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Interbacterial antagonism: at the center of bacterial life

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Abstract

The study of bacteria interacting with their environment has historically centered on strategies for obtaining nutrients and resisting abiotic stresses. We argue this focus has deemphasized a third facet of bacterial life that is equally central to their existence: namely, the threat to survival posed by antagonizing bacteria. The diversity and ubiquity of interbacterial antagonism pathways is becoming increasingly apparent, and the insidious manner by which interbacterial toxins disarm their targets emphasizes the highly evolved nature of these processes. Studies examining the role of antagonism in natural communities reveal it can serve many functions, from facilitating colonization of naïve habitats to maintaining bacterial community stability. The pervasiveness of antagonistic pathways is necessarily matched by an equally extensive array of defense strategies. These overlap with well characterized, central stress response pathways, highlighting the contribution of bacterial interactions to shaping cell physiology. In this review, we build the case for the ubiquity and importance of interbacterial antagonism.

Introduction

Most bacteria are likely to exist in a state that could be defined as single-celled for at least a portion of their existence. This includes instances in which kin cells or otherwise cooperating cells are separated by a physical distance that prohibits one or more adaptive group behaviors. In this single-celled state, the bacterium would be more vulnerable to a large range of threats, spanning simple fluctuations in the physical or chemical nature of their immediate environment, to more direct, biotic threats to their existence such as antibiotics, bacteriophage, antagonistic bacteria, and even predatory unicellular eukaryotes. Thus, every bacterial cell must be equipped to cope with such threats, and we argue that the relentless hostility of unicellular life at the micron scale has been a major force shaping

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bacterial evolution. In particular, we suggest that the contribution of interbacterial antagonistic interactions in shaping bacterial physiology might be underappreciated.

Few microbiologists would question the impact of phage in shaping the evolution of bacteria. Estimates suggest that phage outnumber bacteria 10:1, and just one of these particles can decimate an entire population of bacteria [1]. A recent spate of studies describing diverse mechanisms bacteria use to defend against phage attack have brought to the forefront the importance of phage in the life of bacteria [2–7]. In addition to the six distinct forms of CRISPR/Cas, at least 12 other phylogenetically and mechanistically distinct pathways for phage defense have been characterized, and many more that have yet to be studied in detail have been identified in genomic analyses [4, 8, 9]. But simply tallying the number of genes involved in phage defense does not do justice to their role in shaping the physiology of bacteria; these parasites promote horizontal gene transfer between bacteria, their own genes have been co-opted and repurposed to serve critical roles in numerous fundamental cellular processes, and the eons of evolutionary pressure they have exerted on the form and function of the cell surface cannot be overstated [10, 11].

Yet, we contend that other bacteria may be on equal or even higher standing as an omnipresent and unceasing threat to a typical bacterium. We base this, naturally, on our own bias [12–14], but also on observations deriving from multiple disciplines. In a comprehensive analysis that considered and weighted accordingly each of the major habitats of bacteria on earth, Flemming and Wuerz concluded that 20–80% of bacteria are surface associated [15]. Interestingly, it is within this state that carefully conducted *in situ* studies provide insights into the biogeography of microbial assemblages. Such work reliably demonstrates diversity at small length scales, and quite often, seemingly direct cell–cell contacts between bacterial species. These observations extend into habitats such as soil and the deep oceanic and continental substructures, which together account for ~75% of bacteria [16–18], and to host-associated habitats like the human gut and oral cavity [19, 20]. There is thus a reasonable body of “physical evidence” indicating that bacteria typically live in close proximity to one another. Now, to understand what is transpiring in during these interactions, i.e., whether they are antagonistic or cooperative in nature, we must largely extrapolate from *in vitro* experimentation, genomic evidence and theoretical models.

In this review, we present the case that each of these lines of evidence, as well as a handful of *in situ* studies that have emerged, all support the conclusion that interbacterial interactions are largely antagonistic in nature, and we argue that this has had profound consequences for the evolution of bacterial physiology. One of the impacts of this prevalence of antagonism appears to be the diversification of toxins themselves; we highlight below several examples of the nefarious means by which antibacterial toxins efficiently lead to target cell killing. Finally, we conclude this piece by describing the defensive mechanisms bacteria employ to contend with the onslaught of antagonism they face.

The diversity and ubiquity of antagonistic pathways

The ability of bacteria to kill or inhibit the growth of other bacteria has been appreciated since the early twentieth century, when the first antibiotics were isolated from *Streptomyces*

spp. [21, 22]. However, as bacteriology evolved as a discipline focused on the study of organisms in pure culture, characterization of interactions between bacteria fell out of favor, and the study of bacterial antagonism became largely confined to the search for clinically relevant inhibitory molecules. Early in the twenty-first century, it was found that bacteria can also antagonize closely contacting neighbors with antibacterial toxins delivered via the action of specialized secretion systems [23, 24]. It soon became apparent that such mechanisms are widespread [12, 25], and this has galvanized a renaissance in the study of interbacterial antagonism.

With interest growing and genomic data pouring in, the rate of discovery of interbacterial antagonism mechanisms is increasing. All major bacterial phyla have now been shown to possess antagonistic pathways, including both contact-dependent and -independent mechanisms in many cases (Tables 1 and 2). Diffusible toxins identified encompass the classical small molecule antibiotics that were the focus of researchers studying *Streptomyces*, as well as proteinaceous toxins that range in size from peptides to multi-subunit assemblages [26, 27]. Contact-dependent antagonism is mediated by a multitude of specialized secretion systems, including the type IV, V and VI pathways in Gram-negative organisms and the Esx secretion system of Gram-positive bacteria [23, 24, 28–30]. Each of these systems delivers toxic effector proteins to neighboring cells and employs cognate immunity proteins to prevent self and kin intoxication. Contact-dependent interbacterial antagonism can also occur via other routes such as outer-membrane fusion-mediated toxin exchange in *Myxococcus*, surface associated production of amyloid-like bacteriocins in *Caulobacter*, and the production of peptidoglycan anchored YD-repeat proteins with C-terminal toxin domains in *Bacillus* [31–33]. For detailed descriptions of these individual antagonistic mechanisms, we direct readers to recent reviews published on this topic [34, 35].

Not only are interbacterial antagonistic mechanisms diverse when viewed across the phylogenetic spectrum; individual species can themselves encode multifaceted antagonistic arsenals. Diversification occurs at numerous levels, including species carrying multiples of unique antagonistic mechanisms (Figure 1), non-redundant versions of a given mechanism (i.e. multiple toxin-exporting secretory pathways), and a plethora of effectors delivered by a single delivery system [36, 37]. The sum of the antagonistic pathways found in an organism can account for a significant proportion of the total coding capacity of the cell. For example, *Pseudomonas aeruginosa* encodes at least six distinct means of intoxicating competitors, which collectively account for 190 kb of the genome (Figure 1A) [24, 38–52]. Several of these encompass non-redundant versions of related systems, including three type VI secretion systems (T6SSs), each associated with as many as seven unique secreted effectors. This bacterium also possesses genes encoding two contact-dependent inhibition (CDI) and three pyocin systems. The Gram-positive species *Bacillus subtilis* encodes a similarly extensive but non-overlapping array of antagonistic mechanisms (Figure 1B) [32, 37, 53–64].

While tremendous progress unearthing new mechanisms of interbacterial antagonism is being made, it is clear that the field does not yet have the tools necessary to fully leverage available sequence data. These pathways are typically composed of genes without previously

ascribed function; thus, applying thoughtful new genome-wide experimental approaches or heuristic sleuthing efforts (like those undertaken to define novel phage defense mechanisms [4, 5, 7]) may well prove fruitful in uncovering additional means by which bacteria can inhibit the growth of their bacterial competitors. As recent work describing potential antimicrobial biosynthetic pathways in understudied phyla such as Acidobacteria, Verrucomicrobia, Gemmatimonadetes and Rokubacteria illustrates, we suspect such studies will reveal that antagonism is a prevalent trait even in bacterial groups not historically associated with this function [65].

The insidious nature of interbacterial toxins

The effectiveness of an interbacterial antagonism pathway is not merely a function of the inherent potency of its toxin. Toxins must overcome the tolerance resulting from cellular defenses that become activated as a result of the damage they inflict, those that act on cytoplasmic molecules must breach the cellular envelope, and a successful intoxication strategy should be relatively impervious to the emergence of resistance. As described below, recent studies highlight the insidious and complex strategies that have evolved to enable antibacterial toxins to meet each of these requirements.

Many antibacterial toxins act on molecules not readily accessible from the outside of the cell. Delivery by a dedicated secretion machinery is one means by which antimicrobial toxins can access otherwise inaccessible targets. For instance, it appears that the majority of T6SS toxins act in the periplasm, and although there is some debate, evidence suggests that at least in *P. aeruginosa*, the secretion apparatus initially delivers its antibacterial proteins to this compartment [49, 66]. Therefore, T6SS substrates with cytoplasmic targets require a means of crossing the bacterial inner membrane. While the details of cytoplasmic entry remains largely unknown for these proteins, T6SS toxins do not appear to share a requirement for specific inner-membrane receptors with toxins delivered by via the CDI system [67]. Indeed, one T6SS toxin of *P. aeruginosa*, Tse6, appears to surmount this hurdle through a rather unique mechanism [68]. The toxin, which degrades the essential intracellular metabolite nicotinamide adenine dinucleotide (NAD), tightly interacts with one of the most abundant proteins in the cell, elongation factor Tu (Ef-Tu). This interaction is dispensable for both the biochemical activity and secretion of the toxin, yet disruption of the Tse6–Ef-Tu interaction leaves the protein entirely unable to act on recipient cells. A detailed mechanistic understanding is lacking, but parsimony yields a model in which the Ef-Tu interaction promotes Tse6 translocation across the inner membrane, possibly by kinetically trapping the Ef-Tu-interaction motif within the cytoplasm.

Toxin delivery via the T6, T4 and Exs secretion systems requires prolonged cell-cell contact, precluding use of these antagonism mechanisms under fluid conditions [24, 29, 30]. In contrast, the CDI pathway mediated by a T5SS can act between cells grown in liquid culture [25]. A recent study by Hayes and collaborators describes a series of distinctive features shared by CDI toxins that mediate their uptake upon relatively transient contact with target cells [69]. CDI toxins had previously been described as “toxins on a stick”, as they consist of an extended N-terminal filament that remains anchored to the producing cell at the site of secretion through a β -barrel protein, and a C-terminal toxin domain that is delivered to

intoxicated cells [70]. This report demonstrated that in the absence of the target cell, secretion of the filament arrests prior to extrusion of the toxin or adjacent FHA-2 domain. This results in the surface presentation of a hairpin-shaped structure, consisting of the first half of the filamentous portion of the toxin with a domain responsible for receptor binding (RBD) in target cells at its distal end, and with the remaining portion of the protein extending back toward the secretion channel or residing within the periplasm prior to secretion. Upon binding of the RBD to its receptor on target cells, secretion arrest is relieved, and the FHA-2 domain associates with and becomes embedded in the outer membrane of the target cell. By a mechanism that remains to be elucidated, this then facilitates translocation of the C-terminal toxin domain into the periplasm of the target cell. A specific proline-rich domain in the toxin was shown to be responsible for secretion arrest. Remarkably, cells lacking this domain remain able to intoxicate targets under solid media growth conditions but lost the ability to act in liquid culture. This study illustrates how the route by which antibacterial toxins access their target molecules can have profound consequences for the conditions under which they can be effectively delivered.

Freely diffusible proteinaceous toxins that act on periplasmic or cytoplasmic targets face a particularly formidable access barrier. Unlike contact-dependent intoxication mechanisms, where the energy to fuel toxin uptake can be provided by the delivery system, these molecules must either store energy themselves, such as phage-derived tailocins, or they must have a means of harnessing it from the target cell [71, 72]. Many bacteriocins have evolved to accomplish this feat by parasitizing bacterial nutrient uptake systems that rely on outer membrane β -barrel proteins and the TonB or Tol systems for energized transport across the periplasm [72]. The details of this process were recently delineated for the bacteriocin pyocin S2 (pyoS2) of *Pseudomonas aeruginosa* [73]. This toxin is a 74 kD nuclease and must transit the entire cell envelope to reach its site of action. The receptor for pyoS2 is the outer membrane siderophore transporter FpvAI. The bacteriocin appears to follow the same import route through the center of this β -barrel protein as its native ligand, despite the fact that the toxin is more than eight times larger. This mechanism is facilitated by structural mimicry of a loop on the toxin that makes interactions with the receptor surface, mirroring native ligand contacts. The cleavage and transit of the toxin domain of PyoS2 across the inner membrane is then facilitated by the AAA⁺ ATPase FtsH.

The ingenuity of toxins goes well beyond the insidious manner by which they reach their targets; toxins would quickly be disarmed without a means to subvert the emergence of resistance. A “strength in numbers” approach, i.e., simultaneous delivery of multiple toxins acting through distinct mechanisms, may have evolved in contact-dependent toxin delivery systems in part to address just this problem. This strategy has the additional advantages of permitting toxin synergy and facilitating intoxication under a range of environmental conditions that might otherwise nullify the activity of a single toxin [48].

Individual toxins can also harbor their own resistance subversion solutions. For example, the primary intoxication mechanism employed by the ADP-ribosylase and T6SS substrate Tre1 of *Serratia proteamaculans* is to block cell division through modifying the cell-division protein FtsZ [74]. Yet the activity of Tre1 is not restricted to this target; its capacity to promiscuously ADP-ribosylate surface-accessible arginine residues allows Tre1 to modify

several other essential proteins. Accordingly, if FtsZ acquires a mutation rendering it resistant to modification, Tre1 remains, in principle, poised to disable the cell via other means.

Interbacterial antagonism in natural environments

While genomic evidence and laboratory studies leave no doubt as to the prevalence of antagonistic mechanisms across the domain Bacteria, the role these play in natural settings is only beginning to come into focus. Interactions between organisms can be difficult to monitor *in situ*, particularly in habitats with a high degree of diversity or in sites challenging to access in real-time, such as the mammalian gut. Nonetheless, studies employing a range of approaches and diverse model systems have begun to emerge suggesting that antagonism could be a universal feature of bacterial habitats.

Soil contains one of the most diverse microbial assemblages on earth and has long been known to harbor organisms with antagonistic capacity [22, 75]. More recently, soil bacteria from the under characterized phyla Acidobacteria, Verrucomicrobia, Gemmatimodetes and Rokubacteria were shown to encode diverse pathways for the production of secondary metabolites predicted to act as antibacterials [65]. However, cooperative traits such as cross-feeding and degradative synergy are also frequently detected among soil bacteria, leading some to question the role of antagonism in this environment [76]. Classical ecological theory predicts that the more a niche inhabited by two organisms overlaps, the more likely they will engage in competitive behavior, which has led to the hypothesis that antagonism among soil bacteria is most intense between related organisms living in close proximity [77]. Two groups of bacteria for which this idea has been explored are *Streptomyces* sp., which produce small molecule antimicrobials, and *Pseudomonas fluorescens* strains, which can engage in antagonism mediated by bacteriocins. *Streptomyces* isolate pairs from the same sampling site were more likely to exhibit an antagonistic interaction than pairs collected from distal sites and if they exhibited similar nutritional requirements [78]. *P. fluorescens* inhibitory interactions were more prevalent between isolates from different samples than within a sample; however, the distances between sites sampled were on the order of meters, in contrast to the continent-wide scale employed in the *Streptomyces* study [79]. Like *Streptomyces* spp, *P. fluorescens* isolates with similar nutritional requirements were more inhibitory to each other than those with distinct requirements. Strikingly, both studies found that the ability to resist antagonism was a much more widespread trait than the ability to actively inhibit another strain. Two evolutionary outcomes from the interaction between related soil bacteria were posited: 1) acquisition of resistance to the toxin produced by competing strains and/or 2) nutritional specialization to avoid antagonistic interactions. Studies of additional bacterial populations will help in deciphering whether these are general phenomena among soil organisms.

Given the density of microbial colonization in the mammalian gut, it is not surprising that an array of antagonistic pathways are encoded by bacteria that inhabit this ecosystem [80]. Yet in the absence of perturbances, gut microbial communities are remarkably stable [81], raising the question as to how and when antagonistic mechanisms might be employed in this environment. It could be that antagonism in the gut is primarily important for mediating

interbacterial competition during initial colonization of infants. Analysis of metagenomic datasets for T6SS gene abundance in *Bacteroidales* appears to support this; the *B. fragilis*-specific T6SS was more commonly found in infant colonizing strains than in those derived from adults [82]. Experiments employing gnotobiotic mice and a model community support the role of this system during gut colonization [83]. However, measurements of antagonistic pathways, including the T6SS, in adult gut communities suggests they also play a role beyond initial colonization [29, 82, 84]. Indeed, the T6SS of *B. fragilis* contributes to colonization resistance against sensitive strains in gnotobiotic mice [85]. Moreover, two other *Bacteroidales* T6SSs and their respective effectors are encoded on mobile elements and evidence suggests strong selective pressure for strains colonizing the same hosts to acquire and maintain these elements via horizontal transfer, leading to compatibility in effector and immunity gene pairs [82, 84].

Further evidence supporting the critical nature of antagonism in mature gut communities derives from the discovery of acquired interbacterial defense (AID) and recombination associated AID (*rAID*) systems [86]. These *Bacteroidales* elements composed of orphan immunity genes are pervasive in adult gut metagenomes, and are capable of complete neutralization of a corresponding toxin delivered by antagonizing *Bacteroides* strains. Interestingly, *rAID* clusters show hallmarks of active gene acquisition, suggesting they could serve an adaptive immune function – analogous to CRISPR arrays, but for defense against interbacterial antagonism rather than phage attack. The closest homologs to many of the genes encoded within *rAID* clusters are immunity genes associated with toxin delivery pathways other than the T6SS and which are found outside of *Bacteroidales*, raising the possibility that the systems grant protection from disparate antagonists. The prevalence of these orphan immunity gene clusters in gut *Bacteroidales* suggests that the acquisition of resistance to locally produced toxins promotes coexistence between otherwise incompatible strains, which may in turn contribute to community stability.

In an example of the role of diffusible antimicrobials in the mature gut community, a four-member bacterial consortia has been identified that provides colonization resistance against vancomycin-resistant enterococci (VRE) in mice [87]. This preventative effect requires production of a broad specificity lantibiotic peptide by the Gram-positive bacterium *Blautia producta*. Underscoring the important role that colonization resistance can play in the clinic, patients undergoing haematopoietic cell transplantation are rendered sensitive to VRE infections unless they possess genes for the production of this lantibiotic. Diffusible antimicrobials produced by Gram-negative bacteria may similarly have important roles in the gut community. *Bacteroides* sp. secrete antimicrobial proteins, termed BSAPs, that target specific surface molecules on target cells [88]. Resistance to the action of BSAPs is widespread and mediated through the expression of alternative target alleles encoded adjacent to the toxin genes. Within human gut metagenomes, strains with resistant target alleles are enriched when BSAP genes are present; therefore, like antagonism mediated by the T6SS of *Bacteroides*, diffusible antimicrobial proteins produced by these organisms may select for compatibility among strains colonizing an individual.

The examples described above highlight the potential role of interbacterial antagonism during colonization of naïve habitats and in defending established populations. Studies

indicate that a third role for these pathways lies in mediating invasion. The enteric pathogens *Vibrio cholerae*, *Shigella sonnei* and *Salmonella typhimurium* each encode a T6SS that contributes to gut colonization or exhibits commensal species targeting *in vivo* [89–91]. Bacteriocins can also mediate invasion of the gut. *Enterococcus faecalis* producing a plasmid-encoded bacteriocin is specifically able to invade and replace indigenous *E. faecalis* [92], and bacteriocin production by a virulent strain of *Listeria monocytogenes* facilitates mouse gut colonization by altering the commensal microbial community [93]. Antagonistic mechanisms can further function in concert with external disruptions to facilitate pathogen invasion. This is illustrated by the finding that the Enterobacteriaceae-specific bacteriocin colicin 1b of *S. typhimurium* contributes to its ability to compete against commensal *E. coli* strains in the mouse gut only during inflammation, which generally disrupts gut community structure and promotes the growth of Proteobacterial species [94]. Interestingly, gut commensal species can employ a counterstrategy under these conditions. *E. coli* Nissle suppresses the growth of invading *Salmonella* through the production of antimicrobial peptides called microcins, which are induced in response to low iron, a hallmark of the inflamed gut [95]. Furthermore, microcins are often conjugated to siderophores, facilitating their uptake by siderophore receptors in target cells that must actively scavenge iron to persist.

As experimental studies have provided evidence that interbacterial antagonism is a prevalent facet of life in microbial communities, questions have arisen regarding how antagonistic interactions influence community dynamics. Reports suggest that the high level of bacterial diversity present across many habitats can be explained through cooperative metabolic cross-feeding [96, 97]. However, these studies often fail to explicitly account for active antagonistic mechanisms, and rather focus exclusively on competition for nutrients, i.e., exploitation. Mathematical modeling of the impact of cooperation as well as antagonistic and exploitative interbacterial competition on community dynamics in the human gut revealed that, counterintuitively, antagonism promotes stability [98]. In this analysis, cooperation was destabilizing due to its potential to create dependencies between species, which therefore has an overall destabilizing impact on the community. In contrast, antagonism promotes stability across a wide range of diversity levels. This study also identified spatial segregation as a mechanism that promotes community stability by dampening the extent to which species interact. A recent study of the function of the T6SS system from the squid symbiont *Vibrio fischeri* during colonization of its host provides an elegant real-life example of spatial segregation and antagonism working in concert to structure a microbial community [99]. In this example, the authors found that *V. fischeri* strains encoding a T6SS and those susceptible to intoxication could co-colonize the same animal, but only by occupying distinct, spatially segregated crypts within the squid light organ.

Defense against antagonism

If interbacterial antagonism is as ubiquitous and ancient a facet of microbial life as the genomic, mechanistic, *in situ* and theoretical studies described above would suggest, then it stands to reason that bacteria would also have evolved many ways to defend against these attacks. One potential defensive mechanism is simply to mount a better offense; this may be

one factor that has contributed to the diversification and multiplication of antagonistic mechanisms that individual strains can encode [34]. A related strategy an organism could employ would be to encode the capacity to vary the toxins it produces, depending on the selective advantage of a given activity in the presence of particular competitors. An example of the later approach is illustrated by the Rhs toxins. These large, polymorphic toxins consist of a repetitive region linked to a C-terminal toxin (CT) domain that is encoded upstream of gene pairs predicted to encode additional CT domains and cognate immunity proteins [100, 101]. However, these pairs lack the repetitive portion of the toxin required for secretion. In *Salmonella typhimurium*, repeated passaging of the bacterium selected a clone bearing an Rhs toxin in which the ancestral CT domain was replaced by a downstream CT domain [102]. Selection for this strain is achieved by virtue of disruption of the open reading frame encoding this CT toxin and its immunity determinant in the parent strain. The genes clusters encoding the polymorphic MafB toxins of *Neisseria* species exhibit a similar architecture, although recombination-mediated toxin domain exchange has not been experimentally examined in this system [103, 104].

A second means of subverting antagonism lies in the acquisition of immunity determinants that provide protection from specific toxins (Figure 2). As described above, this appears to be a widespread trait of gut Bacteroidales [86]. While these organisms are so far the only group shown to encode arrays of immunity genes that appear to be actively acquired and have been functionally characterized, a number of other examples of so called “orphan” immunity genes predicted to confer protection against toxins not made by the organisms encoding them have also been described [74, 101, 105, 106]. Additionally, genes conferring resistance to small molecule antimicrobials are more abundant than producer species in soil, suggesting they play a broader role in the protection of target cells [107].

Bacterial stress responses are well known to contribute to antimicrobial resistance in a clinical context [108]. However, this has largely been considered an unfortunate side effect of their true function in providing protection from abiotic stressors. Reexamining these pathways in the context of evidence for widespread antibacterial antagonism, we argue that providing protection from biotic attack may in fact be their primary function in nature. This is supported by examples of stress response pathways that provide complex means of countering the action of specific toxins produced by other bacterial species. For example, the soil bacterium *Bacillus subtilis* employs a series of complementary mechanisms to resist intoxication from lantibiotics, polycyclic peptide antimicrobials produced by a number of Gram-positive bacteria [109]. *B. subtilis* defenses against lantibiotics include *i*) synthesis of negatively charged lipotechoic acids that can slow passage of cationic peptides through the cell wall, *ii*) production of the membrane signal peptidase SppA, thought to contribute to peptide antibiotic degradation in the membrane, *iii*) induction of phage shock protein homologs that protect the membrane against pore forming lantibiotics, and *iv*) induction of tellurite resistance gene homologs that contribute to lantibiotic resistance through an unknown mechanism. Stress response pathways can also grant resistance to contact-dependent antagonism. The *E. coli* envelope damage sensing relays Rcs and BaeSR provide protection against a specific T6SS effector of *Vibrio cholerae*, TseH, that likely acts on peptidoglycan (Figure 2) [110].

Another line of evidence linking stress response pathways to interbacterial antagonism is the observation made by Cornforth and Foster that many antagonistic mechanisms are themselves regulated by these pathways, a phenomenon they termed competition sensing [111]. These authors put forth the hypothesis that cellular damage indicates the presence of an antagonistic competitor, and that induction of antagonistic mechanisms in coordination with repair systems provides a means of simultaneously resisting and countering the attack. In the bacterium *Pseudomonas aeruginosa*, stress response mechanisms and antagonistic pathways are also coordinately regulated, but by a different mechanism that has been termed PARA (*P. aeruginosa* response to antagonism) [112]. Self-derived intracellular contents released by the activity of lytic antimicrobial toxins serve as paracrine signals that stimulate activation of the Gac/Rsm global posttranscriptional regulatory pathway in nearby cells (Figure 2). Induction of the T6SS, alongside additional factors that collectively enhance competitiveness toward antagonistic competitors, ensues. Many genes in the Gac/Rsm regulon have no known function, suggesting that additional mechanisms providing defense against antagonism remain to be characterized in this organism.

Conclusions

The diversity and widespread distribution of interbacterial antagonism pathways, the myriad settings in which these mechanisms contribute to bacterial competitiveness, and the evidence supporting an ongoing arms race between insidious toxins and robust defenses all point to the central importance of interbacterial antagonism in bacterial life. One clear implication of this is the expectation that interactions between any two organisms in a given environment are likely to be antagonistic. This is significant, as any attempts to engineer bacterial communities, including the gut microbiome, to alter their properties must include a means by which introduced organisms can contend with the antagonistic interactions they will invariably encounter. A more subtle potential outcome of the diversification of antagonistic pathways is their potential to serve as an evolutionary reservoir of toxins, including those used by bacterial pathogens against mammalian hosts and toxins horizontally acquired by eukaryotes to defend against bacteria. In summary, although this piece has focused on conflict that is necessarily restricted to the microscopic scale and typically taking place around us without detection, its impact on human health and the very foundation of most natural ecosystems should not be overlooked.

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Figure 1. Bacterial genomes are replete with loci encoding diverse interbacterial antagonistic pathways.

Depiction of the chromosomes of *P. aeruginosa* (A) and *B. subtilis* (B) with the position of loci encoding antagonistic factors highlighted. The loci are scaled to accurately reflect their relative lengths. Boxed schematics illustrate the function of genes within the respective element. A dashed cellular outline indicates toxin activity and arrows between two cells show toxin delivery; many loci encode factors that participate in both processes. Bacterial cell shading reflects intraspecies (light) or interspecies (dark) targeting. Values in parentheses indicate the number of genes followed by the cumulative length (in kilobases) of those genes. Factors are grouped by specific pathways (A,B) or more broadly (B), as indicated by box color and the corresponding keys.

Figure 2. Diverse defense pathways of Gram-negative bacteria against the T6SS.

Representatives of three categories of mechanisms are highlighted. The general stress pathways Rcs and Bae respond to envelope damage brought about by cell wall targeting toxins [110], and the danger sensing Gac pathway responds to signals released from neighboring cells undergoing lysis [112]; outputs from these pathways lead to transcriptional and translational changes in the cell, respectively. The defensive component(s) of the Gac regulon are not known. Within the effector-specific defenses in the rightmost portion of the cell are depicted two disparate orphan immunity mechanisms: (r)AID clusters (top) that encode effector-specific immunity proteins and ARH (ADP-ribosylhydrolase) domain proteins (bottom) that cleave the ADP-ribose moiety from targets (e.g. FtsZ) acted upon by ART (ADP-ribosyltransferase) toxins [74, 113].

Table 1.
Contact-independent mechanisms of interbacterial antagonism.

The diversity of antagonistic mechanisms mediated by diffusible agents is presented, along with their distribution across bacterial phyla and key characteristics. Volatile antimicrobial compounds and secreted lytic enzymes are not included.

Mechanism	Distribution ^a	Model Organisms ^b	Target Range ^c	Diagnostic features	Other characteristics
Ribosomally-synthesized and post-translationally modified peptides (RiPPs) [27, 114]	Actinobacteria Bacteroidetes Cyanobacteria Deinococcus Firmicutes Proteobacteria Spirochaetes Thermotogae	<i>Lactobacillus</i> sp., <i>E. coli</i>	Varies from intraspecies to intraphylum	Peptides synthesized as longer precursor peptide, encoded adjacent to immunity determinant, modifying enzymes and transporters	Short, modified peptides (<40 amino acids). In Firmicutes, these are known as class I bacteriocins, and many are lantibiotics. Termed microcins in <i>Enterobacteriaceae</i> .
Pediocin-like bacteriocins [115]	Firmicutes	Lactic acid bacteria	Closely related species	Conserved YGXGV sequence near the peptide N-terminus	Also called class IIa bacteriocins
Circular bacteriocins [116]	Firmicutes	<i>B. subtilis</i> , <i>Enterococcus faecalis</i>	Interspecies within Firmicutes	Similar to RiPPs, but lacking modifying enzymes, and containing a cytoplasmic ATP binding protein	Head-to-tail cyclized peptides lacking other modifications, also called class IIc bacteriocins.
Unmodified peptide bacteriocins [117]	Firmicutes	<i>Enterococcus</i> spp., Lactic acid bacteria	Varies from closely related species to within Firmicutes	Linear, unmodified single peptide bacteriocins in Firmicutes with no sequence similarity to pediocins.	Also called class IId bacteriocins.
Two peptide bacteriocins [118]	Firmicutes	Lactic acid bacteria	Interspecies within Firmicutes	Both propeptides are encoded in an operon, usually with transporter genes.	The individual peptides have low or little activity in isolation. Also called class IIb bacteriocins.
RTX bacteriocins [119]	Proteobacteria	<i>Rhizobium leguminosarum</i>	Closely related species	Proteins containing C-terminal glycine and aspartate-rich repeats, secreted via the type I secretion system	The RTX protein family also consists of anti-eukaryotic toxins.
Colicin-like bacteriocins [120]	Proteobacteria	<i>E. coli</i> (colicins), <i>P. aeruginosa</i> (pyocins)	Closely related species	Proteins containing translocation, receptor-binding and toxin domains.	Release is typically lethal to producers and occurs in response to stress.
Lectin-like bacteriocins [121]	Proteobacteria Firmicutes	<i>P. syringae</i> , <i>Ruminococcus flavegaciens</i>	Closely related species	Proteins containing monocot mannose-binding lectin (MBL) domains	Functions have yet to be ascribed to many bacterial proteins with MMBL domains.
Tailocins [122]	Proteobacteria Firmicutes	<i>P. aeruginosa</i> , <i>Clostridium difficile</i>	Largely intraspecies, with a few exceptions	Multi-subunit structures bearing structural homology to phage tails.	Single particles kill susceptible cells; release requires lysis of producing cell.
MACPF-domain containing proteins [88, 123]	Bacteroides Cloroflexi	<i>Bacteroides fragilis</i>	<i>Bacteroides</i> spp.	Proteins containing a conserved membrane attack complex/perforin (MACPF) domain.	Resistance conferred by expression of a variant form of the surface receptor, encoded adjacent to the toxin gene.

Mechanism	Distribution ^a	Model Organisms ^b	Target Range ^c	Diagnostic features	Other characteristics
NRPS/PKS synthesized antimicrobials ^d [65, 124–127]	Acidobacteria Actinobacteria Bacteroidetes Cyanobacteria Firmicutes Planctomycetes Proteobacteria Rokubacteria Verrocomicrobia	<i>Streptomyces</i> spp.	Varies from intraspecies to broad	NRPS/PKS antimicrobial biosynthetic gene clusters potentially distinguishable from those that synthesize other secondary metabolites by the presence of resistance determinants [65]	NRPS and PKS pathways are widespread in diverse phyla, but the molecules they synthesize are often uncharacterized.

^aThe distribution of antagonistic mechanisms was determined on the basis of published reports of the presence of genes encoding the indicated pathways.

^bModel organisms listed are the species in which the given pathway has been most extensively characterized.

^cRefers to experimentally demonstrated; range could extend beyond that indicated.

^dThe distribution for NRPS/PKS synthesized antimicrobials reflects reports in which pathways were either experimental shown to produce antimicrobial products or were predicted to on the basis of gene homology and/or the presence of antimicrobial resistance determinants within biosynthetic gene clusters.

Table 2.
Contact-dependent interbacterial antagonism pathways.

Pathways shown to mediate interbacterial antagonism that require direct cell-cell contact are listed, along with their distribution across bacterial phyla and key characteristics. Antimicrobial pathways that have been proposed on the basis of genomic analyses but that have yet to be functionally characterized in any organism are not included.

Mechanism	Distribution ^a	Model Organisms ^b	Target Range ^c	Diagnostic features	Other characteristics
Contact dependent inhibition (CDI) [128]	Proteobacteria	<i>E. coli</i> , <i>Burkholderia thailandensis</i>	Intraspecies	Mediated by CdiA/CdiB two-partner secretion proteins	Mechanism requires only transient cell-cell contact, functions under fluid conditions.
Type VI secretion system (T6SS) [36]	Proteobacteria Bacteroidetes Acidobacteria ^d	<i>P. aeruginosa</i> , <i>Serratia marcescens</i> , <i>Vibrio cholerae</i> , <i>E. coli</i>	Interphyla, Gram-negative	Seven structural genes are shared between antibacterial T6SSs in Proteobacteria and Bacteroidetes	Effectors often contain recognizable domains or motifs (VgrG, PAAR, Hcp, RHS, MIX)
Type IV secretion system (T4SS) [129]	Proteobacteria	<i>Xanthomonas citri</i>	Interspecies, within Proteobacteria	Distinguishable from non-antimicrobial T4SSs by C-terminal extensions in the VirB7 and VirB8 subunits and by homology to the <i>X. citri</i> system.	Effectors contain a conserved C-terminal XVIP domain.
Esx secretion system [28, 29]	Firmicutes Actinobacteria ^e	<i>Staphylococcus aureus</i> , <i>Streptococcus intermedius</i>	Intraspecies (<i>S. aureus</i>) or interspecies within Firmicutes	Most antibacterial Esx substrates appear to contain N-terminal LXG domains	Reports differ as to whether cell-cell contact is required for Esx-mediated intoxication.
Contact-Dependent Inhibition by Glycine Zipper Proteins (CDZ) [31]	Proteobacteria	<i>Caulobacter crescentus</i>	Closely related species	One or two small proteins containing glycine zipper motifs encoded adjacent to a type I secretion system homologous to CdzAB.	Secreted bacteriocins that form large aggregates on the cell surface.
WapA proteins [32]	<i>B. subtilis</i>	<i>B. subtilis</i>	Intraspecies	YD-repeat proteins with a C-terminal toxin domain	Distantly related to Rhs toxins of Gram-negative bacteria
Multiple Adhesin Family (Maf) [130]	<i>Neisseria</i> spp.	<i>N. meningitidis</i> and <i>N. gonorrhoeae</i>	Closely related species	MafB toxins containing a signal peptide, an N-terminal DUF1020 and a variable C-terminal toxin domain	Alternative MafB C-terminal toxin domain genes and accompanying immunity genes are often found in Maf genomic islands.
Outer membrane exchange (OME)-mediated toxin delivery [33]	<i>Myxococcus</i>	<i>M. xanthus</i>	Closely related species	SitA toxins, which share a homologous N-terminal domain containing a lipobox	OME is facilitated by TraAB.

^aThe distribution of antagonistic mechanisms was determined on the basis of published reports of the presence of genes encoding the indicated pathways.

^bModel organisms listed are the species in which the given pathway has been most extensively characterized.

^cRefers to experimentally demonstrated; range could extend beyond that indicated.

^dGenes encoding T6SS structural genes and putative effectors have been identified in genomes of Acidobacteria, but the system has yet to be characterized in organisms from this phylum [65, 131].

^ePredicted antimicrobial substrates of the Esx system have been identified in genomes of Actinobacteria, but none have been functionally characterized [106].

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