Characterization of FK506-binding protein of XopAZ from *Xanthomonas cynarae*

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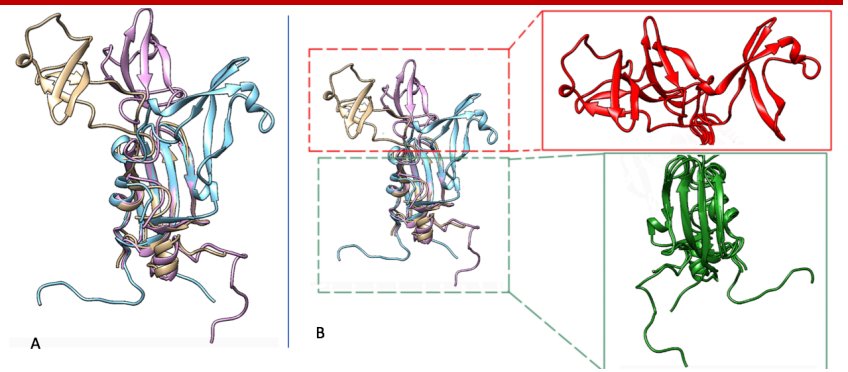
Abstract

Xanthomonas cynarae is a species of proteobacteria that injects avirulence proteins (Avr protein) into the host cell through the Type III Secretion System (T3SS), causing bacterial disease in artichoke leaves. However, the T3SS flagellum has a much smaller diameter than the diameter of a folded protein. Thus, the bacterial proteins must be unfolded before entering the needle complex of the T3SS system. We hypothesized that the protein XopAZ, which is produced in *X. cynarae,* contains a chaperone and FKBP domains. Both of the domains are responsible for refolding the injected Avr protein inside the plant host cell. In this study, we describe the preparation of and results from a FK506 binding assay to determine if XopAZ has an FKBP activity.

Introduction

XopAZ is homologous to the *Escherichia coli* sensitive to lysis protein D (SlyD) (70% amino acid identity) and with the *E. coli* surface layer protein A (SlpA) (50% amino acid identity). Both SlpA and SlyD contain two domains: a chaperone/insert-in-flap domains (IF domain) and prolyl isomerase domain (PPIase/ FKBP domain) (Knappe 2007). The figure A showed the structural alignments of the three homology structures, XopAZ (in tan), SlpA (in magenta), SlyD (in blue). The figure B showed the chaperone/IF domain (in red) and PPIase/FKBP domain (in green) in all 3 structures.



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References

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Materials and Methods

A calmodulin/calcineurin Malachite green FK506-binding assay (R&D Systems (Minneapolis, MN) was conducted using the recombinant *Xanthomonas cynarae* XopAZ protein expressed and purified in our laboratory. The immunosuppressant compound FK506 has been demonstrated to inhibit the phosphorylation activity of calcineurin when activated by calcium bound calmodulin. *Citrus sinensis* cyclophilin A (Cyp A), a known peptidyl-propyl isomerase that binds FK506, was used as positive control. EDTA was used as negative control as it chelated the Ca^{2+} that would normally be bound by calmodulin which would then subsequently activate calcineurin and allow it to dephosphorylate a target substrate. The samples were run in triplicate in a 96-well plate at 37°C. Following incubation, the absorbance of the samples was read at 620 nM to quantitate the released phosphate from the samples.

Results + Discussions

The results indiciated that both Cyp A and XopAZ had FKBP activities. As can be seen in the first column, stripping calmodulin of Ca^{2+}, prevented activation of calcineurin. Similarly,the omission of FK506 binding proteins from allowed calcineurin to bind FK506 and thereby prevent hydrolysis of phosphate groups from the sample. However, the binding of FKBP domains in XopAZ and Cyp A allowed calcineurin to restore its phosphatase activity, as shown in the green and blue columns. Based on the result, the amount of phosphate released with the presence of XopAZ was lower than with the presence of Cyp A. This implies that the FKBP domain of XopAZ was smaller and weaker than the FKBP domain of Cyp A. The figure showed the difference in size between the FKBP domain of Cyp A (in purple) and of XopAZ (in green).