

REVIEW SUMMARY

MICROBIOTA

Bacterial antagonism in host-associated microbial communities

Leonor García-Bayona and Laurie E. Comstock*

BACKGROUND: Microbial communities are ubiquitous on Earth. The microbiota of different habitats are diverse and have distinct functional traits, but there are common ecological principles that govern their composition. The ability of a microbe to compete with other members of its community for resources is paramount to its success. Competition through the production of molecules that harm other members, known as interference competition, is also important in the assembly and maintenance of microbial communities. As new technologies allow for more in-depth analyses of microbial communities and their genetic content, we are better able to identify new antimicrobial toxins and analyze the effects of their production. Here, we explore the range

of antibacterial protein/peptide toxins and toxin-secretion systems, together with the fitness benefits they confer to the producing organisms. Because human-associated microbial communities have been intensely studied over the past decade, our focus is on the growing body of data regarding bacterial antagonism in these and other host-associated microbial communities.

ADVANCES: Studies continue to reveal the large arsenal of antibacterial peptides and proteins that bacteria produce and the secretion systems that they use to deliver these toxins to competing cells. Bacterially produced antimicrobial peptides and proteins are diverse in terms of their structures, cellular targets, mech-

anisms of action, and spatial range. Their antagonistic range also varies; some are limited to intraspecies killing, whereas others are able to kill across genera, families, and orders. Through a combination of mathematical modeling and experimental model systems, the ecological outcomes of bacterial antagonism are being

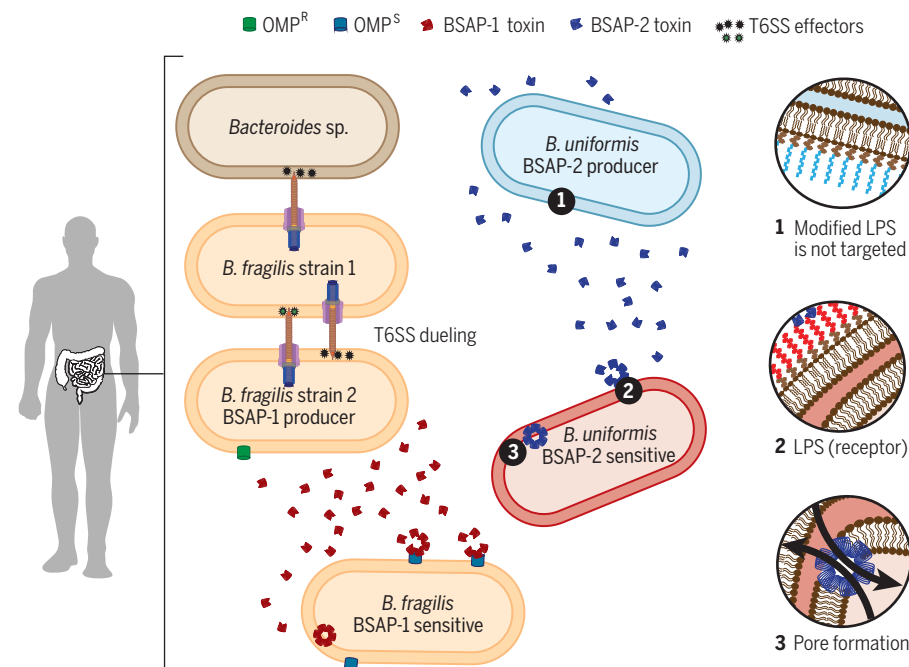
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elucidated. In vivo analyses in host models have shown that some antimicrobial toxins play a role in microbiota-mediated colonization resistance by preventing invasion of path-

ogens. Some pathogens, however, also use toxins to battle with the resident microbiota to invade an ecosystem and cause disease. Antagonism has also been shown to facilitate genome evolution; the DNA released from killed cells can be taken up and incorporated into the aggressor's genome. In some cases, antagonism has been shown to increase rather than reduce microbial diversity, potentially through promotion of spatial segregation of competing strains, facilitating the exchange of signals and secreted products between related cells (kin). The factors that regulate the production and release of some antibacterial toxins are also becoming better understood. Studies are revealing that toxin producers respond to various environmental signals, including signals that indicate host occupancy, that nutrients are limiting, or that they may be attacked by other bacterial community members.

OUTLOOK: Although bacterial antagonism is an active area of research, we are still in the early stages of understanding the impacts of these interactions in natural community settings and how they influence the overall structure, dynamics, and composition of complex microbial communities. The rapid increase in the number of available metagenomic datasets derived from diverse microbial communities and the expanding capability to culture and genetically modify these organisms is allowing for the identification and characterization of new antibacterial toxins. The protective function of microbiota-produced toxins in warding off pathogens indicates a potential for applications in medical, agricultural, and other industrial settings. In addition, the inclusion of antibacterial toxins in genetically engineered bacteria (live biotherapeutics) may allow for specific targeting of harmful community members, including those involved in therapeutic failures, and may also allow a live biotherapeutic to compete with members of the microbiota to deliver various health-promoting functions. ■



Intra- and interspecies antagonism—the example of *Bacteroides* species in the human gut.

Bacteroides fragilis and *Bacteroides uniformis* use MACPF (membrane attack complex/perforin) toxins—BSAP-1 and BSAP-2, respectively—for intraspecies killing. Producer strains carry a modified receptor [outer membrane protein (OMP) or lipopolysaccharide (LPS) glycan] that confers resistance to its cognate toxin. *B. fragilis* can also kill other *B. fragilis* strains and most gut Bacteroidales species via type VI secretion systems (T6SSs).

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Bacterial antagonism in host-associated microbial communities

Leonor García-Bayona and Laurie E. Comstock*

Antagonistic interactions are abundant in microbial communities and contribute not only to the composition and relative proportions of their members but also to the longer-term stability of a community. This Review will largely focus on bacterial antagonism mediated by ribosomally synthesized peptides and proteins produced by members of host-associated microbial communities. We discuss recent findings on their diversity, functions, and ecological impacts. These systems play key roles in ecosystem defense, pathogen invasion, spatial segregation, and diversity but also confer indirect gains to the aggressor from products released by killed cells. Investigations into antagonistic bacterial interactions are important for our understanding of how the microbiota establish within hosts, influence health and disease, and offer insights into potential translational applications.

Bacteria commonly live in complex microbial communities and have properties that allow them to survive, replicate, and compete in their ecosystems (1, 2). Central to their survival is the ability to harvest nutrients and scarce resources, to establish a spatial niche, and to withstand specific environmental conditions. In the human gut, colonizing microbes must survive in the face of various host conditions and factors, such as the low pH encountered during transit through the stomach; bile; host-produced immune factors, including antimicrobial peptides; and growth under anoxic conditions (3). Not least among the challenges of life in the gut are the competitive behaviors of other members of the microbial community and the potentially deleterious effects other organisms may have on the local environment, such as production of molecules that modulate host immunity, toxic metabolic end products, and alteration of oxygen levels (3).

The past decade of research has further highlighted the importance of microbial communities for human, animal, and plant health. The mammalian gut is currently one of the most investigated microbial ecosystems. Many studies report rapid changes in gut communities in response to numerous factors—including diet, drugs, and during infection—yet these are often transient, and over the lifetime of a healthy adult, the microbiota remains relatively stable (4, 5). An understanding of the ecological principles that govern this stability and that contribute to ecosystem fluctuations is essential to successfully alter microbial communities for therapeutic and agricultural benefits.

Broadly, ecological competition can be classified as exploitative competition, in which an

organism consumes the resources required by another member, or interference competition, in which a microorganism inhibits the growth of another through the synthesis of harmful products (6). Interference competition can be mediated through the production of different types of molecules, including small-molecule antibiotics, nonribosomally synthesized peptide antimicrobials, metabolites such as hydrogen peroxide, modification of host-produced molecules (7), signal interference, and the production of ribosomally synthesized peptides and protein toxins (6). This Review will largely focus on interference competition among bacteria mediated by diffusible proteinaceous toxins and toxins deployed by contact-dependent systems (Box 1).

Recent studies have provided insights into the benefits of toxin production to organisms within a community, but we still have a limited understanding of the full range of these competitive interactions and their ecological impact. Studies with an ecological slant have ranged from mathematical modeling, experimental systems, and observations of natural microbial communities. In addition, the ever-increasing number of metagenomes of diverse microbial communities is a growing resource for analyzing the numbers and types of toxins produced by a community.

Although the obvious outcome of toxin production is to provide a competitive advantage by killing a competitor, the overall effects to the community can be diverse. Some toxins may allow a competing member to enter an ecosystem (invasion), and some have been shown to function defensively to prevent invasion and to compete with established members. Several studies show that toxin production tends to increase diversity, by promoting spatial segregation, rather than reduce it. When considering the larger ecological consequences of these antagonistic

systems, the specific constraints of each system must also be considered. For example, antagonistic mechanisms that require physical contact cannot kill cells at a distance. Many toxins only target strains of the same species and therefore may not affect other competitors in the community. Further, the synthesis and secretion of these toxins may be physiologically costly and thus affect fitness (8, 9). Toxins whose release requires cell lysis can only be deployed by a fraction of the population and will have different relative fitness costs and dynamics compared with actively secreted toxins. We will examine these themes in more detail in the next sections.

Antagonism between community members

The complex gut microbiota of humans remains relatively stable over time, with many strains persisting for decades (4, 10, 11). The prevalence of diverse antagonistic systems produced by its bacterial members seems counterintuitive considering the overall stability. However, mathematical models of communities with high species diversity predict that communities dominated by antagonistic interactions are more stable than those in which cooperative interactions are more prevalent. Cooperative interactions tend to decrease resilience to perturbations because interspecies dependencies render members especially vulnerable to perturbations to their cooperative partners, whereas more antagonistic communities are predicted to be comparatively resilient (12). Moreover, in structured environments with highly diverse members, mutually antagonistic competitors could be isolated from each other through buffer zones, reducing the extent to which species interact with one another (2). Theoretical work suggests that antagonistic systems may promote diversity in unmixed environments under certain conditions (13, 14), as was shown experimentally for colicin production by *Escherichia coli* (15, 16) and type VI secretion system (T6SS)-mediated antagonism between *Aeromonas hydrophila* and *Vibrio cholerae* (17). Likewise, in natural soil communities, strong interference competition by bidirectionally antagonizing *Myxococcus xanthus* strains depends on the frequency of each strain and contributes to high levels of local diversity by reinforcing barriers to cross-territory invasion (18).

Many bacterial members of the human gut microbiota—including *Lactobacillus* spp., *Bifidobacterium* spp., *E. coli*, *Enterococcus* spp. and *Bacteroides* spp.—produce one or more types of antagonistic toxin systems, ranging from small-peptide bacteriocins, colicins, other secreted proteins, R-type bacteriocins, and toxins delivered by T6SSs (Box 1). The numerous studies of peptide bacteriocins of *Lactobacilli* and *Bifidobacteria* have largely focused on bacteriocin structures, mechanisms of action, therapeutic potential in preventing infection, or their use in the food industry [reviewed in (19, 20)]. By contrast, there is relatively little known about their impacts in their natural ecosystem. One

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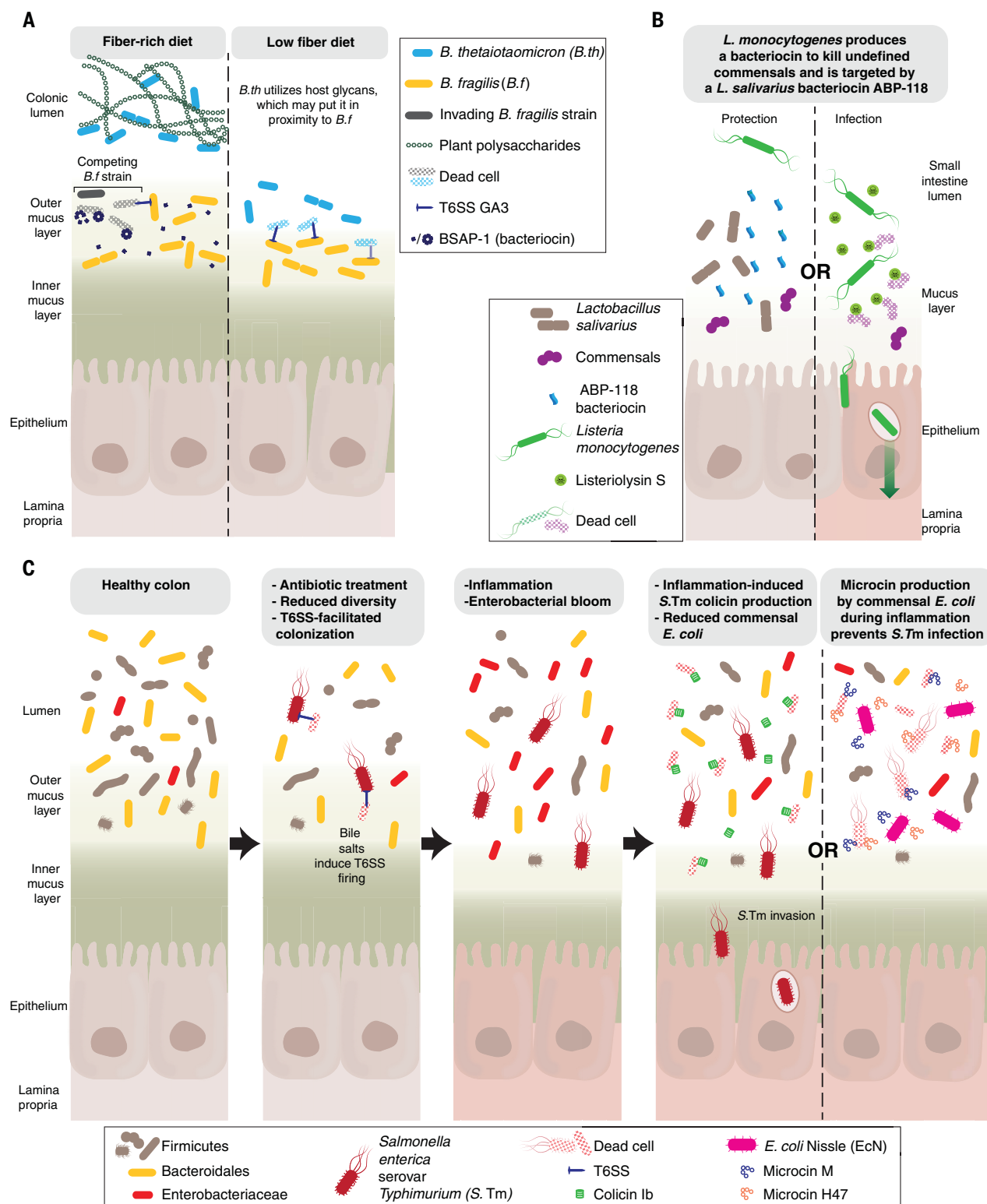


Fig. 1. Examples of interference competition in the gut. (A) *B. fragilis* uses T6SS and bacteriocin-mediated antagonism to compete with other *B. fragilis* strains. It also can use its T6SS to attack other *Bacteroides* species that may localize with *B. fragilis* when dietary polysaccharides are lacking. (B) *L. monocytogenes* colonization of the small intestine is inhibited by the bacteriocin ABP-118 produced by *L. salivarius*. *L. monocytogenes* invasion is facilitated by production of

the bacteriocin listeriolysin S, which targets unknown commensals. (C) Colonization by *S. enterica* serovar Typhimurium (*S. Tm*) can occur when antibiotic treatment alters the commensal microbiota, leading to a proteobacterial bloom and *S. Tm*-mediated inflammation. T6SS antagonism and colicin production help *S. Tm* outcompete other commensal enterobacteriaceae and establish infection. Commensal *E. coli* can also produce microcins that prevent *S. Tm* infection.

study analyzed the effect of bacteriocin ABP-118, a broad-spectrum class IIb bacteriocin produced by gut isolates of *Lactobacillus salivarius*. Compositional analyses of the fecal microbiota of mice and pigs indicated only subtle changes in overall community composition due to this bacteriocin (21).

The most abundant and stable members of the gut microbiota, *Bacteroides* spp. produce membrane attack complex/perforin (MACPF) domain pore-forming toxins (22, 23). Two of the best studied of these, BSAP-1 and BSAP-2, have precise intraspecies targets, and nearly all *Bacteroides fragilis* or *Bacteroides uniformis* strains

examined either contain the respective BSAP gene or are sensitive to it (22, 23). The MACPF bacteriocins do not require cognate immunity proteins because the BSAP gene is adjacent to one or more genes encoding a resistant receptor ortholog that likely replaced the native receptor gene (or genes), rendering the BSAP-producing

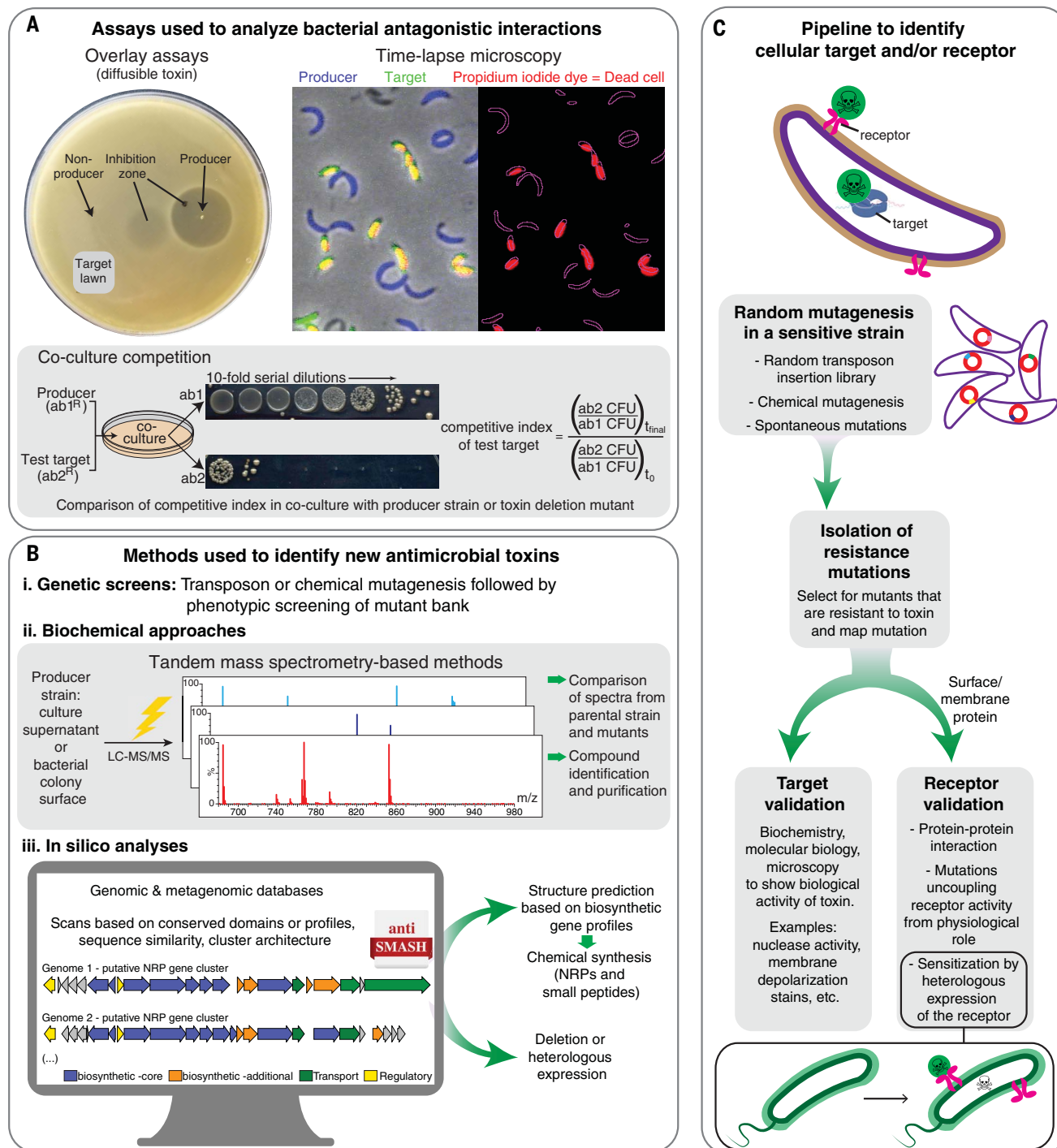


Fig. 2. Experimental approaches to study and characterize new antibacterial toxins. (A) Three phenotypic assays typically used to analyze toxin producer–target cell interactions between two bacteria. ab, antibiotic. (B) Genetic, biochemical, and in silico analyses to identify

new antimicrobial toxins. (C) Identification of surface receptor and cellular target in the sensitive strain can be elucidated by using various methods depending on the strain, its genetic tractability, and whether the receptor is encoded by an essential gene.

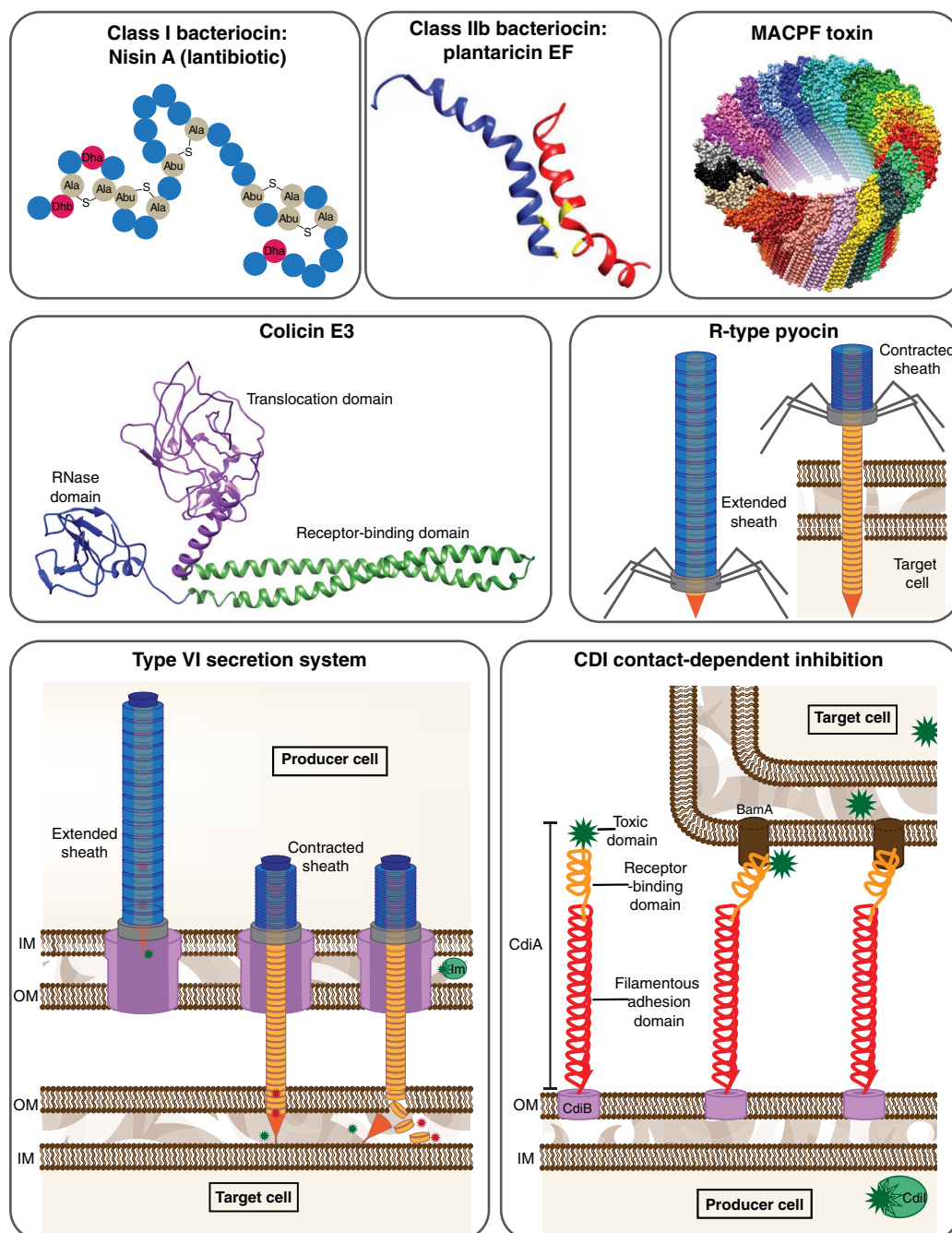
strain resistant. BSAP-producing strains have increased fitness in mice when cocolonized with a sensitive isogenic strain, and human gut metagenomic data indicate that coresidence of BSAP-producer and -sensitive strains is rarer than predicted, suggesting that MACPF bacteriocins provide a strong intraspecies competitive advantage to *Bacteroides* in the mammalian gut (23).

Gut Bacteroidales also synthesize T6SSs with many similarities to those of Proteobacterial species (Box 1) (24–26). T6SSs with three distinct genetic architectures are present in diverse gut Bacteroidales species, with genetic architecture 3 (GA3) T6SSs confined to *B. fragilis* (26). In vitro, GA3 T6SSs antagonize nearly all gut Bacteroidales species analyzed (27). By contrast, when analyzing

these effects in vivo, GA3 T6SS-sensitive Bacteroidales species are able to colonize the gnotobiotic mouse gut to the same extent when co-inoculated with a wild-type *B. fragilis* strain or its isogenic T6SS mutant (27–29). This effect may be due to the different spatial and nutritional niches of these organisms. *B. fragilis* preferentially consumes host glycans over plant polysaccharides

Table 1. Types and characteristics of antibacterial toxins and toxin secretion systems of bacteria.		
	Produced by	Features
Bacteriocins (diffusible toxins)		
Class I	Typically Gram ⁺	Small, 19 to 28 amino acid, <5 kDa Posttranslational modifications, can be cyclical ABC transporter secretion
Class II	Typically Gram ⁺	Small, <10 kDa, typically unmodified Cleaved at GG of leader peptide during secretion Four subclasses (IIa-d), with different structural properties
Microcins	Enterobacteriaceae	Small, <10kDa, linear or knotted peptides Class I <5 kDa, posttranslational modifications Class II 5-10 kDa, modified or unmodified
Colicins and colicin-like bacteriocins	Enterobacteriaceae (colicins), <i>Pseudomonas</i> (S-type)	Require cell lysis for release Three modular domains (Binding, translocation, killing) Colicins A-E, different cellular targets, different cell receptors
F- and R-type bacteriocins (tailocins)	Gram ^{+/–}	Structurally similar to phage tails Rigid and contractile (R) or flexible and noncontractile (F) Require cell lysis for release
Lectin-like bacteriocins	Proteobacteria	High sequence similarity with plant carbohydrate-binding lectins Do not require an immunity protein Lectin domain can be fused to other diverse toxic domains
PT* (also contact-dependent)	Gram ^{+/–}	Diverse toxins with distinct domains and toxin activities Each PT protein typically has three distinct domains Released from the cell using different secretion systems
BSAP	Bacteroidetes	MACPF domains, likely kill by pore formation Mostly intraspecies targeting Do not require immunity protein. Receptor ortholog in producer
Helveticins	Lactobacilli	~ 30 kDa, heat labile, modular Bacteriolytic enzymes (peptidoglycan) Self-export
Cell contact-dependent systems		
T4SS (Antibacterial toxin delivery)	<i>Xanthomonas</i>	Protein complex with a channel that delivers toxic effectors to the periplasm of target cells Likely transfer of diverse toxic effectors Broad range (different orders)
CDI	Proteobacteria	Surface-associated filamentous hemagglutinin domain protein mediates cell-cell adhesion Toxic C-terminal domain cleaved off and imported into target cell Narrow target cell range
T6SS	Gram [–]	A cell-associated retractile phage tail-like apparatus pierces the envelope of the target cell to directly deliver toxic effectors Adenosine 5'-triphosphate (ATP)–dependent process Broad target cell range (often across families)
LXG proteins (Esx type VII secretion)	Gram ⁺	PT domains and immunity proteins toxin likely displayed on producer cell surface
OME	<i>Myxococcus</i>	Outer-membrane lipoprotein toxins exchanged between cells during transient membrane fusion Polymorphic C-terminal toxic domains and cognate immunity protein
*Many PT systems belong to other categories described in this table, such as colicins, pyocins, or CDI.		

Fig. 3. Schematic of some bacteriocins and contact-dependent killing systems of bacteria. Class I bacteriocin (Nisin A): small peptides with modified residues (*Dha* dehydroalanine and *Dhb* dehydrobutyrine), and lanthionine (Ala-S-Ala) and β -methylanthionine (Abu-S-Ala) bridges (shown in gray) (100). Class II bacteriocin (Plantaricin EF): small unmodified two-peptide pore-forming bacteriocin that dimerizes and becomes structured in membranes [Protein Data Bank (PDB) 2JUI and 2RLW] (101). MACPF toxin: MACPF-domain toxins produced by *Bacteroides* species are predicted to form multimeric membrane ring pores similar to those adopted by the MACPF domain human immune complement poly-C9 protein shown (PDB 5FMW) (102). Colicin E3: Crystal structure of the modular colicin E3, produced by *E. coli* that cleaves 16S ribosomal RNA in the target cell (PDB 1JCH) (103). R-type bacteriocins: phage tail-like bacteriocins produced by various bacteria that punch holes in the target cell membrane. Type VI secretion system (T6SS): Toxic effectors (depicted as green and red stars) are delivered directly into the target cell via the contractile phage tail-like T6SS. IM, inner membrane; OM, outer membrane; Im, immunity protein. Contact-dependent inhibition (CDI): CdiB mediates export and surface-anchoring of CdiA, which binds a specific surface receptor in the target cell (BamA) and delivers a C-terminal toxic effector. CdiI, immunity protein (104).



(30) and tends to reside near the mucus layer (31). In comparison, *Bacteroides thetaiotaomicron* preferentially consumes plant polysaccharides and uses host mucin glycans when dietary polysaccharides are not available (30). Therefore, when plant polysaccharides are limited and *Bacteroides* species must consume host glycans, they should come into more frequent contact with *B. fragilis* (Fig. 1A). It is possible that GA3 T6SS-mediated antagonism allows *B. fragilis* to carve out a protected niche and thus allows this relative specialist to thrive in the face of competition from more generalists (Fig. 1A).

In silico analyses further support the pervasiveness of antagonism in human microbial communities. Whitney *et al.* showed that the LXG polymorphic toxin (PT) family (Box 1) present in *Streptococcus intermedius* mediates contact-dependent growth inhibition of diverse Firmicutes species in vitro (32). Their analysis of a human gut metagenomic dataset revealed the presence of numerous LXG toxins. Another in silico analysis that probed human metagenomes for biosynthetic clusters uncovered loci predicted to synthesize two nonribosomal peptide antibiotics named humimycins (33). These

heptapeptides were chemically synthesized and shown to have potent activity against *Streptococcus* spp. and *Staphylococcus* spp. Synthetic humimycin A inhibits lipid II flippase and potentiates β -lactam activity against methicillin-resistant *Staphylococcus aureus*. In an *S. aureus* peritonitis-sepsis model, treatment with humimycin A in combination with dicloxacillin increased the survival of mice after 48 hours compared with treatment with either antimicrobial alone (33). Another in silico study also identified a biosynthetic locus for a thiopeptide called lactocillin (34), which is active against

several Gram-positive bacteria. Lactocillin biosynthetic clusters have been found in several human microbiomes. A metatranscriptomics dataset from the human oral microbiota showed that this region is transcribed in vivo, suggesting functional relevance (34).

Data are also suggesting that there may be opportunities to exploit bacteriocins produced by gut bacteria therapeutically. For instance, an *Enterococcus faecalis* strain that produces the bacteriocin bac-21 was shown to displace indigenous enterococci strains, most notably vancomycin-resistant *E. faecalis* V583 in mice (35). Bac-21, like many bacteriocins, has a narrow activity spectrum, making this type of intervention potentially more targeted and less disruptive than the use of antibiotics.

Interference competition in colonization resistance

Host-associated microbial communities often represent a formidable obstacle to invasion by pathogens. This phenomenon, known as colonization resistance, is multifactorial, including competition for nutrients, niche occupation, immune regulation, and modulation of virulence factors (36). The contribution of secreted antimicrobial toxins by the microbiota to prevent invasion by pathogens has only recently been investigated. Many human enteric pathogens—including pathogenic and toxigenic *E. coli*, *Shigella* spp., and *Salmonella* spp.—are Enterobacteriaceae and sometimes fall within the killing spectrum of the toxins of commensal *E. coli*. The human gut symbiont strain *E. coli* Nissle, first isolated in 1917, has been widely used as a probiotic. A recent study demonstrated that *E. coli* Nissle produces microcins that limit the growth of commensal and pathogenic *E. coli*, as well as *Salmonella enterica* serovar Typhimurium during inflammation in a mouse colitis model (Fig. 1C) (37).

Although most diffusible bacteriocins of gut bacteria do not kill across families and orders, there are exceptions. The ABP-118 bacteriocin of *L. salivarius* targets several Gram-positive pathogens, including *Listeria monocytogenes* (Fig. 1B) (38), which is of a distinct order. Most *Staphylococcus* isolates from nasal passages also produce antimicrobial molecules, especially under stress, such as iron limitation (39). Some of these molecules have a very wide spectrum of activity against other nasal microbes, including Actinobacteria, Proteobacteria, and Firmicutes. In an animal colonization model, a nasal *Staphylococcus lugdunensis* strain produced a nonribosomal thiazolidine-containing cyclic peptide antibiotic that prevented nasal colonization by *S. aureus* and correlates with reduced nasal carriage of *S. aureus* in humans (40).

Similar cross-order and phylum-antagonistic interactions have been reported in the vaginal microbiota. The vaginal symbiont *Lactobacillus rhamnosus* produces a bacteriocin called lactocin 160 that antagonizes the vaginal pathogen *Gardnerella vaginalis* (41). Likewise, *Lactobacillus gasseri* EV1461 produces the bacteriocin gassericin E, which inhibits several common agents of bac-

terial vaginosis, including *Atopobium vaginae*, *G. vaginalis*, and *Prevotella bivia* (42).

In addition to human microbial ecosystems, antagonism among plant symbionts are also well studied. *Pseudomonas putida* is a normal soil organism and symbiont of plants and has three distinct T6SSs that allow it to outcompete known plant pathogens in vitro, including *Pseudomonas syringae*, *Xanthomonas campestris*, *Pectobacterium carotovorum*, and *Agrobacterium tumefaciens* (43). In vivo analyses showed that a *P. putida* T6SS limited plant necrosis by the phytopathogen *X. campestris*, and such antagonistic interactions are predicted to be a major factor in this organism's biocontrol properties (43).

There has been an increased interest in engineering gut symbionts to specifically target pathogens without drastically affecting the microbiota, although to date, studies remain at the proof-of-concept stage. For example, the *E. coli* Nissle microcin H47 gene cluster was modified to be induced by tetrathionate, a reactive oxygen species produced during inflammation, increasing the ability of the strain to target *S. enterica* serovar Typhimurium (44). Similarly, *E. coli* was engineered to detect the *P. aeruginosa* quorum-sensing signal and secrete a chimeric bacteriocin designed to target only this pathogen (45). In addition, *Lactococcus lactis* was programmed to detect the sex pheromone of *E. faecalis* from multidrug-resistant strains and express three bacteriocins (46). These studies highlight the potential for arming bacteria with specific antibacterial toxins for clinical and industrial benefit.

Pathogens and invasion of microbial communities

Although we know a great deal about mechanisms that pathogens use to establish infection, only recently has their antagonism of members of the microbiota been shown to be important during infection. Just as members of host-associated microbiota produce toxins to antagonize each other, which can in some cases act to exclude pathogens, pathogens also synthesize toxins that target members of the microbiota. Bacteriocins, contact-dependent growth inhibition (CDI), and T6SSs have all been shown to facilitate pathogen colonization by antagonizing the resident microbiota (Box 1).

Studies have demonstrated a role for *S. enterica* serovar Typhimurium (S.Tm) colicins and bacterially targeted T6SSs in colonization of the mammalian gut. S.Tm mouse infection models often require mice to be treated with antibiotics in order to allow S.Tm colonization, where it then induces inflammation (Fig. 1C). Low iron concentrations in the gut induce expression of colicin Ib by the salmonellae and the siderophore receptor to which it binds. Inflammation allows *Salmonella* and commensal enterobacteria to bloom relative to strict anaerobes (47–49). Under these conditions, S.Tm has a competitive advantage over most commensal *E. coli* because of colicin Ib production (Fig. 1C) (50). A similar

colonization advantage is conferred by one of the T6SSs of S.Tm (SPI-6) because of its antagonism of commensal Enterobacteriaceae (51). A related study showed that a SPI-6 T6SS mutant of *S. enterica* serovar Dublin was attenuated for colonization in birds and mice (52).

The enteric pathogens *Shigella sonnei*, *V. cholerae*, and *L. monocytogenes* also antagonize members of the gut microbiota to establish infection. The T6SS of *S. sonnei* targets commensal *E. coli* in the mouse gut (53) but also targets its congener *Shigella flexneri*. This effect was suggested to possibly explain the increased global prevalence of *S. sonnei* and the decline of *S. flexneri* (53). Similarly, the T6SS of *V. cholerae* antagonizes commensal *E. coli* (54). The resulting *E. coli* cell death was shown to further drive the host innate immune response, enhancing diarrhea and transmission of the organism, demonstrating an additional benefit of antagonism to *V. cholerae* (54). An additional example is the class I bacteriocin of *L. monocytogenes* that alters the gut microbiota to enhance its intestinal colonization (Fig. 1B) (55). It will be interesting to identify what this organism is targeting because the *Listeriaceae* do not have commensal representatives in the gut microbiota.

It is likely that the ability of pathogens to produce antibacterial toxins to bypass colonization resistance by the endogenous microbiota is common in host-associated communities. For example, plant pathogens *A. tumefaciens*, *Xanthomonas citri*, *Xanthomonas fuscans*, or *Erwinia chrysanthemi* use T6SS, T4SS, or CDI to target plant symbionts (56–59), which may facilitate infection and disease.

Antagonism and genome evolution

The dead cells resulting from these lethal interactions provide genetic material that some transformable organisms take up and incorporate into their genomes. Many streptococcal species, including *Streptococcus pneumoniae* and *Streptococcus mutans* coordinately regulate synthesis of the competence machinery and bacteriocin production through quorum sensing (60). In a biofilm model of *S. pneumoniae* mucosal colonization, DNA exchange was enhanced by bacteriocin secretion (61). Therefore, bacteriocin production can inhibit competitors (62), while also increasing access to DNA during competence. Borgeaud *et al.* showed that the T6SS of *V. cholerae* is part of the competence regulon and that DNA released from killed cells could be taken up into toxin producer cells, notably antibiotic resistance genes and pathogenicity islands (63). Thomas *et al.* also showed that variable genes of *V. cholerae* T6SS loci, encoding toxic effectors and immunity proteins (E-I pairs), can be transferred between mutually antagonistic *V. cholerae* strains via transformation (64). Furthermore, newly acquired E-I pairs replace “ancestral” effectors via homologous recombination, yet the recipient strains often retain the ancestral immunity genes, providing immunity to a wider array of toxins, which suggests an ongoing evolutionary arms race between competing strains (65).

Box 1. Types of bacterial toxins/antagonistic systems.

The repertoire of characterized antibacterial toxins and antagonistic systems of bacteria has substantially increased in recent years. Some traditional and newer strategies used in the discovery and characterization of bacterially produced and targeted toxins are summarized in Fig. 2. The arsenal of bacterial antagonistic systems is extremely diverse (Fig. 3 and Table 1). Antimicrobial toxins or systems can be broadly classified into (i) diffusible molecules that are released into the extracellular milieu and (ii) toxins that are delivered directly to the target cell via cell-to-cell contact through a variety of secretion systems.

Bacteriocins

“Bacteriocin” is a broad term used to describe a very heterogeneous group of diffusible bacterially produced peptides or protein antibacterial toxins. These include small peptide toxins that are often posttranslationally modified (PTM), large proteins, and R-type bacteriocins that are multiprotein complexes with similarities to phage (20). Bacteriocins are ubiquitous, with evidence of their production across all major groups of bacteria and many archaea (20, 105). In contrast to antibiotics, bacteriocins typically have a narrow killing spectrum, targeting close relatives of the producing strain (19, 106). This range is most often dictated by the requirement for a specific cell-surface receptor on the target cell that mediates attachment and sometimes import into the cell (107, 108). Bacteriocins use a variety of mechanisms to kill sensitive cells, including pore-formation, inhibition of cell-wall synthesis, degradation of peptidoglycan, inhibition of protein synthesis, nuclease activity, and gyrase inhibition (19, 109, 110).

A distinguishing feature of peptide bacteriocins relative to small-molecule antibiotics and other small-peptide toxins is that bacteriocins are ribosomally synthesized. In general, bacteriocins are encoded within gene clusters that comprise all the products required for synthesis, PTM, and export, along with a gene that encodes an immunity protein that protects the producer cell from its own bacteriocin (107, 111). Often, these bacteriocin biosynthesis regions contain genes involved in regulation, signaling molecules, and other genes of unknown function (111, 112). Peptide bacteriocins are grouped into a few major classes; however, within those classes there is tremendous variety, leading to subclassifications on the basis of structure, PTMs, and conserved cleavage sites.

Bacteria also produce numerous larger-protein bacteriocins, including the well-studied colicins produced exclusively by Enterobacteriaceae [reviewed in (113)]. Colicin secretion is dependent on cell lysis rather than active secretion, so only a subpopulation secretes the toxin. Colicins typically have a common three-domain organization: an N-terminal region involved in membrane translocation, a central receptor binding domain, and a C-terminal variable toxin domain (Fig. 3). Colicins bind specific outer-membrane molecules, which are typically involved in nutrient uptake, and are transported into the recipient cell.

Until recently, nearly all of the studies of bacteriocins of Gram-negative bacteria have been from members of the phylum Proteobacteria. Interest in host-associated micro-

bial communities has resulted in an intense study of gut Bacteroidales species, which are the most abundant Gram-negative bacteria of the human gut microbiota. Studies of these organisms have revealed completely distinct types of diffusible antibacterial toxins. The first class of diffusible toxins identified in *B. fragilis* (BSAP-1) and *B. uniformis* (BSAP-2) have MACPF domains present in host immune molecules that kill bacteria through pore formation (Fig. 3) (22, 23). A second type of diffusible secreted antimicrobial molecule is produced by a subset of *B. fragilis* strains. This molecule is very similar to human ubiquitin and targets a subset of *B. fragilis* strains (114).

In addition to small-peptide and larger-protein toxins, another distinct type of bacterial killing apparatus given the designation bacteriocin is the R-type bacteriocins [reviewed in (115)]. Like the T6SSs discussed below, these structures are similar to phage in that they contain a needlelike structure contained within a contractile sheath (Fig. 3). This complex is coupled to a phage baseplate-like structure and tail fibers that specifically recognize a target molecule on sensitive cells. Unlike T6SSs that are cell-associated and use cellular ATP hydrolysis for sheath contraction, R-type bacteriocins are released extracellularly from the producing cell upon lysis, and their binding to the receptor molecule drives the needle into the target cell. The R-type bacteriocins do not deliver toxic effectors; rather, the pore created by needle penetration of the membrane dissipates the membrane potential, leading to cell death.

Type IV secretion system toxins

Type IV secretion systems (T4SSs) can transfer DNA, proteins, and protein-DNA complexes into neighboring cells in a contact-dependent manner (116). This ATP-dependent multiprotein complex has a translocation channel that spans the whole cell envelope and an extracellular pilus involved in conjugative DNA transfers between bacteria or translocation of effectors largely into eukaryotic cells (116). Recently, a T4SS of the plant pathogen *X. citri* was shown to secrete toxic effectors into bacterial cells and was shown to antagonize two different Proteobacterial species (57). It has been suggested that T4SSs may play a larger role in interbacterial antagonism than currently appreciated (57).

Polymorphic toxins

Polymorphic toxins (PTs) are a diverse group of modular proteins with distinct domains generated through recombination. The N terminus is typically involved in secretion or cell anchoring, the central domain is often involved in receptor

binding, and the C terminus contains the toxin domain. Genomic analyses revealed a tremendous diversity of PTs and found that they are distributed across all major bacterial lineages (117, 118). One abundant family of PT, the recombination hotspot proteins (Rhs), comprises large proteins with a central region of Rhs repeats that adopts a filamentous fold. Other common families of PT include colicins, CDI toxins, and the LXG toxins described below. Some PTs are released in a diffusible form from a producing cell, others are filamentous structures attached to the cell-surface, whereas others are secreted by means of specific secretion systems as well as through outer-membrane exchange (117, 118).

CDI (type V secretion)

The first contact-dependent antagonistic system to be characterized, *cdiBAI*, was discovered in *E. coli*, but similar systems are widespread in Proteobacteria (118–120). CdiA and CdiB are the components of a type V secretion system subclass called two-partner secretion. CdiB is an outer-membrane β -barrel transporter that mediates translocation of CdiA to the cell surface. CdiA is a large, modular filamentous PT similar to a stick with a variable toxin tip (Fig. 3). Toxin delivery into the target cell requires stable cell-to-cell contact and specific surface receptor molecules on the target cell (104). Once contact is established, the toxin moiety is cleaved from the rest of the protein and imported into the cell (121–123). Rhs proteins of Gram-positive bacteria are thought to mediate attachment and toxin delivery through a mechanism similar to CDI (124, 125).

Type VI secretion system

T6SSs are widespread in Proteobacteria (126) and Bacteroidetes (24, 26). The T6SS apparatus allows producer bacteria to inject toxins directly into adjacent bacteria or eukaryotic target cells in a contact-dependent manner (127, 128). The T6SS multiprotein complex shares structural homology to the T4 contractile bacteriophage tail with a portion spanning the cell envelope of the producing cell. The core components are assembled in the cytosol and membrane and are loaded with toxic effectors before firing. A spike-tipped needle (Fig. 3) is surrounded by a contractile sheath, which through a rapid conformational change propels the needle outward, piercing the membrane of the target cell and delivering the cargo, which can include a variety of different toxins, including PTs (118, 129–131). Bacterially targeted T6SSs have only been shown to be produced by and to antagonize Gram-negative bacteria, but the target cells can be of distinct genera and families than the producer.

Box 1. continued**Type VII secretion (Esx pathway)**

The Esx pathway (T7SS) is widespread in Mycobacteria and other Gram-positive bacteria and exports substrates with a variety of biological roles, including antibacterial toxins [reviewed in (132)]. The LXG polymorphic toxins, broadly distributed in Firmicutes, were recently shown to be Esx clients (32, 117). The LXG architecture is analogous to Rhs toxins: a conserved N terminus (LXG domain) necessary for secretion, a middle domain of variable length, and a C-terminal variable toxin domain. In both *S. aureus* and *S. intermedius*, the T7SS was shown to deliver LXG or non-LXG toxins to target cells of diverse Gram-positive species (32, 133).

Other contact-dependent toxin systems

Another contact-dependent toxin system is the surface-associated glycine-zipper toxins (Cdz) recently described in the aquatic oligotrophic α -proteobacterium *Caulobacter crescentus*. Cdz toxins are secreted via a Type I secretion system and form amyloid-like aggregates on the surface of producer cells, which are delivered to target cells upon contact (134). Yet another distinct toxin delivery system is that of OME described in the social bacterium *Myxococcus xanthus*. OME is regulated by a cell-surface receptor, TraA, that is polymorphic between different strains. TraA interactions between cells lead to transient outer-membrane fusion and exchange of lipopolysaccharide, lipid, and lipoprotein (135, 136). The polymorphic SitA lipoprotein toxins are delivered during OME, in which they are serially transferred between cells, killing those lacking the immunity protein (69).

Antagonism as a facilitator of cooperation

The production of a diffusible shared resource (public good) often incurs a fitness cost to the producing cell; therefore, a mechanism to exclude freeloading cheaters is necessary in order to maintain cooperation over evolutionary time (66). Some bacteriocins have been shown to be a mechanism with which to minimize the impact of spontaneous mutant cheaters. For example, in natural populations of *Pseudomonas fluorescens*, strains that produce the siderophore pyoverdine are less susceptible to exploitation by cheaters when they also produce bacteriocins (67). Similarly, in *Burkholderia thailandensis*, a quorum-sensing regulatory circuit controls a T6SS, constraining the proliferation of quorum-sensing mutants that do not synthesize the T6SS immunity genes and would otherwise have a competitive advantage associated with avoiding the metabolic cost of synthesizing and firing the T6SS (68).

Furthermore, in natural communities in which several strains from the same species co-reside, antagonism can discriminate between kin and competitors, therefore assisting in the production of public goods, which are only evolutionarily advantageous if they benefit individuals who share genes for helping. For example, the soil organism *Myxococcus xanthus* displays an array of coordinated behaviors (such as collective swarming, fruiting body formation, and predation) that require division of labor. Myxobacteria exchange large amounts of surface material through contact-dependent outer-membrane exchange (OME) (Box 1), which not only discriminates and supplies resources to kin but also acts as the mechanism by which a PT is delivered (Box 1) (69). Non-kin are killed because they lack the cognate immunity protein, hence limiting cooperation to kin. Cooperation in *Bacillus subtilis* biofilms is similarly coordinated by kin discrimination facilitated by the combinatorial effect of an array of contact-dependent and diffusible antimicrobial toxins, thus facilitating cooperation at different spatial scales (70). Related examples are found in *V. cholerae* biofilms and during multicellular swarming of *Proteus mirabilis*, where T6SS-mediated antagonism between two strains drives spatial segregation, enabling cooperation within each clonal patch (71, 72). Interestingly, the CDI system deployed in biofilm-forming communities of *Burkholderia thailandensis* acts as a “helping greenbeard” to foster cooperation between individuals that express high levels of the same gene, independent of global genetic similarity. In this case, the toxin moiety that kills cells that lack the cognate immunity gene can also act as a signaling molecule among resistant kin to induce expression of cooperative genes (73, 74).

Regulation of antagonism

Competition experiments and mathematical modeling suggest that there are large trade-offs between deploying aggressive phenotypes and investing resources in growth. Therefore, the outcome is strongly dependent on environmental conditions and metabolic cost of each particular antagonism system (2, 75, 76). Interestingly, this cost can be mitigated through tight regulation of arm deployment (77) and recycling of macromolecular complex components (78).

Our current understanding of the *in vivo* regulation of bacteriocin production and contact-dependent systems is limited. In some cases, antimicrobial toxin systems are induced by environmental signals indicating that the bacterium is inside a host—such as gastric fluid (79), bile (51, 80), iron starvation, and hydrogen peroxide (39)—or by other host products such as mucins (80), as well as signals derived from the microbiota (81, 82). Many bacteriocins require a critical cell density of producer cells to achieve toxic environmental concentrations, and therefore, many bacteriocin gene clusters encode quorum-sensing pheromones and their cognate two-component response systems (83). For example, the gene cluster involved in production of

gassericin E by the vaginal symbiont *L. gasseri* EV1461 encodes an autoinducer peptide, a histidine kinase, and a response regulator, ensuring production only at high cell densities (42).

Regulation of bacterial antagonism systems through different general stress responses can be broadly classified as “competition sensing,” in which toxin production is triggered in response to biotic damage or stress caused by competitors, such as starvation, envelope stress, or DNA damage (83). By contrast, abiotic stress responses, such as heat shock or osmotic stress, tend not to induce expression of antimicrobials (83). Many colicins are part of the LexA regulon (LexA is the master regulator of the SOS response to DNA damage). *Pseudomonas aeruginosa* and *Serratia marcescens* fire their T6SS in response to attack by a T6SS-mediated attack from other species (77, 84, 85). Some antimicrobial systems are regulated by specific signals, such as the absence of a nutrient, and others use a Trojan horse strategy (by modifying the toxin to look like a nutrient) to target competitors that consume a specific resource. For example, microcin MccE492 from *Klebsiella pneumoniae* and MccH47 from *E. coli* have a siderophore moiety attached to the toxic peptide. These bacteriocins are produced during iron starvation and taken up by target cells via their siderophore receptor, leading to death of cells that lack the immunity protein (86–88).

Moreover, bacteria can survey their environment for incoming threats by detecting specific exogenous molecules, such as volatile organic compounds and diffusible secondary metabolites, in a manner akin to pathogen-associated molecular pattern recognition by the mammalian immune system (89, 90). This type of “danger sensing” allows the cell to respond by anticipating attacks, therefore minimizing damage or eliminating the source of the threat. Many diffusible bacteriocins and contact-dependent systems may be regulated in this manner (89). For example, debris from lysed *P. aeruginosa* acts as a danger signal that triggers deployment of its T6SS in nearby unharmed cells (91). Some bacteria, including *L. gasseri* EV1461, can “eavesdrop” on the quorum-sensing signals of competing species and respond by expressing broad-spectrum antibiotics, as well as by generating antimicrobial resistance strategies (42, 89, 92, 93). Notably, the *bfp* quorum-sensing system that regulates bacteriocin production in *S. pneumoniae* is highly polymorphic, and instances of cross-talk and eavesdropping between strains are common (9). Simulations using four strains with varying ability to sense neighbor's quorum-sensing signals show that eavesdropping can confer a fitness advantage with a negative frequency dependence (a strain present at low frequency can induce bacteriocin production to invade a more abundant population). Westhoff *et al.* postulated recently that bacterial responses to danger may be fine-tuned to the proximity of the perceived threat, indicating that more distant threats may be countered by diffusible antimicrobial products, whereas response to contact-dependent

antagonism may be an immediate counter-attack (90).

Perspectives

Antibacterial toxin production is ubiquitous in microbial communities. Bacteria synthesize a diverse array of antimicrobial toxins and systems for their secretion. In agreement with the principle of “habitat-filtering,” phylogenetically related species tend to reside in similar environments and compete for resources; therefore, narrow range antagonism systems are common (2, 94). Yet many organisms are armed with multiple antibacterial toxins, some that kill strains of the same species and others that kill across species, genera, families, and orders. Furthermore, a single-secretion apparatus (such as T6SS) can be used to simultaneously deploy an array of toxic effectors that target different cellular processes, giving these producer cells an upper hand in an evolutionary arms race and increasing the range of possible competitors that can be targeted (65, 95). There are also numerous examples in which reciprocal fighting should occur, such as the battles between armed pathogens and armed members of the microbiota or between two natural members of a microbial community. The outcome of such reciprocal antagonism likely depends on density (strain frequency) (18), species range, and its local distribution (diffusible versus contact dependent) and mechanism of toxicity.

The studies highlighted in this Review demonstrate numerous fitness benefits to toxin producers in microbial communities. The most obvious benefit is the killing of competitors that may use the same nutrients and scarce resources, but there are distinct ecological outcomes to these interactions. Antagonism can prevent organisms from invading ecosystems but can also assist invasion. Killed cells release DNA that can be taken up by the killer, leading to genome evolution. Material released from dead cells can also modulate host responses, increasing transmission of a pathogenic aggressor (54). Antagonism can also maintain microbial diversity, promote spatial segregation of different genotypes, and facilitate cooperation between kin cells.

Despite numerous advances in this field, there is still limited knowledge of the ecological effects of antagonism owing to an incomplete understanding of the spatial architecture of many natural communities, regulatory factors governing toxin synthesis and secretion, diffusion capabilities of various toxins in these natural communities, and the combinatorial effects of the deployment of multiple toxins. Analyses to further probe these interactions will benefit from a combination of mechanistic analyses, experimental model studies, and mathematical modeling as well as analyses of metagenomic and metatranscriptomic datasets of natural microbial communities. This combination of analyses is key to understanding how antagonistic interactions allow an organism to establish and compete in a community and, therefore, influence its overall structure and composition.

Continued analyses of these ecological effects will help to facilitate the translation of these antimicrobial toxins into clinical, agricultural, and industrial applications. Such toxins could potentially allow a genetically engineered beneficial organism to get a foothold in an ecosystem to deliver a therapeutic property or to prevent invasion of pathogens into host-associated communities. Antimicrobial toxins could also be used to selectively remove strains with pathogenic potential such as *Clostridium difficile*, strains carrying resistance genes to clinically relevant antibiotics, and organisms associated with various drug failures such as to the cardiac drug digoxin (96) and cancer immunotherapies (97–99). The next decade is likely to usher in an era of selective microbiota engineering in which antimicrobial toxins may play an important role.

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Bacterial antagonism in host-associated microbial communities

Leonor García-Bayona and Laurie E. Comstock

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Interspecies competition shapes communities

The gut microbiota of mammals is diverse and dynamic, and gut bacteria respond sensitively to diet and drug intake. Nevertheless, in a healthy adult, microbial community composition remains remarkably stable over time, despite being highly individual. García-Bayona and Comstock review the mechanisms that gut bacterial species use to jostle for space and resources and maintain their populations in the face of intense and varied competition. Bacteria have evolved a range of antibiotics, bacteriocins, toxins, and delivery devices to enable interspecies conflict. These interbacterial weapons possess a spectrum of specificities and range from those that target strains of their own species to broad-acting bacteriocides. This toxic armamentarium provides a valuable resource for potential therapeutic development.

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