INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* (PARATRANSGENESIS FOR CONTROL OF PIERCE'S DISEASE): IDENTIFICATION OF ENDOPHYTIC BACTERIA CYCLED BY GLASSY-WINGED SHARPSHOOTERS TO HOST PLANTS

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Reporting Period: The results reported here are from work conducted from April 1, 2001 to October 31, 2002.

INTRODUCTION

Homalodisca coagulata Say, the glassy-winged sharpshooter (GWSS) is known to transmit the etiological agent of Pierce's disease, *Xylella fastidiosa*. A paratransgenic approach designed to disrupt the ability of the insect vector to transmit the pathogen involves finding bacterial candidates that possess some degree of intimacy either within the insect and/or host plant xylem. Once a candidate(s) is/are found, then avenues open for finding strategies aimed at controlling *X. fastidiosa* infection and/or transmission, such as paratransgenesis. This report details the survey for, and isolation and identify of candidate bacteria for use in a paratransgenic strategy to control Pierce's disease. It also includes information about the possible relationship internal extracellular bacteria have with GWSS.

OBJECTIVES

1. Identify bacterial candidates for use in a paratransgenic strategy for control of Pierce's disease.
2. Understand the relationship(s) between and among bacteria in sharpshooters and their host plant xylem.

RESULTS AND CONCLUSIONS

Glassy-winged sharpshooters, captured in nature, were aseptically dissected for their alimentary canal organs, particularly, cibarial pumps, fore- and midguts. Bacterial inhabitants were retrieved using dilute nutrient media held at 22-24^{o}C. The lower concentration of nutrients in bacteriological media mitigated the typical problems associated with growing endophytic bacteria under laboratory conditions (Bell et al. 1995). Isolates were identified using standard biochemical tests and morphological methods. Three primary bacterial species were isolated and identified from the cibarial region and fore-and midguts as (in order of dominance): *Alcaligenes xylosoxidans denitrificans, Chryseomonas luteola*, and *Ralstonia pickettii.* These bacteria are typical of plants (endophytes), soil, and water (Holt and Krieg, 1992). Two *Bacillus* spp., *Bacillus coagulans and Bacillus brevis* were infrequently isolated from midgut samples. Another isolate, tentatively identified as *Sporosarcina* sp., and a yeast-like organism were also infrequently isolated from pump samples.

Twenty-four biochemical tests were performed on the three primary isolates. The data suggest that these bacteria may be participating in nitrogen and hydrogen cycling within GWSS. To begin to determine the extent of nitrogen and hydrogen activity within the gut of GWSS and the possible contribution of bacteria with these activities, we performed a cytochemical assay using transmission electron microscopy (McLean et al. 1985). We found that nitrogenous compounds are concentrated within the midgut of GWSS (Figure 1) and that where bacteria were present, they participated in nitrogen catabolism within the insect gut (Figure 2). Metabolism of nitrogenous and/or other organic compounds by sharpshooters has been examined (i.e. Anderson et al. 1989; Brodbeck et al. 1993, 1995, 1996, 1999), however, the contribution of bacteria in xylem and/or in sharpshooters to these processes has not been addressed.

The mouthparts and guts of wild-captured GWSS were also examined for microorganisms using fluorescent techniques. Dissection and transection of GWSS tissues were stained using the ViaGram^{TM} Red+ Bacterial Gram Stain and Viability Kit (Molecular Probes Inc., Eugene, OR). Images were obtained using fluorescent and confocal laser scanning microscopes and revealed the presence of stationary and motile viable bacteria within the mouthparts and gut regions of GWSS samples. In addition, the yeast-like microorganism was found within mouthpart samples. Similar images were acquired using scanning electron microscopy of GWSS samples.

The three primary bacterial isolates, *Alcaligenes* sp., *Chryseomonas* sp., and *Ralstonia* sp., were screened for their response to a battery of antibiotics in preparation for genetic manipulation experiments performed by Dr. D. Lampe (the reader is referred to the report presented by D. Lampe for details). Subsequently, we received numerous GWSS from Dr. B. Bextine.

Dr. Bextine used his plant-based delivery system (the reader is referred to the report presented by B. Bextine for details) for the introduction of DsRed *Alcaligenes xylosoxidans denitrificans* (RAX) to both GWSS and plants. In both cases, we often detected (typically in 40% of samples) RAX in treated samples with no detection of RAX in controls. Therefore, RAX was found to cycle from plants to GWSS and additionally, from GWSS containing RAX to plants.

We are currently engaged in experimentation designed to determine the physiological parameters that facilitate optimal establishment and cycling of RAX in plant and insect samples. We are working toward determining the accuracy of our detection level of RAX in insect and plants, i.e. is our current detection methodology using fluorescent microscopy an underestimate of the actual presence of cells within the samples? Concurrently, we are attempting to determine the optimal delivery dose and physiological state of RAX necessary for use in an effective paratransgenic strategy. We aim to conduct similar experiments using the transformed *Chryseomonas* sp. in the near future.

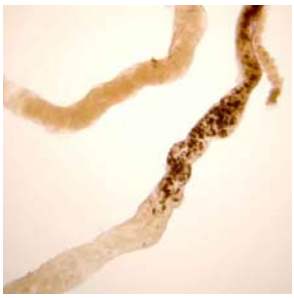

Figure 1. Midgut from a wild GWSS after partial treatment for detection of nitrogenous compounds. Dark areas reveal the presence of localized nitrogen.

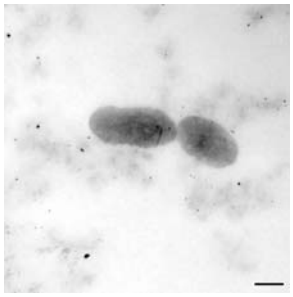

Figure 2. Transmission Electron micrograph showing bacteria in the same wild GWSS midgut participating in nitrogen catabolism. Dark areas are indicative of nitrogen catabolism and nitrogenous products.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service.

Genetics of Xylella fastidiosa / Miscellaneous