

INSECT-SYMBIOTIC BACTERIA INHIBITORY TO *XYLELLA FASTIDIOSA* IN SHARPSHOOTERS: TOXIC PEPTIDES AGAINST *XYLELLA*

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ABSTRACT

Eleven strains of the pathogen, *Xylella fastidiosa*, and the glassy-winged sharpshooter (GWSS) gut bacterium, *Alcaligenes xylosoxidans denitrificans*, were screened for sensitivity to 41 antimicrobial peptides (in addition to 18 screened previously), and more detailed studies of effective inhibitory concentrations of these peptides were conducted. Of 28 additional peptides found to have toxicity toward *X. fastidiosa*, 25 were also toxic to *Alcaligenes*, leaving three as additional candidates for engineering of this GWSS gut bacterium in addition to one found last year. Genes encoding these peptides are being designed and constructed with appropriate promoters and signal peptides for expression and secretion by *Alcaligenes*. Another 89 antimicrobial peptides derived from a combinatorial peptide library were also recently obtained and will be tested against *Xylella* and *Alcaligenes xylosoxidans denitrificans*. The results support the idea that this glassy-winged sharpshooter gut bacterium could be engineered to produce a peptide with toxicity toward the Pierce's disease pathogen.

INTRODUCTION

The destructive potential of *Xylella fastidiosa* in grapevine and other hosts has been greatly increased by the appearance and rapid spread of the glassy-winged sharpshooter (GWSS) in California. This insect vector acquires and carries the pathogen in its mouthparts and transmits the disease to other plants during subsequent feeding. In addition to control measures directed toward reducing populations of the insect, a reduction of the ability of the insect to acquire, maintain, and transmit the pathogen could greatly enhance control. The overall goal of this project is to genetically transform glassy-winged sharpshooter endosymbionts to produce toxic substances that would inhibit or kill *Xylella fastidiosa* and reduce disease transmission. In our component of the project, we have been screening antimicrobial substances against *Xylella*, as well as against the endosymbionts that have been selected for transformation.

Antimicrobial peptides represent one of the most widely distributed forms of natural defense against bacteria and fungi and are now being developed for a variety of medical and agricultural applications. Examples of their use against bacterial pathogens of plants include the transformation of a synthetic cecropin gene into tobacco plants to produce the cecropin peptide *in planta* in an attempt to provide resistance to the leaf pathogen *P. syringae* pv. *tabaci* (Hightower et al., 1994). Harakava et al. (1999) infected tobacco plants with a PD strain of *X. fastidiosa* and used this host pathogen system as a model for testing expression of cloned genes that may give resistance to *X. fastidiosa*. Two antibacterial peptides, cecropins A and B effectively killed a PD strain in an *in vitro* assay. A preliminary study also reported sensitivity of *X. fastidiosa* to Magainin 2 (Momol et al., 2000).

OBJECTIVES

1. Identify toxic peptides effective against *Xylella fastidiosa* but non-toxic to selected endosymbiotic bacteria.
2. Design and construct genes encoding antimicrobial peptides to be expressed and secreted by endosymbiotic bacteria.

RESULTS

Last year, we reported that six strains of *X. fastidiosa* from grape and almond had been used in screening 18 antimicrobial peptides, with four peptides found to be toxic to all strains. One of these was non-toxic to *A. xylosoxidans denitrificans*, one of the natural gut bacteria from the glassy-winged sharpshooter that we are targeting for genetic transformation. During this past year, more extensive studies of antimicrobial peptide sensitivity of 11 strains of the pathogen, *X. fastidiosa*, and of the GWSS gut bacterium, *A. xylosoxidans denitrificans* were conducted, including 41 additional antimicrobial peptides and more detailed studies of effective inhibitory concentrations of these peptides. Of 28 additional peptides with toxicity toward *X. fastidiosa*, 25 were also toxic to *Alcaligenes*, leaving three as additional candidates for engineering of this GWSS gut bacterium. Genes encoding these peptides are being designed and constructed with appropriate promoters and signal peptides for expression and secretion by *Alcaligenes*.

Another 89 antimicrobial peptides derived from a combinatorial peptide library were also recently obtained and will be tested against *Xylella* and *A. xylosoxidans denitrificans*.

CONCLUSIONS

Several antimicrobial peptides were found with toxicity toward *X. fastidiosa* but not against the glassy-winged sharpshooter gut bacterium, *A. xylosoxidans denitrificans*, suggesting that this bacterium could be engineered to produce a peptide with toxicity toward the Pierce's disease pathogen.

REFERENCES

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