PARATRANSGENESIS TO CONTROL PIERCE'S DISEASE: THE "SOCIAL LIFE" OF *ALCALIGENES XYLOSOXIDANS DENITRIFICANS*

Project Leaders:

Carol Lauzon
Department of Biological Sciences
California State University, Hayward
Hayward, CA 94542

Thomas A. Miller
Department of Entomology
University of California
Riverside, 92521

Collaborators:

Blake Bextine
Department of Entomology
University of California,
Riverside, 92521

David Lampe
Department of Biological Sciences
Duquesne University
Pittsburgh, PA 19219

Don Cooksey
Department of Plant Pathology
University of California
Riverside, 92521

Frank Richards
Yale Medical School
New Haven, CT 06520

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ABSTRACT

Characteristics of *Alcaligenes xylosoxidans denitrificans* (*Axd*), a bacterium associated with *Homalodisca coagulata* (Say) and several host plants of this sharpshooter, were examined because this bacterium is being considered for use as a paratransgenic vehicle for control of *Xylella fastidiosa* (*Xf*). *Axd* established in sharpshooter vectors of *Xf* but was not found in predators that *Axd*-fed sharpshooters. *Axd* did not establish on or within table grapes, or in a variety of red and white wines. The bacterium also did not establish in soil samples. *Axd* catabolized a variety of nitrogen substrates but did not produce certain extracellular enzymes considered to be virulence factors. An auxotrophic subpopulation of *Axd* that requires cysteine was isolated.

INTRODUCTION

Homalodisca coagulata (Say), the glassy-winged sharpshooter (GWSS), acquires and transmits the causative agent of Pierce's disease (PD), *Xylella fastidiosa* (*Xf*). While much attention has been put forth to characterize, describe, and understand the establishment of *Xf* within the sharpshooter, little effort has been directed toward the isolation of other microbial inhabitants within the alimentary canal of GWSS. If GWSS do possess a normal microbiota, or harbor transient microorganisms for some time period, then these microorganisms may inhibit or possibly facilitate the attachment of *Xf* with the sharpshooter. Determination of either scenario would add to what is currently known about PD transmission and could be useful for the implementation of a paratransgenic strategy to control PD.

Alcaligenes xylosoxidans denitrificans (*Axd*) is one bacterium that has been isolated routinely from GWSS Here we report on research over the past year that examines the use of *Axd* as a paratransgenic candidate for management of PD.

OBJECTIVES

1. Characterize the establishment of *Axd* in GWSS, blue green, and smoke tree sharpshooters.
2. Determine the survival and growth of *Axd* in fruits and wine.
3. Determine if *Axd* is passed horizontally to predators of GWSS.
4. Monitor the growth and survival of *Axd* in soil.
5. Survey *Axd* for auxotrophic subpopulations.

RESULTS

GWSS, captured in nature, were aseptically dissected for their alimentary canal organs, particularly, cibarial pumps, fore- and midguts. *Axd,* was the most frequently isolated bacterial species and subsequently was chosen as the first candidate for use in a paratransgenic strategy. We have spent the last year monitoring the activities of *Axd* and transformed strains of *Axd,* spanning the behavior and biology of *Axd* in host plants (both field and laboratory experiments), in sharpshooters, in predators that fed on sharpshooters and under different culture conditions. In summary, we have found that *Axd* and transformed strains of *Axd* establish in plant xylem of a variety of host plants and sharpshooters that feed on these plants. *Axd* and strains thereof do not, however, grow or grow well in soil with established microbial communities. Similarly, *Axd* and strains thereof do not grow in a variety of grapes or wines. *Axd* strains were also not found in predators that fed on sharpshooters. *Axd* strains lack the expression of typical virulence factors, such as extracellular enzymes and β- hemolysin. A cys^- population of *Axd* was isolated and this population, if deemed fit, would not establish in environments lacking cysteine.

CONCLUSIONS

Axd is a promising candidate for a paratransgenic approach to prevent, control, and/or manage Pierce's disease. *Axd* establishes well in vectors of *Xf* and host plant xylem but does not establish well in non-target environments that we have surveyed. If *Axd* cys^- are shown to be fit, the use of this strain would reduce and/or eliminate certain concerns associated with

the release of a genetically augmented bacterium in the environment. Nevertheless, it appears that the spread of *Axd* strains is limited by biotic and abiotic conditions associated with the host plant environment of Pierce's disease susceptible plants.

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