Xylella fastidiosa Virulence Factor Mutant Strain as a Promising Biocontrol for Pierce’s Disease

Lingyun Hao¹, Kameka Johnson¹, Patricia Mowery², Luciana Cursino², and Thomas J. Burr¹

¹Plant Pathology and Plant-Microbe Biology Department, New York State Agricultural Experiment Station / Cornell University, Geneva NY; ²Biology Department, Hobart and William Smith Colleges, Geneva, NY

Abstract

Xylella fastidiosa is an important phytopathogen that infects a number of important crops including citrus, almonds, and coffee. The X. fastidiosa Temecula strain infects grapevines and causes Pierce’s disease. We recently deleted the X. fastidiosa PD1311 gene and found that the strain was no longer pathogenic. Based on sequence analysis, PD1311 appears to encode an acyl-CoA synthetase, which is a class of enzymes involved in many different processes including secondary metabolite production. Given the critical role of PD1311 in Pierce’s disease development, we are exploring how it induces its phenotype. In addition, we are testing the ΔPD1311 strain as a potential biocontrol for preventing Pierce’s disease.

Background

X. fastidiosa is a Gram-negative, xylem-limited bacterium that is transmitted to plants by xylem sap-feeding insects. X. fastidiosa Temecula induces Pierce’s disease in grapevines. Once in the xylem, X. fastidiosa is postulated to 1) migrate, 2) aggregate, and 3) form biofilm that clogs the vessels, leading to Pierce’s disease.7

While the steps leading to blocked vessels appears to be key to disease, we wanted to explore if additional virulence factors facilitate symptoms. We explored a X. fastidiosa gene, PD1311, that is a putative acyl-CoA synthetase (ACS). The most studied bacterial ACS is the Escherichia coli FadD, which catalyzes fatty acid metabolism.8-10 proteins are implicated in cell signaling, protein transportation, and protein acylation.8-10 Importantly, ACSs are known to be involved in virulence factors.7 We discovered that deleting PD1311 results in a non-pathogenic strain when inoculated in grapevines, indicating that PD1311 is fundamental for Pierce’s disease development. Given our findings with the ΔPD1311 strain, we have two lines of inquiry.

1) Does the ΔPD1311 strain have potential as a biocontrol for Pierce’s disease? If yes, what will be the timing for application to achieve best disease suppression? The weakly virulent X. fastidiosa complement strain EB2-1311 has been studied as a potential Pierce’s disease biocontrol.9-10 Other approaches include naturally resistant rootstocks or transgenic varieties.11-16 However, continued research for Pierce’s disease controls is warranted.

2) In relation to disease, what are the roles of the putative PD1311 protein? These findings may provide key insights into Pierce’s disease development.

Results

Fig. 1. Pierce’s disease. A) X. fastidiosa clogging xylem vessels. B) Grapevines showing Pierce’s disease symptoms.17

Fig. 2. The ΔPD1311 strain is avirulent. Grapevines were inoculated with wild-type X. fastidiosa (diamond), ΔPD1311 strain (circle), or buffer (square). Symptoms were monitored on 10 plants per treatment over 20 weeks and rated on a scale of 0-5.18-19 Plants were started late in the season and the buffer control showed symptoms at week 14, suggesting that the ΔPD1311 symptoms may be due to senescence and not Pierce’s disease.

Fig. 3. The ΔPD1311 strain impacts wild-type cell biofilm formation. Quantification of biofilm in 96 wells plates with agitation with equal amounts of wild-type X. fastidiosa constitutively expressing green fluorescent protein (wt-GFP) plus either wild-type X. fastidiosa (wt) or ΔPD1311 strain (ΔPD1311). Experiment was performed with 24 replicates. Fluorescence in artificial units.

Fig. 4. ΔPD1311 strain reduces PD. Grapevines were inoculated with wild-type X (1), ΔPD1311 strain (2), ΔPD1311 complement strain (3), co-inoculation ΔPD1311 and wild-type X (4), pre-treat with ΔPD1311 strain two weeks before wild-type cells (5), post-treat with ΔPD1311 strain two weeks after wild-type cells (6), and buffer (7). Bold lines represent the median values and circles representing outliers of each data group. Symptoms have been monitored on 12 plants for each treatment for 24 weeks and rated on a scale of 0-5.18

Conclusions and Future directions

X. fastidiosa motility, aggregation, and biofilm formation are key steps in Pierce’s disease development.7 The putative X. fastidiosa PD1311 protein is critical for the development of Pierce’s disease in grapevines and is involved in one or more of the vessel-blocking disease steps. More importantly, ΔPD1311 suppresses Pierce’s disease caused by the WT strain on grapevines, and pre-treating plants with ΔPD1311 before WT inoculation seem to give the greatest disease suppression. In the future, we would like to focus on the following research areas:

1) Optimize timing and conditions for application of the ΔPD1311 strain

2) Continue investigation of the roles of the ΔPD1311 protein and understand the bacterial control mechanism

3) Develop clean deletion strain of ΔPD1311 that would be suitable commercialization

Overall, these studies will help further understanding of disease development and prevention and facilitate the utilization of ΔPD1311 as a biological control agent

References


Acknowledgements

We thank the California Department of Food and Agriculture Pierce’s Disease Program for funding (860779). We thank Prof. Steven Lindoso, University of California, Berkeley, for the X. fastidiosa gfp strain. We also thank Cheryl Genevans and Cherie Reid for technical assistance.