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MINIREVIEW

Multifaceted Interfaces of Bacterial Competition

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Microbial communities span many orders of magnitude, ranging in scale from hundreds of cells on a single particle of soil to billions of cells within the lumen of the gastrointestinal tract. Bacterial cells in all habitats are members of densely populated local environments that facilitate competition between neighboring cells. Accordingly, bacteria require dynamic systems to respond to the competitive challenges and the fluctuations in environmental circumstances that tax their fitness. The assemblage of bacteria into communities provides an environment where competitive mechanisms are developed into new strategies for survival. In this minireview, we highlight a number of mechanisms used by bacteria to compete between species. We focus on recent discoveries that illustrate the dynamic and multifaceted functions used in bacterial competition and discuss how specific mechanisms provide a foundation for understanding bacterial community development and function.

Microbes compete to survive in naturally mixed communities and diverse environments. Microbial communities colonize niches as different as the surface of our teeth to the soils beneath our feet. The taxonomic diversity of organisms within these communities is a complex function of differing nutrients, niches, and interactions between species. In general, the abiotic influences on communities are identified through analysis of the chemical, spatial, and other relevant parameters that define local environments. Abiotic factors are varied, affecting microbial growth in many ways, and can often be manipulated in the laboratory to understand their influence on microbial communities. The interactions between species, on the other hand, are functions of a particular community and are a challenge to identify and resolve. Some broad categorization provides guidelines for outcomes expected during interaction between species. Specifically, when nonneutral interactions occur between species, they are at times cooperative, but this appears to be the exception to the rule (1). More commonly, competition between species appears to define the interactions that may predominate in microbial communities.

Competition is categorized into two modes, exploitative and interference (2). Exploitative competition is passive in the sense that one organism depletes its surroundings of nutrients, thereby preventing competitors from gaining access to those resources. In contrast, interference competition invokes antagonistic factors produced to impede competitors (3). In microbial systems, competition is typically framed in the context of growth limitation or inhibition due to exploitation and interference. However, while species may be sensitive or resistant to growth inhibitory activities, they also may engage in antibiotic synthesis, motility, sporulation, predatory functions, and biofilm formation in response to competition. Although not universal among all bacteria, these physiological changes represent the diversity of mechanisms to enhance the competitive fitness of bacterial species equipped with them. The ability of individual species to employ a spectrum of competitive mechanisms and responses to challenges may be essential to their survival in communities of diverse organisms, where competitive stress may take many forms. To better understand the forces that enable bacteria to thrive in communities, we consider numerous competitive functions that determine the relative fitness of different bacteria within a community.

Direct studies on natural communities, such as those in soils or plant and animal hosts, are notoriously difficult, because they are complex and variable. Also, explanting environmental isolates to the laboratory creates additional complications. For instance, many organisms do not grow under standard laboratory conditions. Recent technological advances, such as the iChip (4), enable the growth of many previously uncultured bacteria, but *in situ* manipulation of whole bacterial communities remains challenging. A frequently used approach to study microbial community interactions is to culture two or more species together under defined conditions. By investigating microbial interactions in defined formats, culture-based studies can provide powerful mechanistic insights into competitive functions.

In recent years, competition studies between bacteria have contributed to a more informed view of the competitive mechanisms used by different species. We focus this minireview on mechanisms of interference and exploitation competition between species involving specialized metabolites, enzymes, and functions associated with the cell envelope, highlighting interaction outcomes that differ from growth inhibition by classical antibiotics. The cell envelope forms the barrier between a bacterial cell and its surroundings, which include competing bacteria. We will parse different competitive mechanisms into those that occur across the envelope due to the exchange of diffusible factors and those that require contact between cell envelopes, either directly or via their embedded proteins.

INTERFERENCE AND EXPLOITATION AT A DISTANCE

Specialized metabolites. Competition between species is often mediated through bioactive metabolites synthesized by competitors. Specialized metabolites (SMs) are molecules produced by bacteria that are not involved in primary metabolism but are involved in other biological processes. Many specialized metabolites

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were previously called "secondary" metabolites because their presence is dispensable under laboratory conditions, and their production often occurs during the late stages of growth (5). However, SMs may be essential for some bacteria to persist in the environment (6) or under competitive stress. In the context of competitive interactions, SMs of primary interest are those affecting the growth and development of competing bacteria. For instance, antibiotics provide some of the clearest mechanistic insights into chemical interactions between competing species of bacteria. However, considering their measurable biological activities at subinhibitory concentrations, even the empirical roles of antibiotics in nature are subject to debate (7–10). Overall, the biological functions of SMs are numerous and, arguably, largely unknown. We will focus, therefore, on several illuminating examples where bacteria use antibiotics and other SMs in precisely targeted mechanisms that affect competing organisms in ways other than inhibition of growth. The ability of bacteria to respond dynamically to a range of chemical stresses may have profound effects on their fitness in competitive multispecies communities.

Exploitation competition due to SMs. In some cases, clearly self-serving functions of SMs indirectly lead to the exploitation of resources, yielding a competitive advantage. Exploitation competition occurs when one organism disrupts the growth of its competitors by using a shared limited resource (11). Exploitation often occurs when one bacterial species alters its external environment through various metabolic functions and prohibits the growth of other bacterial species (3). This exploitation can arise from direct consumption of nutrients, buildup of toxic waste products, or the activity of SMs. An example of SM-mediated exploitation is found in siderophores, which are SMs produced for the capture of iron (12). Iron is essential for cytochromes and iron-sulfur proteins, and competition for iron is driven by its availability. Siderophores are one mechanism to chelate external iron, which is then imported as a complex into the producer cells (13). Siderophore production thus increases the bioavailability of iron and simultaneously depletes the supply available to competitors. The significance of iron is underscored by the numerous examples of siderophore-mediated competition in different environments, including competition for colonization of the light organ in Hawaiian bobtail squid by different strains of *Vibrio fischeri* (14) and between the human opportunistic pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* (15). Bacteria also acquire iron from their environment and engage in exploitation competition by using other iron uptake systems, including transporters (16). However, because siderophores are extracellular SMs, they are also subject to piracy by other species, posing a competitive risk to the producing organism (e.g., see references 17 and 18). These examples of siderophore-mediated interactions illustrate the potential complexity of specialized metabolites and exploitative interactions that are probably pervasive in nutrient-limited environments.

Interference competition due to SMs. (i) Antibiotic activity without antibiosis. The classic view of antibiotics and other SMs as weapons has guided their isolation and characterization since their discovery. In the process of discovery, antibiotic molecules are isolated from bacterial strains grown in the laboratory and tested for growth inhibition of target organisms (19). This approach has been effective for identifying the majority of antibiotics, but it has left gaps in our understanding of the ecological functions of these molecules. For instance, concentrations of antibiotics sufficient to inhibit growth may be rare in natural environments (20, 21). Do antibiotics at lower-than-inhibitory concentrations have functions relevant to competitive interactions? This question has inspired investigation into the effects of subinhibitory concentrations of antibiotics on bacteria, where a wide range of responses have been observed among organisms exposed to different antibiotics. For example, subinhibitory concentrations of jadomycin B cause *Streptomyces coelicolor* to prematurely sporulate and produce a pigmented antibiotic, prodigiosin (22). Subinhibitory concentrations of kanamycin induce the expression of type VI secretion genes in *P. aeruginosa* (23). Numerous other antibiotics induce global transcriptional responses (reviewed in depth in reference 24). Cellular stresses from subinhibitory antibiotic concentrations may trigger these responses as early warning systems of chemical warfare. Alternatively, the natural functions of some antibiotics and SMs may be reflected in the subinhibitory responses of competitors, independent of inhibitory activity (10). Clearly delineated mechanisms of concentration-dependent activities and responses during competition are needed to understand the roles of antibiotics and other SMs in community dynamics.

(ii) Multifunctional metabolites. Bacteria produce many SMs, representing an enormous chemical diversity with poorly understood function (20). Although antibiotic activity is the most common activity ascribed to SMs, many antibiotics also have effects on bacterial competitors that are independent of growth inhibition (see above). There are numerous reports detailing the effects of SMs on the multicellular development of a bacterial species. For example, the soil bacterium *Pseudomonas protegens* produces 2,4-diacetylphloroglucinol, an SM with antifungal activity that is used in biocontrol (25). The cellular differentiation of *Bacillus subtilis* is inhibited by 2,4-diacetylphloroglucinol when cultured with *P. protegens* (26). In contrast, *B. subtilis* biofilm formation is stimulated by the antifungal nystatin (27) and by peptide antibiotics (28). Bacillaene is a *B. subtilis*-produced SM that was originally identified as an antibiotic inhibitor of protein synthesis (29). Bacillaene also interferes with prodigiosin production in *Streptomyces coelicolor* and *Streptomyces lividans* without inhibiting growth (30, 31).

Another mechanism for SM interference in competitor development is to derail normal signaling processes. For example, some marine bacteria produce SMs that interfere with quorum sensing and thus disrupt subsequent downstream processes reliant on communication between competitor cells (32, 33). One challenge is to understand the fitness benefits of such modulatory activities in competitive interactions between bacteria. However, in many cases, the connection between SMs and the responses they elicit in competitors is unknown. Model systems using two or more bacteria cultured together have been developed to investigate how SMs and other factors influence competitive fitness under controlled settings.

Model systems of SM-mediated competition between species. Multispecies model systems are advantageous because they open the door to the diversity of competitive functions used by a single organism, including the production of multiple SMs and different patterns of response to competitor SMs. Soil bacteria provide an illustrative example of diverse competitive functions. Species of *Streptomyces* are ubiquitous in the soil and renowned for their capacity to synthesize SMs (34). In addition, *Streptomyces* species undergo developmental phases of their life cycle, including

aerial growth and sporulation, which may be affected by SM activity (35). For example, sporulation of some streptomycetes depends upon the peptide SapB, which acts as a surfactant and lowers surface tension, enabling aerial hyphae to expand upward (36). *Bacillus subtilis* produces its own lipopeptide surfactant, surfactin. *Bacillus subtilis* requires surfactin for biofilm development and some types of motility (27, 37, 38). Intriguingly, surfactin also antagonizes aerial development of many *Streptomyces* species (39, 40). Insight into the mechanism arose from *S. coelicolor*, which when treated with surfactin was unable to process and secrete SapB to support aerial growth (41). Compared to antibiotics that target growth, inhibition of sporulation is a relatively subtle developmental effect that presumably prevents the spread of *Streptomyces*. Although *B. subtilis* does not likely produce multifunctional surfactin explicitly for competition, the inhibition of *Streptomyces* development may enhance competitive fitness in natural environments. Indeed, some species of *Streptomyces* have acquired enzymatic resistance to surfactin, consistent with a natural competitive function. Using imaging mass spectrometry, it was demonstrated that *Streptomyces* sp. strain Mg1 hydrolyzes surfactin (Fig. 1A and B) (40). The enzyme, surfactin hydrolase, was shown to specifically inactivate surfactin and plipastatin, another lipopeptide produced by *B. subtilis* (40). Hydrolytic inactivation is a common resistance mechanism for many antibiotics (42). Analogous to the emergence of new β-lactamases, the production of surfactin hydrolase and other antibiotic-degrading enzymes promotes the competitive fitness of their bacterial producers, although with surfactin, the selection is against a developmental process.

Competitive culture models enable us to interpret the functions of SMs in new ways that enhance our view of competition dynamics. Several reports show that SMs provide defense against otherwise overwhelming forces. For instance, laboratory strains of *B. subtilis* are preyed upon by *Myxococcus xanthus*, but the undomesticated *B. subtilis* strain NCIB 3610 is resilient (43). Many domesticated laboratory strains of *B. subtilis* lack a gene, *sfp*, required for the production of several SMs, including bacillaene (44, 45). This defect, which renders domesticated *B. subtilis* susceptible to *M. xanthus* predation, was subsequently shown to be specific to the loss of bacillaene production (43) (Fig. 1C and D). Indeed, exogenous application of bacillaene protected sensitive strains of *B. subtilis* and *Escherichia coli* from predation. Thus, under the pressure of predation, bacillaene is essential for the defense of *B. subtilis*. Intriguingly, this is not the only demonstration of a defensive role for bacillaene. Strains of *B. subtilis* deficient in bacillaene production are also hypersensitive to lysis by linearmycins produced by *Streptomyces* sp. Mg1 (46, 47). Bacillaene was originally discovered as an antibiotic inhibitor of protein synthesis (29), and its function is dispensable for the growth of *B. subtilis*. However, competition studies expand our view of bacillaene to include essential defensive functions, the precise mechanisms of which are not known. Nevertheless, examples, such as bacillaene and surfactin, serve to illustrate that SMs provide diverse and important competitive functions for the producer organisms.

As seen in examples ranging from antibiotics to siderophores, SMs have varied and sometimes essential functions in competition between species. However, aside from antibiotics, little mechanistic detail is available for the targets and processes affected by SMs (e.g., see references 32 and 33). The identification of chemically mediated mechanisms of competition will require continued exploration of the competitive dynamics between species. An important consideration is how the SMs operate along with other entities that mediate interactions between competing species.

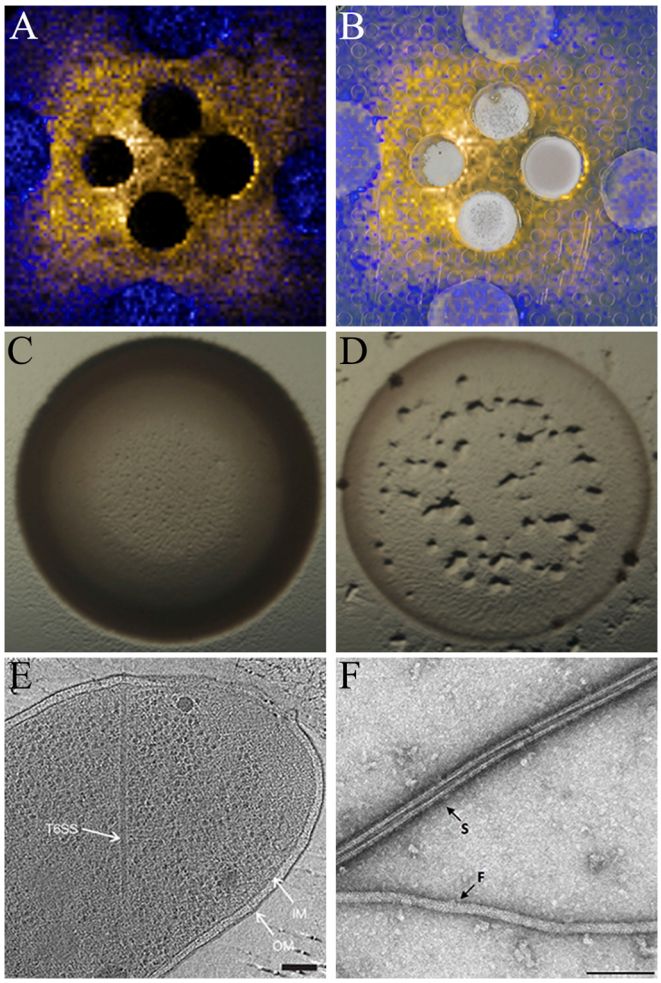

FIG 1 Mechanisms of bacterial competition. (A and B) Detection of patterns of SM production and degradation through imaging mass spectrometry. (A) False-colored extracted ion image showing the distribution of surfactin (blue) produced by *B. subtilis* and hydrolyzed surfactin (yellow) resulting from the activity of surfactin hydrolase secreted by *Streptomyces* sp. Mg1. (B) The extracted ion image from panel A overlaid onto a photograph of a culture of *B. subtilis* and *Streptomyces* sp. Mg1 to highlight the localization patterns of each SM during competition. (C and D) Revealing essential SM functions using predator-prey interactions. (C) Photograph of *M. xanthus* spotted onto the center of a wild-type *B. subtilis* NCIB 3610 colony. The colony is mostly opaque due to intact viable *B. subtilis* cells. (D) A mutant *B. subtilis* strain deficient in bacillaene production becomes transparent as it is consumed by *M. xanthus*, which forms fruiting bodies on the lysed remains of the *B. subtilis* colony. (E and F) Structural features of a contact-mediated competitive apparatus. (E) Cryo-electron micrographs of a T6SS apparatus inside an intact *Vibrio cholerae* cell. Scale bar = 100 nm. IM, inner membrane; OM, outer membrane. (F) Comparison of flagellum (F) and T6SS sheath (S) isolated from *V. cholerae*. Scale bar = 100 nm. Panels C and D were provided by John Kirby. Panels E and F were reproduced with permission from reference 152.

Secreted enzymes. In addition to SMs, bacteria secrete enzymes that participate in competition. Secreted enzymes that confer antibiotic resistance have a clear competitive benefit (40, 42). Additionally, bacteria benefit by interfering with the development of their competitors, e.g., using enzymes to degrade signaling mol-

ecules, like acyl homoserine lactones (48–51). However, surprisingly little is known about how bacteria use secreted enzymes to kill or inhibit their competitors. The predatory bacterium *M. xanthus* is a prolific producer of degradative enzymes and encodes in its genome more than 300 degradative hydrolytic enzymes (52, 53). The functions of many of these enzymes are unknown, but bacteriolytic activity has been demonstrated for some (54). An example of competitive enzyme function is found where *Staphylococcus epidermidis* competes with *S. aureus* for colonization of the human nasal cavity (55). *Staphylococcus epidermidis* secretes a serine protease, Esp, which inhibits *S. aureus* biofilm formation (56). Esp degrades *S. aureus* biofilms by inactivating autolysins and preventing the release of DNA that is an essential component of the biofilm extracellular matrix (57). The presence of *Corynebacterium* spp. in the nasal cavity is often inversely correlated with pathogenic *Streptococcus pneumoniae* (58). Like *S. epidermidis*, *Corynebacterium accolens* also utilizes a secreted enzyme, LipS1, to interfere with a competitor. LipS1 is a triacylglycerol lipase that produces oleic acid from the hydrolysis of a human-produced triglyceride, triolein (59). Oleic acid and other free fatty acids inhibit the growth of *S. pneumoniae* (59, 60). Esp and LipS1 interfere with bacterial competitors but through fundamentally different mechanisms. Thus, secreted enzymes may have many active roles at or near the cell surface of competitors, although this area is in need of further study.

Extracellular vesicles. Extracellular vesicles are of great interest for both bacterial and eukaryotic interaction processes. Vesicles are capable of vectoring proteins, lipids, nucleic acids, and small molecules that function in competitive and signaling processes (61). Many bacteria produce extracellular vesicles (EVs) during normal growth. The precise mechanisms of EV biogenesis and cargo loading are beginning to be identified. Gram-negative bacteria produce EVs (also called outer membrane vesicles) when the outer membrane is "pinched," and the vesicle buds from the cell surface (62). A second vesicle release mechanism is reported to occur within biofilms of *P. aeruginosa* (63). In this system, prophage-encoded endolysins activate cellular lysis, releasing membrane fragments that form vesicles and permeate the extracellular space. The problem for Gram-positive bacteria is more complicated due to the lack of an outer membrane, and the mechanism of EV generation is currently unknown, although several models have been hypothesized (64). After formation, EVs are released into the environment. When an EV encounters a Gram-negative cell, the vesicular membrane and the outer membrane fuse, which delivers the cargo into the recipient's periplasm (65). Extracellular vesicles have been observed to adsorb to the cell wall of Gram-positive bacteria, thereby delivering their contents to target cells (65).

Extracellular vesicles are used by bacteria for diverse processes, including biofilm formation (66), carbon storage (67), virulence (68), and quorum sensing (69). Bacteria also use EVs for defensive measures against several types of antimicrobial insult. For instance, the EVs of *Prochlorococcus* adsorb phages (67), and EVs from *P. aeruginosa* and *S. aureus* protect β-lactamases from proteolytic degradation (70, 71). Although EVs are often characterized for their defensive functions (72), bacteria also use vesicles to deliver antagonistic agents to competing bacteria. These agents can be enzymes, such as the peptidoglycan-degrading hydrolases produced by *P. aeruginosa* (65) and *Lysobacter* sp. strain XL1 (73), or antibiotic SMs, like actinorhodin or prodigiosins found in the EVs produced by *S. coelicolor* (74) and *S. lividans* (75), respectively.

The EVs of *M. xanthus* are a tour de force in regard to their competitive potential. The EVs produced by *M. xanthus* contain not only 29 predicted hydrolytic enzymes (11 of which were not found in the outer membrane) but also 16 SMs, including the myxalamids, which are known antibiotics, and DKxanthene 534 (76). DKxanthene 534 and myxalamids are polyketide and hybrid polyketide-peptide molecules, respectively, both having nonpolar hydrocarbon regions. Consistent with membrane localization, both molecules are typically extracted from cell pellets and have low abundance in supernatants (77, 78). These characteristics highlight an important function of EVs to facilitate the transfer of hydrophobic molecules, including antibiotics, across aqueous environments (69).

Extracellular vesicles also intersect with SMs in intriguing patterns that may affect competition between bacteria. Recently, it was shown that *B. subtilis* disrupts its own EVs by secreting surfactin (79). The targeted lysis of EVs by surfactin may serve as a defensive mechanism against antibiotic-laden vesicles produced by competing organisms or as an offensive tool to prevent nonpolar signaling molecules, including quorum sensors, from reaching their intended targets. Extending on overlapping functions, bacteria reportedly become reversibly resistant to antibiotics when they swarm (80). In *B. subtilis*, swarming motility requires surfactin (81, 82). As an intriguing hypothesis for niche exploration, *B. subtilis* might produce surfactin not only to promote its movement over surfaces but also as a defense mechanism against EVs produced by other organisms.

CONTACT-MEDIATED COMPETITION

Different species of bacteria physically interact at high cell densities in ways that promote information exchange, such as plasmid conjugation, or through competitive interaction mechanisms. Some competitive functions appear to have evolved to function specifically in close proximity. In particular, bacteria use membrane- and cell envelope-embedded functions that are outwardly directed toward competitors. Such mechanisms are likely to be important for survival under crowded conditions through both their inhibitory functions and their contributions to community structure.

Contact-dependent inhibition. As a specific mechanism of interference competition, contact-dependent inhibition (CDI) describes a membrane protein that operates as a delivery system for a cellular toxin. The prototypical CDI system was first described in uropathogenic *E. coli* EC93 and consists of three components, CdiA, CdiB, and CdiI (83). CdiA and CdiB are homologous to the two-partner secretion system proteins TpsA and TpsB, respectively. In two-partner secretion systems, the secreted substrate TpsA is translocated across the outer membrane through its cognate beta-barrel protein TpsB (84). Likewise, in CDI systems, the toxin CdiA is attached to CdiB, which is an outer membrane beta-barrel protein that extends away from the cell. This arrangement leads to CDI being referred to as a "toxin on a stick" (85). CdiI provides the producing cell with immunity toward its own toxin by specifically binding to CdiA and inhibiting its activity (86). When a CDI-producing cell (CDI^+) makes direct contact with a susceptible target cell, its CdiA toxin makes contact with the target cell's outer membrane protein BamA (87). The CdiA protein is then deposited onto the target cell surface and undergoes self-

cleavage, which transports the carboxy-terminal (CT) portion of CdiA into the periplasm (88). Many CdiA toxins are nucleases and require entry into the cytoplasm to exert their effects (86). Translocation of the toxin into cytoplasm requires the proton motive force (89) and interaction with toxin-specific inner membrane protein receptors (90). The requirement for a membrane receptor protein on target cells limits CDI to a narrower range of specificity compared to diffusible agents, like antibiotics. This specificity is due to variability in extracellular loops 6 and 7 of BamA, which form the CdiA-CT-binding site (91). Due to the narrower target range, it has been speculated that CDI systems are a means to inhibit closely related species. This would allow CDI^+ bacteria to inhibit other bacteria that are more likely in direct competition for the same or very similar ecological niches (85).

Biofilms are community structures that form as a result of the concerted effort between many cells. The conditions within a biofilm are inherently stressful to cells. Resources, including nutrients, oxygen, and physical space, are limiting (92). These conditions breed competition between cells within the biofilm and provide strong selection for competition. For example, growth within a biofilm selects for bacteria that engage in exploitation competition by preferentially occupying biofilm surfaces and gaining access to oxygen (93). Biofilm growth has also selected for cells that are able to engage in inference competition with their neighbors. *Burkholderia thailandensis* illustrates the utility of CDI functions for promoting competitive success in a biofilm. Disruption of the CDI system (CDI^-) of *B. thailandensis* both sensitizes cells to CDI from isogenic siblings and abolishes biofilm formation (94). Both functions are tied to BcpA (homologous to CdiA), but the biofilm functions are independent of CDI activity (95). These observations suggest that CDI systems help ensure a competitive advantage by supporting biofilm formation while excluding competitors. CDI-dependent cell adhesion and defects in biofilm production for CDI^- strains have also been reported in *E. coli* (96) and *P. aeruginosa* (97), further solidifying the link between CDI and biofilm development.

Aside from the costs of biofilm formation and the cellular challenges within a biofilm, these structures serve to protect bacteria from various external stresses (98, 99). For instance, bacteria have evolved mechanisms, including CDI, to competitively exclude nonsibling cells from biofilms (100). Developing biofilms contain three-dimensional structures called "pillars" for *B. thailandensis* (101). These structures extend outwards from the biofilm attachment site, providing cells within the pillars better access to oxygen and nutrients than the cells in the biofilm substratum (92). The CDI system of *B. thailandensis* excludes CDI-sensitive cells from developing pillars (101). Cells that produce the same CDI system, presumably siblings, are not killed by CDI due to their cognate immunity genes. This selective killing by CDI provides a kin discrimination mechanism for *B. thailandensis* biofilms and likely protects the biofilm from invaders. Taken together, the CDI functions of *B. thailandensis* demonstrate important competitive advantages that arise in close cellular proximity through the direct inhibition of competitors and through the construction of defensive biofilm structures.

Type VI secretion systems. The type VI secretion system (T6SS) was originally identified as a virulence factor produced by *V. cholerae* against amoebae and macrophages (102). Subsequently, genes encoding T6SS were found in roughly one-quarter of *Proteobacteria* with sequenced genomes (103), including but not limited to opportunistic pathogens, such as *Acinetobacter baumannii* (104) and *Serratia marcescens* (105). The observation that many of the identified T6SS had no apparent effect on eukaryotic cells and that T6SS gene clusters occurred in nonpathogenic bacteria prompted investigation into potential antibacterial activities (105, 106).

The T6SS of Gram-negative bacteria has emerged as a powerful weapon in close-quarters interference competition between bacteria. The basic mechanism of function for the T6SS is to inject toxic effector molecules directly into the cytoplasm of target cells. Structurally and functionally, the T6SS apparatus is homologous to bacteriophage contractile tails (107). The T6SS apparatus is a cylindrical spiked-tipped inner tube that is surrounded by a sheath and anchored to the inner membrane (Fig. 1E and F). When the cell is in physical contact with its target, the sheath contracts, and the inner tube is propelled outward and punctures the membrane of a target cell using its spiked tip. Within the target cell, the spike disassociates from the tube, and the toxic effectors are delivered. Common effectors characterized thus far include phospholipases (108–110), peptidoglycan hydrolases (111–113), and nucleases (114, 115).

In addition to T6SS being an effective delivery system for toxic payloads, one example demonstrates that the sharpened spike of the T6SS is a potent weapon even in the absence of toxic effectors. Using its TagQRST-PpkA-Fha1-PppA sensing system, *P. aeruginosa* detects cell envelope damage caused by the T6SS of other bacteria (116). This detection or "danger sensing" allows the cell to mount a response against its antagonist and minimize future damage to the cell or its siblings (117). In the case of *P. aeruginosa*, the cell retaliates against T6SS-mediated attacks, directing its own T6SS in the same direction as the initial attack in a behavior called "dueling" (118). Duels damage target cells and can cause membrane blebbing, plasmolysis, and even lysis. Strains of *P. aeruginosa* that are deficient in the production of all known T6SS effectors still retaliate against T6SS-mediated attacks and engage in dueling with effective killing activity (116). If *P. aeruginosa* cells lose their duels and are lysed by competitors, they release diffusible danger signals that stimulate T6SS activity and promote the survival of siblings (119).

Like CDI, the T6SS killing mechanism also functions to favor siblings in multicellular activities. Strains of *Proteus* sort self from nonself in mobile multicellular swarms. This kin discrimination is observed as cell-free zones between swarms called Dienes lines (named for their discoverer Louis Dienes) on agar surfaces. In these zones, opposing swarms of *Proteus mirabilis* do not intermingle. The establishment of Dienes line formation was found to be due to the T6SS (120). At the intersection between opposing swarms, *P. mirabilis* use their T6SS to kill and in turn are killed by the T6SS of competitors, creating a demilitarized zone (DMZ) where the Dienes lines exist between mobile populations. As with *B. thailandensis*, strains join the beneficial swarm when they are not killed by the T6SS. An added benefit of this kin discrimination arises because swarming provides increased resistance to antibiotics (80). Thus, entry into the swarm promotes competitive fitness of bacteria by excluding unrelated cells and by enhancing defense against antibiotics. Similar boundary formation has also been reported for *M. xanthus* (121) and *B. subtilis* (122). The observation of discrimination in *B. subtilis* demonstrates that CDI and T6SS are not the only mechanisms that bacteria use for kin discrimination, as *B. subtilis* does not produce CDI or T6SS. The question

remains whether *B. subtilis* demarcates Dienes lines through a contact-dependent or -independent mechanism, although evidence suggests that combinatorial mechanisms are used (123).

The CDI and T6SS are analogous in that a toxin is delivered directly to a target cell. However, like many antibiotics, these toxins are typically soluble molecules. How then, are insoluble effectors delivered? In one case, the T6SS toxin Tse6, produced by *P. aeruginosa*, contains transmembrane domains that are shielded from the aqueous environment by an associated chaperone. The chaperone, EagT6, protects Tse6 until delivery into the target's periplasm (124). This example appears to be the exception, where the majority of membrane-associated effectors lack a chaperone or other clear vectoring mechanism. As described previously, extracellular vesicles are another mechanism for the delivery of otherwise insoluble cargo.

Outer membrane exchange. In addition to CDI, T6SS, and EVs, Gram-negative bacteria appear to use the outer membrane itself as an effective delivery system for otherwise insoluble toxins. Outer membrane exchange (OME) for *Myxobacteria*, for example, is a contact-dependent mechanism for cells to share membrane components, including phospholipids and insoluble lipoproteins, with other cells (125). OME has been demonstrated to extracellularly complement mutants deficient in the production of particular outer membrane products. For example, via OME, the gliding motility of nonmotile *M. xanthus* mutants is stimulated when mutant cells are mixed with wild-type cells (126). OME is also intertwined with colony swarming and sporulation (126). Furthermore, a recent report implicates OME as a powerful defensive mechanism to dilute membrane damage over a population of cells (127).

OME requires the production of an outer membrane protein complex, TraAB, in both the donor and recipient cell (126). TraAB appears to be the only component necessary to mediate OME (128) and, similarly to the BamA receptor in CDI systems, TraAB contains a polymorphic domain that limits OME to a narrow range of related targets (129). Given the functional similarities to CDI systems and the potential of OME to directly deliver toxic effectors into the envelope of target bacteria, it is not surprising that *Myxobacteria* use OME to mediate competition and engage in kin recognition. Motile cells of *M. xanthus* are killed when cultured with their nonmotile siblings. Killing is dependent upon the presence of TraA in the target motile cell and a polyploid prophage in the killer nonmotile sibling (130). Currently, the effector delivered by OME is not known, but it is likely produced from a toxin-antitoxin module encoded on the prophage (130). No further examples of OME-mediated competition have been reported thus far. However, as with EVs, new studies will likely uncover fascinating roles for these membrane-derived strategies in bacterial competition.

CONCLUSIONS

Bacteria use competitive mechanisms that are nearly as diverse as the competitors they encounter (Fig. 2). Inherent in each competitive strategy are advantages and disadvantages. When bacteria use secreted effectors, like antibiotics, enzymes, or vesicles, they are able to compete while minimizing the risks of direct damage during contact-mediated competition. Once a cell exports its competitive molecules across its envelope, those molecules are subject to diffusion, which diminishes their inhibitory effect on competitors at a distance. However, many of these metabolically expensive products operate between inactive and inhibitory concentrations and may act as chemical cues for competitors (131). Exposure to subinhibitory antibiotic concentrations can induce resistant states (132–134), select for resistant competitors (135), stimulate biofilm formation (136–138), and stimulate motility (139). The activation of a resistant state allows a competitor unrestricted access into a previously protected niche. If potential prey senses a cue and escapes predation, the producer loses nutrients in the form of that lysed cell. Thus, if a competitor senses a cue, the producer may suffer the consequences for competitive fitness. However, it is also important to note that our current understanding of the response to subinhibitory concentrations of antibiotics and other SMs in the context of bacterial communities is limited and requires further investigation. The direct delivery of toxins into a target cell by CDI or T6SS circumvents diffusion and the potential costs of subinhibitory antibiotic concentrations. The trade-off is that contact-mediated competition puts a cell in direct contact with its competitor and allows the risk of retaliation, such as in the dueling response (116), or from high concentrations of diffusible SMs.

We have emphasized the differences between competitive mechanisms that are contact mediated and those that occur at a distance. However, bacteria are not mutually exclusive in the systems they employ. For example, *Pseudomonas* species use T6SS but are also prolific producers of SMs, including antibiotics and siderophores (140). Bacteria also use direct contact to deliver secreted factors at high local concentration. Predatory *Bdellovibrio* species physically collide with target cells, pierce their cell envelope, and digest their prey from within using an impressive cocktail of secreted enzymes that includes nucleases and peptidoglycan hydrolases (141, 142). The differences between contact-mediated and at-a-distance approaches may reflect how bacteria use both systems in competition. A cell producing secreted molecules, like antibiotics, creates a chemical or enzymatic protective shell around itself. Within this shell, the cell is also able to simultaneously engage in exploitative competition via its exclusive access to nearby nutrients. The spectrum of inhibitory activities, in concert with small size, low charge, and ease of entrance into target cells (143, 144), places antibiotics at the foundation of such protective chemical shells. However, if a competitor breaches the defenses, the delivery of toxic effectors by CDI or T6SS directly into the target may stop the invasion. A remarkable balance of antibiotic resistance and contact-dependent mechanisms has been shown with *A. baumannii* (145). Several multidrug-resistant *A. baumannii* strains carry a plasmid that provides antibiotic resistance while also inhibiting the expression of T6SS systems. However, the plasmid is unstable, and loss of the plasmid provides a mechanism to activate T6SS at the cost of losing antibiotic resistance in some cells. The net result is a population with shared functions in competitive fitness through defense and through close-quarters exclusion of competitors. Perhaps contact-mediated mechanisms, like CDI, T6SS, or OME, are needed to selectively inhibit closely related competitors with the capacity to pass unharmed across a chemical defensive barrier (91, 129, 145).

Culture-based studies have revealed many mechanistic details of bacterial competition. However, we note that many of the studies highlighted in this minireview used simple small-scale bacterial communities with minimal mixing. To gain a deeper understanding of bacterial competition in natural communities, systems are needed that combine the use of multiple approaches and contain expanded knowledge of diverse competitive mechanisms. Al-

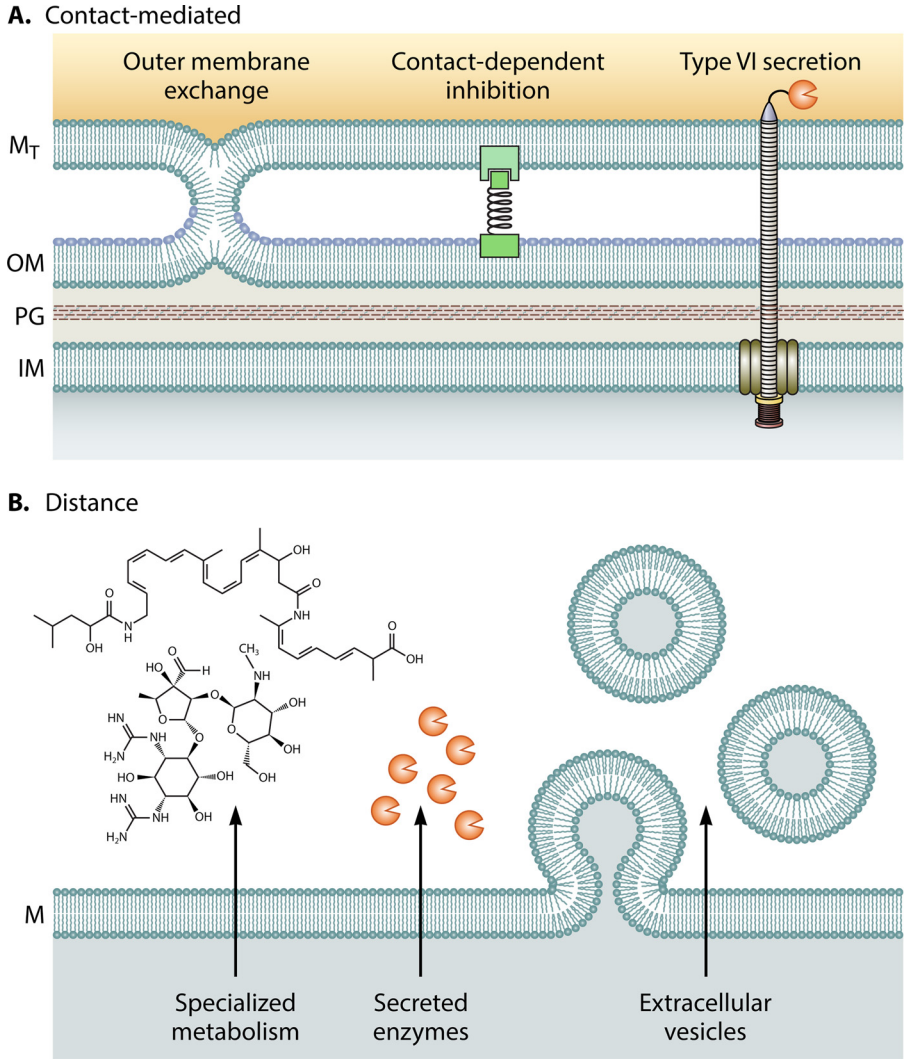

FIG 2 Summary of mechanisms used in bacterial competition. (A) Contact-mediated mechanisms involve either direct contact between cell envelopes (OME) or are facilitated by protein complexes (CDI and T6SS). In the case of CDI and T6SS, toxic effectors (square or wedged circle) are delivered into the target cell. (B) Bacteria compete at a distance using SMs (examples shown are bacillaene and streptomycin), secreted enzymes, and extracellular vesicles. CDI, contact-dependent inhibition; EVs, extracellular vesicles; M, membrane; M_T, target cell membrane; IM, inner membrane; OM, outer membrane; PG, peptidoglycan; T6SS, type VI secretion system.

though beyond the scope of this minireview, mathematical modeling is a powerful approach to understand how bacterial communities are formed and maintained (e.g., see references 146 and 147). Mathematical approaches stand to become more powerful as they incorporate diverse competitive outcomes in addition to killing or survival. For instance, what effects does T6SS-mediated retaliation have in a modeled competition? How does SM-mediated developmental inhibition affect a community? What are the consequences of exposure for cells outside the inhibitory ranges of SMs? Using controlled experiments in the laboratory, new mechanistic details of competition are being identified, despite limitations to our understanding of these mechanisms in natural environments. The genomes of many antibiotic-producing bacteria contain silent SM gene clusters that are not expressed under laboratory conditions (148). Likewise, many studies with CDI and T6SS require artificial expression conditions (149, 150). These obstacles are a central focus of current efforts to understand competitive mechanisms. Meanwhile, models that better mimic the native environment are being developed to provide a clearer view of bacterial interactions under natural conditions (e.g., see references 86, 115, and 151) The examples above and many more innovative studies are expanding our views of the interactive interfaces between two bacterial species. The emerging challenge is to build these interfaces into networks, which will represent the many facets of competition within microbial communities.

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