogen detection and identification.

DNA microarray detection of pathogens is one of the most reliable ways to detect pathogens. From the known Xf genome sequences, unique probes have been designed for each strain. Each probe is a short DNA sequence (60 nucleotides) with no significant match in any of the currently available sequenced genomes of microorganisms. Once these probes have been confirmed by microarray experiments, they will be published in this database.

We have mechanically inoculated Madam Vinous sweet orange trees with non-CVC strains isolated in California in order to determine if any of these can colonize citrus. If any of these strains colonize citrus (with or without causing symptoms), we plan to conduct microarray experiments to test the early responses of citrus to Xf infection by Xf. The knowledge of earlier responses of citrus trees could provide an alternative basis for rapid detection of Xf.

Although the amount of available genomics and bioinformatics data for CTV and HLB bacteria is limited, we will incorporate this information into the database during the next year.