THE INFLUENCE OF THE CELL SUSPENSION REDOX POTENTIAL ON THE CAPACITY OF *XYLELLA FASTIDIOSA* TO AGGREGATE

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ABSTRACT
The Calcium Bridging Hypothesis (CBH) implies that surface redox changes on cells of *Xylella fastidiosa* (*Xf*) may influence the capacity of these cells to aggregate. A series of experiments were designed to challenge the proposed CBH. In this hypothesis, thiols (SH) located at the outer membrane level or in adhesion related structures of *Xf* could increase or decrease the cells attraction to the xylem wall surface and/or other *Xf* cells. The focus of this investigation was to address the possibility to alter the surface status of SH groups by exposing cells to reduced and oxidized forms of the tripeptide gluthathione (commonly found in xylem fluid). CBH also assumes that divalent ions would mediate the interaction between thiols and other negative charges. *Xf* aggregation was measured after the following treatments: deionized water (negative control), $CaCl_2$ 100 mg/L (positive control), reduced glutathione 10 mM (GSH), oxidized glutathione 10 mM (GSSG), GSH 10 µM for 20 min + $CaCl_2$ 50 mg/L and GSSG 10 mM for 20 min + 50 mg/L. Maximum aggregation was obtained with pre-treatment with GSH 10 mM for 20 min followed by exposure of cells to $CaCl_2$ 50 mg/L. Results indicate that a reducing environment is essential for cell aggregation. A reducing environment apparently modified the surface of *Xf* cells and predisposed them to interact with divalent ions.

XYLELLA FASTIDIOSA GROWTH ON CHARD2, 3G10R AND *XF*-26 CHEMICALLY-DEFINED MEDIA

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ABSTRACT
Pierce`s disease (PD) in grapevines is caused by the bacterium *Xylella fastidiosa* (*Xf*). *Xf* is injected into xylem vessels by leafhoppers. *Xf* can grow planktonic (free cells) or can form aggregates or biofilm (colonies). Growth and biofilm formation of UCLA and STL PD strains was compared in three chemically-defined media, *Xf*-26 (22 components), CHARD2 (10 components) and 3G10R (9 components). PW^+, a rich non-defined medium, was used as a control. Both planktonic growth and biofilm formation were assessed during the incubation period. CHARD2, which has the amino acid cysteine as a component, was by far the best medium inducing biofilm formation. CHARD2 and *Xf*-26 differed in planktonic growth; CHARD2 exhibited no detectable planktonic growth, whereas *Xf*-26 cultures were predominantly planktonic. 3G10-R performance was below the expectations, since this medium has performed satisfactorily before as an aggregation inducer. 3G10-R has reduced glutathione (reducing agent), however it contains glucose, which is not present in CHARD2. We hypothesize that the redox environment, in each medium, induced the differences in biofilm architecture verified.

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 form 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 Ca^{++}, and 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 $MgCl_2$ and $CaCl_2$ (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.