GENOTYPIC CHARACTERIZATION OF *ALCALIGENES XYLOSOXIDANS* SUBSP. *DENITRIFICANS* (*AXD* HC01) AND FOUR RELATED STRAINS

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ABSTRACT
In symbiont therapy, an insect's natural symbionts are genetically modified to prevent the transmission of a pathogen, and this strategy is currently under investigation as a way to control the spread of Pierce's disease. The glassy-winged sharpshooter (*Homalodisca coagulata*) symbiont used in this research was identified through metabolic tests as *Alcaligenes xylosoxidans denitrificans* Hc01 (*Axd* Hc01). Since *Axd* Hc01 has the potential to be used agronomically, fully describing it genetically as well as metabolically is important for regulatory purposes. In this study, we used sequence data from two highly conserved prokaryotic genes, the 16S rDNA gene and the gyrase B gene, to genetically characterize *Axd* Hc01 and four of its relatives. These sequences were aligned and used to generate three neighbor-joining phylogenetic trees, two for the 16S gene and one for the gyrase B gene. A preliminary analysis of this data indicates that *Axd* Hc01 is most closely related to members of the genus *Pseudomonas*.

INTRODUCTION
One new potential management strategy for Pierce's disease (PD) of grapevine is the use of symbiont therapy. Symbiont therapy exploits the interactions among a pathogen-transmitting organism, its bacterial symbionts, and the pathogenic organism itself (Beard 2002). First, a bacterial symbiont that occupies the same niche as the pathogen must be identified. These symbionts are genetically modified to produce a molecule that hinders the spread of the pathogen in question. The genetically modified bacteria are re-introduced into the vector so that they can reduce its ability to transmit the pathogen in question. For this approach to be successful, the bacterial symbiont must be easily cultured and manipulated *in vitro*, and the genetic modification cannot alter their value to the host organism or their ability to occupy their niche. In addition, the bacterial symbionts cannot be pathogenic to either their host or to non-target organisms before or after the genetic modification (Durvasula 2003). Symbiont therapy has been investigated as a way to control the spread of Chagas Disease (Beard 2002; Durvasula 2003), murine colitis (Steidler 2000), and HIV (Chang 2003).

For symbiont therapy to be effective in limiting the spread of PD, a culturable symbiont that inhabits the pre-cibarium and cibarium of *Homalodisca coagulata* (*H. coagulata*) is required, since these areas are colonized by the pathogen, *Xylella. fastidiosa*. Three bacterial species that meet these requirements are *Chryseomonas* spp, *Ralstonia* spp, and *Alcaligenes* spp (Bextine 2004). The *Alcaligenes* species were of particular interest because they were frequently isolated from wild *H. coagulata* (Kuzina 2004) and because they could also successfully colonize the xylem of various plants, including citrus (Araujo 2002, Bextine 2005). Using standard morphological and biochemical tests, one of the *Alcaligenes* species isolated from *H. coagulata* was designated as *Axd* Hc01 and selected for further study (Bextine 2004). However, the classification of *Axd* Hc01 remains unsettled.

OBJECTIVES
If *Axd* Hc01 is to be used as part of a symbiont therapy program, the issues surrounding its taxonomic identity must be resolved. One way to help clarify its identity and relationship to other identified *Axd* strains is to construct phylogenetic trees based on the sequences of universally present, highly conserved prokaryotic genes (Laguerre 1994). The goal of this research is to help identify *Axd* Hc01 and its relatives by placing them in phylogenetic trees based on the 16S, gyrase B, and 16S-23S intergenic spacer region sequences.

RESULTS
The phylogenetic tree based on 16S sequences shown in Figure 1 and the tree based on gyrase B sequences in Figure 3, indicate that *Axd* Hc01 groups with members of the genus *Pseudomonas*. In addition, the phylogenetic tree based on 16S sequences shown in Figure 2 indicates that *Axd*1 is more closely related to *Axd* Hc01 than *Axd*3 and *Axd*4. Abbreviations used are as follows: rAxd, *Axd* Hc01; PA, *Pseudomonas aeruginosa*; AP, *Achromobacter piechaudii*; AR, *A. ruhlandii*; AD, *A. denitrificans*; PP, *Pseudomonas putida*; PF, *P. fluorescens*; Pps, *P. pseudoalcaligenes*; PS, P. stutzeri; AF, *Alcaligenes faecalis*; AO, *Alcaligenes odorans*; BC, *Burkholderia cepacia*; SP, *Shewinella putrefaciens*; SM, *Stenotrophomonas maltophilia*; and XM, *Xanthomonas maltophilia*.

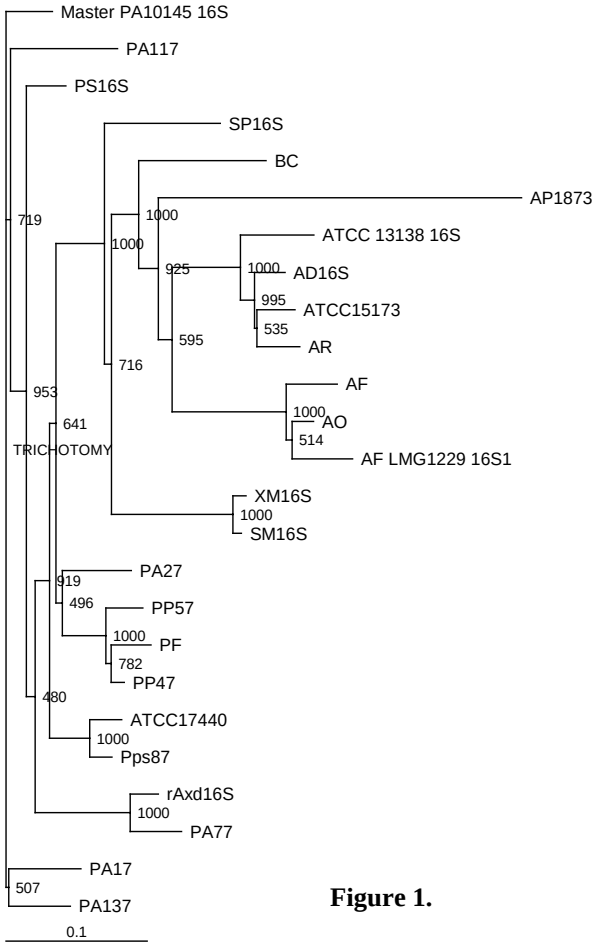

Figure 1.

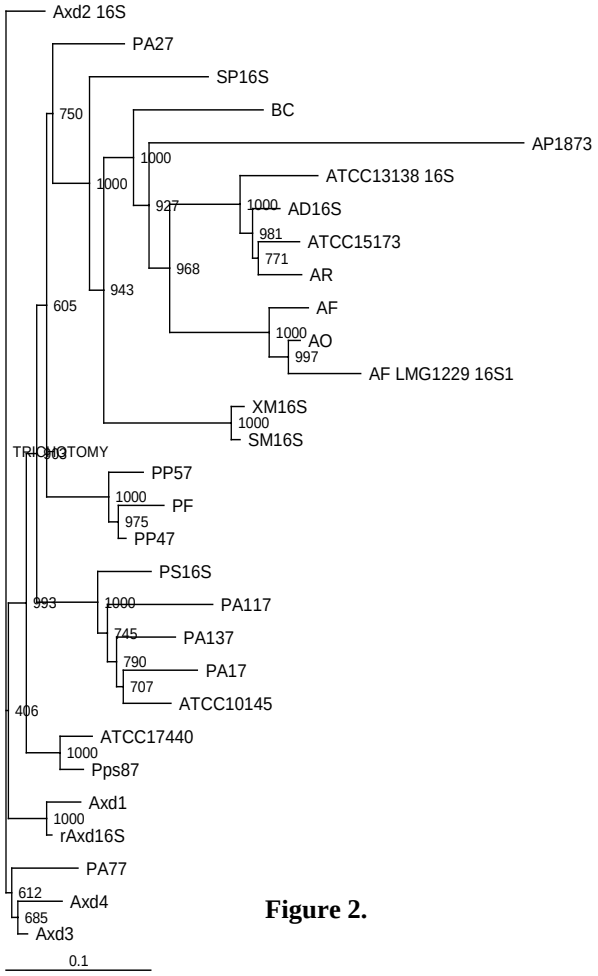

Figure 2.

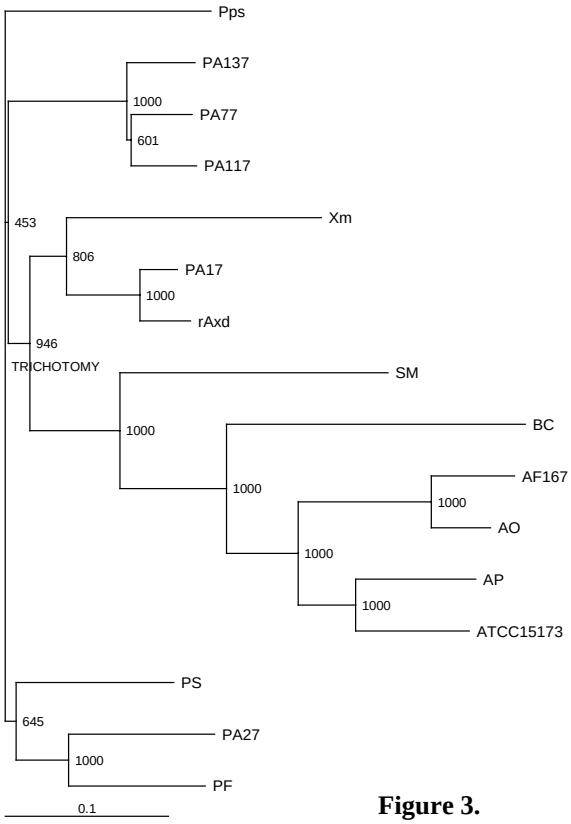

Figure 3.

CONCLUSIONS

From a preliminary analysis of these results, it can be concluded that *Axd* Hc01 and its relatives are related to members of the genus *Pseudomonas*. However, more work will be necessary to provide more information concerning the identity of *Axd* Hc01 at the species and subspecies level and to clarify its relationship to *Axd*1, *Axd*2, *Axd*3, and *Axd*4. The successful identification of the *Axd* Hc01 bacterium and its relatives will help contribute to a strategy based on symbiont therapy to control PD.

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