

In vitro studies of the PilT protein from *Xylella fastidiosa* twitching motility system

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Abstract

The bacteria *Xylella fastidiosa* (Xf), Gram-negative and non-flagellated, is responsible for diseases in important crops, such as Citrus Chlorosis Variegated (CVC) in citrus and Pierce's disease in grapevines. The CVC disease has a manage cost of 100 million dollars/year in Sao Paulo state. It colonizes the xylem vessels of the plants, blocking the water and nutrient flows. PilT protein is a part of the motility system and very important for *Xylella* pathogenicity and our protein target for drug design.

Key words:

Xylella fastidiosa, PilT, twitching motility system.

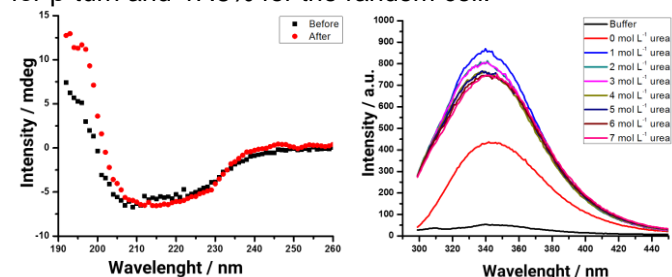
Introduction

The type IV pili has an important role in the pathogenicity of almost all Gram-negative bacteria as it is responsible for the motility and bacterial dispersion in wet surfaces, auto aggregation and biofilm formation. Commonly, the energy required for this movement is provided by ATP hydrolysis that is promoted by the action of PilB and/or PilT proteins. Important phytopathogenic bacteria, such as *Xylella fastidiosa*, have been using this system to colonize important crops. The inhibition of the PilT (Xf_PilT) protein leads to the pathogenicity loss of some bacteria strains. In this project we propose the protein expression and characterization for posterior *in vitro* inhibition tests with *in silico* designed molecules.

Results and Discussion

Bacteria *Xf-9a5c* (citrus pathogen) was used in gene amplification using PCR. The primers employed were: Primer forward: 5'GAC TCA CAT ATG GAT ATT GCT GAA CTG TTA 3' ($T_m = 56.6$ °C) with recognition site for *NdeI* and reverse: 5'TCA GTA CTC GAG TCA AAA ATT CGC TTT ATC 3' ($T_m = 57.0$ °C) with recognition site for *XhoI*. Insert and vector pET-28a(+) were treated with *NdeI* and *XhoI*, ligated using DNA ligase, purified and inserted in *E. coli* (DH10B) for cloning. Plasmidial DNA was then removed from the positive clones and electroporation in *E. coli* BL21(DE3) was performed. The cells were grown in Luria-Bertani (LB) broth until OD₆₀₀ of approximately 0.8 and induced with IPTG (1 mmol·dm⁻³). Expression was carried at 37 °C and 250 rpm during 4 h. The cells were harvested and lysed with sodium phosphate buffer 25 mmol·dm⁻³, NaCl 150 mmol·dm⁻³ and 0.2% Tween-20 under sonication. As Xf_PilT remained in the insoluble fraction, the pellets were washed using sodium phosphate buffer 25 mmol·dm⁻³, NaCl 150 mmol·dm⁻³ and 2 % Tween-20 until only the band for Xf_PilT was observed in the SDS-PAGE. Different protocols for protein solubilizing were tested using urea and different surfactants. After dialysis, protein concentration was measured by UV-Vis Spectroscopy obtaining about 20-45 μmol·dm⁻³. Biophysical characterization was done using Circular Dichroism (Image 1A) and Fluorescence Spectroscopy (Image 1B). As seen by thermal unfolding, Xf_PilT is thermostable in low concentration (2.4 μmol·dm⁻³). Moreover, by deconvolution of the CD spectra by CDNN

were obtained 15.4% for α-helix, 44.4% for β-sheet, 21.1% for β-turn and 47.8% for the random coil.



A.

B.

Image 1 A. Circular Dichroism (CD) spectra of Xf_PilT in sodium phosphate buffer (2.5 mmol·dm⁻³) before and after thermal unfolding (15-95 °C and 95-25 °C). **B.** Fluorescence spectra of Xf_PilT in different urea concentrations.

The Fluorescence experiments showed the tridimensional environment around the Tryptophan residue and can be used for pH and denaturant conditions analyses.

Conclusions

The protein was purified and biophysically characterized by Fluorescence Spectroscopy and Circular Dichroism. In the fluorescence spectra, a red shift in the maximum wavelength can be noticed when the Xf_PilT is submitted to a higher concentration of urea indicating that the aromatic residues were exposed to a less hydrophobic environment, when the protein loses its tertiary structure exposing these residues to the solvent. However, the elevation of the temperature did not lead to the protein unfolding, suggesting that Xf_PilT is thermostable in the tested conditions.

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