Structure of *Xanthomonas citri* complex used to kill other bacterial species revealed

Scientists used cryo-EM and obtained a 3.3 Å resolution model

By Maria Celia Wider

In the struggle for survival, bacteria attack, defend and compete among themselves and with other organisms for the same niche. These interactions led to the evolution of secretion systems used by bacteria to transfer DNA and proteins into prokaryotic and eukaryotic targets. Among them, the ubiquitous type IV secretion (T4S) systems are found in both in bacteria and archaea. These secretory nanomachines are being extensively investigated by scientists interested in understanding their functioning at the molecular level.

Using cryo-electron microscopy, the team of Professor Shaker Chuck Farah, from the Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo (USP), in collaboration with groups at the CNPEM in Campinas and at Birkbeck College in the United Kingdom, characterized the structure of the type IV secretion system core complex from *Xanthomonas citri*, a known bacterial phytopathogen that causes citrus canker disease. This system is important because it is used by *X. citri* to inject lethal toxins into other bacterial species.

"The *X. citri* T4S system is structurally similar to the systems used for bacterial conjugation and horizontal gene transfer, and more distantly related to systems encountered in pathogens such as *Helicobacter pylori*, which causes gastritis and peptic ulcer, or *Legionella pneumophila*, which causes Legionnaire's disease. The characterization of the architecture of the core complex of this system was important for getting information on both type of T4S systems", said postdoctoral fellow Germán Sgro, the first author of the paper published in the journal *Nature Microbiology.*

According to Chuck Farah, the elucidation of this structure expands the knowledge of the molecular details of T4S system organization, assembly, and evolution. "We can now study the structures of large protein complexes that represent the biological context with more fidelity", he said.

Type IV secretion systems

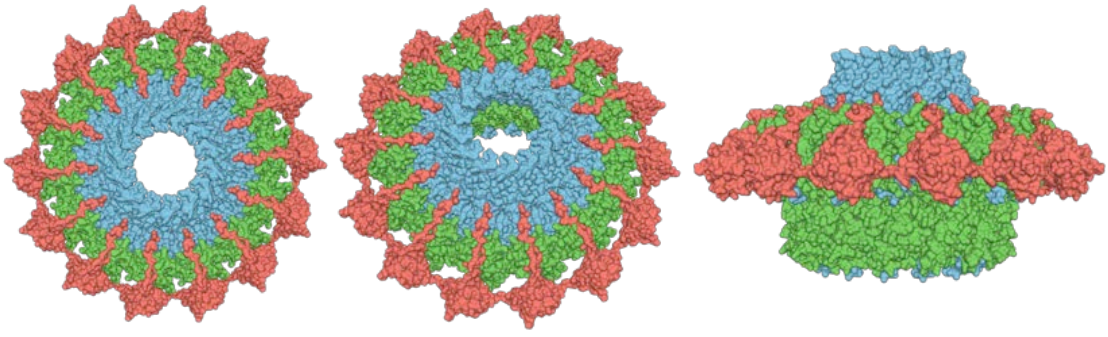
Type IV secretion systems are multiprotein complexes that transport proteins and DNA across the bacterial membranes into other target cells. As mediators of bacterial conjugation, T4S systems play an essential role in the spread of antibiotic resistance genes. In their role of virulence factors in pathogenicity,

they secrete transforming DNAs into plants leading to tumorigenic growths, and also effector proteins in animal cells, causing infectious diseases.

Many bacteria of the family Xanthomonadaceae, which occupy diverse environmental niches, carry a distinct T4S system, different from those found in other bacteria, and whose function until recently was unknown.

In 2015, Chuck Farah's group published an article in the journal *Nature Communications* with results showing that the T4S system of *Xanthomonas citri* provides these cells the ability to kill other bacteria in a contact-dependent manner, by transferring toxins. The researchers demonstrated the versatility of the T4S system and its determinant role in interspecies bacterial competition. “Analysis of the sequenced genomes of other bacteria allowed us to determine that this type of T4S is only found principally in bacteria of the same family (Xanthomonadaceae)”, comments Chuck Farah. However, to understand how these systems work it was necessary to know its molecular structure. This was the goal of the group, which resulted in the characterization of the structure of the core complex as an important first step.

The T4S systems have 12 conserved subunits termed VirB1-11 and VirD4, which are divided into two subcomplexes: the inner membrane complex and the core complex (associated with both bacterial membranes). The T4SS core complex from *X. citri* is composed of 14 copies each of VirB7, VirB9 and VirB10 proteins, forming ring that can be thought of as a tetradecamer of trimers. Characterizing this complex was challenging, both because of the size of the structure (1.13 MegaDaltons) and by the fact that it is associated with both the inner and outer bacterial membranes.



Structure of the X. citri T4S system core complex. Top, tilt, and side views, with colored subnites: VirB7 in red, VirB9 in green, and VirB10 in blue. Image: Germán Sgro.

As Germán Sgro comments about the approach used for this challenging project, the researchers cloned the *X. citri* genes encoding the core complex proteins into the bacterium *Escherichia coli*, which was able to correctly express and assemble the complex in its membrane. The next step was to

process the samples in order to extract the complexes from the bacterial membrane without destroying them.

To obtain the structural model, they initially used the negative-staining electron microscopy technique, in collaboration with the group of physicist Rodrigo V. Portugal, at the Laboratório Nacional de Nanotecnologia (LNNano), Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), in Campinas. This allowed them to be sure that they were indeed isolating intact core complex particles.

The next step was to use cryo-electron microscopy (cryo-EM), a high-resolution structural biology technique only recently pioneered in Brazil by the Rodrigo Portugal group. After the Farah and Portugal teams were able to determine the precise conditions necessary to prepare samples for cryo-EM analysis a collaboration was initiated with the group of Prof. Gabriel Waksman, from University College of London and Birkbeck College, which maintains a center of excellence in the area of cryoEM and where Germán Sgro visited for 12 months in 2016-2017. During his time in the UK, Germán was able to obtain a data set of approximately 1,500 images and 185,000 particles which was used to produce an electron density map with a resolution of 3.3 Å (an Angstrom is equal to 10^{-10}m). This map provided a detailed view of the molecular details of the *X. citri* core complex.

After three-dimensional modeling of the amino acid sequences into the 3D map, the researchers could study the importance of the many different types of interactions between the different subunits of the structure.

Chuck Farah points out that it was important to collaborate with the group of Professor Roberto K. Salinas, of the Departamento de Bioquímica of the Instituto de Química, USP, with whom he was investigating the interaction between VirB10 and VirB9 proteins by nuclear magnetic resonance. The information obtained by this technique was used to interpret a particularly interesting region of the 3D map obtained by cryo-EM.

The researchers then set out to perform a functional analysis of the structural features of the core complex. They mutated VirB7, VirB9 and VirB10 subunits, at different sites, in both wild-type *X. citri* and in a strain in which the VirB10 subunit was fused to a GFP (green fluorescent protein) molecule. The mutants were used by Dr. William Cenens, a post-doc in the Farah lab, in various competition and killing assays and compared to wild-type bacteria for efficacy in killing the target *E. coli*. Dr. Cenens also studied the mutant *X. citri* strains under a fluorescence microscope which allowed him to count the number of complexes in each cell and correlate that number with the killing efficiency.

Biotechnological tool

Studying the structure and function of the *X. citri* T4S system adds to our knowledge about the mechanisms of bacterial secretion systems in general and may find applications in the medical field.

For example, systems similar to *X. citri* are found in bacteria of the genus *Stenotrophomonas*, which is an environmental bacterium, but is sometimes associated with hospital-acquired infections. It is possible that its T4S system is involved in bacterial colonization of a specific niche or host and may therefore be of clinical importance.

The bactericidal activity promoted by this T4S system is also a focus of interest. In the 2015 article, the researchers listed the toxins - or, more formally, the effectors - secreted by *X. citri*, and are now actively studying their molecular targets, which are diverse within the target cell: lipids in the membranes; peptidoglycans, which are polymers that give structure to the bacterial cell wall; and nucleases, which can degrade DNA or RNA within the target cell.

According to Germán Sgro, the T4S system could be used as a biotechnological tool to transfer a set of effectors specifically designed to combat undesired bacterial targets.

The article *Cryo-EM structure of the bacteria-killing type IV secretion system core complex from Xanthomonas citri*, by Germán G. Sgro, Tiago R.D. Costa, William Cenens, Diorge P. Souza, Alexandre Cassago, Luciana Coutinho de Oliveira, Roberto K. Salinas, Rodrigo V. Portugal, Chuck S. Farah & Gabriel Waksman, can be accessed at
https://www.nature.com/articles/s41564-018-0262-z