XYLELLAIN, THE CYSTEINE PROTEASE OF PHYTOPATHOGEN BACTERIUM OF A XYLELLA FASTIDIOSA.

Faro A. R¹, Leite N. R.¹, Oliva G¹, Thiemann O. H.¹ ¹Laboratory of Molecular Biology Struture, Institute of physics of São Carlos, USP.

Xylella fastidiosa is a xylem-limited Gram-negative bacterium responsible for economically important plant diseases, such as the citrus variegated chlorosis (CVC). Proteases might be involved in the infection process by disrupting plant tissue. The Xylellain is a cysteine protease which is differently expressed in strain pathogen and non-pathogen of X. fastidiosa. The xylellain's structure was solved recently by our group with Single Anomalous Dispersion (SAD) technique, using crystals of recombinant protein with incorporated selenometionine. The structure's analysis shows a similarity with others cysteine proteases already reported. It shows that this protein has a pro-protein form. Structural studies had shown a difosfatado ribonucleotideo close to the amine terminal region, which can be related to the enzyme's pos-traductional processing. Punctual mutations in aminoacids which, interacts directly with this ribonucleotideo were produced in order to evaluate it influence in the enzyme's functional activity. The mutant proteins were less soluble than the native ones, which contributes to the hypothesis about the nucleotideo's importance in the structure's fold. Future experiments will help in the comprehension of the xylellain's structure/activity relation and it includes: crystallization of the mutant proteins, analysis of Circular Dichroism spectrum (CD) and fluorescence. In addition, a comparative enzymatic characterization will be carried out, between the native and the mutant proteins, and xylellain's complexes attainment with E64, a composition commonly used for cysteine protease's possible inhibit, will be explored.