Survey of Phages in *Xylella fastidiosa* Almond Leaf Scorch Strains

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Project Leader: Jianchi Chen
USDA/ARS
9611 S. Riverbend Ave.
Parlier, CA 95648
(559) 596-2924
Jianchi.chen@fresno.ars.usda.gov

Project Cooperator: Brad Higbee, Paramount Farming Co., Bakersfield, CA

Objectives:

To survey *X. fastidiosa* ALSD strains in field for the presence and estimate the frequency of phages.

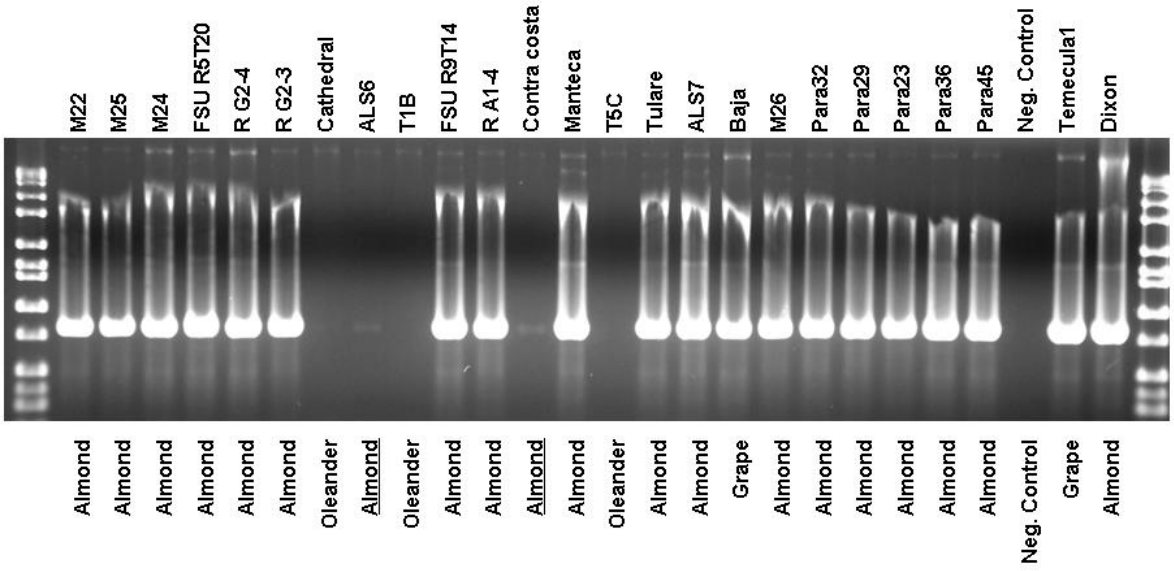
Interpretive Summary:

Xylella fastidiosa causes Almond Leaf Scorch Disease (ALSD) in California. The bacterium is nutritionally fastidious and difficult to culture in artificial media. Therefore, studies on the biology of *X. fastidiosa* are highly challenging. As a result, our knowledge about the pathogen and ALSD is limited, so are the options of the disease control. A better understanding of the biology of *X. fastidiosa* will benefit ALSD control and the almond production industry. Since 2003, this laboratory has been focusing on gathering and analyzing biological information on *X. fastidiosa* strains including ALSD strains. We recently detected and observed phage-like particles in several *X. fastidiosa* strains (Chen et al., 2005; Chen and Civerolo, 2008).

Phages are viruses infecting bacteria. They are obligate intracellular parasites and lack of their own metabolism. Most phages are highly host-specific, infecting only specific species or even strains. There are also phages infecting and killing bacteria within a broad taxonomic group such as at the genus level. In medical microbiology, there have been extensive studies on phages and recently a renew interest in using bacteriophages as a tool for bacterial control to counter the problem of antibiotic resistance. The extreme specificity of phages renders them ideal candidates for applications designed to target only the pathogen. Pathogen-specific phages or phage-derived proteins can be used for quick and specific bacterial identification. Lytic or virulent phages, if identified, have high potential for use in bio-control. Because phages are the natural enemies of bacteria, their uses will not interfere with the natural microflora and, therefore, environmentally sound.

We have recently finished the whole genome sequences of two *X. fastidiosa* ALSD strains isolated from Kern County of California. Analyses of whole genome sequences identified many prophage sequences including putative *Siphoviridae*, *Podoviridae* and *Inoviridae* phages. However, there is no information available regarding the frequency of bacterial phages in *X. fastidiosa* population. Chen et al. (2005a) reported a phage DNA sequence from the genome of a Pierce's disease (PD) strain isolated in Florida. This sequence was, however, absent in the whole genome sequence of a California PD strain Temecula-1, but present in other California PD strains, as well as some ALSD strains.

In this study, a survey of phages in *X. fastidiosa* ALSD strains from orchards in Fresno County and Kern County will be performed. Samples will be collected between June and November when ALSD symptoms are visible. Phages will be detected from pure culture and in infected plant tissues. For *X. fastidiosa* isolation, a previously described procedure (Chen et al., 2005b, 2007) will be followed. To prepare infected tissue samples for PCR, the collected almond leaves will be placed in a labeled paper envelope and freeze-dried in a freeze-drier following the published procedure (Chen et al., 2008). Phage particles will be detected by transmission electron microscopy. Frequency of phages in the bacterial population will be estimated by PCR based on a previously known phage DNA sequence. A preliminary result of a phage frequency estimiation is shown below:



We recently identified a new phage, XFp1109, from a *X. fastidiosa* strain. Primers were designed based on the phage sequence. PCR was performed on 26 *X. fastidiosa* strain culture. Among them, 20 were from ALSD samples collected in California. The absence or high degree of heterogousity was detected in two strains. In another word, phage XFp1109 could be detected in roughly 90% of the ALSD strains. More experiments are currently underway and impact of our work in ALSD research will be discussed at the completion of this project.

References:

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