HEMOLYMPH-ASSOCIATED SYMBIONTS: IDENTIFICATION OF *DELFTIA* SP. IN GLASSY-WINGED SHARPSHOOTERS AND INVESTIGATION INTO THEIR PUTATIVE FUNCTION

Principal Investigator:
Blake Bextine
Dept. of Biology
University of Texas-Tyler
Tyler, TX 75799
bbextine@uttyler.edu

Researchers:
Lucas Craig Shipman
Dept. of Biology
University of Texas-Tyler
Tyler, TX 75799
lshipman@patriots.uttyler.edu

Daymon Hail
Dept. of Biology
University of Texas-Tyler
Tyler, TX 75799
daymon.hail@gmail.com

Cooperator:
Scot E. Dowd
Research and Testing Lab
Lubbock, TX 79407
sdowd@pathogeneresearch.org

Reporting Period: The results reported here are from work conducted March 2009 to December 2010.

ABSTRACT

The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) feeds on a wide variety of host plants including grapes, oleander, and citrus and is the primary vector of *Xylella fastidiosa (Xf)*, the causal agent of Pierce's disease of grapevine, oleander leaf scorch, and citrus variegated chlorosis. Additional bacterial species have been identified within GWSS which may contribute to the insect's survival and ability to adapt to the environment. *Delftia sp*., a gram negative bacterium which belongs to rRNA superfamily III or the β subclass of the *Proteobacteria*, was detected only in the insect's hemolymph. Therefore, in this study, *Delftia sp*. associated with GWSS hemolymph was further identified through direct sequencing, and the relationship between this symbiont and its host was investigated. *Delftia* is a D-amino acid amidase-producing bacterium. D-amino amidases are increasingly being recognized to be important catalysts in the stereospecific production of D-amino acids. *Delftia* may be found in the hemocoel of the GWSS to hydrolyze D-amino acid amides to yield D-amino acid and ammonia which can perform as the insect's chiral building blocks.

LAYPERSON SUMMARY

The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) is a xylem feeding insect pest that has the ability to transmit the bacterium, *Xylella fastidiosa (Xf)*. This bacterium is the causal agent of Pierce's disease in the grapevine which has had a detrimental impact on the wine and grape industries. Among extensive studies done on *Xf* , many other bacteria that reside in GWSS have been identified. In a recent survey where bacterial extractions were taken from different parts of the insect's body, *Delftia* sp. was the only symbiont found only in the hemolymph (Bextine et al., 2009). Identification and isolation of *Delftia* sp. from GWSS hemolymph preludes investigative techniques that will describe the symbiotic role of this particular bacterium with its host.

INTRODUCTION

The bacterium, *Xylella fastidiosa (Xf)*, is the causal agent of several economically important plant diseases. This insect transmitted bacterium has a well understood relationship with its insect vector, the glassy-winged sharpshooter (GWSS). Many other bacteria that survive inside GWSS have been identified through next generation high throughput sequencing and these symbiotic relationships have not been elucidated (Bextine et al., 2009). *Delftia* sp. was found exclusively in the sharpshooter hemolymph and not in the insect's hemolymph. To confirm *Delftia* sp. presence in the insect's hemolymph, the bacterium was isolated in culture media and a genus-specific primer set [Delf63F (5' TAACAGGTCTTCGGACGC 3') and Delf440R (5' CCCCTGTATTAGAAGAAGCT 3')] was used to confirm presence.

Delftia sp. is a known D-amino acid amidase-producing bacterium. Investigating the symbiotic role of this bacterium in the hemolymph can be hypothesized based on this known amidase formation. In one study on amidase production, soil samples were used and based on morphology, physiological traits and 16S rRNA sequence analysis, *Delftia tsuruhatensis* was identified as the only bacterium capable R-enantioselective degradation of 2, 2-dimethylcyclopropanecarboxamide. Two other strains in the genus Delftia have been reported to produce R-stereospecific amidase: *Comamonas acidovorans* and *Delftia* acidovorans (Zheng et al., 2007). In another soil sample study, bacteria were monitored for amidase production and the strain exhibiting the strongest activity was identified as *Delftia acidovorans* strain 16. This strain produced intracellular D-amino acid amidase constitutively (Hongpattarakere et al., 2005). In order to investigate characteristics of the enzyme, amidase, was produced intracellularly, Hongpattarakere et al. (2005) analyzed its activity which was only seen in the supernatant of sonicated cell-free extract and no activity was seen from in the supernatant of culture broth. Obviously, amidase is a major enzyme produced by *Delftia* sp. The relationship between *Delftia* sp. and GWSS hemolymph is unknown Additionally, the possible role of amidase in the insects hemolymph warrents further investigation.

OBJECTIVES

1. Identify *Delftia* sp. in hemolymph.
2. Investigate relationship between *Delftia* sp. and its host.

RESULTS AND DISCUSSION

16S pyrosequencing based upon the bTEFAP methodology (Dowd et al., 2008a; Dowd et al., 2008b) optimized for theTitanium pyrosequencing platform (Roche, Indianapolis, IN) was used to identify bacterial species in GWSS whole

bodies, hemolymph, and alimentary canal. Seventeen orders (**Figure 1**) and at least 38 genera (**Figure 2**) of bacteria were detected in the insect's hemolymph. In a replicated study; sequences were taken from separately prepared extracts of hemolymph suspended in 1X PBS. The sequences were approximately 500 bp (370-820 bp) and were compared to NCBI's basic local alignment search tool (BLAST) for homologies. Some of the shorter sequences aligned to multiple genera and were placed in a separate category called "Other" because it was not clear which identification was appropriate.

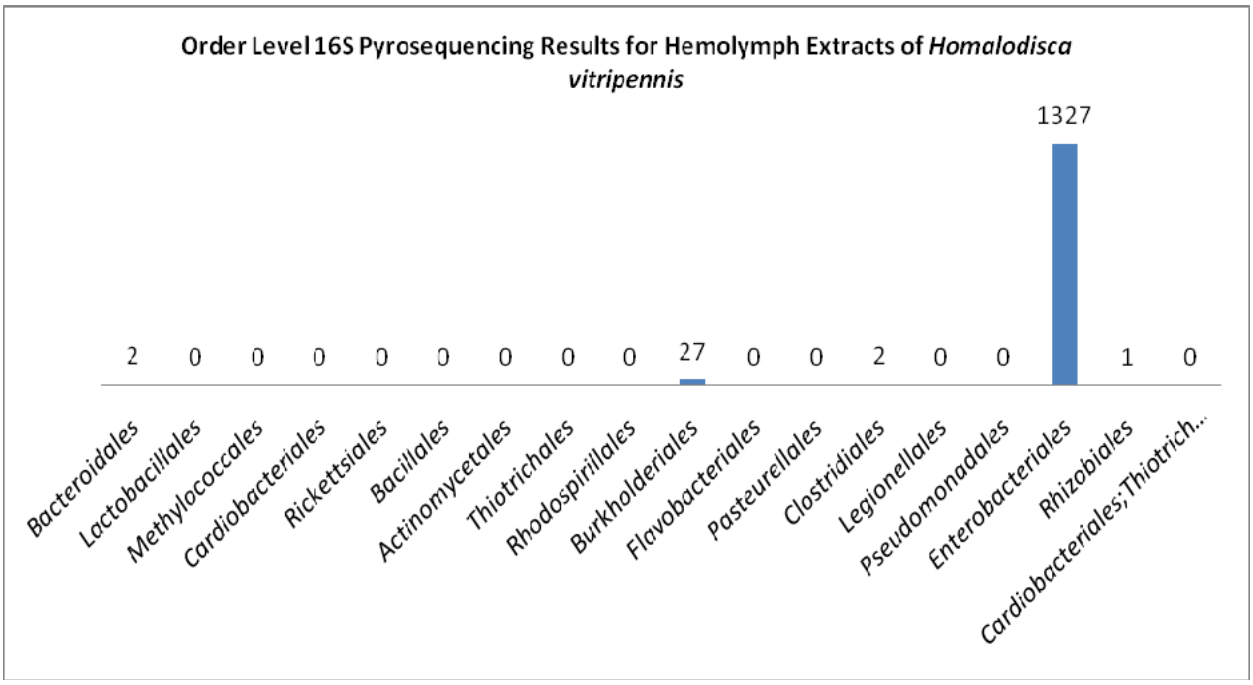

Figure 1. Order level sequencing results for the hemolymph of the GWSS. Larger numbers of sequences related to Enterobacteriales were recovered as well as Bacteroidales and Burkholderiales.

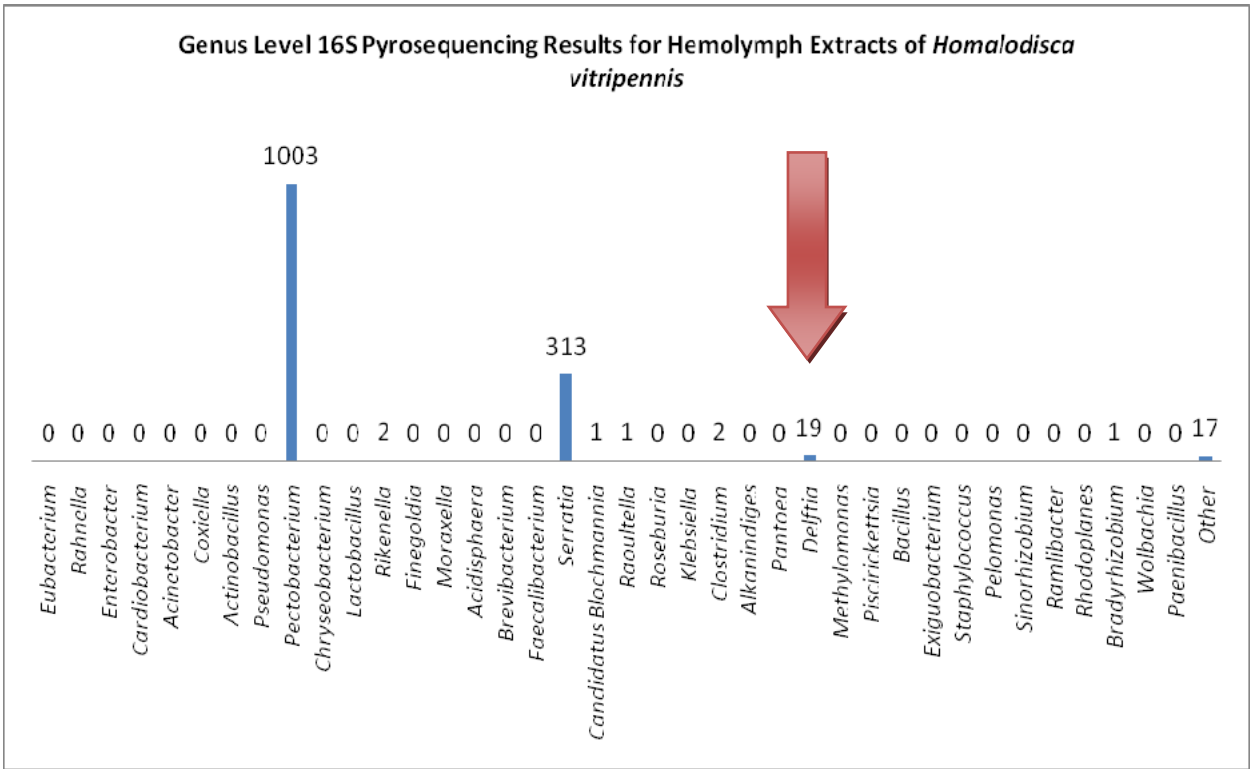

Figure 2. Genus level sequencing results for the hemolymph of the GWSS. Large numbers of *Pectobacterium* and *Serratia* were recovered. Some *Delftia* (formerly *Pseudomonas*) and other non-specific identifications were made. Note that no sequences from *Wolbachia*, an intracellular symbiont, were recovered.

Delftia sp. sequences were recovered in all GWSS hemolymph samples and in whole insect preparations. This genus was never detected in the insect's alimentary canal. *Delftia* sp. is known to be D-amino acid amidase-producing bacteria. An interesting finding was that in contrast to other amidases inhibited by low concentrations (0.5e10 mM) of urea, amidase from *D. tsuruhatensis* ZJB-05174 was not significantly inhibited by urea even at a high concentration (500 mM). (Zheng et al., 2007). Because inhibition of amidase activity was reported to involve binding of urea to the active site of the enzyme, these results suggested that this amidase from *D. tsuruhatensis* ZJB-05174 might have a different active site structure. This specific *D. tsuruhatensis* amidase stability could be a reason *Delftia* has a permanent residence in the hemocoel of GWSS. Even more excitingly, studies done on *Drosophila* have demonstrated that flies exhibit strong hemolymph amidase activity that hydrolyzes peptidoglycan into nonstimulatory fragments and that peptidoglycan recognition protein (PGRP-LB) contributes to this activity in vivo (Zaidman et al., 2006). This information could signify the presence of *Delftia* amidase as an immune defender. However, there was a study that somewhat disproves this hypothesis. In that study, the *D. acidovorans* amidase enzyme was active preferentially toward D-amino acid amides rather than their L-counterparts (Hongpattarakere et al., 2005). It exhibited strong amino acid amidase activity toward aromatic amino acid amides including D-phenylalanine amide, D-tryptophan amide and D-tyrosine amide, yet it was not specifically active toward low-molecular-weight D-amino acid amides such as D-alanine amide, L-alanine amide and L-serine amide. Moreover, it was not specifically active toward oligopeptides. Because the bacterial peptidoglycan layer is made up in part by a short (4- to 5-residue) amino acid chain, containing D-alanine, D-glutamic acid, and *meso*-diaminopimelic acid in the case of a gram negative bacteria or L-alanine, D-glutamine, L-lysine, and D-alanine in the case of a gram positive bacteria, amidase activity would not be seen hydrolyzing these low-molecular weight amino acids. Further studies must be done to prove the importance of *Delftia* sp., its amidase production, and its occurrence in the hemolymph of GWSS.

CONCLUSIONS

Delftia sp. was identified in all hemocoel samples collected from GWSS and not from the alimentary canal. This symbiont's known prominent D-amino acid amidase activity has lead to the hypothesis that this enzyme is playing a key role on the insect's survival. In particular, this amidase could contribute as a means of immune protection by its ability to hydrolyze the peptidoglycan layer of invasive pathogenic organisms. More studies must be done to describe the relationship between this symbiont and its host, but its presence in the GWSS definitely provides another avenue to study when finding an answer for GWSS managment.

REFERENCES CITED

Bextine, B., D. Hail, I. Lauzière. 2009. Non-Culture Dependent Survey of the Microbiota of the Glassy-Winged Sharpshooter Using 454 Pyrosequencing, *CDFA's Pierce's Disease Control Program: PD Research Proceedings for 2009*, p. 3-8.

Hongpattarakere, T., H. Komeda, and Y. Asano. 2005. Purification, characterization, gene cloning and nucleotide sequencing of D-specific amino acid amidase from soil bacterium: *Delftia acidovorans*. J. Ind. Microbiol. Biotechnol. 32: 567-576.

Zaidman- Rémy, A., M. Hervé, M. Poidevin, S. Pili-Floury, M. Kim, D. Blanot, B. Oh, R. Ueda, D. Mengin-Lecreulx, B. Lemaitre. 2006. *Drosophila* Amidase PGRP-LB Modulates the Immune Response to Bacterial Infection. *Immunity*. 24, 4: 463-473.

Zheng RC, Wang YS, Liu ZQ, Xing LY, Zheng YG, Shen YC. (2007) Isolation and characterization of Delftia tsuruhatensis ZJB-05174, capable of R-enantioselective degradation of 2, 2-dimethylcyclopropanecarboxamide. *Res Microbiol*, 158:258–264.

FUNDING AGENCIES

Funding for this project was provided by the Texas Pierce's Disease Research and Education Program, and the USDA Animal and Plant Health Inspection Service.