PARATRANSGENESIS FOR CONTROL OF PIERCE'S DISEASE: MANIPULATION OF ENDOPHYTIC BACTERIA FOR PARATRANSGENIC CONTROL OF PIERCE'S DISEASE

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INTRODUCTION

The Glassy-Winged Sharpshooter (GWSS) is the principal vector of *Xylella fastidiosa*, the causative agent of Pierce's disease, in the California wine country. One strategy to control the spread of *Xylella* by the GWSS is to make the insect refractory to transmission of the bacterium. One can imagine at least two ways this can be done. The first is to genetically engineer the population of sharpshooters directly to become refractory. This transgenic method is being seriously discussed by biologists wishing to halt the spread of malaria in Africa, via the direct genetic manipulation of *Anopheles gambiae* populations (Kiszewski and Spielman 1998). This method is fraught with potential difficulties of many sorts and, in any case, has not been tried to date.

The second method is to manipulate the insect vector indirectly by manipulating its gut flora. This technique is termed paratransgenesis and has many potential advantages over the direct transgenic approach. First, bacteria are far easier to manipulate genetically. Secondly, bacteria can be made to secrete or carry very specific agents of control, like single chain antibodies. Paratransgenesis has been attempted to control Chagas' disease in S. America (Beard et al. 2001) and a form of it is being developed to deliver therapeutic agents in mouse models of human disease, for eventual applications for humans (Beninati et al. 2000).

In attempting to create transgenic gut symbionts of the GWSS several problems present themselves immediately. The final transgenic strain will need to be stable (i.e., the exogenous DNA not contained in a virus or bacterial transposon), the exogenous DNA should be incorporated into the chromosome and not borne on a plasmid, no drug markers should be left in the strain, and as little exogenous DNA should be transferred as possible. We have developed a genetic modification system that meets those requirements based on the *mariner* family of eukaryotic transposable elements. These elements are active in all domains of life when appropriately manipulated, but do not occur naturally in prokaryotes. Thus stable strains of GWSS gut symbionts can be created that should be suitable for release into the wild for the control of *X. fastidiosa.*

OBJECTIVES

1. Construct a genetic DNA insertion system for *Alcaligenes* sp. and *Chryseomonas luteola* based on *mariner* family transposable elements.
2. Identify single chain antibodies that bind specifically to the surface of *Xylella fastidiosa* and express these on the surface of one or more gut symbiotic bacteria of the GWSS as agents of control.

RESULTS AND CONCLUSIONS

A genetic manipulation system for GWSS endosymbionts:

We have constructed a matable transformation system for two GWSS bacterial symbionts based on the *mariner* transposable element, *Himar1*. Below is a figure illustrating the features of the system that help it fulfill the requirements set out in the introduction. All of the necessary requirements are borne on a single suicide plasmid. The plasmid has an RP4 origin of transfer so it can be mated from *E. coli* to *Alcaligenes* or *Chryseomonas*. It also contains a R6K origin of replication so that it can only replicate in special strains of *E. coli*. The drug marker is carried between two FRT sites, the sequences that are used by the FLP recombinase of yeast. Thus, once insertions of the transposon are obtained, the drug marker can be removed by recombination. Since the transposase gene lies outside the inverted terminal repeats (ITRs) the insertions are stable after the plasmids are lost.

Using this system we obtained insertions at random positions in the chromosome of *Alcaligenes* and *Chryseomonas*. Genetic and Southern evidence showed that only the transposon inserted and not any part of the plasmid backbone. Furthermore, we

were able to remove the drug marker by FLP-mediated excision. These systems were used to introduce fluorescent protein genes into each of these species for microbial ecology studies.

Specific modifications to gut symbionts:

The goal of paratransgenesis is to indirectly modify the phenotype of the vector (or host plant) through the modification of a symbiotic organism. There are many traits that could be added to the symbiont, including secreted enzymes, toxins, antibacterial peptides, or single chain antibodies (scFv's). An alternative to secreting a factor is to express it in the outer membrane. We are currently screening phage display scFv libraries to identify scFv's that can bind specifically to the surface of *Xylella fastidiosa* with high affinity. Candidate scFv's will be expressed as OmpA-scFv fusions (inserted into the chromosome with the *mariner* transformation system described above) that will allow the transgenic bacteria to adhere tightly to the surface of *Xylella*, either preventing infection or slowing its spread. Although we have targeted the entire surface of *Xylella* initially, specific outer membrane targets identified from *Xylella* genome project can also be targeted individually, particularly those that are associated with pathogenesis.

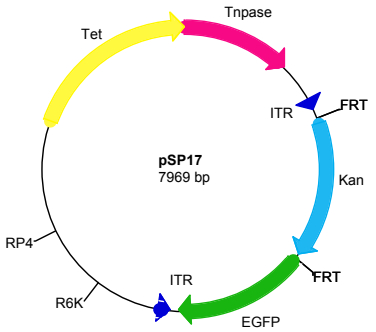

Figure 1. pSP17, a matable suicide plasmid designed to create stable transgenic gut symbiotic bacteria from the glassy-winged sharpshooter.

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