TITLE: Characterization of PstS and PhoX of *Xanthomonas citri* pv. citri

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Uptake and transport of phosphate is one of the important factors for the pathogenicity of many bacteria, including *X. citri*. In *X. citri* were identified two different periplasmic proteins that bind phosphate: PstS and PhoX, which have 68.6% of identity. PstS is a known protein because it belongs to the ATP-Binding Cassette transport system (ABC transporter) called PstSCAB that is able to transport phosphate across the membrane against the concentration gradient energized by ATP. Three-dimensional structure of PhoX bound phosphate of *X. citri* was solved by crystallography and it let know that PhoX and PstS conserve the binding residues. Investigations until now have not determined if there are or not differences of both proteins in their affinity for phosphate and uptake of phosphate. Based on this, the objectives of this work are express, purify, denaturate-renaturate and obtain the constant of dissociation of both proteins to phosphate, as well as obtain the mutant strains *Xac::pstS*, *Xac::phoX* and *Xac:pstS/phoX* to perform phosphate uptake assays. Plasmids pET28a- PstS y pET28a- PhoX, cloned in a previous work, were transformed in *E. coli* BL21(DE3) and cultivated in LB medium with kanamycin, gene expression was induced with 0.1 mM of IPTG during 4 hours at 30°C. Both proteins were purify by immobilized nickel-affinity chromatography with buffer phosphate 50 mM pH 7.2, 200 mM NaCl, 20-500 mM imidazole. To eliminate potentially phosphate, proteins were dialyzed against 10 mM Tris-HCl, 6 M guanidine hydrochloride, pH 8.0, and then were refolded by two consecutive dialyses into 10 mM Tris-HCl pH 8.0. To confirm the renaturation, proteins were evaluated in Tycho-NanoTemper machine. Progress of this work includes expression, purification and validation of the denaturation-renaturation protocol of both proteins to subsequently perform affinity assays.

Key words: *Xanthomonas citri*, PstS, PhoX, phosphate, affinity, uptake.