

## COMPARATIVE STUDY OF *XYLELLA FASTIDIOSA* SURFACE PROTEINS EXHIBITING HIGH CONTENTS CYSTEINE RESIDUES: IMPACT IN PATHOGENICITY

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### ABSTRACT

The Calcium Bridging Hypothesis (CBH) validity is highly dependent on the existence of thiol moieties on the surface of *Xylella fastidiosa* (*Xf*) cells. The major question that remains to be addressed is how surface thiol and divalent ions would mediate aggregation. Strong evidence was revealed from studies with the Cowpea Mosaic Virus (CMV), by the Scripps Research Institute, California. Dissimilar patterns of surface cysteine on the surface of CMV particles resulted in distinct attachment properties. Likewise, cell-cell and cell-xylem interactions may also be mediated by the establishment of ionic bonds involving  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$ . Cysteine residues located on the outer membrane region of *Xf* surface proteins can form covalent disulfide linkages with thiol residues from other cells. Calcium and magnesium ions could also bridge negatively charged surface areas. Our objective in the present work was to search for potential surface proteins with thiols (negative charge) on the *Xf* cell surface. Several adhesion related proteins were investigated. We especially targeted domains localized outside the cell, and focused on the extracellular cysteine-rich residues regions. Hemagglutinin-like proteins presented the desired characteristics to fit the hypothesis. Other surface proteins are discussed, including type IV fimbriae, recently demonstrated to be involved in *Xf* twitching.

## INDUCTION OF AGGREGATION *IN VITRO* OF *XYLELLA FASTIDIOSA* CELLS BY DIVALENT IONS

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### ABSTRACT

*Xylella fastidiosa* (*Xf*) aggregates within xylem vessels. Aggregation is followed by biofilm formation and ultimately vessel plugging. Characteristic Pierce's disease (PD) symptoms are visualized right after vessel plugging. Nutritional and water stress are the most common deficiencies and may result in leaf yellowing, leaf scorching and interveinal chlorosis. We hypothesize that xylem fluid chemical composition strongly influences aggregation and biofilm formation. Divalent ion availability is dissimilar in susceptible and resistant plants. In order to clarify these findings, we assayed aggregation of *Xf* in different concentrations of  $\text{MgCl}_2$  and  $\text{CaCl}_2$  (20, 50 and 100 mg/L) with two *Xf* PD strains (UCLA and STL). Our results indicate that calcium or magnesium induced approximately a 10-fold increase in aggregation of *Xf* cells. Controls were treated with deionized water. Aggregation of UCLA cells was greater than for STL cells either with calcium or magnesium treatments. However, calcium and magnesium induced aggregation. These results support the hypothesis that divalent ion availability is important in determining PD susceptibility and or resistance.

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

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### 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°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*

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### 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.