CHARACTERIZATION OF THE VIRB7-VIRB9 INTERACTION AND INITIAL NMR STRUCTURE DETERMINATION OF VIRB7 FROM *XANTHOMONAS AXONOPODIS* PV. *CITRI* CHROMOSOMAL TYPE IV SECRETION SYSTEM

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Xanthomonas axonopodis pv. *citri* (*Xac*) is a phytopathogen that causes citrus canker in orange trees. Among the possible virulence determinants in the *Xac* genome is the chromosomal Type IV secretion system (T4SS), an important transenvelope apparatus that secrets proteins and DNA in many plant and animal pathogens. The model T4SS found in *Agrobacterium tumefaciens* is constituted by twelve structural proteins: VirB1-VirB11 and VirD4. We expressed the *Xac* $VirB7_{His\text{-}24\text{-}139}$ and $VirB9_{34\text{-}255}$ fragments in *Escherichia coli*. The proteins were purified by standard chromatographic methods and characterized by SDS-PAGE, mass spectrometry, fluorescence and circular dichroism. $VirB9_{34\text{-}255}$ was expressed as an insoluble polypeptide, purified in the presence of urea and refolded by dialysis. The VirB7-VirB9 interaction was assayed by fluorescence titration, indicating a Kd of ~$4x10^{-8}$M. For NMR studies, $VirB7_{His\text{-}24\text{-}139}$ was induced in minimum media for incorporation of ^{15}N and ^{13}C and the His-tag was removed by thrombin proteolysis. Several multidimensional spectra were collected for resonance assignment, including ^{15}N-HSQC, ^{13}C-HSQC, HNCO, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HBHA(CO)NH, ^{15}NTocsy-HSQC and HC(CO)NH-Tocsy. More than 50% of the backbone and the side chain Cβ and Hβ have been assigned. The VirB7-VirB9 interaction was monitored by ^{15}N-HSQC of ^{15}N-labelled VirB7 before and after addition of VirB9, indicating that the VirB7 C-terminal remains highly flexible in the VirB7-VirB9 complex.

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