Bacterial Wars: Functional and Structural Studies on the Bacteria-Killing Type IV Secretion System from Xanthomonadaceae

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Type IV secretion systems (T4SSs) form the most common and versatile class of secretion systems in bacteria, capable of injecting both proteins and DNAs into host cells. The T4SSs from the *Xanthomonadaceae* family of bacteria present several distinguishing features, one of which is their role in the killing of bacterial rivals by injecting toxins into neighboring cells upon contact. Three purified toxins or X-Tfes (Xanthomonadaceae-T4SS effectors) from *Xanthomonas citri* are able to degrade peptidoglycan and lyse *B. subtilis* cells and another X-Tfe is shown to possess phospholipase activity. These toxin activities are inhibited by cognate immunity proteins (X-Tfis) whose genes are found upstream to the X-Tfe genes. We show that X-TfeXAC2609 is secreted by *Xanthomonas citri* on contact with *E. coli* cells in a T4SS-dependent manner. Using time-lapse microscopy, we observe the rapid lysis of *E. coli* cells that were in direct physical contact with *X. citri* WT cells but did not observe lysis when Δ*T4SS* strains are used.

Canonical T4SSs are typically composed of 12 components that form two major assemblies: the inner membrane complex embedded in the inner membrane and the core complex embedded in both the inner and outer membranes. We will present the 3.3 Å resolution cryo-electron microscopy model of the intact T4SS core complex from *Xanthomonas citri*. This unprecedented structure significantly expands our knowledge of the molecular details of T4SS organization and assembly. The vast network of protein-protein interactions in this 1.13 MDa assembly was functionally probed in an exhaustive mutational investigation of interface residues.