# Exploiting interbacterial antagonism for microbiome engineering

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## ABSTRACT

Interbacterial antagonism can significantly impact microbiome assembly and stability and can potentially be exploited to modulate microbes and microbial communities in diverse environments, ranging from natural habitats to industrial bioreactors. Here we highlight key mechanisms of interspecies antagonism that rely on direct cell-to-cell contact or diffusion of secreted biomolecules, and discuss recent advances to provide altered function and specificities for microbiome engineering. We further outline the use of ecological design principles based on antagonistic interactions for bottom-up assembly of synthetic microbial communities. Manipulating microbial communities through these negative interactions will be critical for understanding complex microbiome processes and properties and developing new applications of microbiome engineering.

## Introduction

Microbial communities are made up of diverse sets of microbes that participate in complex interspecies interactions and metabolic processes. Such interactions may include mutualistic cross-feeding, competitive exclusion, or antagonistic killing, which often determine the population dynamics, stability, and resilience of the community [1]. Recent studies to understand these key ecological principles have improved our understanding of microbial community assembly, dynamics, and functions [2,3]. On the other hand, much remains to be explored as new processes, mechanisms and biological machineries are being discovered [4]. On a practical level, targeted modulation of microbial community interaction has the potential to impact numerous emerging microbiome applications spanning medicine, agriculture, and bioproduction [5-7].

Bacterial antagonism is one of the most common phenomena observed in microbial communities. Antagonistic interactions enable bacteria to establish their spatial and nutrient niches by directly inhibiting growth of or killing their neighbors. The study of microbial antagonism has led to key breakthroughs in medicine—for instance, the development of antibiotics [8]. With the ever-growing abundance of (meta)genomic data, a diverse set of mechanisms for bacterial antagonism has been discovered [4]. Most major bacterial phyla possess the capacity to carry out such bacterial warfare, with some strains harboring multiple antagonistic systems that produce synergistic efficacy and lethality [9]. While one might posit that the evolutionary arms race for bacteria, weaponized with antagonistic machineries, would significantly destabilize microbial ecosystems, recent studies have shown that such competitive interactions actually strongly promote diversity and stability by promoting spatial structuring [2,10]. In fact, cooperative interactions, while efficient, are often destabilized upon external perturbations, in contrast to antagonistic interactions that lead to more robust populations [2]. As many antagonistic systems have only been recently described, their roles as mediators of microbial ecology and dynamics are underestimated thus far.

Here, we outline key mechanisms for interbacterial antagonism and highlight recent studies that have utilized these systems to manipulate microbial communities. Specifically, we discuss several promising contact-dependent (cis) and diffusion-based (trans) antagonistic systems. Since there is extensive literature detailing various aspects of bacterial antagonism that cannot be comprehensively covered here, we refer the readers to several excellent reviews on the subject for further reading [1,3,4,11-13]. Instead, we focus here on aspects related to the use of such systems for directed microbiome modulation. We will examine recent examples of engineering efforts to apply and enhance these systems to optimize their performance and specificity in complex microbial consortia. Finally, we will consider how these antagonistic systems can be leveraged to assemble synthetic microbial communities and

control them to exhibit sophisticated and robust phenotypes for several biotechnological applications.

**Contact-dependent microbial antagonism**

Contact-dependent antagonism is based on the direct transfer of toxic protein effectors or protein-DNA complexes from a donor cell to a target cell. Target cells that lack immunity proteins to protect against the toxins are killed. These local antagonistic interactions are generally mediated by multicomponent protein secretion machineries, including Type IV, V, and VI secretion systems (T4SS, T5SS, and T6SS), while some involve direct exchange of outer membranes between cells (**Figure 1a**) [11,13]. Since contact-dependent antagonism only impacts nearest local neighbors, it predominantly serves to partition spatial niches and interspecies boundaries. We outline several examples of how engineering these cis-antagonistic interactions is paving the way for in situ modulation of microbial communities.

The T5SS is one of the first identified mechanisms of interbacterial antagonism where bacterial growth is regulated by direct cell-to-cell contact, termed contact-dependent growth inhibition (CDI) [14]. T5SSs are found in the outer membrane of gram-negative bacteria and have relatively simple architectures, with some subtypes consisting of only one protein component [15]. T5SS proteins are composed of two distinct functional regions, typically a C-terminal β-barrel domain anchored in the outer membrane and an exposed N-terminal passenger domain that can be cleaved and released extracellularly. The functions of the passenger domain can be highly diverse, including adhesion to target cells or extracellular matrix components, autoaggregation for biofilm formation, and cell-to-cell CDI [15]. CDI by the Type Vb secretion system is based on two-partner secretion (TPS), with subunits CdiB and CdiA. CdiB is a translocator that exports the CdiA toxin effector to the cell surface. CdiA proteins range in size from 180 to 630 kDa, but all share the same general architecture [13]. Recent elucidation of the CdiA secretion and toxin delivery mechanism shows that CdiA is exported first into the periplasm through the Sec-dependent secretory pathway, then across the outer membrane through CdiB using an N-terminal TPS transport domain [16]. Upon recognition of its target by its protruding receptor binding domain, CdiA autoproteolytically cleaves its effector domain (CdiA-CT), which is delivered into the target cell. T5SS results in strong inhibition activity against target cells even when cell-cell interactions are transient under planktonic conditions, unlike other contact-dependent antagonistic systems that often require prolonged cell-cell adhesion.

Rational engineering of CDI target specificity could be achieved by altering the receptor binding domains (RBD). Ruhe *et al.* identified a RBD in BamA-specific CdiA from *E. coli* by generating tagged CdiA fragments and performing a binding assay against purified BamA [17]. Interestingly, BamA-specific and OmpA-specific CdiA proteins shared only ~24%

sequence identity for their putative receptor binding regions and swapping the regions altered their specificity accordingly. Beyond modular target specificity, CDI could be loaded with diverse passenger proteins, given that T5SS autotransporter domains have been widely used as potent cell surface display platforms for heterologous proteins in a variety of biotechnological applications [18]. Willett *et al.* demonstrated this potential by showing that CdiA C-terminal toxin domains from different bacterial species are interchangeable and can be redirected through different translocation pathways when fused to N-terminal domains of heterologous CdiA proteins [19].

The T6SS is a prevalent cis-antagonistic system found in gram-negative bacteria, especially in Proteobacteria and Bacteroidetes, that injects target-specific toxin effectors into neighboring cells [11]. Some bacteria, such as *Pseudomonas aeruginosa,* contain multiple evolutionarily distinct T6SSs [9]. T6SSs can be potent against closely related bacteria as well as those in other genera and kingdoms, including their eukaryotic hosts or fungi that are also a part of the ecosystem [20,21]. Recent studies have revealed the role of T6SSs in not only shaping microbial community composition but also augmenting host's resilience to pathogen colonization in the mammalian gut [22,23]. The canonical T6SS from *E. coli* consists of 13 genes (*tssA* through *tssM)* encoding its core structural components*,* and one gene encoding the PAAR (proline-alanine-alanine-arginine repeat) domain-containing protein on its tip [11]. When the T6SS complex sheath-like structure enters a contracted state, the Hcp(TssD)-VgrG(TssI)-PAAR puncturing complex is able to penetrate and translocate into the target cell to deliver the toxin effector (**Figure 1b**) [20,24]. T6SS toxin effectors have a wide range of antibacterial effects and can be grouped by their targets: cell wall (peptidoglycan amidases, peptidoglycan hydrolases), cell membrane (phospholipases, pore-forming effectors), and cytoplasm (nucleases, NADP+ hydrolases, FtsZ inhibitors). There are immunity proteins that can detoxify specific toxin effectors (i.e. effector/immunity pairs) and are generally encoded downstream of the effector loci [11] or sometimes in separate mobile arrays of immunity genes [25].

The diversity and modular nature of T6SS systems and its effector/immunity proteins suggests the possibility for engineering. Loading other protein domains (e.g. from β-lactamase) either directly to the puncturing complex or to other effectors that are associated with the complex has been demonstrated for T6SS-based protein delivery into eukaryotic host cells [26,27]. Wettstadt *et al*. also recently showed in *P. aeruginosa* that fusing the C-terminus of a canonical VgrG with other proteins enabled extracellular secretion of the fused protein by T6SS although direct injection into a target bacteria was not demonstrated [28]. While further work will be needed to assess carrying capacity and extend it towards diverse protein substrates, these examples clearly demonstrate the potential of T6SS as a generalizable platform for interbacterial protein delivery. To improve specificity of cell targeting in a mixed

population, Ting *et al.* sought to develop “programmed inhibitor cells” (PICs) expressing synthetic protein binders that can interact with bacterial surface antigens on target cells to enhance selective killing (**Figure 1c**) [29]. In this study, camelid-derived single domain antibodies (nanobodies) were displayed on the cell surface of T6SS-active *Enterobacter cloacae* to direct the antibacterial activity of the T6SS against *E. coli* cells either in synthetic or natural microbial communities, resulting in specific killing of the target cells at >90% efficiency. While such nanobody cell-surface binders have many desirable characteristics, such as small size, high stability, and strong antigen binding affinity, generation of potent nanobodies against specific novel bacterial strains remains challenging. We expect that ‘reverse genomics’ [30] and continuous directed evolution [31] approaches will be useful to identify surface-exposed target antigens and expedite the discovery and affinity maturation of nanobodies against these antigens. Furthermore, metagenomic mining and characterization of natural binding proteins against diverse microbes, such as phage/prophage receptor binding domains [32,33], could expand the binding repertoire for programmable T6SS antagonism.

The T4SS is arguably the most versatile family of protein secretion systems with functionally diverse subtypes depending on both the class of molecules they export and their biological roles, including contact-dependent interbacterial antagonism [11]. T4SSs are found in both gram-negative and gram-positive bacteria as well as in archaea, and can mediate translocation of cargo molecules including monomeric as well as multi-subunit protein toxins and nucleoprotein complexes. The canonical T4SS systems are all encoded by 12 conserved genes, *virB1* through *virB11* and *virD4*. For conjugative DNA transfer to occur, DNA transfer and replication (Dtr) proteins bind to a cognate origin-of-transfer (*oriT*) sequence to form a DNA-protein complex, termed the relaxosome, and process the DNA into a single-stranded DNA substrate (T-strand) [34]. The T-strand further interacts with Type IV coupling protein VirD4 to be transferred through the T4SS channel. Protein effector substrates interact with VirD4 via a positively charged, C-terminal signal sequence that is hydrophilic and has a net positive charge with a consensus motif of R-X(7)-R-X-R-X-R-X-X(n) [11].

The T4SS’s broad target range and unique capability for DNA transfer have enabled its use for genetic manipulation of diverse microbes and microbial communities (**Figure 1d**). In a recent example, Brophy *et al.* demonstrated T4SS-based DNA transfer from an engineered donor *B. subtilis* strain, called XPORT, into diverse gram-positive bacteria isolated from human gut, skin, and soil samples using integrative and conjugative elements (ICE) [35]. Among 55 bacterial strains tested, 35 gram-positive bacterial strains spanning 26 species and 9 genera yielded mini-ICE transconjugants using XPORT. A 10-kb nitrogen fixation gene cluster could be delivered by the mini-ICE system into four *Bacillus* species. Similarly, our lab developed a technique called “metagenomic alteration of gut microbiome by in situ conjugation”

(MAGIC), where mobile plasmids are delivered from a donor *E. coli* probiotic strain to resident microbes in the mammalian gut *in situ* through broad host range RK2/RP4-based T4SS conjugation system [36]. MAGIC could deliver genetic payloads (e.g. a green fluorescent protein or an antibiotic-resistance gene) into over 5% of the diverse murine gut microbiota spanning multiple major bacterial phyla. Beyond these general T4SS gene transfer applications, CRISPR-Cas systems can also serve as programmable effectors when delivered by T4SS to mediate directed antagonism (**Figure 1e**). CRISPR-Cas9, Cas13a, as well as recently characterized CRISPR-transposon systems, could be delivered as vectors encoding Cas genes and associated guide RNAs against specific genomic loci to mediate sequence-specific killing or enrichment of target cells [37-40].

Bacterial cells can also interact with each other by direct outer membrane exchange (OME). OME was first identified and most extensively studied in *Myxococcus xanthus* [41]. Neighboring cells in the Myxobacterial population transiently fuse their outer membrane using TraA-TraB cell surface proteins. The outer membrane fusion allows diffusion of outer membrane lipids and proteins between cells, homogenizing the cell populations with heterogeneous outer membranes until they get separated again [42]. Interestingly, it was shown that this bacterial social behavior improves the overall fitness of mixed populations of healthy and damaged cells by complementation of cellular damage or mutational defects in the damaged subpopulation. Furthermore, this multicellular cooperation is precisely limited to their kin through polymorphic toxin, SitA, that is transferred during OME and requires a cognate immunity gene, SitI, for neutralization [13]. OME-based interbacterial interaction mechanisms are seemingly widespread given that other bacteria have also been found to exchange their membrane and associated contents between cells either directly [43] or even remotely through outer membrane vesicles (OMVs) or membrane-derived nanotubular structures [44]. The unique capabilities associated with direct modification of outer membranes of target cells and translocation of diverse substrates, including protein, DNA, and metabolites, suggest potential utility of OME mechanisms for modulating microbial communities in manners distinct from protein secretion systems.

**Diffusion-based microbial antagonism**

Fierce interbacterial competition has led to the evolution of long-range warfare systems in bacteria, such as soluble small molecules, peptides, proteins, and even viral particles that can diffuse into surrounding environments and mediate interactions between distant bacterial cells [12]. Diffusion-based antagonistic systems have long been used as antimicrobials in medical therapeutics and the food industry. Beyond traditional antibiotics, peptide- or protein-based diffusible antagonistic systems are poised to become next-generation antimicrobials for microbiome modulation owing to their relatively simple production process, adjustable target

spectrum, widespread natural biodiversity, and vast combinatorial sequence space (**Figure 2a**) [45].

Bacteriocins represent a broad and large family of ribosome-synthesized bacterial toxins with bactericidal or bacteriostatic effects, found in all major phyla including archaea [12]. Bacteriocins can antagonize target cells at the cell envelope (e.g. pore formation and inhibition of cell wall synthesis) or in the cytoplasm (e.g. inhibition of DNA gyrase and RNA polymerase) [45] and are generally classified into either small peptide and larger protein groups. Peptide bacteriocins either undergo extensive post-translational modifications (class I or lantibiotics from gram-positive bacteria and class I microcins from gram-negative bacteria) or are unmodified (class II from gram-positive bacteria and class II microcins from gram-negative bacteria) [12]. Nisin, produced by *Lactococcus lactis,* is a representative pore-forming class I peptide bacteriocin that effectively inhibits wide range of bacteria. Nisin-like class I bacteriocins range from 21 to 38 amino acids in size and are generated from gene clusters that encode the prepeptide (*nisA*), modification of amino acids (*nisB, nisC*), cleavage of leader peptide (*nisP*), secretion (*nisT*), immunity (*nisI, nisFEG*), and gene regulation (*nisR, nisK*) [46]. Unmodified class II bacteriocins, ranging from 30 to 60 amino acids, have relatively simple biosynthesis due to limited post-translational modifications and constitute the largest group of bacteriocins. Class II bacteriocins act on a variety of essential cellular machineries, such as sugar transporters and ribosomal subunits [45]. Colicin, produced by *E. coli,* is a representative group of protein bacteriocins [13]. Colicins are typically encoded on plasmids as a gene cluster that produces the colicin toxin, a cognate immunity protein, and a lysis protein for release of the toxin. Colicins are divided into many different subtypes, but generally composed of three functional domains, an N-terminal domain for translocation through the membrane of the target bacteria, a central receptor binding domain for recognition of specific surface receptors on target bacteria, and a C-terminal domain responsible for the toxic activities such as pore formation or nucleic acid degradation [13].

Currently, the throughput to characterize and engineer natural and synthetic bacteriocins is limited because individual bacteriocins need to be assayed in individual wells. Droplet-based miniaturization and parallelization of assay reactions offers a greatly improved and less expensive approach to characterize a large number of bacteriocins simultaneously [47]. Alternatively, the cells themselves could act as such microassay reactors. Tucker *et al.* devised a technique called “surface localized antimicrobial display” (SLAY) where individual antimicrobial peptides are anchored on the surface of bacterial cells, only affecting the viability of the expressing cells [48]. SLAY allowed up to 800,000 peptides to be assayed in a single tube using multiplexed sequencing readouts. The method identified thousands of fully synthetic peptide sequences with antimicrobial activities. Interestingly, in contrast to natural antimicrobial peptides that are dominated by cationic and amphipathic residues, the synthetic

peptides covered a wider sequence space with potentially different inhibitory mechanisms. While 20-mer random peptides against *E. coli* were tested in the study, we expect that SLAY and similar approaches could be applied to natural bacteriocins and their variants with cell envelope-associated mechanisms against diverse bacterial species (**Figure 2b**). Furthermore, host genetic determinants of bacteriocin sensitivity could also be systematically investigated using barcoded transposon-insertion mutant libraries [49]. To improve bacteriocin production, which is often very challenging due to their cellular toxicity, Liu *et al.* demonstrated a rapid cell-free framework for reconstructing and screening multi-gene biosynthetic pathways for nisin and its analogs [50]. Such cell-free approaches that decouple cell viability from production provide an alternative and powerful route to synthesize and screen toxins at scale that are otherwise difficult to generate.

Long-range interbacterial interactions can also be mediated by prophages integrated in bacterial genomes that are conditionally activated. The wide prevalence of temperate bacteriophages and prophages in nature suggests that lysogenic phages might increase host competitiveness in the ecosystem, despite a fitness burden of prophage carriage [51]. For example, in the two-species microbial community of slow-growing *Curvibacter* sp. and fast-growing *Duganella* sp. that colonize *Hydra vulgaris*, an inducible prophage in the *Curvibacter* sp. that can lytically infect *Duganella* sp. plays a key role in coexistence of the two bacterial species by switching its life cycle between lysogenic and lytic pathways (**Figure 2c**) [52]. In addition, recent studies have shown that dietary compounds, such as sugars, and microbiota-derived short-chain fatty acids can often induce prophages from various bacterial species in a species-selective manner [53,54]. Given that phage genomes can now be extensively engineered with synthetic lytic-lysogenic regulatory circuits [55], modified host ranges (**Figure 2d**) [56,57], or diverse genetic payloads for any desired functions [38,58], new prophage activation mechanisms could be used as a system for phage-mediated microbial interactions by leveraging lysogenic bacteria as a stable and programmable vehicle for natural or engineered phages to modulate microbial communities.

**Antagonistic modulation of synthetic microbial communities**

Bottom-up approaches have gained recent attention for assembling synthetic communities with defined microbes and their interactions [59]. Beyond their utility as a minimal model system to study the organization and dynamics of complex natural microbiomes, synthetic communities with unique qualities, such as specific divisions of labor or spatial organization, can be useful in a variety of biotechnological applications. Furthermore, synthetic complex communities could be used to replace dysbiotic microbiota in certain applications, such as during pathogen infections in the gut, for safer and more predictable therapeutic outcomes [60]. In practice, however, the utility of such synthetic microbial communities

depends heavily upon the robustness, scalability, and programmability of the underlying interbacterial interactions among the members, which require extensive characterization and engineering.

Interbacterial antagonistic mechanisms have recently been adopted to address challenges in assembling and modulating robust synthetic microbial communities. In a recent example, Kong *et al.* demonstrated that synthetic communities of *L. lactis* could be programmed with all possible modes of pairwise microbial interactions (i.e., commensalism, amensalism, neutralism, cooperation, competition, and predation) by reconfiguring biosynthetic pathways for bacteriocins [61]. Both signaling and antimicrobial features of nisin were extensively utilized to design and construct these pathways. To create a cooperative two-strain community, the multi-gene nisin biosynthetic pathway was divided into two steps: (i) synthesis and secretion of precursor, and (ii) post-translational modification. Each strain in the community was assigned with a single synthetic step so that they could produce active nisin and survive in tetracycline-supplemented media by nisin-inducible tetracycline resistance only when they cooperate. Quantitative models derived from the two-strain synthetic communities was used to design and build more complex ecosystems with three and four members. In another work, Liao *et al.* showed that cyclical 'rock-paper-scissor' ecology among three bacterial strains can extend the lifetime of genetic integrity and community-wide function of the system [62]. Each strain of the community was designed to produce both a toxin (colicins) that can kill one of the other strains and the corresponding immunity proteins to protect themselves. Serial introduction of a strain that can displace a previously existing strain population prolonged the desired function of the microbial community by removing potential mutants with nonfunctional genetic circuits and effectively resetting the gene pool. While these examples clearly demonstrate the utility of interbacterial antagonism in building robust and functional synthetic microbial communities, further work will be needed to assemble communities at much larger scales for different applications. We expect high-throughput methods that can rapidly resolve microbial interaction mechanisms will accelerate the discovery and characterization of novel antagonistic mechanisms [63,64].

## Outlook and conclusions

Engineered bacteria with programmable antagonistic capabilities to target and manipulate any specific bacteria at the strain level in complex microbial communities will be an enabling platform for microbiome engineering. Engineered interbacterial antagonistic systems could be used for: (i) killing or growth inhibition, (ii) engraftment or growth activation, (iii) replacement, (iv) spatial structuring, and (v) genetic engineering of bacteria in their native environments. We expect that building upon the previous approaches using protein binders, modular domain swapping or mutagenesis, and programmable sequence-specific nucleases

will be key to modulating target specificity of both contact-dependent and diffusion-based antagonistic mechanisms [17,29,33,37-39,57]. Selecting a suitable antagonistic system for specific applications will be needed as spatial scale of interference is a critical parameter that determines organization of microbial ecosystems [65]. For example, interaction scales of contact-dependent antagonistic systems are highly limited compared to those of diffusion-based antagonistic systems due to their requirement for direct cell-to-cell contact. However, the cis-antagonistic systems generally exhibit stronger interference within their confined spatial niches as they are less prone to dilution effects that often reduce the efficacy of trans-antagonistic systems. It is also important to consider interbacterial interactions when engineering microbiomes. Recently, Hsu *et al.* showed phages with narrow target spectrum can significantly impact even species that are not directly targeted [66]. Better understanding of the functional mechanisms and ecological roles of antagonistic systems in shaping microbial communities will be needed for further development of programmable cellular and molecular microbiome engineering tools while considering such collateral damages that can be induced from manipulating target bacteria in complex microbial communities. Recent advances in high-throughput DNA synthesis and sequencing technologies as well as massively parallel assays will facilitate the systematic exploration of the vast biodiversity of the antagonistic systems and will provide a foundation for a variety of new powerful tools to modulate diverse natural and synthetic microbial communities.

## Conflict of interest

H.H.W. is a scientific advisor to SNIPR Biome and Kingdom Supercultures. The authors declare no additional competing interests.


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## REFERENCES AND RECOMMENDED READING


1. Little AE, Robinson CJ, Peterson SB, Raffa KF, Handelsman J: **Rules of engagement: interspecies interactions that regulate microbial communities**. *Annu Rev Microbiol* 2008, **62**:375-401.

2. Coyte KZ, Schluter J, Foster KR: **The ecology of the microbiome: Networks, competition, and stability**. *Science* 2015, **350**:663-666.

3. Garcia-Bayona L, Comstock LE: **Bacterial antagonism in host-associated microbial communities**. *Science* 2018, **361**:eaat2456.

4. Peterson SB, Bertolli SK, Mougous JD: **The Central Role of Interbacterial Antagonism in Bacterial Life**. *Curr Biol* 2020, **30**:R1203-R1214.

5. Inda ME, Broset E, Lu TK, de la Fuente-Nunez C: **Emerging Frontiers in Microbiome Engineering**. *Trends Immunol* 2019, **40**:952-973.

6. Lawson CE, Harcombe WR, Hatzenpichler R, Lindemann SR, Loffler FE, O'Malley MA, Garcia Martin H, Pfleger BF, Raskin L, Venturelli OS, et al.: **Common principles and best practices for engineering microbiomes**. *Nat Rev Microbiol* 2019, **17**:725-741.

7. Tshikantwa TS, Ullah MW, He F, Yang G: **Current Trends and Potential Applications of Microbial Interactions for Human Welfare**. *Front Microbiol* 2018, **9**:1156.

8. Hutchings MI, Truman AW, Wilkinson B: **Antibiotics: past, present and future**. *Curr Opin Microbiol* 2019, **51**:72-80.

9. LaCourse KD, Peterson SB, Kulasekara HD, Radey MC, Kim J, Mougous JD: **Conditional toxicity and synergy drive diversity among antibacterial effectors**. *Nature Microbiology* 2018, **3**:440-446.

10. Kelsic ED, Zhao J, Vetsigian K, Kishony R: **Counteraction of antibiotic production and degradation stabilizes microbial communities**. *Nature* 2015, **521**:516-519.

11. Klein TA, Ahmad S, Whitney JC: **Contact-Dependent Interbacterial Antagonism Mediated by Protein Secretion Machines**. *Trends Microbiol* 2020, **28**:387-400.

12. Riley MA, Wertz JE: **Bacteriocins: evolution, ecology, and application**. *Annu Rev Microbiol* 2002, **56**:117-137.

13. Ruhe ZC, Low DA, Hayes CS: **Polymorphic Toxins and Their Immunity Proteins: Diversity, Evolution, and Mechanisms of Delivery**. *Annu Rev Microbiol* 2020, **74**:497-520.

14. Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA: **Contact-dependent inhibition of growth in Escherichia coli**. *Science* 2005, **309**:1245-1248.

15. Meuskens I, Saragliadis A, Leo JC, Linke D: **Type V Secretion Systems: An Overview of Passenger Domain Functions**. *Front Microbiol* 2019, **10**:1163.

16. Ruhe ZC, Subramanian P, Song K, Nguyen JY, Stevens TA, Low DA, Jensen GJ, Hayes CS: **Programmed Secretion Arrest and Receptor-Triggered Toxin Export during Antibacterial Contact-Dependent Growth Inhibition**. *Cell* 2018, **175**:921-933 e914.

17. Ruhe ZC, Nguyen JY, Xiong J, Koshiniemi S, Beck CM, Perkins BR, Low DA, Hayes CS: **CdiA Effectors Use Modular Receptor-Binding Domains To Recognize Target Bacteria**. *mBio* 2017, **8**:e00290-00217.

18. van Ulsen P, Rahman S, Jong WS, Daleke-Schermerhorn MH, Luirink J: **Type V secretion: from biogenesis to biotechnology**. *Biochim Biophys Acta* 2014, **1843**:1592-1611.

19. Willett JL, Gucinski GC, Fatherree JP, Low DA, Hayes CS: **Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways**. *Proc Natl Acad Sci U S A* 2015, **112**:11341-11346.

20. Coulthurst S: **The Type VI secretion system: a versatile bacterial weapon**. *Microbiology (Reading)* 2019, **165**:503-515.

21. Trunk K, Peltier J, Liu YC, Dill BD, Walker L, Gow NAR, Stark MJR, Quinn J, Strahl H, Trost M, et al.: **The type VI secretion system deploys antifungal effectors against microbial competitors**. *Nat Microbiol* 2018, **3**:920-931.

22. Sana TG, Lugo KA, Monack DM: **T6SS: The bacterial "fight club" in the host gut**. *PLoS Pathog* 2017, **13**:e1006325.

23. Hecht AL, Casterline BW, Earley ZM, Goo YA, Goodlett DR, Bubeck Wardenburg J: **Strain competition restricts colonization of an enteric pathogen and prevents colitis**. *EMBO Rep* 2016, **17**:1281-1291.

24. Unterweger D, Kostiuk B, Pukatzki S: **Adaptor Proteins of Type VI Secretion System Effectors**. *Trends Microbiol* 2017, **25**:8-10.

25. Ross BD, Verster AJ, Radey MC, Schmidtke DT, Pope CE, Hoffman LR, Hajjar AM, Peterson SB, Borenstein E, Mougous JD: **Human gut bacteria contain acquired interbacterial defence systems**. *Nature* 2019, **575**:224-228.

411 26. Jiang F, Waterfield NR, Yang J, Yang G, Jin Q: **A Pseudomonas aeruginosa type VI**
412 **secretion phospholipase D effector targets both prokaryotic and eukaryotic cells**.
413 *Cell Host Microbe* 2014, **15**:600-610.

414 27. Ma AT, McAuley S, Pukatzki S, Mekalanos JJ: **Translocation of a Vibrio cholerae type**
415 **VI secretion effector requires bacterial endocytosis by host cells**. *Cell Host*
416 *Microbe* 2009, **5**:234-243.

417 28. Wettstadt S, Filloux A: **Manipulating the type VI secretion system spike to shuttle**
418 **passenger proteins**. *PLoS One* 2020, **15**:e0228941.

419 29. Ting SY, Martinez-Garcia E, Huang S, Bertolli SK, Kelly KA, Cutler KJ, Su ED, Zhi H, Tang
420 Q, Radey MC, et al.: **Targeted Depletion of Bacteria from Mixed Populations by**
421 **Programmable Adhesion with Antagonistic Competitor Cells**. *Cell Host Microbe*
422 2020, **28**:313-321 e316.

423 30. Cross KL, Campbell JH, Balachandran M, Campbell AG, Cooper SJ, Griffen A, Heaton M,
424 Joshi S, Klingeman D, Leys E, et al.: **Targeted isolation and cultivation of**
425 **uncultivated bacteria by reverse genomics**. *Nat Biotechnol* 2019, **37**:1314-1321.

426 31. Wellner A, McMahon C, Gilman MSA, Clements JR, Clark S, Nguyen KM, Ho MH, Shin
427 JE, Feldman J, Hauser BM, et al.: **Rapid generation of potent antibodies by**
428 **autonomous hypermutation in yeast**. *bioRxiv* 2020.

429 32. Mageeney CM, Sinha A, Mosesso RA, Medlin DL, Lau BY, Rokes AB, Lane TW, Branda
430 SS, Williams KP: **Computational Basis for On-Demand Production of Diversified**
431 **Therapeutic Phage Cocktails**. *mSystems* 2020, **5**.

432 33. Ando H, Lemire S, Pires DP, Lu TK: **Engineering Modular Viral Scaffolds for Targeted**
433 **Bacterial Population Editing**. *Cell Syst* 2015, **1**:187-196.

434 34. Alvarez-Martinez CE, Christie PJ: **Biological diversity of prokaryotic type IV secretion**
435 **systems**. *Microbiol Mol Biol Rev* 2009, **73**:775-808.

436 35. Brophy JAN, Triassi AJ, Adams BL, Renberg RL, Stratis-Cullum DN, Grossman AD, Voigt
437 CA: **Engineered integrative and conjugative elements for efficient and inducible**
438 **DNA transfer to undomesticated bacteria**. *Nat Microbiol* 2018, **3**:1043-1053.

439 36. Ronda C, Chen SP, Cabral V, Yaung SJ, Wang HH: **Metagenomic engineering of the**
440 **mammalian gut microbiome in situ**. *Nat Methods* 2019, **16**:167-170.

37. Hamilton TA, Pellegrino GM, Therrien JA, Ham DT, Bartlett PC, Karas BJ, Gloor GB, Edgell DR: **Efficient inter-species conjugative transfer of a CRISPR nuclease for targeted bacterial killing**. *Nat Commun* 2019, **10**:4544.

38. Kiga K, Tan XE, Ibarra-Chavez R, Watanabe S, Aiba Y, Sato'o Y, Li FY, Sasahara T, Cui B, Kawauchi M, et al.: **Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria**. *Nat Commun* 2020, **11**:2934.

39. Rubin BE, Diamond S, Cress BF, Crits-Christoph A, He C, Xu M, Zhou Z, Smock DC, Tang K, Owens TK, et al.: **Targeted Genome Editing of Bacteria Within Microbial Communities**. *bioRxiv* 2020.

40. Vo PLH, Ronda C, Klompe SE, Chen EE, Acree C, Wang HH, Sternberg SH: **CRISPR RNA-guided integrases for high-efficiency, multiplexed bacterial genome engineering**. *Nat Biotechnol* 2020.

41. Nudleman E, Wall D, Kaiser D: **Cell-to-cell transfer of bacterial outer membrane lipoproteins**. *Science* 2005, **309**:125-127.

42. Vassallo C, Pathak DT, Cao P, Zuckerman DM, Hoiczyk E, Wall D: **Cell rejuvenation and social behaviors promoted by LPS exchange in myxobacteria**. *Proc Natl Acad Sci U S A* 2015, **112**:E2939-2946.

43. Zhou X, Rodriguez-Rivera FP, Lim HC, Bell JC, Bernhardt TG, Bertozzi CR, Theriot JA: **Sequential assembly of the septal cell envelope prior to V snapping in Corynebacterium glutamicum**. *Nat Chem Biol* 2019, **15**:221-231.

44. Pande S, Shitut S, Freund L, Westermann M, Bertels F, Colesie C, Bischofs IB, Kost C: **Metabolic cross-feeding via intercellular nanotubes among bacteria**. *Nat Commun* 2015, **6**:6238.

45. Cotter PD, Ross RP, Hill C: **Bacteriocins - a viable alternative to antibiotics?** *Nat Rev Microbiol* 2013, **11**:95-105.

46. Cheigh CI, Pyun YR: **Nisin biosynthesis and its properties**. *Biotechnol Lett* 2005, **27**:1641-1648.

47. Lei W, Demir K, Overhage J, Grunze M, Schwartz T, Levkin PA: **Droplet-Microarray: Miniaturized Platform for High-Throughput Screening of Antimicrobial Compounds**. *Adv Biosyst* 2020, **4**:e2000073.

48. Tucker AT, Leonard SP, DuBois CD, Knauf GA, Cunningham AL, Wilke CO, Trent MS, Davies BW: **Discovery of Next-Generation Antimicrobials through Bacterial Self-Screening of Surface-Displayed Peptide Libraries**. *Cell* 2018, **172**:618-628 e613.

49. Carim S, Azadeh AL, Kazakov AE, Price MN, Walian PJ, Chakraborty R, Deutschbauer AM, Mutalik VK, Arkin AP: **Systematic Discovery of Pseudomonad Genetic Factors Involved in Sensitivity to Tailocins**. *bioRxiv* 2020.

50. Liu R, Zhang Y, Zhai G, Fu S, Xia Y, Hu B, Cai X, Zhang Y, Li Y, Deng Z, et al.: **A Cell-Free Platform Based on Nisin Biosynthesis for Discovering Novel Lanthipeptides and Guiding their Overproduction In Vivo**. *Adv Sci (Weinh)* 2020, **7**:2001616.

51. Kim MS, Bae JW: **Lysogeny is prevalent and widely distributed in the murine gut microbiota**. *ISME J* 2018, **12**:1127-1141.

52. Li XY, Lachnit T, Fraune S, Bosch TCG, Traulsen A, Sieber M: **Temperate phages as self-replicating weapons in bacterial competition**. *J R Soc Interface* 2017, **14**.

53. Boling L, Cuevas DA, Grasis JA, Kang HS, Knowles B, Levi K, Maughan H, McNair K, Rojas MI, Sanchez SE, et al.: **Dietary prophage inducers and antimicrobials: toward landscaping the human gut microbiome**. *Gut Microbes* 2020, **11**:721-734.

54. Oh JH, Alexander LM, Pan M, Schueler KL, Keller MP, Attie AD, Walter J, van Pijkeren JP: **Dietary Fructose and Microbiota-Derived Short-Chain Fatty Acids Promote Bacteriophage Production in the Gut Symbiont Lactobacillus reuteri**. *Cell Host Microbe* 2019, **25**:273-284 e276.

55. Atsumi S, Little JW: **A synthetic phage lambda regulatory circuit**. *Proc Natl Acad Sci U S A* 2006, **103**:19045-19050.

56. Yehl K, Lemire S, Yang AC, Ando H, Mimee M, Torres MT, de la Fuente-Nunez C, Lu TK: **Engineering Phage Host-Range and Suppressing Bacterial Resistance through Phage Tail Fiber Mutagenesis**. *Cell* 2019, **179**:459-469 e459.

57. Yosef I, Goren MG, Globus R, Molshanski-Mor S, Qimron U: **Extending the Host Range of Bacteriophage Particles for DNA Transduction**. *Mol Cell* 2017, **66**:721-728 e723.

58. Lu TK, Collins JJ: **Dispersing biofilms with engineered enzymatic bacteriophage**. *Proc Natl Acad Sci U S A* 2007, **104**:11197-11202.

59. Rapp KM, Jenkins JP, Betenbaugh MJ: **Partners for life: building microbial consortia for the future**. *Curr Opin Biotechnol* 2020, **66**:292-300.

60. Xiao Y, Angulo MT, Lao S, Weiss ST, Liu YY: **An ecological framework to understand the efficacy of fecal microbiota transplantation**. *Nat Commun* 2020, **11**:3329.

61. Kong W, Meldgin DR, Collins JJ, Lu T: **Designing microbial consortia with defined social interactions**. *Nat Chem Biol* 2018, **14**:821-829.

62. Liao MJ, Din MO, Tsimring L, Hasty J: **Rock-paper-scissors: Engineered population dynamics increase genetic stability**. *Science* 2019, **365**:1045-1049.

63. Hsu RH, Clark RL, Tan JW, Ahn JC, Gupta S, Romero PA, Venturelli OS: **Microbial Interaction Network Inference in Microfluidic Droplets**. *Cell Syst* 2019, **9**:229-242 e224.

64. Sheth RU, Li M, Jiang W, Sims PA, Leong KW, Wang HH: **Spatial metagenomic characterization of microbial biogeography in the gut**. *Nat Biotechnol* 2019, **37**:877-883.

65. Celik Ozgen V, Kong W, Blanchard AE, Liu F, Lu T: **Spatial interference scale as a determinant of microbial range expansion**. *Sci Adv* 2018, **4**:eaau0695.

66. Hsu BB, Gibson TE, Yeliseyev V, Liu Q, Lyon L, Bry L, Silver PA, Gerber GK: **Dynamic Modulation of the Gut Microbiota and Metabolome by Bacteriophages in a Mouse Model**. *Cell Host Microbe* 2019, **25**:803-814 e805.

**Special interest (•)**

[3] Garcia-Byona L, Comstock LE, *Science* 2018, 361:eaat2456 – This review focuses on bacterial antagonistic interactions in host-associated microbial communities.

[4] Peterson SB, Bertolli SK, Mougous JD, *Curr Biol* 2020, 30:R1203-R1214 – This review details the diversity and ubiquity of interbacterial antagonistic pathways.

[6] Lawson et al., *Nat Rev Microbiol* 2019, 17:725-741 – A review of current methods used for engineering microbiomes, including top-down and bottom-up design approaches and emerging tools to analyze microbiome functions.

[11] Klein TA, Ahmad S, Whitney JC, *Trends Microbiol* 2020, 28:387-400 – A review of contact-dependent antagonistic mechanisms based on protein secretion machineries detailing current knowledge about the secretion mechanisms and effector repertoire used by each system.

[13] Ruhe ZC, Low DA, Hayes CS, *Annu Rev Microbiol* 2020, 74:497-520 – This review details structures, functions, and delivery mechanisms of polymorphic toxin effectors that mediate interbacterial antagonism.

**Outstanding interest (••)**

[26] Ting et al., *Cell Host Microbe* 2020, 28:313-321 – This study demonstrates modulating target specificity of T6SS activity using cell surface-displayed nanobodies that are specific to surface receptors on target bacteria.

[36] Rubin et al., *bioRxiv* 2020 – This study demonstrates the use of CRISPR-transposon systems for targeted genome editing of specific bacteria within microbial communities.

[45] Tucker et al., *Cell* 2018, 172:618-628 – The authors describe highly multiplexed assay for screening hundreds of thousands of peptides for antimicrobial activity.

[46] Carim et al., *bioRxiv* 2020 – This study demonstrates high-throughput screening of host genetic factors involved in sensitivity to bacteriocins.

[47] Liu et al., *Adv Sci* 2020, 7:2001616 – The authors show the development of a cell-free platform to synthesize and screen nisin and its analogs.

[53] Yehl et al., *Cell* 2019, 179:459-469 – This study describes structure-informed engineering of tail fiber domains to alter host range of phages.

[59] Liao et al., *Science* 2019, 365:1045-1049 – The authors demonstrate three-strain microbial ecosystem where each strain could kill or be killed by one of the other two strains for prolonged stability of genetic circuit functionality.

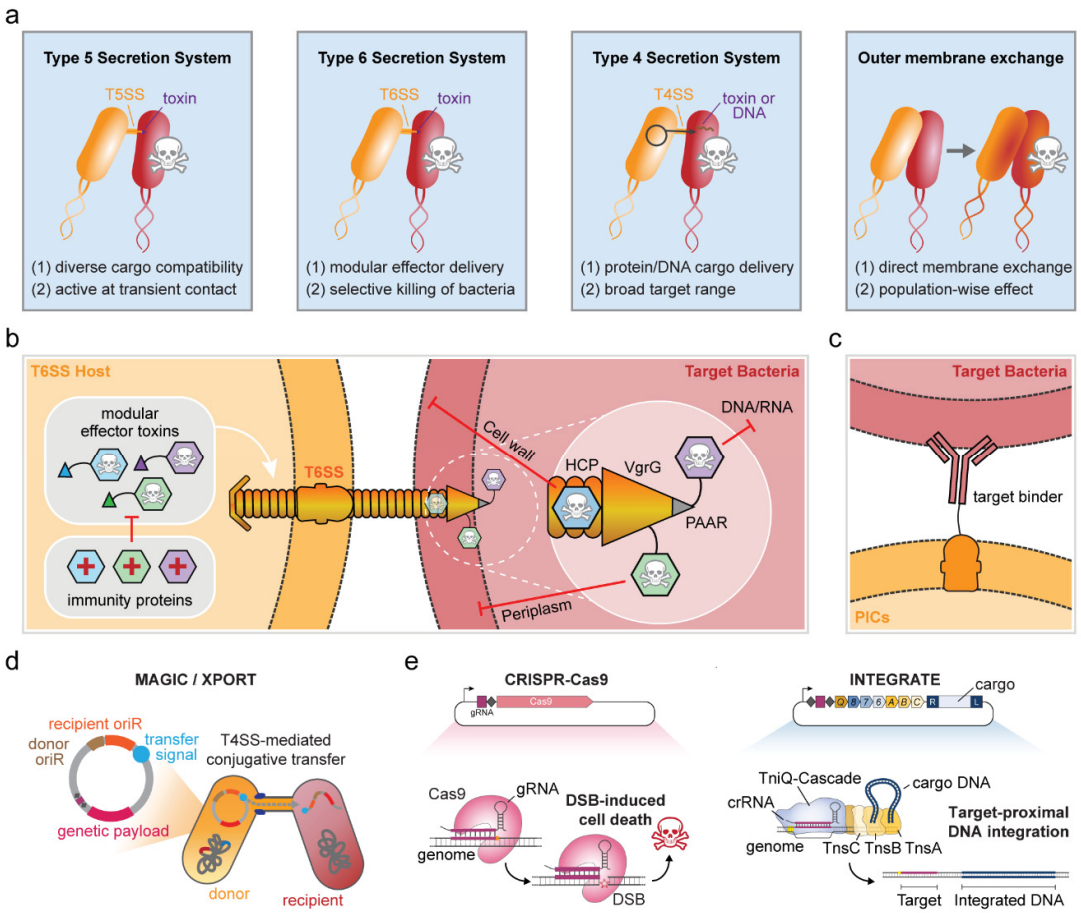
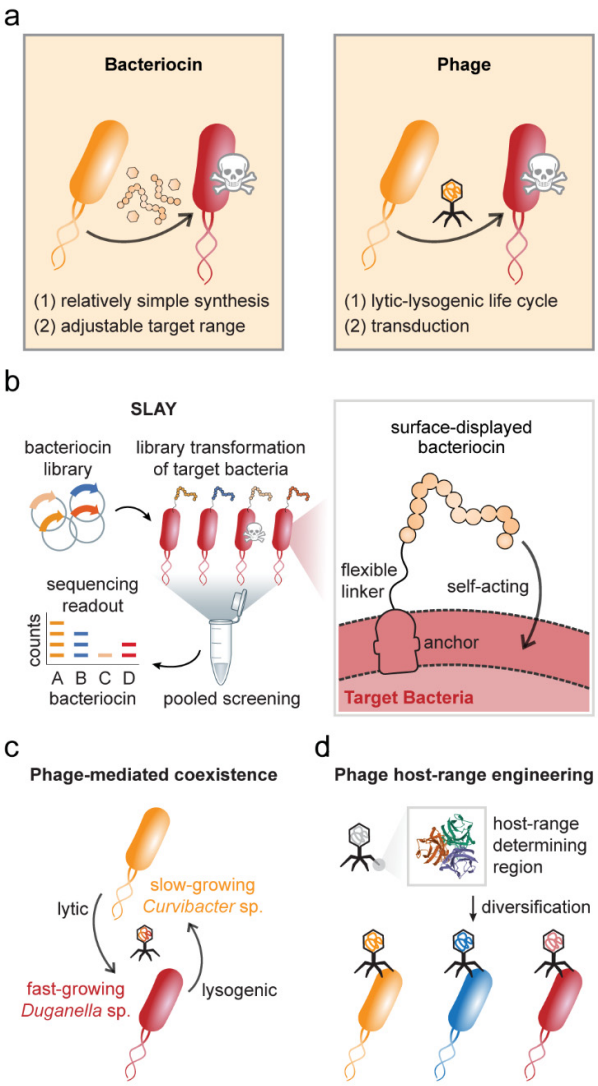



**Figure 1. Representative contact-dependent interbacterial antagonistic systems and their applications. (a)** Schematic diagram of the systems and their key features. **(b)** Modular effector delivery mechanisms of T6SS. **(c)** Target specificity of T6SS can be modified by introducing binding protein specific to target bacteria. **(d)** T4SS-based conjugative gene transfer has been applied to engineering of diverse microbes in their native environments. **(e)** CRISPR-based systems can be delivered as genetic payloads for sequence-specific manipulation of microbial communities.

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576 **Figure 2. Representative diffusion-based interbacterial antagonistic systems and their**
577 **applications. (a)** Schematic diagram of the systems and their key features. **(b)** SLAY method
578 could be applied to high-throughput characterization of natural and synthetic bacteriocins
579 against diverse bacteria. **(c)** Coexistence of slow-growing *Curvibacter* sp. and fast-growing
580 *Duganella* sp. are mediated by an inducible prophage in *Curvibacter* sp. that can lytically infect
581 *Duganella* sp.; **(d)** Phage host-range can be modulated by diversification of tail fiber proteins.