

VIRULENCE ANALYSIS OF THE PIERCE'S DISEASE AGENT *XYLELLA FASTIDIOSA*

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INTRODUCTION

The bacterium, *Xylella fastidiosa* (*Xf*), is accepted as the causative agent of Pierce's disease of grape. In our survey of plants for their reaction to *Xf* cell suspensions pressure infiltrated into leaves, we found that *Chenopodium quinoa* (Cq) developed a chlorosis in 24-48hr that conformed to the area infiltrated with suspensions of 10^6 to 10^8 *Xf* cells/mL. Comparisons of infiltrated opposite leaf halves for the intensity of the developed chlorosis provided a useful semi-quantitative assessment of the relative potency of *Xf*-derived preparations. The chlorosis-inducing activity was associated with *Xf* cells, not washings of cells, and heating *Xf* cells at 100°C for 6 min slightly enhanced the activity. We observed that the chlorosis-inducing activity survived treatment with sodium dodecyl sulfate (SDS). Although the indicated stabilities do not suggest a protein as the active agent, the chlorosis-inducing activity was sensitive to each of three proteases and was lost after treatment with chloroform or acetic acid. These results suggested that *Xf* possesses a protein elicitor that is recognized in the intercellular spaces of Cq plants even when the protein is in a denatured state. Presumably metabolic events of Cq, subsequent to recognition, result in chlorosis.

Chenopodium ambrosioides (Ca) is known to be a natural host of *Xf* and a source of *Xf* inoculum that can be transmitted to grape under experimental conditions (Freitag 1951). We were able to infect Ca with *Xf* after inoculation by petiole injection. However, infiltrated leaves of Ca failed to develop chlorosis or other reaction. In contrast, Cq, which developed chlorosis after infiltration, did not become detectably infected after inoculation with *Xf*. In several systems, a pathogen protein that acts as an elicitor in one species may act as a virulence factor in a closely or distantly related line or species (De Wit, Joosten et al. 1994; van't Slot and Knogge 2002). Therefore, the *Xf* elicitor of Cq chlorosis may be a virulence factor in other, susceptible plant species, e.g., Ca and *Vitis vinifera*.

OBJECTIVES

1. Identify gene product(s) and gene(s) of *Xf* that contribute to its virulence.
2. Exploit knowledge of *Xf* virulence factor(s) in strategies for control of Pierce's disease.

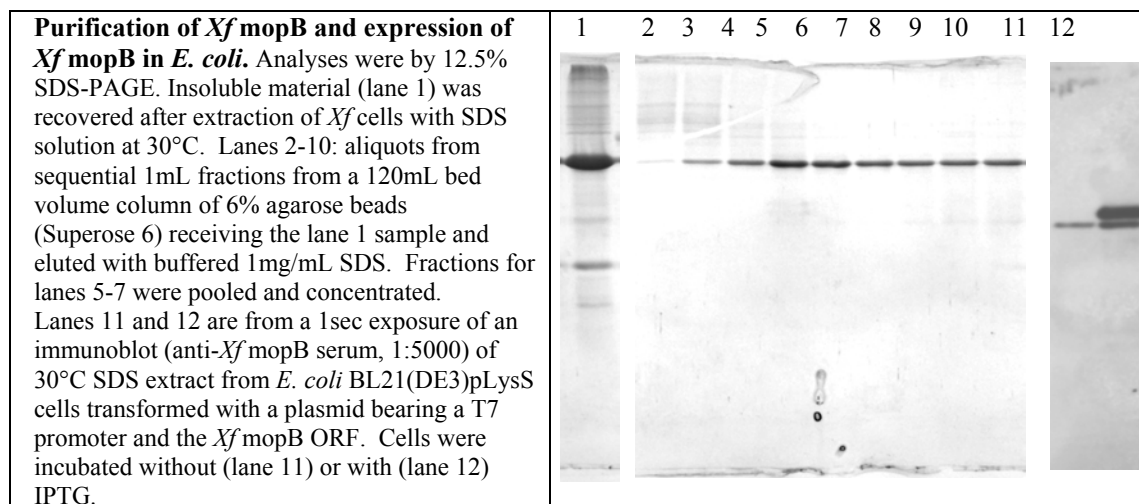
RESULTS AND CONCLUSIONS

A precipitate was collected by high speed centrifugation after incubating washed and suspended *Xf* cells for 30 min at 30°C in Tris-buffered sodium dodecyl sulfate (SDS) solution at pH approx. 8.6. Compared to intact *Xf* cells, the precipitate after SDS extraction presented a greatly simplified pattern of proteins after SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Extracts of gel regions were assayed by infiltration into Cq leaves. The bulk of the chlorosis-inducing activity was associated with material with mobility corresponding to molecular weight of about 40K. A prominent band (Coomassie blue stained) from the region was excised and subjected to digestion with trypsin and analysis by mass spectrometry (Young Moo Lee, UC Davis Molecular Structure Facility). Peptides were identified that correspond to about 40% of the *Xf* outer membrane protein mopB. The pyroglutaminyl-terminated peptide pyro-QEFDDR mapped to the mopB gene sequence (Simpson, Reinach et al. 2000) to predict a mopB protein of molecular weight 38.5K. Results from other experiments suggest that the pyroglutaminyl residue is the natural end of mature *Xf* mopB protein and is not created as an artifact of our analysis by cyclization of an amino-terminal glutamine residue. Edman degradation gave in very low yield the sequence MKKKILT..., consistent with a mopB protein of molecular weight 40.7K as a minor component. The 22 amino acid residue sequence at the amino end of the minor 40.7K protein, and not present in the abundant 38.5K protein, has the characteristics of a signal peptide. We conclude from the above results that the translation product of the *Xf* mopB gene is the 40.7K protein and that release of its 22 residue signal peptide results in insertion of the 38.5K mopB protein so tightly into the *Xf* outer membrane that it remains insoluble during a SDS treatment that releases most other *Xf* proteins.

translated mop B: MKKKILTAALLGGIAHQVASAQEFDDRWYLAGSTG... 40.7K
mature mop B: pyro-QEFDDRWYLAGSTG... 38.5K

The *Xf* mopB amino acid sequence differs from the citrus strain *Xf* mopB at only seven sites. The next most similar proteins in databases form a group of more than 15 “ompA” proteins of Gram-negative bacteria. The ompA proteins show close to 30% similarity to *Xf* mopB, confined mostly to the carboxyl terminal region. The *Pseudomonas fluorescens* ompA protein OprF and *Xf* mopB, unlike most other ompA proteins, have a proline-rich region preceding the carboxyl end region of similarity. *P. fluorescens* competes against certain root-pathogenic fungi because of its ability to colonize root surfaces. De Mot and Vanderleyden (1991) purified OprF, a major outer membrane protein, and demonstrated that OprF binds tightly to roots and probably is responsible for some aspect of the root-adhesion capabilities of *P. fluorescens*, a supposition also consistent with mutational studies (Deflaun, Marshall et al. 1994). Therefore, we postulate that mopB may contribute to *Xf* virulence by adhering to xylem element interior surfaces.

The insoluble fraction obtained after 30°C SDS extraction of *Xf* cells was solubilized in hot SDS and chromatographed on 6% agarose beads. As indicated in the figure, fractions (lanes 2-10) were analyzed by SDS-PAGE to identify those showing greatest purity of *Xf* mopB. Pooled fractions were concentrated for production of rabbit anti-mopB. Attempts at cloning *Xf* mopB in *E. coli*, using constructions that encompassed the entire *Xf* mopB gene, including its putative promoter, were not successful. Therefore, the *Xf* mopB open reading frame (ORF) was placed under control of a bacteriophage T7 RNA polymerase promoter in *E. coli* strain BL21(DE3)pLysS, which bears a *Lac* promoter-driven T7 RNA polymerase gene, as well as a T7 lysozyme gene to prevent accumulation of active T7 RNA polymerase prior to induction of the *Lac* promoter. Induction of cultures with IPTG resulted in appearance of a new band with *Xf* mopB mobility as detected by immunoblotting (lanes 11,12; work of Paul Feldstein), but not to levels readily detected by staining with Coomassie blue. That is, *Xf* mopB accumulation may sicken *E. coli*. A band (lane 11), from uninduced culture and reacting with anti-*Xf* mopB antibody, may be due to cross reaction with the *E. coli* outer membrane protein ompA, which is slightly smaller than *Xf* mopB.



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