DESIGN OF CHIMERIC ANTI-MICROBIAL PROTEINS FOR RAPID CLEARANCE OF *XYLELLA*

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INTRODUCTION

Globally, one-fifth of potential crop yields is lost due to plant diseases primarily of bacterial origin. *Xf* is a devastating bacterial pathogen that causes PD in grapevines, citrus variegated chlorosis (CVC) in citrus, and leaf scorch disease in numerous other agriculturally significant plants including almonds in California (http://danr.ucop.edu/news/speeches). Since the glassy-winged sharpshooter (an insect vector) efficiently transmits PD, a great deal of effort has been focused on using insecticides to localize and eliminate the spread of this disease. However, the availability of the whole genome sequences of PD and CVC strains of *Xf* offer new avenues to directly target and inactivate the pathogen. In this project, we propose a structure-based approach to develop chimeric anti-microbial proteins for rapid destruction of *Xf*. The strategy is based upon the fundamental principle of innate immunity that plants recognize and clear pathogens in rapid manner (Cohn et al., 2001; Magor and Magor, 2001). Pathogen clearance by innate immunity occurs in three sequential steps: pathogen recognition, activation of anti-microbial processes, and finally pathogen destruction by anti-microbial processes. Different sets of plant factors are involved in different steps of innate immunity. Our strategy of combining the pathogen recognition element (SRD) and the pathogen killing element (defensin) in the chimeric molecule is a novel concept and has several short and long term impacts.

ABSTRACT

Xylella fastidiosa, a gram-negative xylem-limited bacterium is a causative agent of Pierce's disease (PD) in California grapevines. During very early stages of *Xylella fastidiosa* (*Xf*) infection, specific proteins/carbohydrates/lipids on the outer membrane of *Xf* interact with plant cells and are important for virulence (Pieters, 2001). Design of a protein inhibitor that interrupts this step of the plant-*Xf* interaction will be useful in combating virulence and controlling PD. Traditionally, antibiotics are prescribed as a preferred therapy. Antibiotics target the enzymes involved in the biogenesis of the bacterial outer membrane and diminish pathogen viability. However, a pathogen often develops antibiotic resistance without the corresponding loss of virulence and pathogenicity (Baquero and Blazquez, 1997). In this UC/LANL project, we offer a novel countermeasure against *Xf* as a viable alternative to antibiotic therapy. We propose to develop chimeric anti-microbial proteins with two functional domains. One domain (referred hereafter as the surface recognition domain or SRD) will be designed to target either a protein or a carbohydrate moiety on the outer membrane whereas the other (a defensin molecule) will be designed to insert and lyse the *Xf* lipid membrane. For protein binding, the SRD will contain either an elastase or a single chain full-length variable region (scFv) antibody targeted against the newly discovered *Xf* outer membrane protein, mopB (Bruening et al., 2002). For carbohydrate binding, the SRD will contain the carbohydrate recognition domain (CRD) of a lectin (Barre et al, 2001; Feinberg et al., 2001; Sharma and Surolia, 1997) to specifically bind to the carbohydrate on the lipid head or on the X*ylella* surface. The defensin molecule will be chosen from group IV plant defensins that exhibit strong anti-bacterial activity (Segura et al., 1998).

OBJECTIVES

1. Design of SRDs (15-20KDa) and defensins (5 KDa) by utilizing the literature data on *Xylella* lipids, surface carbohydrates, or outer membrane proteins.
2. Expression of SRDs and defensins in insect and plant cells.
3. *In vitro* testing of *Xylella* binding by SRD and anti-*Xylella* activity by defensins.

RESULTS AND CONCLUSIONS

The focus of our experiments will be objectives 1, 2 and 3 respectively. We will begin by examining the potential of the 218 amino acid residue long Leukocyte elastase a human neutrophil granular protein in order to target surface proteins (Sinha et al., 1987). Neutrophils contain a variety of proteins that enable the cells to migrate toward and eliminate microbial pathogens (Elsbach and Weiss, 1988). Until 1991, no specific antibacterial activity had been ascribed to Elastase (Wasiluk et al., 1991). However recent research has established that Elastase is the only human neutrophil protein, which is capable of individually killing *Borrelia burgdorferi*, the causative agent of Lyme disease (Garcia et al., 1998; Lusitani et al., 2002). Furthermore, it is known that Elastase can augment the cidal properties of other active proteins. This has been proven to be the case for *C. sputigena*, where very high concentrations of Azurocidin, an antimicrobal granule protein, became cidal upon addition of Elastase (Miyasaki and Bodeau, 1991). Currently, we are studying the efficacy of Leuckocyte Elastase and antibacterial peptides Cecropin and Defensin HNP-2 in killing *X. fastidiosa*. Their killing capability will be tested by establishing kill-curves, which show the number of colony forming units remaining after bacterium was exposed to these proteins individually or in combination. We will also define the protein on the bacterial surface that is a target for elastase activity. We will begin culturing grape embryo cultures as well as callus cultures expressing PGIP using membrane bioreactors CELLine 350 (Integra Biosciences, Inc.). In this bioreactor the plant cells are contained in a relatively low volume, rectangular chamber (5 mL) bounded by an oxygen-permeable membrane on one side and a protein-impermeable, 10kD molecular weight cut-off membrane on the other side that separates the cell compartment from a much larger nutrient medium compartment (350 mL). The use of a bioreactor will serve several important purposes. First, it will enable us to develop and optimize strategies for growing transgenic grape cell cultures. Second, it will allow us to monitor the total protein concentration in the cell chamber as a function of time and to characterize the proteins secreted into the medium in the cell chamber using SDS-PAGE. Third, we will be able to replace the entire contents of the medium compartment under sterile conditions. We will be using these bioreactors to express SRDs and Defensins. and to study the effects of environmental conditions (nutrient medium composition, temperature, pH, oxygen, light etc) on biomass growth, sucrose consumption, total protein concentration in the extracellular medium and recombinant protein production.

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