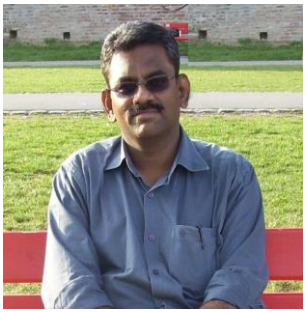
Structure and functional analysis of type 3 secretion system pilus proteins of plant pathogenic bacteria



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Abstract

Type 3 secretion system (T3SS) is an essential virulence system utilized by many gram-negative bacteria including *P. syringae* and *R. solanacearum* to deliver effector proteins into host cells. The extracellular, long, needle-like proteinaceous complex (pilus) of T3SS transports effectors. In *P. syringae*, HrpA, an 11 kDa protein assembles to form the pilus structure whereas in *R. solanacearum* the 8.6 kDa HrpY protein assembles into a large needle like apparatus. The structure and stability of these proteins remain poorly understood. To address this, recombinant HrpA and HrpY proteins were prepared and carried out the biophysical characterization. The native PAGE and dynamic light scattering analysis showed higher-order oligomerization of rHrpA and rHrpY with hydrodynamic radii in the range of 1-1000 nm. Transmission Electron Microscopy revealed that both proteins spontaneously form needle-like filaments. CD spectroscopic analysis showed the predominantly helical nature of these proteins. We examined the effects of detergents, denaturants, pH and temperature on rHrpA and rHrpY assemblies. Detergents such as SDS and sarkosyl effectively disrupted the oligomers, whereas urea and guanidine hydrochloride had no effect. The sequence analysis of HrpA from different pathovars of *P. syringae* revealed that, for the first time, two groups of HrpA proteins (108 aa and 113 aa) that are sequence-wise unrelated. This study contributes to our understanding of the T3SS pilus structure and its stability. The results of this study could lead to new approaches for T3SS pilus protein structure-function investigation.