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Opinion

Making the Best of Aggression: The Many Dimensions of Bacterial Toxin Regulation

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Most bacteria use toxins to exclude competitors. As the synthesis and delivery of these molecules entail considerable costs for the producers, their expression is tightly regulated, often by molecular systems detecting physiological stresses or environment-specific cues. However, the ecological connection between such systems and competitive behaviors is not always clear. Here, we review the regulation of antibacterial toxins and propose a conceptual framework organizing the decision-making processes controlling toxin production. As bacteria are unable to precisely identify their competitors, we argue that toxin regulation primarily responds to cues directly or indirectly associated with the presence of competing strains. The density and fitness of the producing population also play a role in the decision-making process. Overall, we contend that optimal toxin production strategies involve monitoring of both self and foe.

Highlights

Toxin synthesis and delivery entail significant costs to bacteria.

Antibacterial toxin production is under strict regulatory control.

Numerous molecular mechanisms of toxin regulation have been elucidated, but the underlying decision-making strategies are often ignored.

Decisions behind toxin production aim at maximizing long-term fitness. They rely on inferences about the presence of competitors and the physiological state of the producing population (density and fitness of the producers).

To detect competitors, bacteria mostly depend on generic competition cues informing them of damage to themselves or their siblings, lack of nutrients, or competitive environments.

Toxin-Mediated Interbacterial Competition

Since the first report of toxin-mediated bacterial warfare at the beginning of the 20th century [1], the repertoire of known bacteriocins has been ever expanding. Currently it encompasses a wide range of diffusible toxins, from large multidomain proteins to post-translationally modified oligopeptides [2–4]. In addition to classic bacteriocins, several contact-dependent toxin-delivery systems have been discovered in the past 15 years. These include the type VI secretion system (T6SS) [5,6] and the contact-dependent inhibition (CDI) systems [7,8] found in many Proteobacteria, as well as other, less well-studied, membrane-associated toxins [9–12]. According to comparative genomics data, most bacteria encode at least one type of toxin, whilst some of them produce dozens [2,13,14]. Moreover, it has been demonstrated that several contact-dependent and contact-independent systems can be found in the same genome, and that the toxin complement can vary widely between different strains of the same species [2,13]. This suggests that interference competition is a key aspect of bacterial life and that toxins are crucial for the survival and success of bacterial populations.

The expression of most known antibacterial toxin types is tightly regulated [15–20], consistent with the fact that the overall costs of toxin production are expected to be significant. Release of many diffusible toxins, like colicins or pyocins, occurs through lysis and death of the producing cells [17,21,22]. In this case, the frequency of lysing producers, which sometimes is as high as 8% of the population, gives a conservative estimate of toxin production costs [23,24]. Contact-dependent systems also generate a burden for the producer as they rely on large, highly antigenic protein scaffolds (CDI) or complex ATP-driven machineries, some components of which are abundantly secreted in the extracellular milieu (T6SS) [25]. These unavoidable operating costs make it essential for bacteria to choose wisely the timing of toxin production in order to maximize the benefits of interference behaviors.

Although the literature on toxin regulation is abundant, and often meticulous on molecular mechanisms, the underlying decision-making strategies have been discussed in very few studies. Here, we propose a conceptual framework which defines and classifies the ecological principles behind the regulation of bacterial interference competition. According to this framework, decisions on toxin production ultimately rely on the assessment of two main variables, the presence of competitors in the immediate surroundings and the fitness and density of the producing population (Figure 1, Key Figure). The necessity to monitor both siblings and competitors stems from the altruistic nature of toxin production, as often toxins do not benefit the producing cell, but are advantageous to its kin. Therefore, the producer must not only make sure that there are competitors nearby, but also that there are clonemates worth saving.

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Monitoring Competitors

Even though some toxins have acquired functions which are independent of their capacity to harm competitors, such as communication between siblings (CDI) [26], contribution to biofilm formation (T6SS, CDI) [27,28], or nutrient acquisition (T6SS) [29,30], their principal purpose is interference competition. Consequently, for toxin release to be beneficial, sensitive competitors should be growing in the vicinity of the producers. It is almost impossible for bacterial cells to unequivocally identify competitors in their immediate surroundings or to obtain accurate information on the toxin resistance profile of their neighbors. As a result, they tend to use proxies to infer the presence of competing strains and species. Specific cases of such inferences have been described as 'competition sensing' and 'danger sensing'. The concept of 'competition sensing' was proposed to describe an overlooked dimension of bacterial stress responses [31]. Physiological stressors, like DNA damage or nutrient shortage, are often connected to the presence of competitors which can harm the focal strain through direct attacks or depletion of resources. These stressors can, therefore, function as cues for competition. 'Danger sensing' [32] has been used for cases where the regulatory networks controlling toxin production rely on cues which are not stressors themselves, but are linked to the presence of competitors, like the detection of molecules released by dying siblings. Here, we present a more comprehensive picture of the regulation of interference behaviors and propose that four main

Key Figure

Decision-Making Factors Affecting Toxin Production

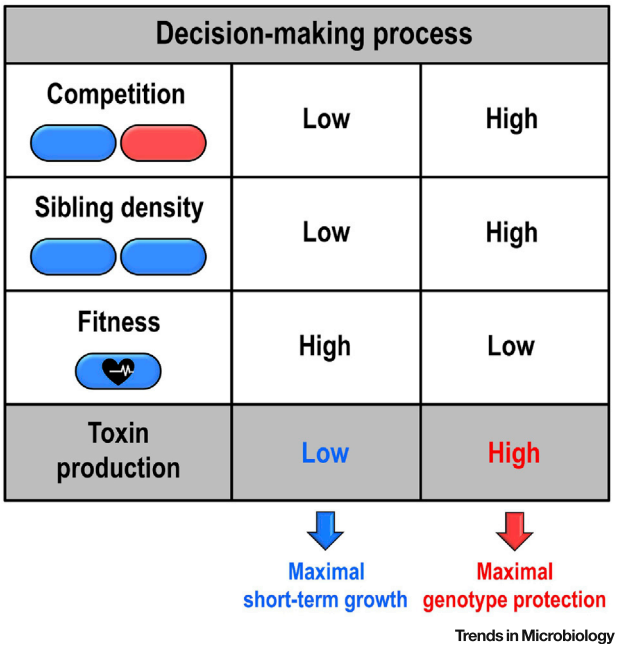

Figure 1. The regulation of antibacterial toxins involves inferences about the presence of competitors (first row) and the state of the producing population (second and third row); the focal toxin-producing strain is shown in blue, and the competing strain in red. For toxin production to be beneficial, competitors must be present in the surroundings, therefore the first parameter has the foremost importance. Additionally, depending on the environmental context, the density and fitness of the producing population might need to be considered in order to optimize the investment in toxins. Low toxin production promotes a maximal short-term growth rate of the colony (blue arrow), whereas high toxin production leads to maximal protection of the producing genotype from incoming threats (red arrow). Ultimately, this decision-making process aims at maximizing long-term fitness by orchestrating the timely release of toxins.

types of cues enable bacteria to efficiently respond to competition (Figure 2); we base this classification on the nature of the cue and its relationship to the competitor or the producer.

Direct Interference Sensing

In specific cases, bacteria can detect toxin-mediated attacks from competitors by directly sensing damage (Figure 2, top left). Indeed, loss of cellular integrity has been shown to trigger the production of colicin-like toxins as well as the activation of the T6SS. Colicins are often controlled by a housekeeping regulatory system, the SOS response, which senses DNA damage. Since many antibacterial toxins target DNA as an essential component of the cell, this regulatory system can prompt direct

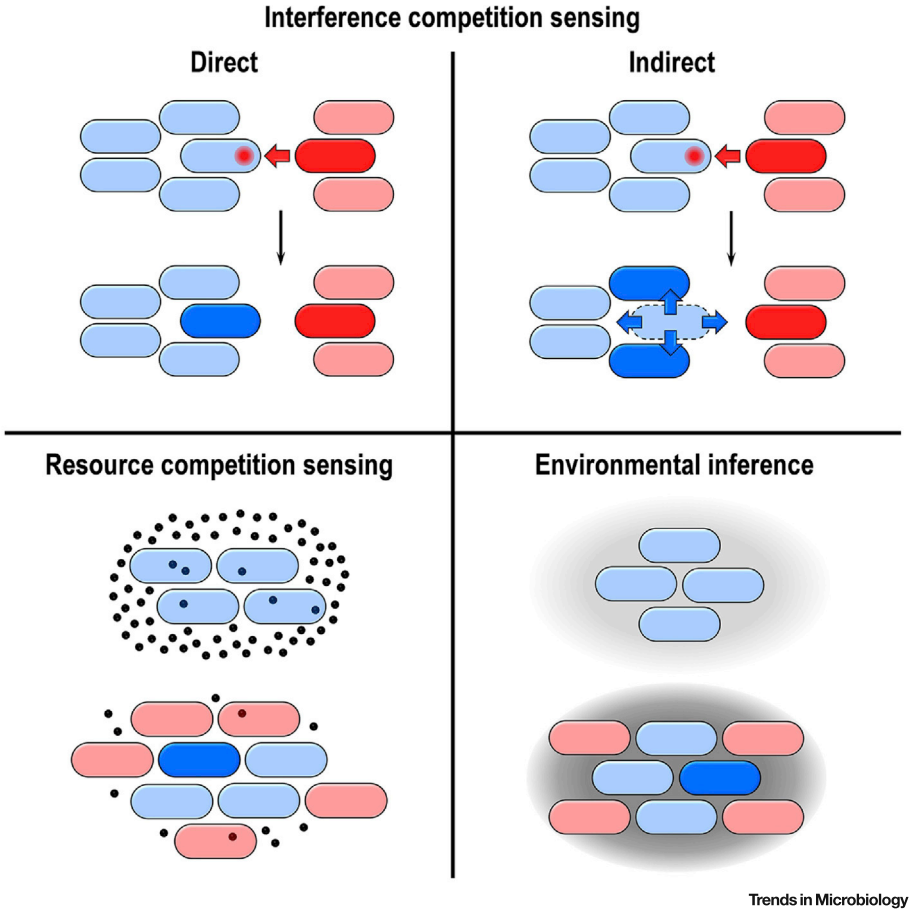

Figure 2. Overview of the Types of Cues That Bacteria Can Use to Infer the Presence of Competitors.
Direct interference sensing (top left panel) occurs when a focal cell in a toxin-producing population senses direct damage (red dot) caused by a competitor; examples of direct damage include DNA or RNA degradation or membrane perturbations. This results in a counterattack mediated by toxin production and release. By contrast, indirect interference sensing (top right panel) refers to cases where toxin producers detect incoming attacks via 'alarm cues' (blue arrows) produced by harmed siblings. This allows toxin producers to detect competitors in advance and respond by expressing toxins before becoming damaged themselves. Alternatively, bacteria can use nutrient shortage (bottom left panel), often associated with resource competition, as a cue to drive toxin expression. Finally, in the life cycle of many bacterial strains, abiotic factors, such as temperature or salinity, robustly correlate with noncompetitive or competitive niches (light and dark gray fields, respectively) and can lead to meaningful inferences about competition (bottom right panel). The focal toxin-producing population and a competing genotype are represented in light blue and red colors, respectively. Focal cells that are actively producing toxin, and competitor cells that are causing direct damage, are depicted in darker blue and red colors, respectively.

reciprocation to toxin-mediated antagonism [33–35]. Another case of direct interference sensing has been described in *Pseudomonas aeruginosa*. In this bacterium, one of its three type VI secretion systems, the H1-T6SS, is primed for firing in a stepwise process, encompassing transcriptional and post-transcriptional control [6,36].The last activation step involves sensing of membrane perturbations caused by T6SS attacks of competitors, via the TagQRST signaling cascade [37]. In this way *P. aeruginosa* T6SS producers can retaliate in a 'tit-for-tat' manner by rapidly assembling the toxic apparatus at the exact cellular location where damage was initially sensed. Although these two examples are quite striking, direct sensing of toxin-mediated damage is unlikely to be the rule in the bacterial world. Given the diversity of antibacterial toxins, it would be extremely challenging for producers to develop, or even repurpose, regulatory systems able to respond to every kind of attack. Moreover, even for cases where direct attacks are efficiently detected, as in the H1-T6SS example, it is not clear whether the focal cell would ultimately benefit; due to delays in signal transduction, retaliation may often come too late to save the producer.

A second mode of direct interference sensing likely complements the narrow-range regulatory approaches described above. Many bacteria release generic anticompetitor molecules, such as broad-range antimicrobials (e.g., hydrogen cyanide, phenazines, or antibiotics) or metal chelators, which are harmful to surrounding microorganisms and can serve as competition or even aggression cues [38–42]. In agreement with this, subinhibitory concentrations of several antibiotics have been found to enhance the production of both bacteriocins and secretion systems [43–46]. In addition, a common indirect effect of many antimicrobials is the increased production of endogenous reactive oxygen species (ROS) [47,48]. ROS can be detected via widespread stress–response regulatory networks and can also trigger defense mechanisms before their concentration becomes life-threatening [49]. Indeed, oxidative stress has been repeatedly linked to competitive traits, such as the production of colicin-like toxins by Enterobacteria and pyocins by *P. aeruginosa,* or the induction of the T6SS in *Yersinia pseudotuberculosis* and *Burkholderia thailandensis* [18,50,51]. It is worth noting that ROS-inducing compounds will work as cues across phylogenetically distant strains and species because their harmful effects can be detected by ubiquitous bacterial stress response systems.

Indirect Interference Sensing

To minimize harm from interference competition, toxin producers would benefit from sensing incoming attacks well in advance. One way to achieve this is for cells to be able to detect 'alarm' cues released by attacked siblings (Figure 2, top right).

DNase colicins, in addition to their role as toxins, can also function as alarm cues [52]. When a colicinogenic cell detects DNA-damaging toxins it produces colicins via direct interference sensing, and then lyses to release them. While the released colicin molecules are primarily intended to kill competitors, they are also internalized by siblings. Upon reaching a certain concentration in the cytoplasm, they saturate the immunity proteins of the receivers, damage their DNA, and trigger further colicin production. This results in a spatial wave of toxin release that sweeps through colicin-producing colonies and allows the producers to eradicate invading competitors. In this case, toxin molecules function as information carriers, prompting the colony to invest more in defense mechanisms. Another example of toxin production instigated by an alarm cue has been reported for the *P. aeruginosa* H1-T6SS, the structural components of which are regulated post-transcriptionally by the Gac/Rsm system [25,53]. This regulatory system detects so far still unknown signals stemming from lysing siblings [54]. Thus, it is activated in cases of collective cell death, for example when the colony is too dense or when it is under attack from competitors. Under these circumstances, the H1-T6SS components are translated and the system only requires an additional 'tit-for-tat' cue to assemble and fire [37].

Resource Competition Sensing

Nutrient shortage can also function as an indirect cue for the presence of competitors (Figure 2, bottom left). This is especially true for bacteria whose lifestyle involves phases of rapid growth in nutrient- and competitor-dense environments. Strains synthesizing toxins upon starvation benefit directly, as lysing competitors release nutrients, and indirectly, because they reduce the competitive pressure in their surroundings.

The production of several characterized toxins is known to increase upon carbon or nitrogen starvation or when micronutrients become scarce. Colicin K, for example, is transcribed in response to (p)ppGpp [55], a molecule synthesized when carbon or nitrogen sources are depleted. In addition, cell lysis and release of some colicins is controlled post-transcriptionally by the carbon-shortage regulator protein CsrA [56]. Colicin V, generated by Enterobacteria and plant pathogens such as *Xylella fastidiosa* [57,58], as well as some pyocins [59], are expressed when iron is limited. Interestingly, to enter the target cells, these toxins often use iron receptors whose production is also increased upon iron starvation [60], thus the benefits of toxin production in this regulatory mode are maximized. The synthesis of T6SS is also enhanced in the absence of micronutrients, iron in *Escherichia coli* (detected via FUR) and zinc in *Y. pseudotuberculosis* (detected via ZntR) [51,61].

It is important to note that detection of nutrient shortage, although common, is likely a less reliable signal for the presence of competitors than sibling lysis or cellular damage. This is because the actual cue is not directly linked, but only correlates, with highly competitive environments. Starvation could also be caused by a high density of siblings or simply by environmental nutrient shortage.

Environmental Inference

Many bacteria have predictable life cycles and alternate between niches associated with different types and intensities of competition. This is the case for pathogens and commensals with a host-independent and a host-associated stage, but also for environmental bacteria which regularly switch between free-living and sessile lifestyles. For natural habitats with predictable competitor profiles and densities, toxin producers can rely on environmental cues for the regulation of their defense mechanisms. Environmental inference can, therefore, be driven by abiotic indicators which correlate dependably with competition (Figure 2, bottom right).

Temperature is known to be a major factor in the regulation of colicins [62,63]. The production of these toxins peaks at 37°C, presumably because they are much more useful for enterobacteria in the gut of mammals where competition is high, than in the environment, where density and growth rates are lower. Moreover, organisms alternating between niches containing different sets of competitors are likely to evolve differentially regulated toxin-delivery systems and effector complements. For example, in *Y. pseudotuberculosis*, T6SS1 is expressed at 37°C, whereas T6SS2–4 are active at 26°C [64], suggesting that different secretion systems, and possibly different associated effectors, are beneficial in distinct niches. Further supporting this, T6SS4 is additionally regulated by OmpR and is expressed in high-osmotic environments and in the presence of bile salts [65], which also hints to this particular system being active in specific environments within the host. Similarly, the *Vibrio parahaemolyticus* T6SS1 is expressed in high-salt concentrations, while the T6SS2 is active under low-salt conditions [66]. This would point towards T6SS1 being functional when the bacterium is in free-living form, residing in sea water with high salinity, while T6SS2 is active in the gut of mollusks where the salt concentration is significantly lower.

Environmental inference can also correlate toxin production with other bacterial adaptations which are niche dependent. When *Vibrio cholerae* grows on chitin, for example, it induces both its competence genes and its T6SS operon [67,68]. This links the killing of competitors with DNA uptake, promoting horizontal gene transfer in an environment where many similar strains and species are present. In this case, the benefits of interference competition and competence are likely synergistic, illustrating one of the advantages of relying on indirect environmental cues for the regulation of different but congruent functions.

Monitoring Self

Ensuring that toxins are not expressed in the absence of competitors is probably the easiest way for bacteria to minimize their production costs. However, costs and benefits can be further optimized by restricting toxin production to specific cells in a community, to particular growth phases, or to certain life cycle stages (Figure 1).

Monitoring Fitness

Bacterial populations are not homogeneous in terms of physiology and fitness. Variations among individuals within clonal populations have been recorded for many traits, such as carbon-source utilization [69], phage resistance [70], antibiotic resistance [71], and growth rates [72]. Individuals growing at different rates over several generations, that is, having different fitness, do not contribute equally to the population. From a theoretical standpoint, the potential of a cell to generate descendants in a given amount of time should be a deciding factor for whether it should invest its resources into toxin production and protection of its clonal siblings, or into growth and division [73,74]. Therefore, if cells are indeed able to assess their own fitness, it would be beneficial to include this information in the decision-making process controlling toxin production.

Experimental results show that the expression of some toxins, especially toxins whose release requires cell death and lysis, is confined to a fraction of the growing population (usually in the range of 0.1–1%). This has been shown for numerous type I colicins [34,52,75], but has also been observed in T6SS [76] and CDI systems [15]. For colicin E2, the fitness of toxin-producing cells is significantly lower than the average population fitness [52]. This suggests that individuals which express colicins do not do so at random but based on their lower potential to contribute to population expansion. The correlation of low fitness and toxin production strengthens the link between stress responses and toxin regulation. By definition, bacteria which are exposed to physiological stresses have a lower short-term fitness than ones that are not. Detection of stress might, therefore, not only be used as a proxy for competition, but also as a cue of low reproductive value, justifying a higher investment into toxins that will protect the rest of the community. Unfortunately, detailed experimental data on toxin production and cell physiology at the single-cell level are sparse for most toxin systems, making the question of how general this connection is debatable.

Monitoring Siblings

In environmental settings, toxin production is, to some degree, always a cooperative behavior. This is obvious for toxins released through cell lysis, that can only benefit the producer's siblings. Membrane-attached toxins mainly protect the producer, but in structured polymicrobial communities they also indirectly protect the colony by keeping harmful competitors at bay. As a result, the density of siblings in the vicinity of the producer is a parameter worth considering when deciding on toxin investment. Furthermore, bacterial density can be key for maximizing the benefits of toxin production, particularly when efficient killing only happens above a toxin concentration threshold or the impact of toxin production on strain fitness is crippling during population expansion [31].

For all these reasons, population density has often been observed to affect toxin expression. Bacteriocin regulation through quorum sensing is common in Firmicutes [77]. In Proteobacteria, several toxin-delivery systems are also known to be regulated by bona fide quorum sensing, for example, the CDI system of *B. thailandensis* [78] and the T6SS4 of *Y. pseudotuberculosis* [64]. In these cases, the producers directly assess population density through the detection of specific acyl homoserine lactones and express toxins only when the density of siblings is high enough. However, the regulation of many other toxins involves indirect and less specific ways of assessing population density. Colicinogenic cells have been shown to become more prone to produce toxins in response to the toxin molecules released by their siblings [52]. Similarly, molecules such as ROS, and reactive nitrogen species (RNS), whose concentration correlates with bacterial density, can induce pyocin expression [79]. While these regulatory systems can detect changes in cell density, they are not as specific as most quorum-sensing systems since they could also be triggered by competitors rather than by siblings. Nonetheless, in the context of toxin regulation these nonquorum-sensing molecules probably achieve the same goal, acting either as competition cues or as indicators of population density.

Concluding Remarks

In the model presented here (Figure 1), detection of competitors as well as interactions between cells of a producing population control most regulatory decisions behind toxin production. Whilst these principles are general in scope, they cannot account for all the complexity of bacterial lifestyles.

Therefore, we would like to highlight two potentially fertile fields of investigation that we did not have the opportunity to discuss (see Outstanding Questions).

First, as already mentioned, the information that bacteria can gather on the identity of their immediate competitors is, at best, incomplete and ambiguous. Producers can minimize this problem by hedging their bets and delivering several toxins in one release event, thus increasing their chances of eradicating a competitor. To this effect, some strains carry multiple colicin-producing plasmids or plasmids encoding more than one colicin [52]. The epitome of multiple toxin delivery is clearly the T6SS, whose tip and inner tube are loaded with many effectors [80]. Some *P. aeruginosa* H1-T6SS effectors have been shown to have different activity levels and different interactions with one another (synergies and antagonisms) depending on environmental conditions [81]. However, much remains to be discovered about how selection tunes expression levels and toxin combinations depending on the ecology of bacterial strains.

Second, the decision-making process of toxin expression rarely relies on a single regulator. Most toxins are controlled at multiple levels (transcriptional, translational, post-translational, and delivery) and often, at each level, by multiple factors. Regulatory systems can be hierarchical, with several successive cues required for the toxins to finally be released, as in the case of the *P. aeruginosa* H1-T6SS [6,36,37]. Alternatively, the regulation can be partially overlapping, as seen for the transcription of colicin K, controlled synergistically by the SOS response and by (p)ppGpp [55,82]. Such multilayered regulation ensures that toxin release will be either restricted to very specific contexts (H1-T6SS) or will occur in a broader range of environments (colicin K). Nonetheless, the way several regulatory systems converge to ensure optimal timing of toxin release and fine-tuning of toxin production is still broadly unknown, and so far only very specific cases have been modeled [83]. More experimental and theoretical work is required to unravel how discreet regulatory networks interact to productively monitor both competitors and siblings in complex and changing environments.

Our conceptual framework is based on examples of toxin systems mainly found in Gram-negative bacteria. The discovery of new toxins, particularly through the expansion of our knowledge on polymorphic toxin systems in Gram-positive bacteria [84], as well as further studies on toxin regulation at the single-cell level, will no doubt reveal unprecedented regulatory mechanisms of toxin expression (see Outstanding Questions). Even so, the basic principles discussed here (Figure 1) are likely to be broadly applicable, since they are based on widespread, if not universal, ecological and evolutionary constraints.

Outstanding Questions

How does bacterial ecology impact the complement and expression of bacterial toxins (including T6SS effectors)?

How do the multiple layers of regulation of each toxin system interact to fine-tune toxin production and optimize the timing of toxin release?

Is there a robust link between toxin production and cell fitness at the single-cell level?

How are polymorphic toxin systems regulated in organisms other than Proteobacteria? Can the regulatory principles derived from proteobacterial colicin-like toxins, CDI, and T6SS be extended to evolutionarily distant organisms and systems?

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References

1. Gratia, A. (1925) Sur un remarquable exemple d'antagisme entre deux souches de collibacilles. *C. R. Soc. Biol.* 93, 1041–1042
2. Jamet, A. and Nassif, X. (2015) New players in the toxin field: polymorphic toxin systems in bacteria. *mBio* 6, e00285-15
3. Riley, M.A. and Chavan, M.A. (2007) Bacteriocins – Ecology and Evolution (Springer)
4. Scholl, D. (2017) Phage tail-like bacteriocins. *Annu. Rev. Virol.* 44, 453–467
5. Ho, B.T. *et al.* (2014) A view to a kill: the bacterial type VI secretion system. *Cell Host Microbe* 15, 9–21
6. Hood, R.D. *et al.* (2010) A type VI secretion system of *Pseudomonas aeruginosa* targets a toxin to bacteria. *Cell Host Microbe* 7, 25–37
7. Aoki, S.K. *et al.* (2010) A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. *Nature* 468, 439–442
8. Aoki, S.K. *et al.* (2005) Contact-dependent inhibition of growth in *Escherichia coli*. *Science* 309, 1245–1248
9. Cao, Z.P. *et al.* (2017) The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. *Nat. Microbiol.* 2, 16183
10. Garcia-Bayona, L. *et al.* (2017) Contact-dependent killing by *Caulobacter crescentus* via cell surface-associated, glycine zipper proteins. *eLife* 6, e24869
11. Russell, A.B. *et al.* (2014) A type VI secretion-related pathway in *Bacteroidetes* mediates interbacterial antagonism. *Cell Host Microbe* 16, 227–236
12. Souza, D.P. *et al.* (2015) Bacterial killing via a type IV secretion system. *Nat. Commun.* 6, 6453

13. Steele, M.I. *et al.* (2017) Diversification of type VI secretion system toxins reveals ancient antagonism among bee gut microbes. *mBio* 8, e01630-17
14. Zhang, D.P. *et al.* (2012) Polymorphic toxin systems: comprehensive characterization of trafficking modes, processing, mechanisms of action, immunity and ecology using comparative genomics. *Biol. Direct* 7, 18
15. Anderson, M.S. *et al.* (2012) The *Burkholderia bcpAIOB* genes define unique classes of two-partner secretion and contact dependent growth inhibition systems. *PLoS Genet.* 8, e1002877
16. Bernard, C.S. *et al.* (2010) Nooks and crannies in type VI secretion regulation. *J. Bacteriol.* 192, 3850–3860
17. Cascales, E. *et al.* (2007) Colicin biology. *Microbiol. Mol. Biol. Rev.* 71, 158–229
18. Ghazaryan, L. *et al.* (2014) The role of stress in colicin regulation. *Arch. Microbiol.* 196, 753–764
19. Matsui, H. *et al.* (1993) Regulation of pyocin genes in *Pseudomonas aeruginosa* by positive (*prtN*) and negative (*prtR*) regulatory genes. *J. Bacteriol.* 175, 1257–1263
20. Miyata, S.T. *et al.* (2013) Type VI secretion system regulation as a consequence of evolutionary pressure. *J. Med. Microbiol.* 62, 663–676
21. Penterman, J. *et al.* (2014) Biological cost of pyocin production during the SOS response in *Pseudomonas aeruginosa*. *J. Bacteriol.* 196, 3351–3359
22. Turnbull, L. *et al.* (2016) Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. *Nat. Commun.* 7, 11220
23. Kamensek, S. *et al.* (2010) Genes regulated by the *Escherichia coli* SOS repressor LexA exhibit heterogeneous expression. *BMC Microbiol.* 10, 283
24. Spriewald, S. *et al.* (2015) Reporters for single-cell analysis of colicin Ib expression in *Salmonella enterica* serovar Typhimurium. *PLoS One* 10, e0144647
25. Mougous, J.D. *et al.* (2006) A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* 312, 1526–1530
26. Garcia, E.C. *et al.* (2016) Interbacterial signaling via *Burkholderia* contact-dependent growth inhibition system proteins. *Proc. Natl. Acad. Sci. U. S. A.* 113, 8296–8301
27. Gallique, M. *et al.* (2017) Contribution of the *Pseudomonas fluorescens* MFE01 type VI secretion system to biofilm formation. *PLoS One* 12, e0170770
28. Garcia, E.C. *et al.* (2013) *Burkholderia* BcpA mediates biofilm formation independently of interbacterial contact-dependent growth inhibition. *Mol. Microbiol.* 89, 1213–1225
29. Lin, J.S. *et al.* (2017) A *Pseudomonas* T6SS effector recruits PQS-containing outer membrane vesicles for iron acquisition. *Nat. Commun.* 8, 14888
30. Wang, T. *et al.* (2015) Type VI secretion system transports Zn^{2+} to combat bultiple stresses and host immunity. *PLoS Pathog.* 11, e1005020
31. Cornforth, D.M. and Foster, K.R. (2013) Competition sensing: the social side of bacterial stress responses. *Nat. Rev. Microbiol.* 11, 285–293
32. LeRoux, M. *et al.* (2015) Bacterial danger sensing. *J. Mol. Biol.* 427, 3744–3753
33. Butala, M. *et al.* (2009) The bacterial LexA transcriptional repressor. *Cell. Mol. Life Sci.* 66, 82–93
34. Gillor, O. *et al.* (2008) The role of SOS boxes in enteric bacteriocin regulation. *Microbiology* 154, 1783–1792
35. Janion, C. (2008) Inducible SOS response system of DNA repair and mutagenesis in *Escherichia coli*. *Int. J. Biol. Sci.* 4, 338–344
36. Lesic, B. *et al.* (2009) Quorum sensing differentially regulates *Pseudomonas aeruginosa* type VI secretion locus I and homologous loci II and III, which are required for pathogenesis. *Microbiology* 155, 2845–2855
37. Basler, M. *et al.* (2013) Tit-for-tat: type VI secretion system counterattack during bacterial cell–cell interactions. *Cell* 152, 884–894
38. Blumer, C. and Haas, D. (2000) Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.* 173, 170–177
39. Harrison, F. *et al.* (2008) Interspecific competition and siderophore-mediated cooperation in *Pseudomonas aeruginosa*. *ISME J.* 2, 49–55
40. Hibbing, M.E. *et al.* (2010) Bacterial competition: surviving and thriving in the microbial jungle. *Nat. Rev. Microbiol.* 8, 15–25
41. Hider, R.C. and Kong, X. (2010) Chemistry and biology of siderophores. *Nat. Prod. Rep.* 27, 637–657
42. Pierson, L.S., 3rd and Pierson, E.A. (2010) Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. *Appl. Microbiol. Biotechnol.* 86, 1659–1670
43. Abrudan, M.I. *et al.* (2015) Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proc. Natl. Acad. Sci. U. S. A.* 112, 11054–11059
44. Fajardo, A. and Martinez, J.L. (2008) Antibiotics as signals that trigger specific bacterial responses. *Curr. Opin. Microbiol.* 11, 161–167
45. Jauri, P.V. *et al.* (2013) Subinhibitory antibiotic concentrations mediate nutrient use and competition among soil *Streptomyces*. *PLoS One* 8, e81064
46. Liu, L. *et al.* (2017) Identification and characterization of an antibacterial type VI secretion system in the carbapenem-resistant strain *Klebsiella pneumoniae* HS11286. *Front. Cell. Infect. Microbiol.* 7, 442
47. Dong, T.G. *et al.* (2015) Generation of reactive oxygen species by lethal attacks from competing microbes. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2181–2186
48. Dwyer, D.J. *et al.* (2015) Unraveling the physiological complexities of antibiotic lethality. *Annu. Rev. Pharmacol. Toxicol.* 55, 313–332
49. Dubbs, J.M. and Mongkolsuk, S. (2012) Peroxide-sensing transcriptional regulators in bacteria. *J. Bacteriol.* 194, 5495–5503
50. Si, M.R. *et al.* (2017) Manganese scavenging and oxidative stress response mediated by type VI secretion system in *Burkholderia thailandensis*. *Proc. Natl. Acad. Sci. U. S. A.* 114, E2233–E2242
51. Wang, T.T. *et al.* (2017) ZntR positively regulates T6SS4 expression in *Yersinia pseudotuberculosis*. *J. Microbiol.* 55, 448–456
52. Mavridou, D.A.I. *et al.* (2018) Bacteria use collective behavior to generate diverse combat strategies. *Curr. Biol.* 28, 345–355
53. Goodman, A.L. *et al.* (2004) A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev. Cell* 7, 745–754
54. LeRoux, M. *et al.* (2015) Kin cell lysis is a danger signal that activates antibacterial pathways of *Pseudomonas aeruginosa*. *eLife. Published online February 2, 2015. https://doi.org/10.7554/eLife.05701*.
55. Kuhar, I. *et al.* (2001) Codon-usage based regulation of colicin K synthesis by the stress alarmone ppGpp. *Mol. Microbiol.* 41, 207–216

56. Lechner, M. *et al.* (2016) Hierarchical post-transcriptional regulation of colicin E2 expression in *Escherichia coli*. *PLOS Comp. Biol.* 12, e1005243
57. Boyer, A.E. and Tai, P.C. (1998) Characterization of the *cvaA* and *cvi* promoters of the colicin V export system: iron-dependent transcription of *cvaA* is modulated by downstream sequences. *J. Bacteriol.* 180, 1662–1672
58. Zaini, P.A. *et al.* (2008) The iron stimulon of *Xylella fastidiosa* includes genes for type IV pilus and colicin V-like bacteriocins. *J. Bacteriol.* 190, 2368–2378
59. Dingemans, J. *et al.* (2016) Identification and functional analysis of a bacteriocin, pyocin S6, with ribonuclease activity from a *Pseudomonas aeruginosa* cystic fibrosis clinical isolate. *Microbiologyopen* 5, 413–423
60. Denayer, S. *et al.* (2007) Pyocin S2 (Sa) kills *Pseudomonas aeruginosa* strains via the FpvA type I ferripyoverdine receptor. *J. Bacteriol.* 189, 7663–7668
61. Brunet, Y.R. *et al.* (2011) An epigenetic switch Involving overlapping *fur* and DNA methylation optimizes expression of a type VI secretion gene cluster. *PLoS Genet.* 7, e1002205
62. Cavard, D. (1995) Effects of temperature and of heat-shock on the expression and action of the colicin A lysis protein. *J. Bacteriol.* 177, 5189–5192
63. Kennedy, C.K. (1971) Induction of colicin production by high temperature or inhibition of protein synthesis. *J. Bacteriol.* 108, 10–19
64. Zhang, W.P. *et al.* (2011) Modulation of a thermoregulated type VI secretion system by AHL-dependent quorum sensing in *Yersinia pseudotuberculosis*. *Arch. Microbiol.* 193, 351–363
65. Gueguen, E. *et al.* (2013) Expression of a *Yersinia pseudotuberculosis* type VI secretion system is responsive to envelope stresses through the OmpR transcriptional activator. *PLoS One* 8, e66615
66. Salomon, D. *et al.* (2013) *Vibrio parahaemolyticus* type VI secretion system 1 is activated in marine conditions to target bacteria, and is differentially regulated from system 2. *PLoS One* 8, e61086
67. Borgeaud, S. *et al.* (2015) The type VI secretion system of *Vibrio cholerae* fosters horizontal gene transfer. *Science* 347, 63–67
68. Meibom, K.L. *et al.* (2005) Chitin induces natural competence in *Vibrio cholerae*. *Science* 310, 1824–1827
69. Nikolic, N. *et al.* (2017) Cell-to-cell variation and specialization in sugar metabolism in clonal bacterial populations. *PLoS Genet.* 13, e1007122
70. Bull, J.J. *et al.* (2014) Phenotypic resistance and the dynamics of bacterial escape from phage control. *PLoS One* 9, e94690
71. Bergmiller, T. *et al.* (2017) Biased partitioning of the multidrug efflux pump AcrAB-TolC underlies long-lived phenotypic heterogeneity. *Science* 356, 309–311
72. Kell, D. *et al.* (2015) Individuality, phenotypic differentiation, dormancy and 'persistence' in culturable bacterial systems: commonalities shared by environmental, laboratory, and clinical microbiology. *F1000Res.* 4, 179
73. Hamilton, W.D. (1964) The genetical evolution of social behaviour. I. *J. Theor. Biol.* 7, 1–16
74. Nadell, C.D. *et al.* (2009) The sociobiology of biofilms. *FEMS Microbiol. Rev.* 33, 206–224
75. Bayramoglu, B. *et al.* (2017) Bet-hedging in bacteriocin producing *Escherichia coli* populations: the single cell perspective. *Sci. Rep.* 7, 42068
76. Troselj, V. and Wall, D. (2018) Metabolic disharmony and sibling conflict mediated by T6SS. *Microb. Cell* 5, 256–258
77. Shanker, E. and Federle, M.J. (2017) Quorum sensing regulation of competence and bacteriocins in *Streptococcus pneumoniae* and *mutans*. *Genes* 8, E15
78. Majerczyk, C. *et al.* (2014) Global analysis of the *Burkholderia thailandensis* quorum sensing-controlled regulon. *J. Bacteriol.* 196, 1412–1424
79. Sun, Z.Y. *et al.* (2014) PrtR homeostasis contributes to *Pseudomonas aeruginosa* pathogenesis and resistance against ciprofloxacin. *Infect. Immun.* 82, 1638–1647
80. Basler, M. (2015) Type VI secretion system: secretion by a contractile nanomachine. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 370, 20150021
81. LaCourse, K.D. *et al.* (2018) Conditional toxicity and synergy drive diversity among antibacterial effectors. *Nat. Microbiol.* 3, 440–446
82. Kuhar, I. and Zgur-Bertok, D. (1999) Transcription regulation of the colicin K *cka* gene reveals induction of colicin synthesis by differential responses to environmental signals. *J. Bacteriol.* 181, 7373–7380
83. Niehus, R. *et al.* (2015) Migration and horizontal gene transfer divide microbial genomes into multiple niches. *Nat. Commun.* 6, 8924
84. Jamet, A. *et al.* (2018) Antibacterial toxins: Gram-positive bacteria strike back! *Trends Microbiol.* 26, 89–91