RNA and ssDNA Phages against Multi-Drug Resistant Bacteria

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RNA and single-stranded DNA (ssDNA) phages make up an understudied subset of bacteriophages that have been rapidly expanding in the last decade thanks to advancements in metaviromics. Antibiotics play a crucial role in treating bacterial infections, but their overuse and misuse have led to the emergence of antimicrobial resistance (AMR), resulting in reduced drug efficiency and persistent infections. Given the rapid evolution of AMR and the challenges of developing novel drugs, exploring alternative strategies becomes imperative. Phage therapy, utilizing phage-derived products and both natural and engineered phages for infection treatment, along with infection prevention through vaccines targeting antimicrobial-resistant pathogens, stands out as a promising frontier in the battle against AMR.

RNA phages   ssDNA phages   metaviromics   phage-based applications

1. Phage-Derived Lytic Enzymes as Antibacterial Agents

Phage-derived lysins, also known as "enzybiotics," present a promising alternative to conventional antibiotics. These enzymes exert their antibacterial effects by lysing host bacteria through the cleavage of peptidoglycan (PG), the primary structural component of the bacterial cell wall. Phage-derived lytic enzymes fall into two categories: virion-associated peptidoglycan hydrolases (VAPGHs) and endolysins. VAPGHs initiate degradation of PG at the onset of the phage infection [1][2], whereas endolysins act at the conclusion of the phage infection cycle, facilitating the release of mature phages [3]. In most cases, dsDNA phages encode distinct VAPGHs and endolysins, whereas dsRNA phages feature a single lytic protein that serves as both VAPGH and endolysin [4]. In contrast, ssDNA and ssRNA phages employ a single-gene lysis (Sgl) protein—an impactful degradative protein that induces cytolysis without enzymatically breaking down PGs [5][6] (**Figure 1**) .
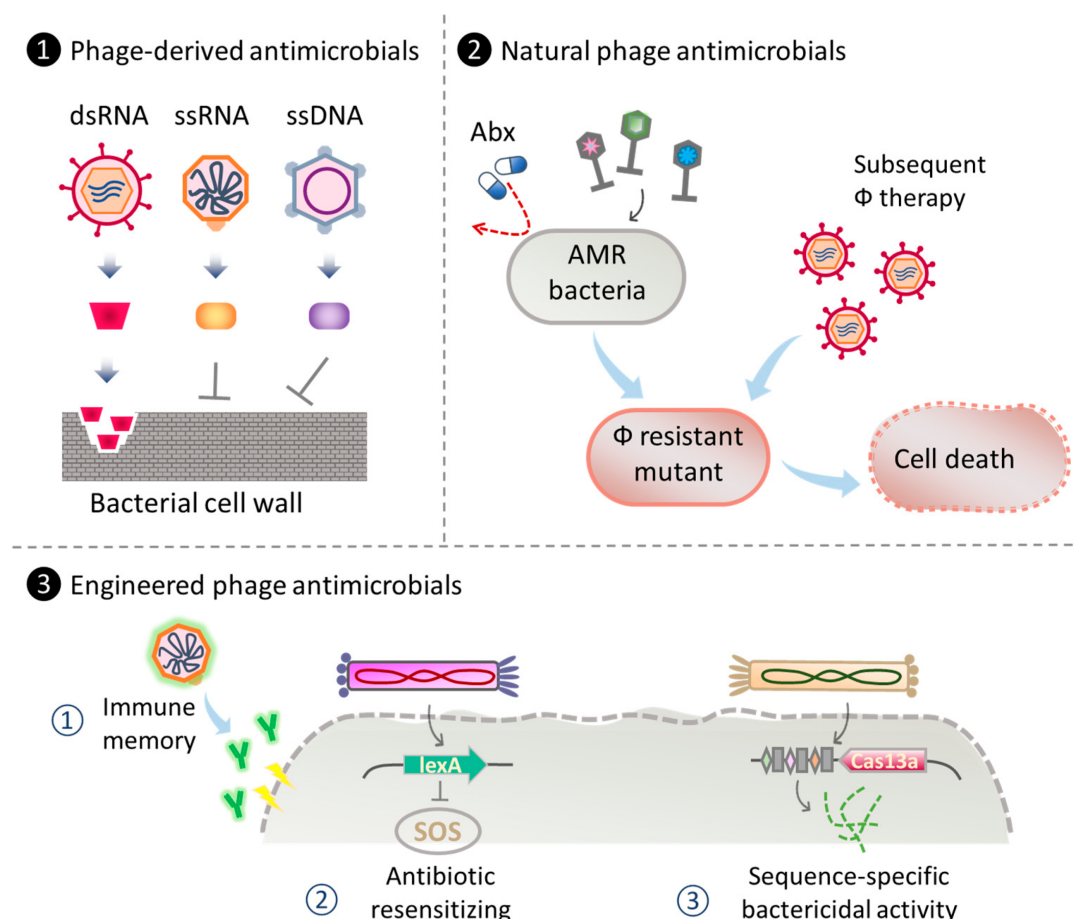
Figure 1. Strategies for phage therapy using ssDNA and RNA phages and their products. (1) Utilization of phage-derived components as antibacterial agents. For instance, the degradation of bacterial cell wall by dsRNA phage lytic enzyme [7] or inhibition of cell wall synthesis using single-gene lysis (Slg) protein from ssDNA and ssRNA phages [5][6][8]. (2) Phage cocktail employing natural ssDNA and RNA phages as antimicrobial therapeutics against AMR bacteria and phage-resistant mutants [9][10]. (3) Engineering ssDNA and RNA phages for antibacterial therapies. Examples include ① engineered phages displaying specific antigens as vaccine against drug-resistant bacteria [11][12]; ② engineered phages to enhance antibiotic efficacy in phage-antibiotic combinatory therapy [13]; ③ engineered antibacterial capsids carrying CRISPR/Cas13a for sequence-specific-based bacterial gene detection, bacterial flora modification, and treatment for AMR bacterial infections [14]. Abx: antibiotic.

Ply17, a lytic enzyme produced by *Pseudomonas* dsRNA phage phiYY, has demonstrated the capability to reduce the number of viable Gram-negative bacteria such as *P. aeruginosa* and *E. coli*, as well as Gram-positive bacteria including *S. aureus* and *S. epidermidis,* by approximately 2 logs when treated with an outer membrane permeabilizer such as EDTA [7]. Notably, recent genome analyses of the animal dsRNA viruses from the *Picobirnaviridae* and *Partitiviridae* families have unveiled potential antibacterial and antifungal lysis genes [15]. Upon cloning and expression of the lytic genes from *Picobirnavirus* and *Partitivirus* in *E. coli* DH5a, the observed growth inhibition was comparable to that induced by the *Enterobacteria* MS2 phage lysin L [16].

The bacteriolytic activity of ssRNA *Leviviridae* phages varies among different phages. For example, Sgl_M of phiM and Sgl_{PP7} of *Pseudomonas* phage PP7 exhibit bacteriolytic activity by inhibiting MurJ, the transporter responsible for moving lipid-binding peptidoglycan precursors from the inside to the outside of the plasma membrane [5][6]. On the other hand, $Sgl_{Q\beta}$ from Qβ phage exhibits bacteriolytic activity by non-competitively inhibiting MurA, the first enzyme in the PG biosynthetic pathway [17]. MS2 Sgl_{MS2} (lysin L) possesses an N-terminal heat shock-responsive chaperone, DnaJ, but its specific mechanism of action remains unclear. Other known Sgls include Sgl_{PRR1} (*Pseudomonas* phage PRR1), Sgl_{KU1} (Enterobacteria phage KU1), and Sgl_{Hgal1} (Enterobacteria phage Hgal1), whose mechanisms of action have not yet been elucidated [6].

$Sgl_{\phi174X}$, a bacteriolytic enzyme produced by the ssDNA phage phiX174, inhibits MraY, an enzyme catalyzing the initial step of the lipid cycle reaction in PG biosynthesis. This inhibition results in bacteriolysis [6][8]. As the cellular targets and modes of action of many ssRNA and ssDNA phage Slgs are still unidentified, future genome analyses hold great capacity for the discovery of new therapeutic candidates.

2. Natural ssDNA and RNA Phages as Antibacterial Agents

Pseudomonas phages phiYY [18] and phiZ98 [19], which belong to the dsRNA *Cystoviridae* family, utilize the lipopolysaccharide (LPS) core oligosaccharide of *P. aeruginosa* as the binding receptor. Smooth-colony-type *P. aeruginosa* strains exhibit resistance to phiYY due to the presence of the *galU* gene, which confers O-antigen to the LPS core oligosaccharide [9]. However, LPS-deficient *P. aeruginosa*, a strain that emerged during dsDNA phage therapy and lacks the *galU* gene [20], exposes its LPS core oligosaccharide on the cell surface, making it susceptible to phiYY [9]. Phage therapy employing phiYY has been reported to reduce bacterial load and alleviate clinical symptoms in patients diagnosed with interstitial lung disease (ILD) and chronic lung infections [10]. Moreover, a phage cocktail, including phiYY among five phages, has demonstrated effectiveness against a broad spectrum of *P. aeruginosa* clinical isolates and significantly impedes the emergence of phage-resistant mutants [9].

dsRNA phages have also been effectively applied as plant therapeutics. *Pseudomonas* phage phi6 has been employed to manage various plant diseases, including controlling *P. syringae pv. phaseolicola* (Pph), the causal agent of halo disease in common bean (*Phaseolus vulgaris*) [21]. Additionally, phi6 has been utilized against *P. syringae pv. actinidiae* (Psa), responsible for kiwifruit psyllid [22], and *P. syringae pv. syringae* (Pss), which causes early leaf symptoms of bean brown spot [21]. Other *Pseudomonas* phages, including phi8, phi12, phi13, phi2954, phiNN, and phiYY, have also shown efficacy against Pph [23]. Collectively, the application of dsRNA phages holds significant promise for complementing and enhancing phage therapy for both human and plant diseases.

3. Engineering ssDNA and RNA Phages for Antimicrobial Therapy

RNA phages exhibit significant potential as antigen carriers in the development of vaccines targeting drug-resistant pathogens. Huo and colleagues pioneered the development of Qβ phage presenting Qβ-glycan 1, a synthetic

tetrasaccharide from *Salmonella enteritidis*. This construct successfully induced robust IgG antibody responses in both mice and rabbits [11]. Notably, carbohydrate-based antigens, such as tetrasaccharide, do not directly interact with helper T cells, leading to limited immune memory. However, the conjugation of glycan antigens to a potent carrier such as Qβ enables engagement with both T-helper and B cells, resulting in a vigorous antibody response. Mice treated with post-immunization serum recovered from rabbits vaccinated with Qβ-glycan 1 demonstrated increased survival rates following administration of lethal doses of *S. enteritidis*. In another study, Rashidijahanabad and colleagues reported the production of Qβ-OSP, a Qβ phage presenting O-specific polysaccharide (OSP) 1 of *Vibrio cholerae*. Employed in mouse immunization, the Qβ-OSP conjugate elicited strong IgG antibody responses against *V. cholerae* O1 Inaba, reaching sufficient IgG antibody levels after just two administrations. Remarkably, the titer of IgG antibodies remained detectable up to day 265 [12].

Phagemid vectors derived from the filamentous ssDNA M13 phage serve as a highly efficient engineering platform for diverse therapeutic applications, including engineering phages for enhanced antibacterial activity. The delivery of antibacterial therapeutics can be achieved either through surface display on the virion or incorporated into the M13 phagemid itself. These displayed therapeutics may target vital cell components, virulent factors, or entire bacterial pathogens. Notably, several potential candidates have been identified for combating *S. aureus*, *P. aeruginosa*, *H. pylori*, and other pathogens. In another example, the dual display of two functional peptides enables M13 to (1) undergo endocytosis by eukaryotic cells and (2) impede infection caused by the intracellular pathogen *Chlamydia trachomatis* [24]. For an in-depth exploration of anti-infective development using phage display, refer to [25].

Antibacterial efficacy can also be achieved through cargoes loaded onto M13 phagemid. Lu and Collins cleverly designed an indirect antibacterial method using M13 phages overexpressing *lexA3*, a repressor of the SOS DNA repair system [13]. The authors demonstrated that suppressing the SOS network in *E. coli* with engineered *lexA3*-M13 significantly enhanced quinolone killing and increased the survival of infected mice. The engineered phage holds promise for targeting antibiotic-resistant bacteria and biofilm cells and modulating the bacterial population resistant to antibiotics post-treatment. In a parallel approach, Prokopczuk and colleagues employed a filamentous phage Pf for an indirect strategy to interfere with *P. aeruginosa* pathogenesis at burn wound sites [26]. Given that *P. aeruginosa* is a major cause of burn-related infections and sepsis, often exhibiting multi-drug resistance, the authors engineered a superinfective Pf phage (eSI-Pf). Administration of eSI-Pf effectively attenuated *P. aeruginosa* virulence, reduced bacterial load at the wound site, minimized bacterial dissemination from the burn site to internal organs, alleviated septicemia symptoms, and ultimately improved mouse survival.

Direct bactericidal effects have been achieved through several successful therapies, including the delivery of programmed CRISPR/Cas by recombinant M13 phages. In a proof-of-concept study on phage therapy for strain-specific depletion and genomic deletions in the gut microbiome, Lam and colleagues engineered M13 to deliver *gfp*-targeting CRISPR/Cas9 to bacteria residing in the gastrointestinal tract [27]. The engineered phage showed efficient sequence-specific targeting of GFP-expressing *E. coli* in the gut, confirming the capability of CRISPR/Cas9 to induce genomic deletions at the designated target site.

References

1. Rodríguez-Rubio, L.; Martínez, B.; Donovan, D.M.; Rodríguez, A.; García, P. Bacteriophage virion-associated peptidoglycan hydrolases: Potential new enzybiotics. Crit. Rev. Microbiol. 2013, 39, 427–434.

2. Latka, A.; Maciejewska, B.; Majkowska-Skrobek, G.; Briers, Y.; Drulis-Kawa, Z. Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process. Appl. Microbiol. Biotechnol. 2017, 101, 3103–3119.

3. Gerstmans, H.; Criel, B.; Briers, Y. Synthetic biology of modular endolysins. Biotechnol. Adv. 2018, 36, 624–640.

4. Caldentey, J.; Bamford, D.H. The lytic enzyme of the Pseudomonas phage b6. Purification and biochemical characterization. Biochim. Biophys. Acta (BBA)-Protein Struct. Mol. Enzymol. 1992, 1159, 44–50.

5. Chamakura, K.; Young, R. Phage single-gene lysis: Finding the weak spot in the bacterial cell wall. J. Biol. Chem. 2019, 294, 3350–3358.

6. Adler, B.A.; Chamakura, K.; Carion, H.; Krog, J.; Deutschbauer, A.M.; Young, R.; Mutalik, V.K.; Arkin, A.P. Multicopy suppressor screens reveal convergent evolution of single-gene lysis proteins. Nat. Chem. Biol. 2023, 19, 759–766.

7. Yang, Y.; Le, S.; Shen, W.; Chen, Q.; Huang, Y.; Lu, S.; Tan, Y.; Li, M.; Hu, F.; Li, Y. Antibacterial activity of a lytic enzyme encoded by Pseudomonas aeruginosa double stranded RNA Bacteriophage phiYY. Front. Microbiol. 2018, 9, 1778.

8. Orta, A.K.; Riera, N.; Li, Y.E.; Tanaka, S.; Yun, H.G.; Klaic, L.; Clemons, W.M. The mechanism of the phage-encoded protein antibiotic from ΦX174. Science 2023, 381, 180.

9. Yang, Y.; Shen, W.; Zhong, Q.; Chen, Q.; He, X.; Baker, J.L.; Xiong, K.; Jin, X.; Wang, J.; Hu, F.; et al. Development of a Bacteriophage Cocktail to Constrain the Emergence of Phage-Resistant Pseudomonas aeruginosa. Front. Microbiol. 2020, 11, 327.

10. Li, L.; Zhong, Q.; Zhao, Y.; Bao, J.; Liu, B.; Zhong, Z.; Wang, J.; Yang, L.; Zhang, T.; Cheng, M.; et al. First-in-human application of double-stranded RNA bacteriophage in the treatment of pulmonary Pseudomonas aeruginosa infection. Microb. Biotechnol. 2023, 16, 862–867.

11. Huo, C.-X.; Dhara, D.; Baliban, S.M.; Nick, S.T.; Tan, Z.; Simon, R.; Misra, A.K.; Huang, X. Synthetic and immunological studies of: Salmonella Enteritidis O-antigen tetrasaccharides as potential anti- Salmonella vaccines. Chem. Commun. 2019, 55, 4519–4522.

12. Rashidijahanabad, Z.; Kelly, M.; Kamruzzaman, M.; Qadri, F.; Bhuiyan, T.R.; McFall-Boegeman, H.; Wu, D.; Piszczek, G.; Xu, P.; Ryan, E.T.; et al. Virus-like Particle Display of Vibrio cholerae O-

Specific Polysaccharide as a Potential Vaccine against Cholera. ACS Infect. Dis. 2022, 8, 574–583.

13. Lu, T.K.; Collins, J.J. Engineered Bacteriophage Targeting Gene Networks as Adjuvants for Antibiotic Therapy. Proc. Natl. Acad. Sci. USA 2009, 106, 4629–4634.

14. Kiga, K.; Tan, X.-E.; Ibarra-Chávez, R.; Watanabe, S.; Aiba, Y.; Sato'o, Y.; Li, F.-Y.; 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, 1–11.

15. Neri, U.; Wolf, Y.I.; Roux, S.; Camargo, A.P.; Lee, B.; Kazlauskas, D.; Chen, I.M.; Ivanova, N.; Allen, L.Z.; Paez-Espino, D.; et al. Expansion of the global RNA virome reveals diverse clades of bacteriophages. Cell 2022, 185, 4023–4037.e18.

16. Gan, T.; Wang, D. Picobirnaviruses encode proteins that are functional bacterial lysins. Proc. Natl. Acad. Sci. USA 2023, 120, e2309647120.

17. Bernhardt, T.G.; Wang, I.-N.; Struck, D.K.; Young, R. A Protein Antibiotic in the Phage Q Virion: Diversity in Lysis Targets. Science 2001, 292, 2326–2329.

18. Yang, Y.; Lu, S.; Shen, W.; Zhao, X.; Shen, M.; Tan, Y.; Li, G.; Li, M.; Wang, J.; Hu, F.; et al. Characterization of the first double-stranded RNA bacteriophage infecting Pseudomonas aeruginosa. Sci. Rep. 2016, 6, 38795.

19. Li, D.; Li, Y.; Li, P.; Han, Q.; Zhang, T.; Yang, B.; Wu, W.; Yang, H. Phage phiZ98: A novel tri-segmented dsRNA cystovirus for controlling Pseudomonas strains with defective lipopolysaccharides in foods. Food Res. Int. 2022, 162, 112197.

20. Shen, M.; Zhang, H.; Shen, W.; Zou, Z.; Lu, S.; Li, G.; He, X.; Agnello, M.; Shi, W.; Hu, F.; et al. Pseudomonas aeruginosa MutL promotes large chromosomal deletions through non-homologous end joining to prevent bacteriophage predation. Nucleic Acids Res. 2018, 46, 4505–4514.

21. Pinheiro, L.A.M.; Pereira, C.; Frazão, C.; Balcão, V.M.; Almeida, A. Efficiency of phage φ6 for biocontrol of pseudomonas syringae pv. Syringae: An in vitro preliminary study. Microorganisms 2019, 7, 286.

22. Pinheiro, L.A.M.; Pereira, C.; Barreal, M.E.; Gallego, P.P.; Balcão, V.M.; Almeida, A. Use of phage φ6 to inactivate Pseudomonas syringae pv. actinidiae in kiwifruit plants: In vitro and ex vivo experiments. Appl. Microbiol. Biotechnol. 2019, 104, 1319–1330.

23. Villalpando-Aguilar, J.L.; Matos-Pech, G.; López-Rosas, I.; Castelán-Sánchez, H.G.; Alatorre-Cobos, F. Phage Therapy for Crops: Concepts, Experimental and Bioinformatics Approaches to Direct Its Application. Int. J. Mol. Sci. 2022, 24, 325.

24. Bhattarai, S.R.; Yoo, S.Y.; Lee, S.W.; Dean, D. Engineered phage-based therapeutic materials inhibit Chlamydia trachomatis intracellular infection. Biomaterials 2012, 33, 5166–5174.

25. Huang, J.X.; Bishop-Hurley, S.L.; Cooper, M.A. Development of anti-infectives using phage display: Biological agents against bacteria, viruses, and parasites. Antimicrob. Agents Chemother. 2012, 56, 4569–4582.

26. Prokopczuk, F.I.; Im, H.; Campos-Gomez, J.; Orihuela, C.J.; Martínez, E. Engineered Superinfective Pf Phage Prevents Dissemination of Pseudomonas aeruginosa in a Mouse Burn Model. mBio 2023, 14, e0047223.

27. Lam, K.N.; Spanogiannopoulos, P.; Soto-Perez, P.; Alexander, M.; Nalley, M.J.; Bisanz, J.E.; Nayak, R.R.; Weakley, A.M.; Yu, F.B.; Turnbaugh, P.J. Phage-delivered CRISPR-Cas9 for strain-specific depletion and genomic deletions in the gut microbiome. Cell Rep. 2021, 37, 109930.

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