DEVELOPMENT OF SYMBIOTIC CONTROL OF PIERCE'S DISEASE

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Pierce's Disease (PD) is a lethal infection of grapevine xylem by the bacterium *Xylella fastidiosa* (*Xf*) for which there is no cure. Control of the glassy-winged sharpshooter (GWSS), the principle vector of PD, is currently the only effective strategy to manage this disease, however it is very costly.

Limiting the spread of *Xf* by rendering GWSS incapable of pathogen transmission or by interfering with the replication of *Xf* in the plant may stop the spread of PD. These goals can be accomplished by genetically modifying bacteria that live in the sharpshooter, the plant, or both in a method called symbiotic control. Symbiotic control seeks to modify the phenotype of an organism indirectly by modifying its symbiotic bacteria.

We set out to apply symbiotic control to Pierce's Disease by carefully considering several criteria that would ensure safety and efficacy of the resulting symbiotic control strain. These criteria were the choice of ecologically-significant yet non-pathogenic symbiotic bacterial species, development of specific anti-*Xylella* effector proteins, an effective means to deliver those proteins, and a method to reduce the likelihood of horizontal transfer of anti-*Xylella* genes from the symbiont to non-target bacteria.

Choice of symbiont. The digestive tracts of field-caught GWSS were screened for the presence of bacteria and several were isolated routinely. One of these was *Alcaligenes xylosoxidans denitrificans (Axd),* a Gram negative pseudomonad-like species that was isolated from GWSS foregut and cibarium. These anatomical regions are precisely the ones that *Xf* occupies in the sharpshooters as it is vectored from plant to plant by the insect. *Axd* can also colonize various plant tissues, including grape and citrus xylem. Importantly, *Axd* is non-pathogenic in insects, plants and healthy humans[1].

Anti-*Xylella* effector proteins and their delivery. Anti-*Xylella* proteins should be as specific as possible for the target organism. Unfortunately, we still know relatively little about the biology of *Xf* so specific targets for anti-*Xylella* factors remain largely unknown. With this in mind, we screened a single chain antibody (scFv) library against intact *Xf* cells to isolate scFvs that bound to the surface of the cell. We were able to isolate one scFv, called S1, that binds to to an unknown target on the surface of Pierce's Disease strains of *Xylella* only. S1 by itself has not shown any anti-*Xylella* activity, but it can be modified to carry anti-bacterial toxins (e.g., peptide antibiotics) that could kill *Xylella* in the plant or its insect vector while still providing the same specificity inherent to single chain antibodies.

Delivery of anti-*Xylella* factors inside the insect vector or grapevines offers another challenge and we have developed two systems to do this. The first is surface display[2]. Surface display is a method to anchor an anti-*Xylella* protein in the outer

membrane of *Axd* where it is exposed on the surface of the bacterium. We used the ice nucleation protein *inaZ* from *Pseudomonas syringae* to anchor the anti-*Xylella* scFv S1 to the surface of *Axd* and could successfully detect it by ELISA and a physical test for ice nucleation ability. Anchored to the surface of the cell, anti-*Xylella* factors have the opportunity to interact with *Xf* should *Axd* come into contact with it in either GWSS or grapevines.

The second delivery system is secretion. Secreted anti-*Xylella* factors have the opportunity to circulate throughout the plant, reaching foci of infection across physical xylem boundaries. Secretion from Gram-negative bacteria, however, is complicated by the fact that these species have two membranes that a protein must cross before appearing outside the cell. One system that seems to have wide applicability is the α-hemolysin autotransporter from *E. coli* [3] This protein is secreted in a single energy-dependent step across both membranes of Gram negative bacteria when the other components of the system are also present (the proteins HlyB, HlyD, and TolC). Fusion of the last 60 amino acids of the protein is sufficient to target any N-terminal passenger protein for secretion. We have successfully secreted functional scFvs using this system in *E. coli* and are currently testing it in *Axd*. Once the system is established it can be quickly adapted to nearly any anti-*Xylella* protein factor.

Suppression of horizontal gene transfer. A critical aspect of any genetically modified organism developed for field release is the suppression of horizontal gene transfer to non-target species. We have taken two complementary approaches to dealing with this challenge. First, we developed a system to incorporate genes into the chromosome of *Axd* based on the transposable element *Himar1*. Horizontal transfer from the chromosome is orders of magnitude less likely than when genes are carried on plasmids. Secondly, we tested a system to actively suppress horizontal gene transfer based on the ColE3 plasmid addiction system. We added the bacterial toxin gene *colE3* (which encodes the antiribosomal protein, colicin) to a plasmid and incorporated the immunity factor for this toxin, *immE3* into the chromosome of *Axd*. When these two factors exist in the same cell, the cell can survive. The presence of the colicin toxin alone kills the cells. We attempted to transfer the colicin plasmid from *Axd* (*immE3*) strains to strains of *E. coli* that either did or did not contain *immE3*. The colicin plasmid was readily transferred to *E. coli* (*immE3*) but we could detect no transfer at all to *E. coli* without *immE3*, indicating that this genetic system can suppress horizontal gene transfer from *Axd* by at least a factor of $1X10^7$ [4]. Tightly linking the *colE3* gene to whatever anti-*Xylella* genes are inserted into the chromosome of *Axd* should eliminate any chance of horizontal transfer to non-target species.

References

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