Design of inhibitors for the PilT protein from *Xylella fastidiosa* using *in silico* and *in vitro* approaches

Fulaz, S.F.[1,3]; Cabrini, F.M.[2]; Carvalho, D.J.P.[1,3]; Borro, L.[3,4]; Santos, C.A.[5]; Beloti, L.[5]; Neshich, G.[3]; Souza, A.N.[5]; Tasic, L.[1].

[1] Chemical Biology Laboratory, Institute of Chemistry, Unicamp, Campinas; [2] Institute of Chemistry, Unicamp, Campinas, Brazil; [3] Embrapa Agricultural Informatics, Computational Biology Research Group, Campinas, Brazil; [4] Institute of Biology, Unicamp, Campinas; [5] Molecular Biology and Genetic Engineering Center, Unicamp, Campinas, Brazil.

Xylella fastidiosa (Xf) is a Gram-negative xylem-limited bacterium responsible for diseases (in approximately 200 hosts) such as Pierce's disease (grapevines-EUA), citrus variegated chlorosis (Brazil) and leaf scorch diseases (olive tree-Italy). The type IV pili has an important role in the bacteria pathogenicity, since it is responsible for the motility and dispersion in surfaces, auto aggregation and biofilm formation. Commonly, the energy required for this movement is provided by ATP hydrolysis promoted by PilB and/or PilT proteins. Xf_PilT protein was chosen for the inhibitors design as we can assume that damages in the motility system can lead to loss of bacteria pathogenicity. The Xf-9a5c (citrus pathogen) was used for gene amplification (PCR). The insert and the vector pET-28a(+) was treated with *Nde*I and *Xho*I, ligated, 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. We tested different expression conditions in an attempt to obtain a soluble protein. Ni-affinity column was employed in the protein purification. For the inhibitor design, computational biology tools were employed in the molecular modeling (Discovery Studio), hexamer formation (Swiss PDB Viewer), model refinement (Yasara) and docking (Pipeline Pilot). We used as template the PilT protein from *Pseudomonas aeruginosa* (PDB ID: 3JVU and 3JVV) with 75% of identity with Xf_PilT. With ClustalW, the primary structure was aligned with PilT proteins from other organisms to find specific targets for Xf_PilT. The specific residues E89, D184 and K187 were identified and E89 was chosen as the first target, since it is in the interface between monomers. Dockings, using virtual high throughput screening (HTVS) with commercial compounds from Zinc database and manually designed molecules, were performed. The best inhibitors were chosen for organic synthesis and posterior *in vitro* inhibition tests with the target Xf_PilT protein.

Keywords: *Xylella fastidiosa*, PilT, inhibitors.