

PURIFICATION AND PARTIAL CHARACTERIZATION OF A PUTATIVE NUCLEOTIDASE SurE FROM PHYTOPATHOGEN *XYLELLA FASTIDIOSA*

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The bacterium *Xylella fastidiosa* was the first phytopathogen to have its genome completely sequenced. This bacterium provokes a disease called Citrus Variegated Chlorosis (CVC) in orange trees that causes tremendous losses to Brazilian citriculture. In this work, it is studied the protein correspondent to *orf XF0858* that contains high similarity with nucleotidases SurE of other bacteria. These nucleotidases dephosphorylate diverse intracellular substrates, especially nucleoside monophosphates. The XF-SurE protein was expressed in *Escherichia coli* BL21(DE3) using the vector pET32Xa/LIC and was purified by two nickel metal affinity chromatographies. The identity of the protein was confirmed by mass spectrometry (MALDI-TOF) and analysis of its secondary structure by circular dichroism spectroscopy indicates that the protein has alpha-helix predominance. Preliminary studies with SAXS (Small-Angle X-Ray Scattering) indicate that the protein is globular and it has a dimeric organization. Functional assays using the pNPP (p-nitrophenol phosphate) as substrate show that the protein was strictly dependent on the presence of divalent metal cations, with greater affinity by Mn⁺². Studies with pH dependence demonstrate that this enzyme is more active at neutral pH. In addition, kinetics assays were performed against pNPP and inorganic phosphate (Pi, a natural inhibitor). These results contribute for a better understanding of the mechanisms of dephosphorilation of substrates, a basic process for the maintenance of the bacterial metabolism.