Trends in Microbiology

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Spotlight

T6SS: killing two bugs with one stone

Dukas Jurėnas[1,*,@] and Eric Cascales[1,*,@]

Bacteria deploy the type VI secretion system (T6SS) to inject effectors into bacterial rivals. Contrary to the prevailing model, a recent study (Le *et al*.) expands the target range of the T6SS by demonstrating that it delivers and potentializes a peptidoglycan-targeting bifunctional toxin into Gram-positive bacteria.

Bacterial secretion systems deliver effector proteins into the surrounding medium or directly into target cells. The T6SS is a fascinating contractile injection nanomachine that uses a spring-like mechanism to propel an effector-loaded syringe into eukaryotic or bacterial cells [1]. Since the discovery of the proficiency of this secretion apparatus to destroy bacterial rivals, the dogma was that only Gram-negatives were targeted and that Gram-positive bacteria remained resistant to T6SS attacks, beside the fact that T6SS effectors are enzymatically competent to elicit damage in all cells [2–4]. It was proposed that the thick, multilayered, peptidoglycan coat of Gram-positives acts as a shield, preventing needle penetration and effector delivery [3,4], although a study reported that the T6SS could drill through even thicker fungal cell walls [5]. In a recent article, Le and coauthors [6] identified Tse4, a T6SS effector targeting both Gram-negative and Gram-positive bacteria (Figure 1), delivered by *Acinetobacter baumannii*, a multidrug-resistant opportunistic human pathogen responsible for hospital-acquired infections.

The T6SS: breaking barriers

In their study, Le *et al.* show that Tse4 is active against Gram-positive bacterial species such as *Bacillus subtilis*, *Listeria monocytogenes*, and methicillin-resistant *Staphylococcus aureus* (MRSA) [6]. As previously documented for other T6SS peptidoglycan-targeting effectors [7], the exogenous addition of Tse4 into the cell culture is not sufficient to induce cell lysis; rather, Tse4 has to be delivered via the T6SS in a contact-dependent manner into or across the peptidoglycan layer [6]. The expanse of the T6SS tail tube (about 500 nm) and the strength of sheath contraction (about 15 000 kcal.mol^{-1}) [8] are readily sufficient to penetrate the 30 nm width and stiffness of the Gram-positive cell wall. For comparison, contractile bacteriophages pierce the entire cell envelope, beside the fact that bacteriophage sheath lengths (about 90 nm) are much smaller than T6SS tail sheaths (about 800 nm). Many reasons can explain why Tse4 needs to be delivered into the interstitial compartment localized between the cytoplasmic membrane and the peptidoglycan mesh. First, the physiological levels of Tse4 translocated by the T6SS in culture supernatants are probably far below the effective concentration required to degrade the thick peptidoglycan layer. By being translocated into the interstitial compartment, the local concentration of Tse4 would enable peptidoglycan damage. Second, the surface of the Gram-positive cell wall is coated by teichoic and lipoteichoic acids, neutral polysaccharides, and exposed proteins that are covalently anchored by sortases, preventing accessibility of the effector to its peptidoglycan substrate.

Tse4, a two-sided effector

The *A. baumannii* Tse4 effector is a modular protein and carries two distinct domains with enzymatic activities on glycan strands (lytic transglycosylase) and peptide crosslinks (endopeptidase) of the peptidoglycan. In both Gram-positive and Gram-negative bacteria, the peptidoglycan sugar backbone is composed of alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic (NAM) acid residues. Thus, the Tse4 lysozyme-like lytic transglycosylase domain that cleaves the 1,4-β linkage between NAM and NAG could be universal. On the contrary, the peptide stems significantly differ in bacterial species, in terms of composition and modifications. The currently described Tae amidase effectors cleave off the peptide stems or digest the peptidoglycan crosslinks but bear different substrate specificities [9]. Unlike these amidase effectors, Tse4 was shown to be active against Gram-positive bacteria that have different types of peptide crosslinks [6]. While it remains to define the specificity spectrum of its endopeptidase domain, *A. baumannii* Tse4 can be considered as a highly promiscuous enzyme. However, it would be interesting to determine the contribution of each enzymatic domain (through substitutions of individual catalytic sites) for Tse4 activity on Gram-positive bacteria in order to clarify whether this dual activity strategy is a secure and efficient way to shoot as many different bugs with one stone.

The Tse4 LysM domain: anchoring the toxin

In addition to the lytic transglycosylase and endopeptidase enzymatic domains, the Tse4 effector presents a third node – a LysM-type peptidoglycan-binding domain. LysM domains are composed of a series of conserved LysM sequences that noncovalently bind the NAG moiety of the glycan strands as well as chitin. While the role of the Tse4 LysM domain remains to be determined, several interesting possibilities can be foreseen. Peptidoglycan-targeting effectors delivered into the periplasm of Gram-negative bacteria remain confined between the two membranes, preventing their dispersion. By contrast, holes created by its enzymatic activities in the Gram-positive peptidoglycan may cause Tse4 leakage out of the cell. By anchoring Tse4 to the cell wall, the LysM domain could thus prevent its

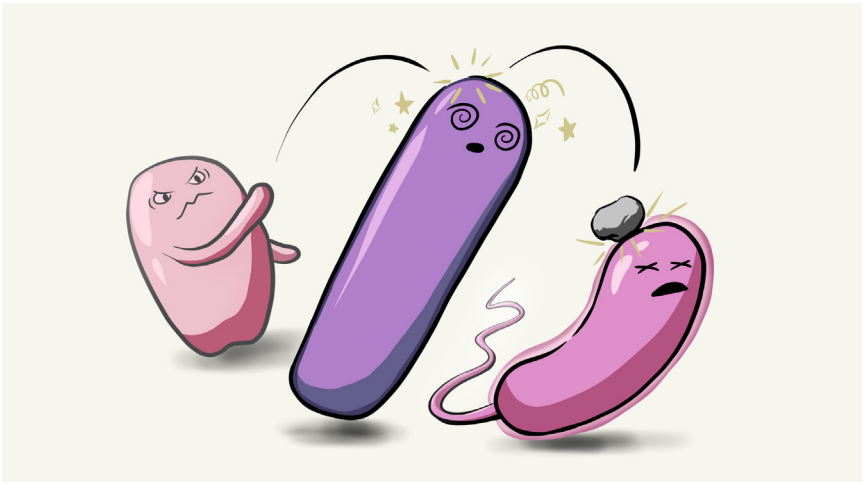

Figure 1. An *Acinetobacter* T6SS effector targets both Gram-negative and Gram-positive bacteria. *Acinetobacter baumannii* (left) throws the Tse4 bifunctional effector (gray stone) onto both Gram-positive (purple stain) and Gram-negative (pink stain) bacteria. Illustration by Clothilde Rousseau (Université Libre de Bruxelles, Belgium).

diffusion, increase its local efficiency, improve the cooperativity between the two enzymatic domains, and reinforce its overall effect. Interestingly, Le *et al.* reported that other T6SS lytic-transglycosylase/endopeptidase effectors have a different modular architecture, comprising an additional domain conferring peptidoglycan anchorage. In addition, LysM domains can be found associated with other T6SS effector/adaptor domains, such as PAAR-like or MIX, suggesting that anchoring an effector module to the cell wall is a valuable strategy to increase its efficiency. Further experiments with LysM domain deletion, or effectors grafted onto LysM, will likely establish whether this domain plays a crucial role in targeting the Gram-positive cell wall.

D-Lysine: optimizing the environment

In addition to injecting this universal effector, *Acinetobacter* also adjusts the battlefield. In the same study, Le and coauthors demonstrated that the concomitant synthesis and secretion of D-lysine is important for efficient killing by buffering the environment in order to reach the optimal pH for the activity of the Tse4 secreted effector [6]. Interestingly, the same group has previously shown that a fraction of the D-lysine is used by the secreting bacterium to edit its own peptidoglycan. Incorporation of this unusual amino acid into the cell wall provides the protective barrier against peptidoglycan-targeting enzymes secreted by other competing bacteria [10].

This study not only broke a dogma into pieces, it also provided a new picture on how bacteria have developed sophisticated strategies to optimize their armory – from building a protective shield to potentializing an effector for attacks.

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Declaration of interests

There are no interests to declare.

[1]Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), Institut de Microbiologie, Bioénergies et Biotechnologie (IM2B), Aix-Marseille Université – CNRS, UMR 7255, Marseille, France

*Correspondence:
djurenas@imm.cnrs.fr (D. Jurėnas) and
cascales@imm.cnrs.fr (E. Cascales).
@Twitter: @DukasJu (D. Jurėnas) and @CascalesLab (E. Cascales).

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