ANALYSIS OF THE BACTERIAL COMMUNITY INHABITING GLASSY-WINGED SHARPSHOOTER FOREGUT BY CULTURE-DEPENDENT TECHNIQUES AND DGGE

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ABSTRACT

The glassy-winged sharpshooter (GWSS) is an important vector of *Xylella fastidiosa* (*Xf*), the bacterial pathogen that causes several economically important plant diseases, including citrus variegated chlorosis (CVC), oleander leaf scorch (OLS) and Pierce's disease (PD) of grapevines. In recent years there has been an increasing interest in the potential use of biological control agents to halt the spread of *Xf*. One such strategy is the exploration of symbiotic microorganisms to reduce the spread of the pathogen (symbiotic control). In a symbiotic control strategy a bacterium symbiont that occupies the same niche as the pathogen must be identified. The study of the bacterial community of GWSS foreguts by isolation and DGGE revealed the presence of several potential symbiotic candidates such as *Bacillus* sp., *Pseudomonas* sp., *Methylobacterium* sp. and *Curtobacterium flaccumfaciens* (*C. flaccumfaciens*). Members of genus *Methylobacterium* and *C. flaccumfaciens* are frequently isolated as endophytes from citrus plants with CVC symptoms and without disease symptoms. Recently, an interaction between *Methylobacterium*, *C. flaccumfaciens* and *Xf* was strongly indicated, reinforcing that these bacteria could interact inside the host plant and vector insect. In the future, the genus *Methylobacterium* and *C. flaccumfaciens* could be an interesting candidate in a strategy of symbiotic control to *Xf*.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) is one of the main vectors of *Xylella fastidiosa (Xf)*. It is a xylophagous insect that has a wide array of host plants, including many ornamental and crop plants (Purcell and Hopkins 1996, Purcell and Saunders 1999). One new potential management strategy for Pierce's disease (PD) of grapevine is the use of symbiotic control. Symbiotic control exploits the interactions among a pathogen-transmitting organism, its bacterial symbionts, and the pathogenic organism itself (Beard 2002). For symbiotic control to be effective in limiting the spread of PD, a culturable symbiont that inhabits the pre-cibarium and cibarium of GWSS is required, since these areas are colonized by the pathogen, *Xf*. A previous biochemical analysis of the GWSS foregut microdiversity encountered three bacterial species that meet these requirements: *Chryseomonas* spp, *Ralstonia* spp, and *Alcaligenes* spp (Bextine 2004). The *Alcaligenes* species were of particular interest because they were frequently isolated from wild GWSS (Kuzina 2004). Although *Alcaligenes* spp. can colonize GWSS, this specie does not colonize grapevines well (Bextine 2005). According to Bextine et al (2005), the amount of colonization by *Alcaligenes xylosoxidans* subsp. *denitrificans* decreased in the following order: orange (*Citrus sinensis* ''sweet orange'') > chrysanthemum (*Chrysanthemum grandiflora* cv. ''White Diamond'') > periwinkle (*Vinca rosea*) > crepe myrtle (*Lagerstroemia indica*) > grapevine (*Vitis vinifera* cv. Chardonnay). Therefore, steps in a symbiotic control strategy should be directed in finding a bacterial symbiont that colonizes well the pre-cibarium and cibarium of GWSS as well as the target host plant: grapevines. The CelectrophoreticCT separation of PCR products of variable regions of genes encoding 16S rDNA (by use of primers homologous to conserved regions of the gene) in a polyacrylamide matrix over a denaturing gradient (DGGE) is a technique recently introduced in microbial ecology (Muyzer et al. C1993CT). The denaturing gradient can be achieved either chemically with urea and formamide in DGGE. This technique is reported to be interchangeable, giving comparable fingerprints of microbial communities. Recently, Reeson et al. (2003) demonstrated the efficiency of DGGE to study the bacterial communities associated to insects (wasp *Vespula germanica*).

OBJECTIVES

1. The aim of this work was to characterize the bacterial community inhabiting GWSS foreguts by using isolation in culture medium and DGGE techniques.

RESULTS

The study of bacterial community by isolation and DGGE (Figures 1, 2, and 3) revealed the presence of several genera of bacteria such as *Bacillus* sp., *Cryocola* sp., *Microbacterium* sp., *Micrococcus* sp., *Pedobacte* sp.r, *Pseudomonas* sp., *Methylobacterium extorquens*, *C. flaccumfaciens*, *Baumannia cicadellinicola*, and *Wolbachia* spp. Members of genus

Methylobacterium are frequently isolated as endophytes from citrus plants with CVC symptoms and recently, an interaction between *Methylobacterium* spp., *Curtobacterium flaccumfaciens* and *Xf* was strongly indicated (Araújo et al. 2002, Lacava et al. 2004) reinforcing that these bacteria could interact inside the host plant and vector insect. Besides, Lacava et al. (2004) suggests that the CVC symptoms in citrus plants could be a result of the population balance between *Methylobacterium* spp., *C. flaccumfaciens* and *Xf.*

CONCLUSIONS

Our study of bacterial diversity associated with GWSS foreguts was initiated by using culture-dependent methods as well as based on sequence (DGGE) polymorphisms of 16S rRNA gene, using total DNA extracted from GWSS foreguts. The diversity profiles obtained with isolation methods indicated a low bacterial diversity. On the contrary, a high bacterial diversity in GWSS foreguts was observed with the use of DGGE (culture independent technique). The preliminary results show that DGGE is suitable for the analysis of the bacterial diversity in GWSS and in the future, bacteria such as *Methylobacterium* spp. *C. flaccumfaciens*, found as part of the bacterial community of GWSS, could be used as potential candidates in a symbiotic control strategy against *Xf*. Further analyses of the data collected in the present study are still being conducted and they will be presented at the symposium.

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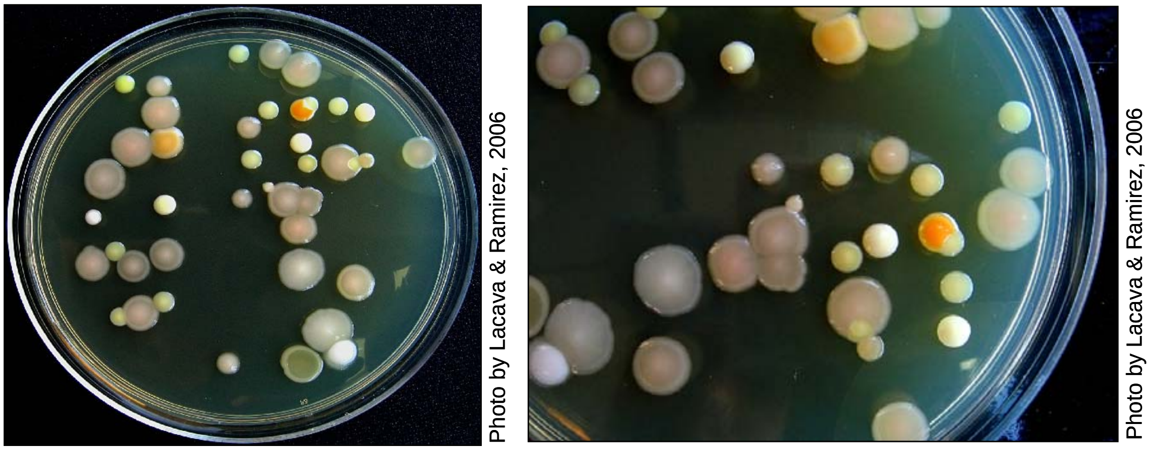
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Figure 1. Petri dishes containing bacterial colonies isolated from GWSS heads.

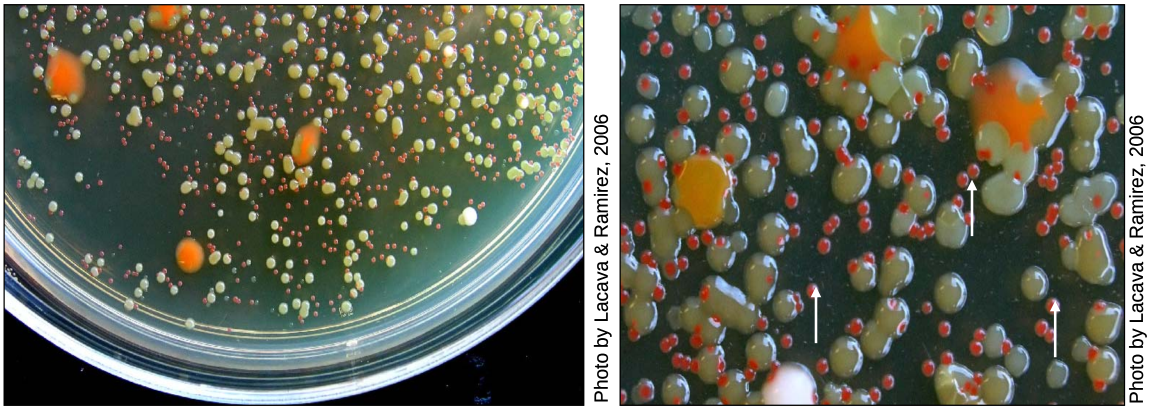
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Figure 2. Petri dishes containing bacterial colonies isolated from GWSS heads. The white arrows (right photo) indicate *Methylobacterium extorquens* isolated from GWSS heads.

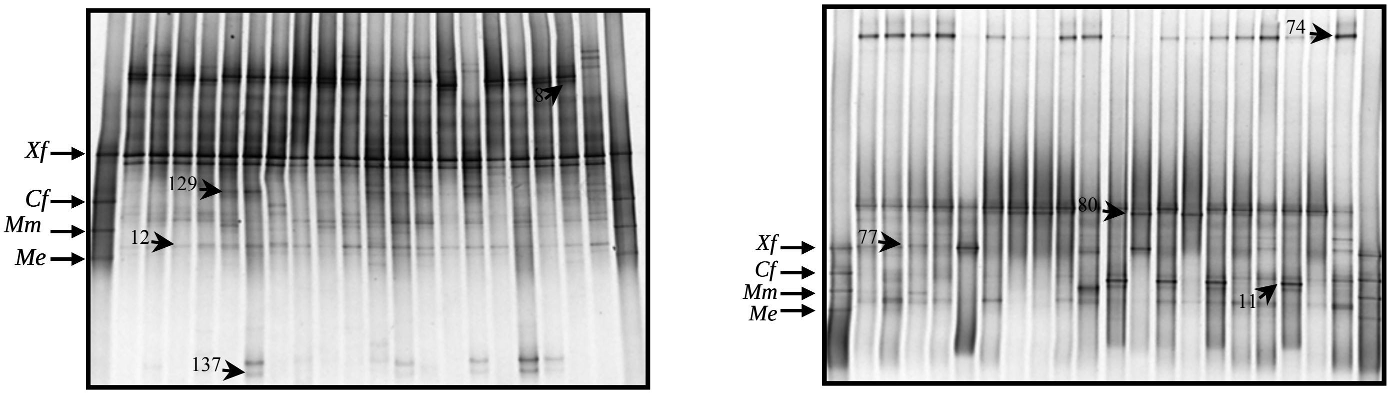

Figure 3. DGGE fingerprints of the bacterial communities from GWSS. **(A)** Young GWSS collected in asymptomatic *Citrus* sp. ; **(B)** Adults of GWSS collected in PD infected grapes and then transferred and reared in healthy grapevines. *Xf: Xylella fastidiosa* (PD strain); *Cf: Curtobacterium flaccumfaciens; Mn: Methylobacterium mesophilicum; Me: Methylobacterium extorquens.*

SPECIES DIVERSITY, DISTRIBUTION, AND ABUNDANCE OF XYLEM FLUID FEEDING HEMIPTERA IN VINEYARDS THROUGHOUT TEXAS

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ABSTRACT

A survey of xylem fluid feeding insects (Hemiptera: Auchenorrhyncha) exhibiting potential for transmission of *Xylella fastidiosa* (*Xf*) the bacterium causing Pierce's disease (PD) of grapevine was initiated in Texas in 2003 and continues to the present. Twenty five insect species belonging to 4 families and 14 genera were collected from yellow sticky traps and identified. Among these, three species, two leafhoppers and one spittlebug, comprised over 90% of the xylem fluid feeding insects caught. *Homalodisca vitripennis* (formerly *H. coagulata*), the most commonly known vector of PD of grapevine in the U.S. is the most common and abundant insect captured across the state. Insect abundance varied significantly over seasons, with most of captures taking place between June and August, and per location, with 5 to 15 species caught at any given vineyard. Residual populations found overwintering near vineyards will be the focus of future studies. A grape strain and an ornamental strain of *Xf* have been detected in insects processed by real time PCR. These initial observations provide critical information to vineyard managers for timely applications of insecticides prior to insect feeding and vectoring to susceptible grapevines.

INTRODUCTION

Pierce's disease (PD) of grapevine has become the most limiting factor to grape production in Texas, as it has in California during the past decade. It is transmitted by certain insects which feed on xylem fluid of plants where the bacterium develops. During the 1990's, the grape growing region of Central Texas witnessed an increase in the incidence and severity of PD (Texas Pierce's Disease Task Force 2004). The insect vectors were not thoroughly identified but glassy-winged sharp-shooters (GWSS), *Homalodisca vitripennis* (Cicadellidae), were suspected. A modest research program was initiated in Texas, funded by the U.S. Department of Agriculture starting in 2002-2003. Within this program, researchers are provided an opportunity to study glassy-winged sharpshooters in their natural habitat. During the past years, intensive studies to identify the vectors and their vectoring potential have been taking place in a growing multi-disciplinary, multi-institutional research program. Exploration for insect species involved, their ecology, host plants used, molecular characterization of *Xf*, vectoring capacity of the Hemiptera captured, natural enemies and population dynamics are well underway.

OBJECTIVES

1. Monitor xylem feeding insect populations in vineyards across Texas. Identify all putative insect vectors of PD. Determine the most common vectors requiring population management, make observations on vector distribution, density and seasonality.
2. Explore for host plants used as breeding sites by insect vectors throughout the year, assess the reproductive state of adult females and determine the age structure composition of the vectors
3. Characterize the proportion of insects carrying *Xf* and the pathotypes involved. Investigate evolution of infection levels throughout the year.

RESULTS

Insect populations in the Hemiptera were monitored on a bi-weekly fashion in 45 vineyards for over 3 years. We placed particular interest in those populations of xylem fluid feeding insects that may play a role in the transmission of *Xf*, causal agent of PD in grapevines. Data indicate the presence of xylem fluid feeding leafhoppers-treehoppers (Membracoidea), spittlebugs (Cercopoidea) and cicadas (Cicadoidea). Of 160 Hemiptera species captured, 25 species have been identified so far as xylem fluid feeding insects present in the vineyards and adjacent natural habitat, all with the potential to carry and transmit *Xf* when feeding on susceptible host plants. Captured insects in the family Cicadellidae (leafhoppers) were the most abundant with a total of 15 species recovered; this family contributed to about 75% of all individuals caught. Predominant species were *Homalodisca vitripennis*, *Graphocephala versuta* and *Clastoptera xanthocephala*, two leafhoppers and a spittlebug. These species together comprised over 90% of all xylem fluid feeding insects identified. *Homalodisca vitripennis* and *C. xanthocephala* were present at each of the surveyed locations. At certain locations, a specific Hemiptera species clearly dominated. Insect species diversity varied from 5 to 15 per location, with an average of 9.18 ± 2.87species per location. Abundance of major xylem fluid feeding Hemiptera species varied greatly throughout time. Monthly variations of the adult populations of xylem fluid feeding Hemiptera well correspond to mean temperatures. These insects' populations increased significantly from April to June when they peaked. After June, insect densities, indicated by trap captures, decreased gradually until they reached their minimal levels between the months of November and April. A similar pattern of