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Bacterial communication mediated by T4SS effectors

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Abstract

Bacteria have the ability to inhibit the growth of competitors by using contact-dependent killing devices, such as the bacterial-killing type IV secretion system (T4SS). A recent publication in *The ISME Journal* by Wang et al. (ISME J, 2023. https://doi.org/10.1038/s41396-023-01533-7) uncovered that T4SS could deliver a unique "non-toxic" effector protein, LtaE, into interspecies bacterial cells. The delivery of LtaE by the bacteria *Lysobacter enzymogenes* induces the antifungal antibiotic 2,4-DAPG production by binding to the transcriptional repressor PhlF in another bacterial species, *Pseudomonas protegens*. As a result, *P. protegens* regains the capacity to protect plants from nearby fungal infections. This finding uncovered a novel role of T4SS in mediating interactions of interkingdom cooperation to kill microbial competitors in soil microbiomes.

The soil microbiome harbors both beneficial and pathogenic species. Due to cohabitation, beneficial soil members have great frequency to "meet" pathogens and inhibit their growth to protect crop health via secreting diverse bioactive metabolites and assembling contact-dependent killing devices (Lugtenberg and Kamilova 2009; Green and Mecsas 2016). The bacterial-killing type IV secretion system is such a powerful contact-dependent killing device. This system is a subgroup of the widespread T4SS that is commonly utilized by bacteria for DNA delivery and protein translocation to eukaryotic cells (Costa et al. 2021). It is known that plant and animal pathogenic bacteria, such as *Xanthomonas citri* and *Stenotrophomonas maltophilia*, use the bacterial-killing T4SS to translocate lethal T4SS effector (T4E) proteins into competitor cells to kill the cells (Souza et al. 2015; Wolfgang et al. 2019), thereby gaining competitive advantages over other microbes during infections. Plant beneficial bacteria, such as *Lysobacter enzymogenes*, also use this system to kill the pathogenic bacteria via injecting toxic effectors (Shen et al. 2021). The discovery of the bacterial-killing T4SS leads microbiologists to hypothesize that this system may often deliver toxic effector proteins into competitor cells (Fig. 1a). Interestingly, Qian and colleagues previously reported that *L. enzymogenes* could use the bacterial-killing T4SS to inject a non-toxic T4E (also known as LqqE1) into the cytoplasm of the antifungal bacteria *Pseudomonas fluorescens* for quorum quenching, which blocks the competitor *P. fluorescens* to form biofilms, a community-like bacterial lifestyle (Fig. 1b). The LqqE1-mediated beneficial interaction allows *L. enzymogenes* to efficiently kill the free-living competitors in soil (Liao et al. 2023).

In a recent issue of *The ISME Journal* (Wang et al. 2023), Qian and colleagues present another intriguing story that refreshes the traditional roles of bacterial-killing T4SS-delivered T4E. They found that *L. enzymogenes* and *P. protegens*, two ubiquitous soil beneficial bacteria, could establish a previously unknown cooperative interaction (Fig. 1c). They showed that under certain soil nutrient conditions, *P. protegens* is blocked from producing the antifungal metabolite 2,4-DAPG, resulting in a loss of plant protection against fungal pathogen

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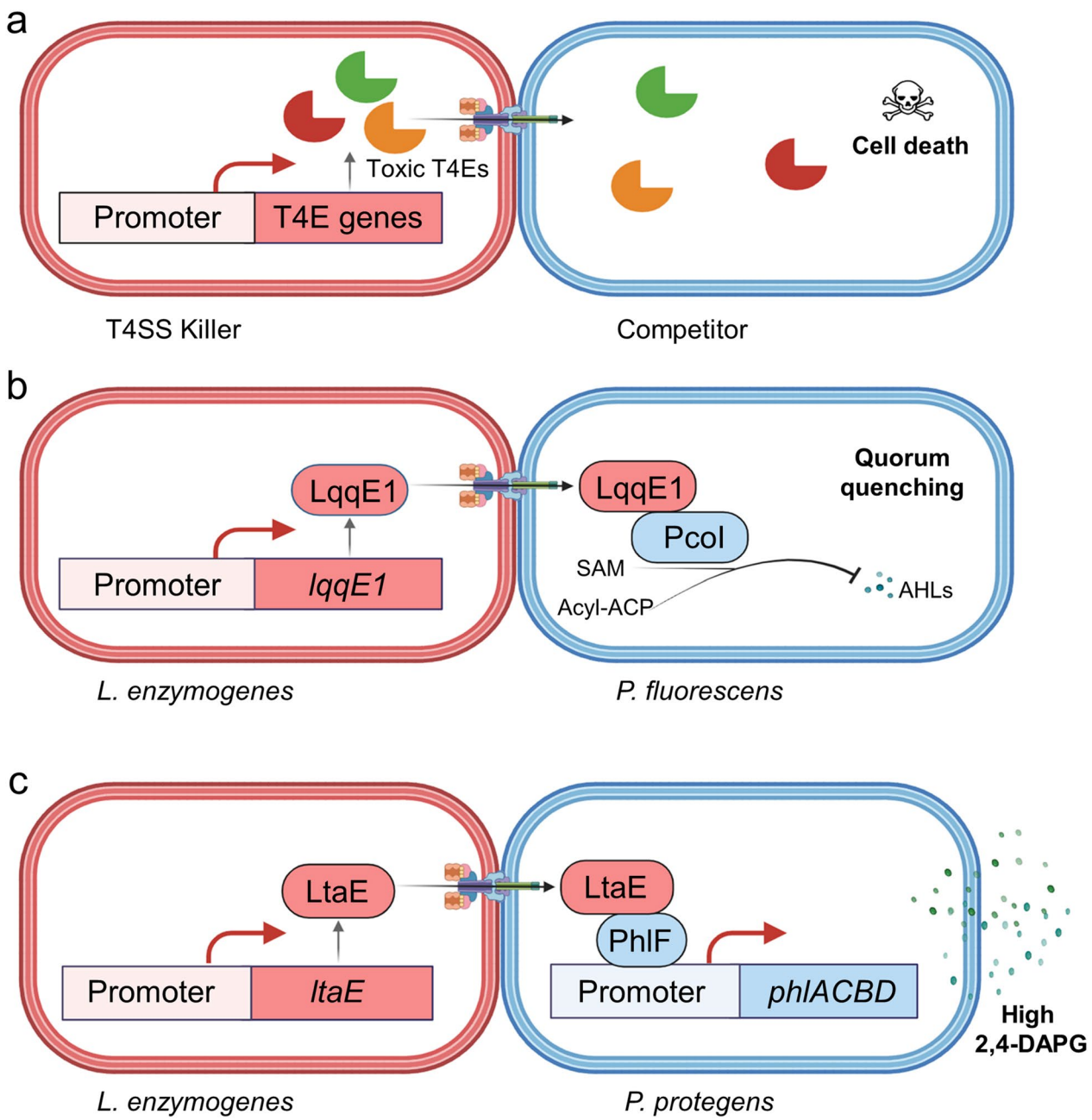

Fig. 1 Diversified functions of T4SS effectors translocated by the bacterial-killing T4SS. **a** Donor species carrying the bacterial-killing T4SS kill bacterial competitors by translocating lethal T4Es. **b** Quorum quenching by a non-toxic T4E, LqqE1. *L. enzymogenes* delivers LqqE1 into *P. fluorescens*. Upon delivery, LqqE1 binds to the AHL synthase PcoI directly, disrupting its recognition with SAM, a crucial substrate for AHL synthesis, resulting in quorum quenching. **c** T4E-triggered bacterial cooperation. *L. enzymogenes* delivers another non-toxic T4E, LtaE into *P. protegens*. Once LtaE enters the cytoplasm of *P. protegens*, it forms a protein complex with PhlF, a transcriptional repressor of 2,4-DAPG production. The binding of LtaE relieves the repressor function of PhlF, which leads to an activation of antifungal 2,4-DAPG production, enabling *P. protegens* to regain the capacity to protect plants from fungal pathogen infections

infections. However, *P. protegens* adopts an adaptive strategy by establishing an interspecies interaction with *L. enzymogenes* through direct cell-to-cell contact. This pivotal contact enables *P. protegens* to sense and acquire a "non-toxic" T4E, named LtaE, that is translocated by the bacterial-killing T4SS of *L. enzymogenes*. After entering the cytoplasm of *P. protegens*, LtaE binds to PhlF, a pathway-specific transcriptional repressor of 2,4-DAPG

expression (Abbas et al. 2002). The formation of the LtaE-PhlF complex disrupts the ability of PhlF to bind to the promoter region of the *phl* operon, leading to the relief of PhlF transcriptional repression and the expression of 2,4-DAPG operon. The production of 2,4-DAPG helps *P. protegens* regain the capacity to protect plants from fungal pathogen infections. Therefore, their finding expands the bacterial interspecies interactions from predation, antagonism, and competition to cooperation, uncovering a special pattern of bacterial-bacterial-fungal interactions in the rhizosphere.

In addition to their canonic role in interbacterial competitions, the role of bacterial secretion systems in cell-to-cell communications has been reported recently. The contact-dependent growth inhibition (CDI) system of *Burkholderia thailandensis* can inject the BcpA toxin into immune bacteria, resulting in the changes of their gene expression. This discovery highlights the role of CDI systems in regulating interbacterial signal transduction (Garcia et al. 2016). In addition, we reported a *Yersinia pseudotuberculosis* type VI secretion system (T6SS) delivered bifunctional effector CccR that mediates interbacterial competition by AMPylating the cell division protein FtsZ in nonself cells and mediates cell-to-cell communication by acting as a transcriptional regulator in kin cells (Wang et al. 2022). Qian and colleagues convincingly demonstrated the role of T4SS effectors in cell-to-cell communication via modulating the activity of a transcriptional repressor (Wang et al. 2023), or via quenching of quorum sensing signals (Liao et al. 2023), providing additional evidence for the role of bacterial secretion systems in cell-to-cell communication. While quorum sensing is considered the main chemical communication process in bacteria, these findings provided new perspectives for understanding bacterial communication. Given the diversity of bacterial secretion systems and their prevalence in bacterial genomes, future research will uncover more effectors in mediating cell-to-cell communication and bacterial cooperation.

The discovery of the bacterial-killing T4SS and the T4E-triggered interspecies cooperation between soil-beneficial bacteria provides a valuable clue for biological and microbial applications. First, a common feature shared by most bacterial biocontrol agents is that the bacteria can produce and secrete antimicrobial metabolites, while T4SS-mediated bacterial killing is independent of such a common feature. Therefore, T4SS-mediated contact-dependent killing could be designed as a promising strategy for isolation and/or re-assessment of agricultural bacteria that are ignored as biocontrol agents due to their deficiency in producing antimicrobial metabolites. The T4SS-mediated contact-dependent killing strategy has been adopted as an efficient approach to design and engineer compatible biocontrol communities with multifunctional and/or synergistic biocontrol alliances (Wu et al. 2021). Second, the generation of a LatE-based engineered *P. protegens* is a promising strategy to resume its function in producing the antifungal 2,4-DAPG under certain soil nutrient conditions and hence enhance the *P. protegens* environmental adaptation and biocontrol efficacy. Third, to design a synthetic microbiome (Syncom) for biocontrol of crop diseases, T4Es may not be ignored despite potential species compatibility because they possibly alter the biosynthesis of antimicrobial metabolites and/or colonization-related biofilm formation in the bacterial partners.

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References

Abbas A, Morrissey JP, Marquez PC, Sheehan MM, Delany IR, O'Gara F. Characterization of interactions between the transcriptional repressor PhlF and its binding site at the *phlA* promoter in *Pseudomonas fluorescens* F113. J Bacteriol. 2002;184(11):3008–16. https://doi.org/10.1128/JB.184.11.3008-3016.2002.

Costa TRD, Harb L, Khara P, Zeng L, Hu B, Christie PJ. Type IV secretion systems: advances in structure, function, and activation. Mol Microbiol. 2021;115(3):436–52. https://doi.org/10.1111/mmi.14670.

Garcia EC, Perault AI, Marlatt SA, Cotter PA. Interbacterial signaling via *Burkholderia* contact-dependent growth inhibition system proteins. Proc Natl Acad Sci USA. 2016;113(29):8296–301. https://doi.org/10.1073/pnas.1606323113.

Green ER, Mecsas J. Bacterial secretion systems: an overview. Microbiol Spectr. 2016. https://doi.org/10.1128/microbiolspec.VMBF-0012-2015.

Liao J, Li Z, Xiong D, Shen D, Wang L, Lin L, et al. Quorum quenching by a type IVA secretion system effector. ISME J. 2023;17(10):1564–77. https://doi.org/10.1038/s41396-023-01457-2.

Lugtenberg B, Kamilova F. Plant-growth-promoting rhizobacteria. Annu Rev Microbiol. 2009;63:541–56. https://doi.org/10.1146/annurev.micro.62.081307.162918.

Shen X, Wang BX, Yang ND, Zhang LL, Shen DY, Wu HJ, et al. *Lysobacter enzymogenes* antagonizes soilborne bacteria using the type IV secretion system. Environ Microbiol. 2021;23(8):4673–88. https://doi.org/10.1111/1462-2920.15662.

Souza DP, Oka GU, Alvarez-Martinez CE, Bisson AW, Dunger G, Hobeika L, et al. Bacterial killing via a type IV secretion system. Nat Commun. 2015;6:6453. https://doi.org/10.1038/ncomms7453.

Wang B, Zhang Z, Xu F, Yang Z, Li Z, Shen D, et al. Soil bacterium manipulates antifungal weapons by sensing intracellular type IVA secretion system effectors of a competitor. ISME J. 2023. https://doi.org/10.1038/s41396-023-01533-7.

Wang D, Zhu L, Zhen X, Yang D, Li C, Chen Y, et al. A secreted effector with a dual role as a toxin and as a transcriptional factor. Nat Commun. 2022;13(1):7779. https://doi.org/10.1038/s41467-022-35522-9.

Wolfgang MC, Bayer-Santos E, Cenens W, Matsuyama BY, Oka GU, Di Sessa G, et al. The opportunistic pathogen *Stenotrophomonas maltophilia* utilizes a type IV secretion system for interbacterial killing. PLoS Pathog. 2019;15(9):e1007651. https://doi.org/10.1371/journal.ppat.1007651.

Wu Q, Wang B, Shen X, Shen D, Wang B, Guo Q, et al. Unlocking the bacterial contact-dependent antibacterial activity to engineer a biocontrol alliance of two species from natural incompatibility to artificial comptability. Stress Biol. 2021;1:19. https://doi.org/10.1007/s44154-021-00018-x.