STRUCTRAL AND FUNCTIONAL ANALYSIS OF MULTI-PROTEIN SYSTEMS INVOLVED IN THE PATHOGENICITY OF *Xanthomonas axonopodis* pv *citri*

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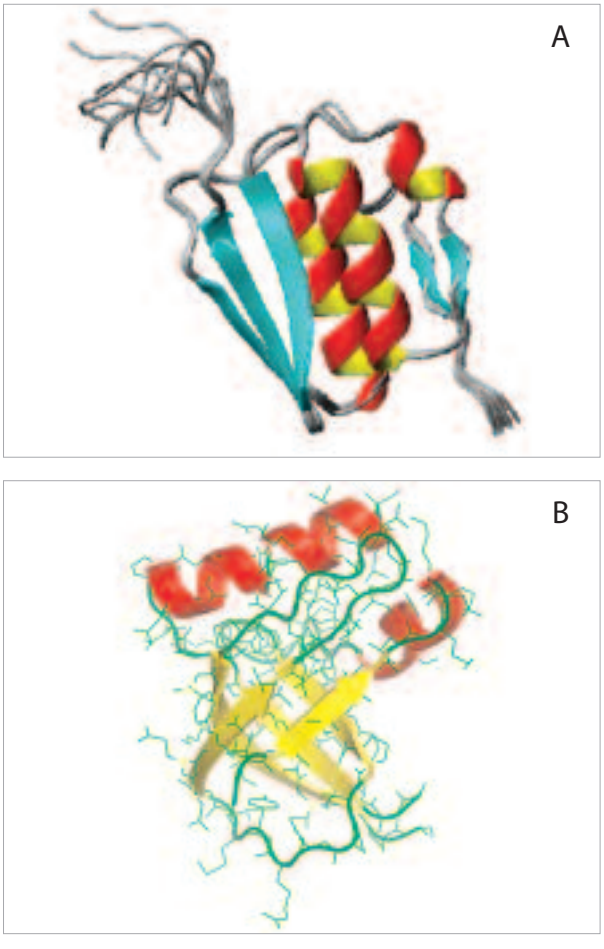

A) Solution structure of VirB7, a periplasmatic component of the Xanthomonas *T4SS. B) Crystal structure PILZ1133, of one of four PILZ domain-containing proteins coded by the* Xanthomonas *genome. PILZ1133 homologs are implicated in the control of bacterial motility. While some PilZ domains have been shown to bind c-diGMP, unique topological features in PILZ1133 structure abolish the nucleotide binding site*

In this project we propose to study some specific molecular systems that contribute to the pathogenicity of *Xanthomonas axonopodis* pv. citri *(Xac)*, the cause of citrus canker disease. We plan to use biochemical, spectroscopic and genetic techniques, in combination with the production of recombinant proteins to study the type III (T3SS) and type IV (T4SS) secretion systems and quorum sensing signal transduction pathway in *Xac*. We also plan to employ structural biology techniques to determine the structures of subunits and subunit complexes of the T3SS, T4SS and quorum sensing pathway. As well as providing details regarding the molecular mechanisms used by this phytopathogen to cause disease, these structures promise to reveal details regarding the function of orthologous systems found in other pathogens, including human pathogens with significant impact on public health.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Our current activities are focused on understanding the following systems:

i) Quorum sensing and c-diGMP signaling – Bacteria do not exist as solitary cells but instead coordinate their behaviors by means of extracellular chemical signals (autoinducers). We have recently shown that proteins involved in the detection of the *Xanthomonas* autoinducer DSF interact with proteins involved in intracellular signaling mediated by cyclic diGMP (c-diGMP), a second messenger implicated in the control of complex bacterial behaviors including motility, biofilm production and virulence. We are using biochemical and genetic approaches to characterize the molecular and physiological functions of several *Xac* proteins with roles in quorum sensing (RpfF, C, G) as well as proteins containing GGDEF, EAL, HD-GYP and PilZ domains involved in c-diGMP production, degradation and binding. We have recently determined the crystal structure of *Xac* PILZ1133 and are using NMR to study its interactions with other polypeptides.

ii) Type III secretion system – This multiprotein secretion complex works together with auxiliary cytosolic proteins to recognize virulence factors and transport them into the host cell. We are characterizing *Xac* strains with mutations in T3SS components and have demonstrated specific interactions between T3SS secretome components, cytosolic chaperones and secreted polypeptides. In one study we have shown that HrcU, an inner membrane T3SS component suffers autolysis and that the liberated fragment interacts with HrpB2 which is itself secreted. Another study is characterizing the involvement of specific *Xac* sigma factors in T3SS regulation.

iii) Type IV secretion system – We have identified a network of interactions among *Xac* T4SS cell envelope components and putative secreted factors. We are currently using NMR to resolve the solution NMR structure of the periplasmatic subunit VirB7 and study conformational transitions that occur upon its interactions with VirB9. The VirB7 structure revealed intriguing characteristics not observed in VirB7 proteins from other bacterial species.

iv) Structure and function of YAEQ – This new structure presents a variation of the PD-(D/E)XK motif encountered in a superfamily of metal-dependent nucleases that includes restriction enzymes and proteins involved in nucleic acid recombination and repair. YAEQ mutants have been produced and crystallized and *Xac* YAEQ knockout strains have been produced to study YAEQ function.

v) Structure and function of SUFE – We have determined the structure of *Xac* SUFE by molecular replacement. SUFE is a component of cysteine desulphurase in which it acts as an activator and transient receptor for elemental sulphur destined to be incorporated in Fe-S clusters of a variety of redox enzymes. We are currently characterizing its interactions with other components of this pathway.

MAIN PUBLICATIONS

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