SYMBIOTIC CONTROL OF PIERCE'S DISEASE: THE BIOLOGY OF THE SHARPSHOOTER SYMBIONT, *ALCALIGENES XYLOSOXIDANS* SUBSP. *DENITRIFICANS*

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ABSTRACT
Alcaligenes xylosoxidans denitrificans (Axd) is closely associated with *Homalodisca coagulata*, the glassy-winged sharpshooter (GWSS), and xylem fluid of host plants. The bacterium has long been characterized as a nitrogen and hydrogen recycler in nature, and was recently recognized as an important decomposer of cyanogenic glycosides in plant material (Ingvorsen et al. 1991). Few studies exist that describe the fitness of Axd when it is introduced to competitive environments, such as established soil or plant microbial communities. Such studies lend important information for assessment of the potential use of Axd for symbiotic control of *Xylella fastidiosa*, the causal agent of Pierce's disease. We have found that Axd and Axd containing DsRed fluorescent protein (Raxd) do not establish when introduced into soil, but can be recovered from soil that was sterilized before inoculation with Axd or Raxd. Axd and Raxd can also be recovered from established phylloplane communities of basil, strawberry, and sage, although recovery is scant to low. Current studies underway include the recovery of Axd and Raxd from lake water microbial communities. Co-culture experiments showed that Axd and Raxd growth is negatively affected by the presence of *Escherichia coli* and the pathogen *Pseudomonas aeruginosa.* Raxd was modified to express an S1 scFv (single chain antibody variable region fragments) antibody (Axd 7.7) that binds specifically to a strain of *X. fastidiosa* that infects grape. Axd 7.7 growth in culture was compared to that of the wild type Axd and to Raxd. All strains exhibited similar growth patterns in tryptic soy broth (TSB). All strains demonstrated longer lag phases in Luria Bertani medium (LB) than for TSB. Cell numbers remained fairly constant for each strain at each growth phase. Growth studies are underway that monitor the growth of Axd, Raxd, and Axd 7.7 in dilute, R2A medium. Current studies also include using enzyme linked immunosorbent assays to monitor the expression of S1 scFv from Axd 7.7 under environmental challenges, such as poor nutrient availability and energetic demands.

INTRODUCTION
Alcaligenes xylosoxidans subsp. *denitrificans* (Axd) is currently being tested for use in symbiotic control of Pierce's disease. While the bacterium naturally resides in terrestrial and aquatic environments, little is known about the fitness of Axd when it is artificially introduced to either allocthonous or autocthonous environments with established microbial communities. Therefore, some indication of the fitness of Axd in competitive biotic scenarios must be acquired to begin to assess the potential of Axd to control *Xylella fastidiosa* (*Xf*) under natural conditions. This point also holds true for any strain of Axd that is modified to express anti-*Xf* products. In most cases, a genetically modified bacterium (GMB) is less fit than the wild type counterpart (Velicer, 1999). In an ideal case, a GMB should remain in an ecosystem for a limited but effective period of time and cause minimal or no disruption to a host or ecosystem. Here we report on the recovery of Axd and Raxd when introduced onto plant surfaces and in soil using semi-natural experimental conditions. In addition, we provide information regarding the growth of Axd and Raxd when grown under strict laboratory conditions in the presence of human and plant-associated bacteria. We also provide a comparison of the growth of Axd, Raxd, and Axd genetically modified to express a synthetic antibody construct on its cell surface (Axd 7.7) under different growth conditions.

OBJECTIVES

1. Study the behavior of strains of *A. xylosoxidans* subsp. *denitrificans* (Axd and Raxd) when grown under various biotic influences and,
2. Investigate and compare the growth of *A. xylosoxidans* subsp. *denitrificans* (wild type) and Raxd to that of Axd modified to express a short chain antibody against *X. fastidiosa* (Axd 7.7) that infects grape under different physiological conditions, such as in response to nutrient availability and energetic demands.

RESULTS

We have found that Axd and Raxd do not establish when introduced into soil, but can be recovered from soil that was sterilized before inoculation with Axd and Raxd. Axd and Raxd, when applied to leaf surfaces, can be recovered from established phylloplane communities of basil, strawberry, and sage, although recovery is scant to low. Co-culture experiments showed that Axd and Raxd growth are negatively affected by the presence of *E. coli* and *P. aeruginosa.* The growth of Axd modified to express an S1 scFv (single chain antibody variable region fragments) antibody (Axd 7.7) that binds specifically to a strain of *X. fastidiosa* that infects grape was compared to that of the wild type Axd and Raxd. Axd, Raxd, and Axd 7.7 exhibited similar growth patterns in tryptic soy broth (TSB). Axd, Raxd, and Axd 7.7 also demonstrated longer lag phases in Luria Bertani medium (LB) than for TSB. Cell numbers remained fairly constant for each strain at each growth phase. Growth studies are underway that monitor the growth of Axd, Raxd, and Axd 7.7 in dilute, R2A medium. Current studies also underway include using enzyme linked immunosorbent assays to monitor the expression of S1 scFv from Axd 7.7 under environmental challenges, such as poor nutrient availability and energetic demands.

CONCLUSIONS

From earlier work we have found that Raxd establishes within the mouthparts of *H. coagulata* (Bextine et al. 2004a) and within the xylem of several of this sharpshooter's host plants (Bextine et al. 2004b). The bacterium, however, does not establish within soil if soil communities are in place. If the soil is sterilized and biotic competition is eliminated, then Axd and Raxd grow relatively well. Conversely, Axd and Raxd can survive and be retrieved from the leaf surfaces of plants other than citrus, such as basil, sage, and strawberry plants for up to two weeks. These data suggest that Axd and Raxd are more suited to the plant environment than to a soil environment. We conclude that Axd and Raxd will remain in the plant environment long enough to exert is anti-*Xylella* effect with little to no disruption of any relevant ecosystem. Raxd did not grow well in the presence of *E. coli* and *P. aeruginosa* compared to Raxd grown in pure culture. Thus, compared to a ubiquitous bacterium and a pathogen, respectively, Raxd is not as fit under standard growth conditions.

Axd 7.7 growth compared to Axd and Raxd differed little under our experimental conditions. All data collectively suggest that Axd 7.7 shows potential for delivery of an anti-*Xylella* product with little impact on nontarget bacterial ecosystems. This statement is qualified by the fact that field tests must be implemented to assess the true behavior of strains of Axd in the environment. Laboratory studies are not suitable for a genuine assessment of risk assessment and environmental impact; nevertheless, they provide important insight.

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