

1467-2 Cryo-Electron Microscopy Structures Of The Twitching Motility Apparatus Of *Xanthomonas citri*

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Resumo:

Type IV pili (T4P) are retractable filaments composed mostly of a major pilin subunit (PilA) that promote surface-associated twitching motility in bacteria. The outer membrane channel through which the pili emerge from the cell is formed by a PilQ oligomer, a member of the secretin superfamily which, in some bacteria, associates with the peptidoglycan-binding protein TsaP. We chose to use cryo-electron microscopy (cryoEM) to study these complexes in the phytopathogen *Xanthomonas citri*, where T4P also contribute to biofilm development and adherence to host leaves, as well as serving as phage receptors. The native chromosomal copy of PilQ was fused with msfGFP plus a Strep-tag, solubilized from *X. citri* membranes, and affinity-purified for cryoEM single particle analysis. An initial refinement resulted in a 3.2 Å resolution map with C7 symmetry representing the N0, N3, and Secretin domains of the tetradecameric PilQ and both C-terminal domains of the surrounding heptameric TsaP ring. Further refinement and polishing enabled a 2.7 Å resolution map to be achieved, the most detailed map yet of a PilQ-TsaP complex, though the flexible N0 domains were lost from the reconstruction. In parallel, T4P filaments were sheared from *X. citri* cells and collected by ammonium sulfate precipitation for cryoEM analysis. A helical refinement resulted in a 2.6 Å resolution map, representing a filament about 59 Å-wide, with a 9.9 Å helical rise and a 93° twist, containing about 3.9 PilA subunits per turn. To date, this is the highest resolution achieved by cryoEM for any bacterial or archaeal pilus of the type IV filament superfamily. We are continuing to advance in the structural biology of the twitching motility apparatus by studying recombinantly expressed PilP, the lipoprotein that bridges the T4P assembly platform in the inner membrane to PilQ.

Palavras-chave:

type IV pilus, *Xanthomonas*, cryo-electron microscopy, secretin

Agência de fomento:

FAPESP, CAPES