RESPONSES OF *NICOTIANA TABACUM* CV. SR-1 TO *XYLELLA FASTIDIOSA* STRAINS

Project Leaders:
Marta Francis
Department of Plant Pathology
University of California
Davis, CA 95616

Edwin L. Civerolo
USDA, ARS
SJV Ag. Sci. Center
Parlier, CA 93648

George E. Bruening
Department of Plant Pathology
University of California
Davis, CA 95616

ABSTRACT
Nicotiana tabacum genotype (SR-1), was evaluated as a susceptible host for the bioassay of *Xylella fastidiosa* strains. Readily transformable *N. tabacum* cv. SR-1 plants were propagated *in vitro*. Transplanted plants were inoculated with various *Xf* strains. Inocula consisted of aqueous suspensions of bacterial cells harvested from 7-10 day old cultures on solid PWG medium. Inoculations were made by needle puncture through 20µL of inoculum (10^8 bacteria/mL) placed in the axils of three basal leaves. Inoculated plants were maintained in a growth room (27-28^{o}C, 12 hour photoperiod provided by GE High Output fluorescent lights) for 1 month, and subsequently transferred to a greenhouse. Generally, symptoms on plants inoculated with *Xf* strain Temecula-1 included necrosis at the margins with chlorotic zones extending toward the midvein after 6-8 weeks. Some affected leaves became cupped and curled downward. As infections became systemic, leaves that developed on new shoots were chlorotic and smaller. These symptoms did not develop on water-inoculated control plants. The presence of *Xf* in stems and leaf petioles of affected plants was confirmed by ELISA and real-time (RT) PCR. ELISA and RT-PCR assays of similar tissues from water-inoculated control plants were negative. Bacteria were observed by TEM and SEM in xylem cells in affected plants. No bacterial cells were observed in control plants. *Xf* was isolated from systemically infected tobacco leaf petioles from plants inoculated with *Xf* strain Temecula-1 and re-inoculated into grape plants cv. Ruby Seedless. Typical Pierce's disease symptoms developed four weeks post-inoculation in the greenhouse, confirming the retention of pathogenicity of this strain to grapes after passage through *N. tabacum* cv. SR-1. *N. tabacum* cv. SR-1 plants with other *Xf* strains are being evaluated. Several factors, including plant age at the time of inoculation, method, and plant handling after inoculation, are being determined.

TWITCHING MOTILITY AMONG VARIOUS WILD-TYPE ISOLATES AND PILUS-DEFECTIVE MUTANTS OF *XYLELLA FASTIDIOSA*

Project Leaders:
Harvey C. Hoch and Thomas J. Burr
Department of Plant Pathology
Cornell University, NYSAES
Geneva, NY 14456

Researchers:
C. D. Galvani, Y. Li, De La Fuente, and G. Hao
Department of Plant Pathology
Cornell University, NYSAES
Geneva, NY 14456

ABSTRACT
The genome of *Xylella fastidiosa* (*Xf*) contains at least thirty genes responsible for pilus assembly or function. Recently, it was shown that *Xf* possesses two distinct types of polar pili: long, type IV pili and short, type I pili. It was also demonstrated that the bacteria of the Temecula strain are able to move on a solid agar surface via type IV-pilus mediated twitching motility that results in the presence of a 'fringe' surrounding the expanding bacterial colony. Since our research had been limited to the Temecula strain, and since such colony morphologies had not been previously reported it was not known whether the fringe we observed in culture was an anomaly of the Temecula strain or if it was also a characteristic of other wild-type strains. We therefore examined fourteen isolates from California, Texas, and South Carolina. All but one *Xf* isolate developed a fringe around the colony periphery, suggesting that twitching motility may be a critical factor in the spread of the bacteria *in planta* and development of Pierce's disease. We further discovered that fringe formation on PW agar is dramatically affected by the concentration of bovine serum albumin (BSA) in the medium. Type IV pilus-defective mutants, e.g., *pilB* did not develop a colony fringe. Mutants defective for the shorter type I pili, e.g., *fimA* continued to exhibit a fringe; and, in fact had a wider fringe.

Finally, to facilitate future stability studies *en planta*, we have also included in the new stability plasmids a copy of *gfp*, which encodes a bacterial optimized green fluorescent protein (GFP) (Tables 1 and 2). Although this phenotypic marker does not aid in plasmid stability, it provides a convenient marker for tagging individual cells and provides an alternative tool for researchers to track the location of *Xf* during an infection. GFP has been used by others, such as the Lindow lab at UC-Berkeley, for tracking *Xf* during plant infections (Newman *et al.* 2003) and its inclusion in this new generation of stable *Xf* plasmids will expand the usefulness of this valuable molecular biology tool.

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