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Feature

Renaissance for Phage-Based Bacterial Control

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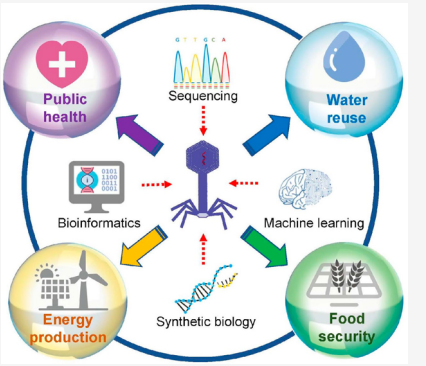
ABSTRACT: Bacteriophages (phages) are an underutilized biological resource with vast potential for pathogen control and microbiome editing. Phage research and commercialization have increased rapidly in biomedical and agricultural industries, but adoption has been limited elsewhere. Nevertheless, converging advances in DNA sequencing, bioinformatics, microbial ecology, and synthetic biology are now poised to broaden phage applications beyond pathogen control toward the manipulation of microbial communities for defined functional improvements. Enhancements in sequencing combined with network analysis make it now feasible to identify and disrupt microbial associations to elicit desirable shifts in community structure or function, indirectly modulate species abundance, and target hub or keystone species to achieve broad functional shifts. Sequencing and bioinformatic advancements are also facilitating the use of temperate phages for safe gene delivery applications. Finally, integration of synthetic biology stands to create novel phage chassis and modular genetic components. While some fundamental, regulatory, and commercialization barriers to widespread phage use remain, many major challenges that have impeded the field now have workable solutions. Thus, a new dawn for phage-based (chemical-free) precise biocontrol and microbiome editing is on the horizon to enhance, suppress, or modulate microbial activities important for public health, food security, and more sustainable energy production and water reuse.



KEYWORDS: *bacteriophages, microbiome editing, pathogen control, indirect targeting, chemical-free disinfection*

INTRODUCTION

Bacteriophages (phages) are the most abundant, diverse, and underutilized biological resource in the biosphere.[1] These viruses exclusively infect bacteria and utilize different life cycles to shape microbial communities through predation, transduction, and reprogramming of bacterial metabolism.[2] Lytic phages function as highly selective antimicrobial agents that can control target bacteria with limited impact on the surrounding microbial community. Conversely, temperate phages can stably integrate their genomes into the bacterial host genome (a process referred to as lysogeny[3]) and have the potential to introduce genes that alter host function or fitness. Beyond this, phages possess many innate characteristics that make them attractive for "chemical-free" microbial control, including specificity, replicative potential, the capacity to mutate and coevolve with their host, a lack of residual toxicity, and sustainable production.[4]

Since the realization by Félix d'Herelle in 1917 that phages could kill bacteria,[5] phage research has largely focused on developing therapies to treat a small number of well-characterized pathogens, with renewed interest primarily driven by concerns over the emergence of multidrug resistant bacteria.[6,7] While phages are also being increasingly applied in the food and agricultural industries,[8,9] their adoption for environmental engineering—including applications for more sustainable energy production and water reuse—has received limited attention (Figure 1). Expansion of phage applications has been partly hampered by recent well-publicized failures of phage therapy in clinical trials.[10−13] Nevertheless, there are many potential applications beyond their traditional use for pathogen control in which phages could be an effective and precise tool for manipulating more complex and dynamic microbial communities to enhance, suppress, or modulate specific microbial processes.

This article examines the current status of phage technology and analyzes the main barriers preventing the transition from proof-of-concept research to commercialization and expansion of phage applications. We also discuss how the convergence of advances in sequencing, bioinformatics, microbial ecology, and synthetic biology is enabling microbiome editing and the development of novel phage-based microbiome editing strategies that contribute to sustainable development.

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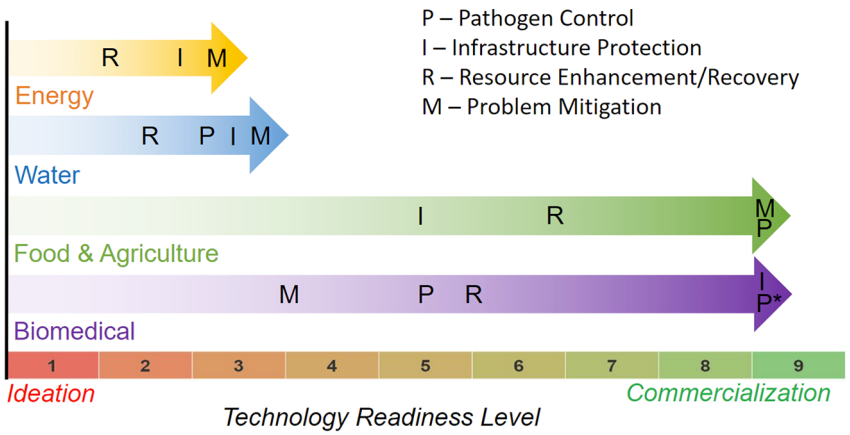

Pathogen Control Targeted elimination of bacteria known to cause disease in humans	Reduction of pathogen load in water systems [118 119] Pathogens in food supply chains [23 120 121 122 123 124] Recurrent antibiotic resistant infections (pre-clinical and compassionate use) [101 108 109 125 126] P* denotes approved phage products for human use [46 105 106 107 127 128 129 130 131]
Infrastructure Protection Mitigate microbial-related deterioration or monitor process performance	Corrosion of energy related infrastructure [116 132] Water pipe corrosion and clogging [133] Membrane biofouling [15 16] Biosensors in water distribution systems [113] Replace corrosive/toxic cleaning disinfectants [134] Point of care diagnostics [135 136]
Resource Enhancement/Recovery Improve resource production & utilization	Extraction of bio-lipids [137] Nanomaterial synthesis [83 84] Protect marine systems (e.g. coral pathogens, harmful algal blooms) [138 139 140] Feed supplementation for livestock growth enhancement [141 142] Antibiotic use reduction or replacement [143]
Problem Mitigation Prophylactically mitigate interference with resource recovery or process performance	Hydrocarbon reservoir souring [14] Control lactic acid bacteria in bioethanol fermentation [143 144 145] Activated sludge bulking and foaming [17 18] Phytopathogen treatments [8 9 22 103] Broad gut microbiome manipulation [147]

Figure 1. Technology Readiness Level progress of phage applications in various fields. TRL 1-Ideation, TRL 2-Basic research, TRL 3-Proof-of-concept, TRL 4-Small prototype, TRL 5-Pilot scale, TRL 6-Prototype system, TRL 7-Demonstration system, TRL 8-Commercial system, and TRL 9-Full commercialization. Selected applications are expanded in the accompanying table.

EMERGING OPPORTUNITIES FOR PHAGE-BASED BIOCONTROL

Sequencing with Higher Taxonomic Resolution May Broaden Phage-Based Biocontrol Applications. Phage-based biocontrol strategies have been proposed for numerous challenges within environmental engineering, such as hydrocarbon reservoir souring,[14] biofouling,[15,16] activated sludge foaming and bulking,[17,18] agricultural methane emissions,[19] and harmful algal blooms.[20] However, few of these proposed applications have progressed further than lab-scale demonstrations. The foremost exceptions to this are in the agricultural sector in the use of phages as alternatives to antibiotic feed additives,[21] pesticides,[22] or disinfectants,[23] with some products now commercially available and several more in development (Figure 1). These products, and most biomedical phage applications, are generally developed to control a single well-defined bacterial target, while many proposed environmental phage applications seek to address a specific property or function of a microbial community, such as biofouling, hydrocarbon reservoir souring, and microbial-induced corrosion. As most characterized phages are species- or strain-specific,[24] uncertainty of target species identity or the need to control multiple species substantially increases implementation difficulty relative to broad-spectrum antibiotics and biocides.

Understandably, the difficulty of designing a phage-based biocontrol strategy is proportional to the number of species encoding the metabolic function or property of concern. For example, there are over 60 genera and 220 species of sulfate-reducing bacteria[25] that could be targeted to mitigate corrosion or hydrocarbon reservoir souring. While phage cocktails that target single species have been commercialized, attempting to develop predefined cocktails for problems caused by multiple species is impractical. In such circumstances, the use of system-specific sequencing may be necessary to characterize the microbial community at sufficient taxonomic resolution to determine how many relevant species are present and in what proportions. While just a few years ago this may have been costly and challenging, recent advances in sequencing technology and simultaneous reductions in cost have now made this an accessible and routine task.[26,27]

Beyond knowledge of the microbial community composition, phage-based approaches benefit from detailed knowledge of the target bacterium, recognizing that major functional differences can exist between strains of the same bacterial species. For example, *E. coli* Nissle 1917 is a probiotic strain used to treat inflammatory intestinal diseases,[28] while *E. coli*

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Environ. Sci. Technol. 2022, 56, 4691−4701

O157:H7 is a serotype that causes severe, acute hemorrhagic diarrhea.[29] Indeed, many strains of the same species share a common core genome but may contain vastly different accessory genomes, putatively as a result of extensive horizontal gene transfer.[30] Thus, high taxonomic resolution of microbiome data is critical for informing and broadening phage-based biocontrol or microbiome editing applications. Previously, most microbiome studies utilized partial 16S rRNA gene amplicons that typically only provide genus-level resolution, which is insufficient for developing more selective microbial control approaches.[31−33] However, the development of shotgun metagenomic sequencing and long-read sequencing technologies has facilitated species-level analyses (in some cases even strain-level) while also increasing data throughput, reducing costs, and enhancing *de novo* genome assembly accuracy.[34] Indeed, starting from a phage lysate or environmental sample, it is now possible to produce a fully annotated phage genome or conduct a microbial community analysis in less than a day, at higher accuracy and resolution than previously possible.[35,36] Such advances in sequencing are enhancing the use of phages by providing detailed information on their specific targets as well as their interactions with the surrounding microbial community.

Network Analysis May Expand Phage Applications. Historically, the analytical techniques used to study microbial communities have focused on a standardized set of properties, predominantly diversity metrics. Recently, the ever-increasing size and number of high-resolution metagenomic data sets have facilitated the application of network analysis toward better understanding of complex microbial associations. Network analysis enables the exploration of direct or indirect interactions between coexisting microorganisms and possible identification of keystone species.[37,38] Indeed, several recent microbiome studies have incorporated ecological network analysis to explain the relationships between different taxa and identify keystone species that are critical for community stability and function.[39−41] For example, *Arthrobacter*, *Acidobacteria*, *Burkholderia*, *Rhodanobacter*, and *Rhizobium* were identified as keystone taxa across three agroforestry systems and correlated with soil organic carbon content.[42] Another study in seawater found that biofilm formation on iron plates coated with antifouling paint was initiated by *Alteromonas genovensis*.[43] Harnessing network analysis to target pertinent bacterial taxa may expand phage applications, though current models that predict microbial interactions are often generated from pairwise experiments and co-occurrence networks, and the relationships within microbial communities (which can include hundreds if not thousands of taxa) are often inferred based on the simplifying assumption that such interactions are fixed rather than dynamic.[44]

More robust species-level network models have the potential to transform phage-based biocontrol by identifying microbial associations that can be disrupted to elicit desirable shifts in microbial community structure or function. For example, phage interventions are generally considered a "subtractive technology" in that they can be used to clear niches and/or suppress a particular species. However, the identification of strong correlations via network analysis enables the use of phages to indirectly increase species abundance by targeting competitors, predators, or amensalistic bacteria (Figure 2a). Multilayered biocontrol strategies can also be developed to provide stronger or more durable bacterial inhibition by creating phage cocktails that simultaneously target various

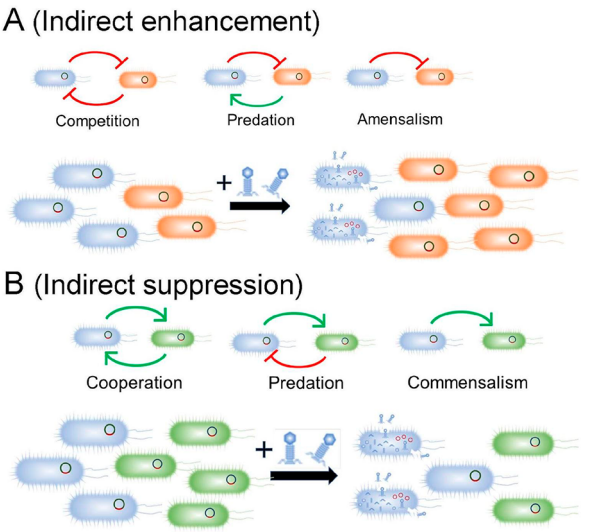

Figure 2. Indirect fostering or suppression of growth of species of interest by phage biocontrol. Novel phage biocontrol strategies to (A) foster or (B) suppress the growth of other species of interest, informed by advanced sequencing and omics analysis.

species with mutualistic, syntrophic, or commensalistic interactions with the primary target for indirect suppression (Figure 2b). Importantly, several studies have reported the indirect modulation of species abundance after phage application,[45] which provides some precedent for this approach. For example, ingestion of a probiotic in combination with an *E. coli* phage cocktail decreased *Desulfovibrio* concentrations and increased *Lactobacillus* colonization relative to treatment with the probiotic alone.[46] Indirect modulation of species abundance can also be used in circumstances where the primary target is too difficult to culture (for phage isolation and production) or present at cell densities insufficient for sustaining phage replication. In such cases, highly abundant and culturable species could be aimed at by phages to disrupt interactions benefiting the unculturable target species (e.g., cross feeding). In circumstances where an unwanted metabolic pathway or activity is encoded by too many different species to practically target, network analysis could be used to identify hub or keystone species integral to the stability of that network module or niche.

With current advances in accessibility and volume of metagenomic sequencing,[34] network models should be built *de novo* for any environment in which phages might be used, and phage-based perturbation studies should be conducted to directly validate causation instead of relying on correlations. Better understanding of the ecological relationships between taxa and their respective phages not only advances general scientific inquiry but also could concretely improve the ability to edit complex microbiomes to improve the efficiency and sustainability of some industrial processes.

Temperate Phage Selection, Applications, and Manipulation Are Facilitated by Phage Genome Sequencing and Improved Annotation. Historically, lytic phages have been used to directly target pathogens or other detrimental species of concern, while temperate phages were typically avoided for therapeutic or biocontrol purposes. This is due to their innate propensity to enter lysogeny, which protects the lysogen (a bacterium containing a prophage) from further infection and limits the initial bactericidal effect.[3]

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Additionally, as prophage survival is linked to host survival, many phages have acquired genes that enhance host fitness, including some that may present safety issues. Despite these concerns, temperate phages possess several advantageous features, including the ability to deliver or disrupt specific genes and propagate in environments suboptimal for lytic lifecycles. Notably, the isolation of purely lytic phages can be difficult for certain species (and in some cases has proven impossible), while temperate phages are generally highly abundant, with the majority of bacterial genomes deposited in the National Center for Biotechnology Information database containing prophage sequences.[47] Thus, they can be much easier to isolate and sometimes the only practical source of phages for a given species. Once isolated, the editing of temperate phages can be much more straightforward using strategies such as recombineering instead of more conventional cloning methods.[48,49]

Advances in sequencing technology have enabled rapid and cost-effective characterization of phage genomes, which has become an integral component of product development to ensure the absence of genes encoding virulence factors, toxins, or antibiotic resistance determinants. This enhanced capacity to assemble and analyze phage genomes has renewed interest in the use and development of temperate phages. Several studies have reported successful inhibition when utilizing temperate phages, individually,[50,51] in cocktails,[52] or in combination with antibiotics. Moreover, virulent mutants of temperate phages which have lost the capacity to enter lysogeny through mutations or indels (genomic insertions or deletions) have similar propagation dynamics and behavior to lytic phages. Such phages occur spontaneously at low frequency, though this process can be accelerated using various *in vitro* methods.[53−55] Low-cost sequencing makes it feasible to screen such mutants and ensure they are truly lytic and have a low probability of reversion. However, as many phage genes have yet to be characterized, the use of temperate phages should be constrained to low-risk applications or situations where appropriate risk mitigation measures can be implemented.

Phages as Gene Delivery Vectors. Beyond their use for biocontrol, phages can also be harnessed for gene delivery, to enable the host to produce natural or transgenic proteins, including enzymes. For example, phages could be used to deliver (or increase transcription of) genes for contaminant biodegradation, biofilm disruption, or increased killing efficiency of competing bacteria. Alternatively, unwanted gene activity can be repressed without necessarily killing the host to avoid selective pressure that might result in phage resistance.[56,57] When gene delivery strategies are informed by prior microbial community characterization, the most abundant species within a community can be targeted to ensure phage proliferation and the highest levels of gene expression. Moreover, harnessing the native community circumvents challenges associated with survival of exogenous species[58] −the most common cause of bioaugmentation failure. Yet, despite the vast potential to engineer phages for such purposes, transgenic manipulations would create significant regulatory barriers for many environmental applications and need to be carefully considered.

Though phage-mediated gene delivery has been proposed to enhance the biodegradation capabilities of indigenous bacteria,[59] the vast majority of engineered phages is derived from model phages (e.g., T7, M13) that infect only *E. coli*.[60] The development of more efficient and universal methods of phage engineering is needed to enable gene delivery to a wider range of environmentally and industrially relevant species.

Alternatively, metagenomic sequencing has made it possible to identify naturally occurring phages that already encode important metabolic pathways. Such phages have the potential to be utilized within a much more permissive regulatory framework. For example, environmental viromes from arsenic- and chromium-impacted soils were found to be enriched in auxiliary metabolic genes (AMGs) involved in transport and speciation of those metals.[61,62] Theoretically, phages containing such AMGs could be isolated and used to enhance microbial community resistance to metal-induced stress or to control metal speciation for remedial purposes. Once identified in a data set, AMG-containing phages could be isolated from samples as prophages using media or enrichment cultures selective for their host. Interestingly, recent studies investigating the effects of virome transplants in a murine model,[63] between people,[64−66] and even in soils[45] have demonstrated large shifts in microbiome composition and function, with expansion of previously low abundance species possibly the result of AMG acquisition. This highlights the role phages can play as "additive" microbiome editing tools (e.g., by increasing species growth) rather than simply serving as subtractive or inhibitory agents.

Synthetic Biology Can Accelerate, Standardize, and Enhance Phage Development for Broader Applications. Through the application of engineering principles to biological systems, synthetic biology stands to expand phage-based technology by facilitating the creation of novel phage chassis and modular genetic components. Several phage genomes have been completely assembled using only synthetic DNA oligonucleotides,[67,68] allowing for rapid and large-scale genome modification[69] and refactoring[70] while simultaneously circumventing low recombination efficiency associated with *in vivo* genome engineering. Moreover, synthetic phage genomes can be "rebooted" in nonhost[71] or cell-free systems,[72] which suggests the potential for phage production against unculturable hosts.

The significant relationship between phage research and synthetic biology cannot be understated, as it has generated various tools that advanced the ability to manipulate biological organisms and biological systems. One example is the clustered regularly interspaced short palindromic repeat (CRISPR)-associated systems (Cas),[73,74] a tool that enables precise genetic manipulations in various of organisms for numerous applications. Other examples are the use of integrases for the generation of genetic circuits and sensors,[75,76] the use of phage RNA polymerases to control gene expression,[77] and transcriptional regulators for creation of biological switches and oscillators.[78] On the other hand, phage genome manipulation has enabled the creation of phages harboring depolymerases with enhanced ability to enzymatically disperse biofilms[79] and phages with extended host range and stability useful in biomedical applications.[80,81] There are also multiple examples of phage proteins and substructures that can be used to design biomaterials with highly tunable properties. A few worth mentioning are phage-based nanomaterials for lithium-ion batteries,[82,83] phage capsid nanoparticles that can block viral infection,[84] and the use of M13 bacteriophage as piezoelectric material to generate electrical energy[85] among many others.

Overall, the ability to move, minimize, and refactor phage genomes to later reboot them in wall-less bacteria or yeast[71]

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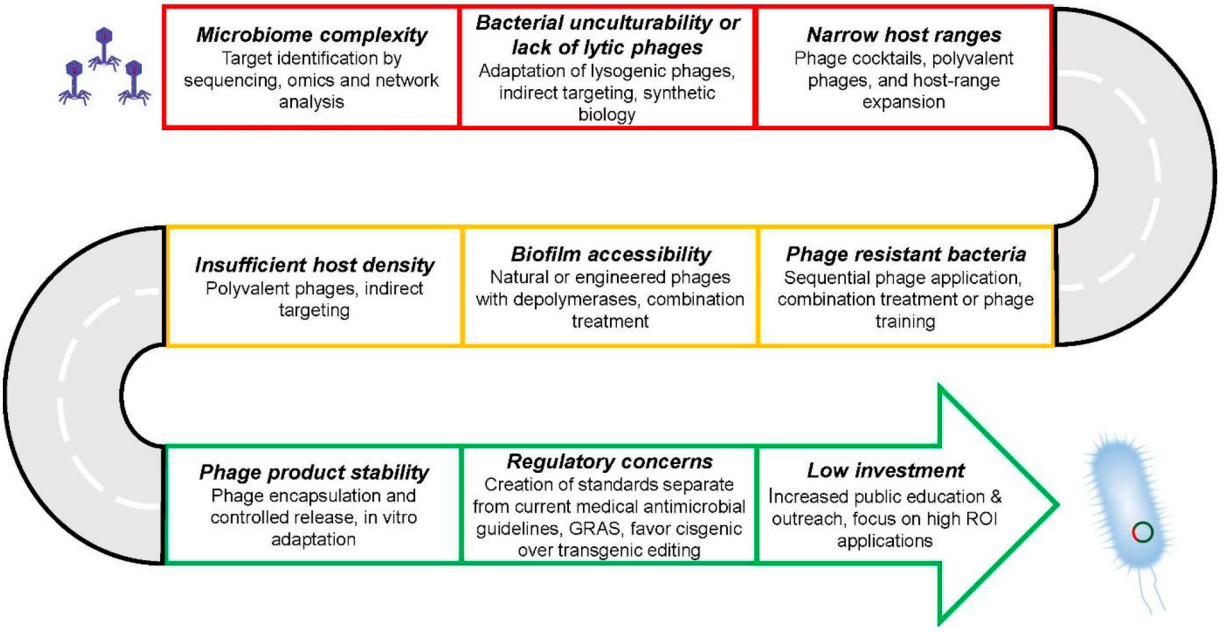

Figure 3. Potential roadblocks and challenges facing phage applications on the road to commercialization. The challenge is in bold italics, and possible strategies and solutions to overcoming these challenges are below.

has reinvigorated interest and opened new avenues for high-precision microbial manipulation. While the creation of engineered phages presents substantial technical and regulatory challenges, it also yields major benefits for intellectual property protection, and standardization greatly accelerates new product development. For example, imagine a universal phage chassis that can be selectively targeted by only swapping out the receptor binding domain. This would not only reduce the need to isolate new phages, but culture optimization, scale up, stability testing, purification process design, and formulation would only need to be conducted once. The feasibility of such a system is within reach because the mosaic nature of phages[86] makes them well suited to swapping components through promiscuous recombination. Though a streamlined system has yet to be brought to market, there is a robust body of work demonstrating that tail-fiber mutagenesis can broaden phage host range,[87] as well as design principles and strategies that could be used to this end.[88]

IMPLEMENTATION BARRIERS AND ENABLING STRATEGIES

Fundamental and Technological Implementation Challenges. Many studies have demonstrated phage applications in the laboratory but fail to translate these benefits into the field. Issues that need to be considered include whether phages can reach their hosts due to environmental challenges (e.g., poor diffusion through biofilms or survival at low pH), whether host concentrations are sufficient to sustain lytic phage replication, and whether phages isolated and developed under laboratory conditions are suitable for field use (Figure 3). The combination of improved environmental characterization and network analysis alongside strategies such as *in vitro* adaptation, selection, or engineering of polyvalence[89] to combat the challenge of narrow host ranges and the use of natural or engineered phages conjugated with other nanomaterials[90] may enable phage applications to succeed where they have heretofore failed in industrial and environmental systems.

As with any emerging technology or material, unintended consequences need to be considered to ensure that phage applications evolve as a tool for sustainability rather than a liability. This includes a proactive assessment of potential disruption of microbial ecology. One common concern is the potential for transduction and enhanced dissemination of pathogenic or antibiotic resistance genes[91−93] or other genes that endow host bacteria with a competitive advantage that results in detrimental consequences. For example, phages from arsenic-resistant bacteria can transduce arsenic-resistance genes such as *arsC*, which codes for As(V) reduction to excretable (via efflux pumps) but more toxic As(III).[61] This transduction was observed to change arsenic speciation and increased soil toxicity. Another unintended consequence is the counter-intuitive stimulation of biofilm growth and densification by polyvalent phages applied at relatively low concentration (e.g., 10^4 pfu/mL),[94] which might accelerate biofouling, biocorrosion, or other biofilm-related water quality problems. Another concern is the fear of extensive phage use leading to widespread phage resistance, ushering in another problem akin to that of the spread of antibiotic resistance.[95] However, the tendency for phages to have a narrow host range near eliminates the chance of horizontal gene transfer to distant taxa, and bacterial immunity to phage via clustered regularly interspaced short palindromic repeats (CRISPR) or modification of surface receptors is highly specific and unlikely to provide adaptive value even in the unlikely event it is disseminated to other genera.[96]

Regulatory Concerns and Commercialization Roadblocks. Regulators want assurances that products will be safe, effective, and standardized. The safety of eukaryotic organisms from phages is inherent in phage biology; these viruses are only able to infect and reproduce inside bacteria.[97] Indeed, the healthy human gut is estimated to host at least 10^{15} phage particles at any given time, and investigations of interactions between phages on eukaryotic immune and neurological systems found no harmful effects.[99] In terms of effectiveness,

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phage-based biocontrol applications in environmental and industrial processes should recognize that phage therapy using a single phage resulted in resistance development by the target host in 7.5 to 85.7% of the cases, depending on the pathogen.[99] Therefore, in the face of bacteria developing resistance to phages, synergistic cocktails[98] or sequential treatment should be established as standard practice.

Well documented shortcomings and failures[99−101] in clinical phage therapy such as rapid resistance development and the long and complicated road to approval as a medicine are of lower concern in industrial and environmental applications. Designating certain phage products as GRAS (Generally Recognized as Safe) is a particularly favorable strategy to overcome potential regulatory or public acceptance barriers (Figure 3). In the case of engineered phages, differentiating between cisgenic and transgenic modifications (which is the strategy used by Pivot Bio to develop nitrogen fixing biofertilizers[102]) may help streamline commercialization.

There is great potential that further research and testbed demonstrations will overcome commercialization roadblocks (Figure 3). A precedent was set by promising or proven phage products currently on the market for medical and agricultural applications. For example, an EPA approved phage product[103] (XylPhi-PD) targets the etiologic agent of Pierce's disease in grapes (*Xylella fastidiosa*) and was shown to reduce the abundance of this phytopathogen by several orders of magnitude and eliminate this disease when administered prophylactically.[104] Another example is PreforPro, a phage plus probiotic product produced by Deerland Probiotics & Enzymes[105] which was tested in a series of human clinical studies[46,106] and found to selectively reduce target organisms without significant disruption of the gut community as well as a reduction in gastrointestinal inflammation. Interest is clearly resurging in the medical field also, through compassionate use of phage therapy at the George Eliava Institute of Bacteriophage, Microbiology and Virology (active since the 1930s in Tbilisi, Georgia and cofounded by D'Herelle),[107] IPATH (the Center for Innovative Phage Applications and Therapeutics - the first phage therapy center in the United States in 2016 at the University of California, San Diego),[108] and the recent efforts of the TAILΦR (Tailored Antibacterials and Innovative Laboratories for phage (Φ) Research) initiative at Baylor Medical School (Houston, Texas).[109]

OUTLOOK FOR FUTURE OPPORTUNITIES

Many success stories in the clinical realm are limited to tailored treatment which is far removed from the vision of widely distributed broad range phage preparations that make attractive investments.[110] However, as the sequencing and network analysis technology advances, the use of phages as microbiome editing tools could be approached more holistically for faster innovation and broader commercialization. Expanding phage applications to environmental, industrial, and more nuanced agricultural niches would be a logical next step.

Advances in nucleotide sequencing technologies, omics analyses, and data sciences are facilitating system-specific characterization of microbiomes and associated ecological networks to discern bacterial targets for customized (direct or indirect) microbiome editing. Eventually, accessible "personalized" manipulations might even be possible for gut microbiome development to enhance public health or for other nontraditional applications discussed below. Realizing this potential, however, will require obtaining and broadly sharing species level microbiome and virome data from various systems, which would be facilitated by more frequent sequencing and publishing of isolated phage sequences, and utilizing tools such as HI-C to better understand phage-host linkages.[111] The creation and expansion of public phage libraries and banks, similar to those that exist for bacteria, would also facilitate selection and formulation of phage cocktails for various applications.

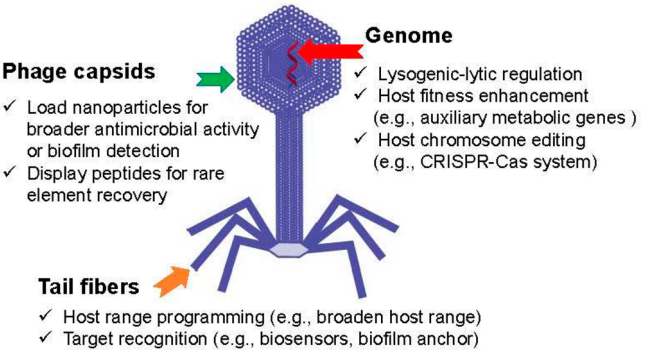

Figure 4. Phage components that could be modified by synthetic biology to enable phage-based microbiome editing and other functions.

Precise microbiome editing opens unprecedented opportunities to enhance or suppress specific microbial activities, which would expand phage applications beyond the traditional use for controlling antibiotic-resistant pathogens in clinical settings. Broader phage-based biocontrol applications could include enhanced food security through higher crop productivity and resilience to climate-related stress (e.g., rhizosphere or phyllosphere[112] microbiomes edited to increase water and nutrient retention or nitrogen fixation in soil or to produce *in planta* growth-stimulating hormones), increased feed efficiency for livestock production (e.g., rumen microbiome manipulations to mitigate nonproductive feed utilization by methanogens), and mitigation of antibiotic resistance propagation by animal agriculture (e.g., by replacing or minimizing the use of antibiotics that exert selective pressure for resistance development). Phage-based biocontrol could also enhance chemical-free water treatment and reuse[113] (e.g., to control *Nocardia* foaming in activated sludge systems[114] and harmful algal blooms[115] in source waters, as well as biofouling of filtration membranes, contactor surfaces,[116] or storage tanks). Microbiome manipulation could also bring significant benefits to energy production, ranging from enhanced carbon sequestration by increasing plant productivity, to mitigation of methane or sulfide emissions. Other microbial activities important to the energy industry that could be controlled theoretically via phages include those associated with hydrocarbon reservoir souring (mainly associated with sulfidogenic bacteria) and associated infrastructure corrosion (Figure 1).[117]

Synthetic biology could be a revolutionary approach to empower lysogenic and filamentous phages as gene delivery vectors to endow indigenous bacteria with enhanced fitness or novel metabolic capabilities for bioremediation or biorefining purposes. Other properties that could be engineered in phages include altering tail fibers to be used as biosensors or enabling phages to display proteins that serve as selective adsorbents to recover rare earth metals or other high-value elements (Figure 4). Nevertheless, genetic engineering of phages will likely face

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some regulatory and public acceptance hurdles, particularly if the genetic manipulations are transgenic rather than cisgenic.

Similar to other emerging technologies, phage-based biocontrol for nontraditional applications will need to carefully consider and mitigate potential system-specific failure modes and unintended consequences. Proactive risk assessment will be important to enhance public and regulatory acceptance. Overall, we have come a long way since d'Herelle first proposed phage therapy, and phage-based biocontrol is likely to experience a renaissance inspired by novel, chemical-free strategies to edit microbiomes and enhance, suppress, or modulate microbial processes important for sustainable development.

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Notes

The authors declare no competing financial interest.

Biography

Jacques Mathieu is the Chief Executive Officer of Sentinel Environmental, an environmental biotechnology company focused on developing microbiome engineering and editing tools. He obtained his PhD in environmental engineering from Rice University (2011) under the direction of Pedro J. Alvarez. Currently, he and his research team are developing a bacteriophage-based alternative to livestock antibiotic feed additives and a product to improve cattle feed efficiency through targeted rumen microbiome manipulations.

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