Abstract
Bacteriophages (phages) are viruses that infect bacterial hosts, and since their discovery over a century ago they have been primarily exploited to control bacterial populations and to serve as tools in molecular biology. In this commentary, we highlight recent diverse advances in the field of phage research, going beyond bacterial control using whole phage, to areas including biocontrol using phage-derived enzybiotics, diagnostics, drug discovery, novel drug delivery systems and bionanotechnology.

Keywords
bacteriophage, phage, phage research, biocontrol, bionanotechnology, bacterial diagnostics, phage-based drug delivery, biotechnology
Corresponding author: Aidan Coffey (aidan.coffey@cit.ie)

Competing interests: The authors declare that they have no competing interests.

Grant information: L. O'Sullivan is supported by a Teagasc Walsh Fellowship Ref. 2013003. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2016 O'Sullivan L et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: O'Sullivan L, Buttimer C, McAuliffe O et al. Bacteriophage-based tools: recent advances and novel applications [version 1; peer review: 3 approved] F1000Research 2016, 5(F1000 Faculty Rev):2782 https://doi.org/10.12688/f1000research.9705.1

First published: 29 Nov 2016, 5(F1000 Faculty Rev):2782 https://doi.org/10.12688/f1000research.9705.1
Introduction

Bacteriophages (phages) are viruses that specifically infect bacteria. After their discovery in 1915 by Twort and 1917 by d’Herelle, these agents were initially used to treat bacterial infections, although widespread acceptance was limited owing to lack of understanding of phage biology and the development of antibiotic therapy in the 1940s. With antibiotic resistance becoming problematic in the late twentieth century, there was a renewed interest in phage therapy research. Alongside this application, and indeed the fundamental role that phage research played in the understanding of molecular biology, phage research has led to the development of new technologies not only for therapy and biocontrol but also for bacterial detection, drug delivery, drug discovery, and nanotechnology.

Antibacterials and biocontrol

In addition to the well-documented cases of using wild-type phages as tools to eliminate pathogenic bacteria in infected humans and in foods, the phage-encoded peptidoglycan hydrolases called endolysins have also been exploited in purified form to rapidly lyse bacterial cells. The Gram-positive phage endolysins generally contain at least one enzymatic domain and a cell-wall-binding domain. Chimeric endolysins have recently been developed by fusing enzymatic domains to alternative cell-wall-binding determinants, thus altering endolysin behaviour and host range. In the case of Gram-negative bacteria, the outer membrane is a barrier to exogenously added endolysin reaching the peptidoglycan target. Thus, the fusion of polycationic peptides to the Gram-negative endolysin facilitates outer membrane penetration allowing these new so-called Artilysin®s access to the Gram-negative peptidoglycan. Recent research has also reported a phage endolysin (from a Streptococcus pyogenes phage) with the ability to cross mammalian cell membranes. Its endolysin, PlyC, was found to consist of two subunits, one of which is proposed to bind to the eukaryotic cell membrane, facilitating entry by endocytosis. These are major breakthroughs in endolysin research, and, with further investigation and testing, similar enzymes may be discovered/engineered and used in the future to, respectively, treat infections caused by Gram-negative bacteria and intracellular bacterial infections.

A recent advance in antibiotic therapy has been the exploitation of phages to control antibiotic-resistant bacteria. Phages have been engineered to deliver CRISPR-Cas nucleases into antibiotic-resistant bacterial cells, and, in doing so, researchers have been able to harness the specific DNA-cleaving capacity of CRISPRs to knock out antibiotic resistance sequences, rendering resistant organisms antibiotic sensitive. The use of phages as delivery vehicles ensures the specificity required in biocontrol. The wider exploitation of phages as delivery systems is discussed below.

Bacterial diagnostics

Phage virions and their encoded proteins can also be useful for the detection and specific identification of bacteria. The simplest of these is where a standard number of specific phages are incubated with a food material or some other test sample. If the bacterial target is present and viable, detectable phage numbers will increase through amplification on the pathogen. Modifications of this method can generate results more rapidly, and in the case of Yersinia pestis, Sergueev et al., for example, developed a quantitative real-time PCR to detect the increase in phage DNA instead of traditional plaque assays. Reporter phages can also detect bacteria through infection without needing cell lysis and progeny phages. In this case, the phage genomes are modified to carry a bioluminescence or fluorescence gene that the phage alone cannot express. Upon injection of the phage DNA into its host, active bioluminescent or fluorescent proteins are synthesized, facilitating visual detection. Recently, Zhang et al. engineered an Escherichia coli 0157:H7 reporter phage containing Luciferase NanoLuc (Nluc) and with it detected as few as five CFU of the E. coli by bioluminescence in a complex food matrix within nine hours.

Reported receptor-binding proteins (RBPs) have also been used successfully in bacterial detection and identification. The receptor-binding domain of the RBP in Campylobacter phage NCTC12673 was used to create a simple glass slide agglutination test for Campylobacter, and when fused to green fluorescent protein, the receptor-binding domain allowed the detection of Campylobacter jejuni and Campylobacter coli isolates through fluorescent microscopy. Phages, because of their vast diversity, provide a plentiful source of host-specific proteins to create simple identification tests such as the agglutination assay mentioned above specifically for Campylobacter. In this regard, whole phage and phage RBPs have been successfully attached to biosensing surfaces for bacterial detection, allowing for high specificity. Of the two, the RBPs are simpler and easier to attach. In addition, they can be recombinantly produced and are reported to have better stability than antibodies. Optimization of phage densities and attachment to biosensing surfaces is still ongoing.

In the context of detection, the phage endolysins (discussed earlier) can also have a role when used instead of traditional DNA extraction reagents. It was shown that the peptidoglycan of Staphylococcus aureus is degraded more rapidly by staphylococcal endolysin ClyH than by lysostaphin, thus shortening the DNA sample preparation for real-time PCR when the endolysin was employed. Phage display, which involves genetically modifying a phage virion so that a foreign peptide is displayed on the surface (discussed further below), can also be exploited in bacterial detection systems. Lee et al. created a phage that displayed two different peptides, one with an affinity to gold nanoparticles and another with specificity to a target protein. By measuring the
ultraviolet absorbance of this phage, they could detect as little as 25 femtomoles of their target antigen. These modified phages have also been incorporated into systems capable of in-the-field real-time detection using engineered phage displaying peptides capable of binding to a magnetoelectric resonator as well as the target analytes, such as bacteria and endospores.

Drug discovery and phage-based drug delivery systems

Since phage display was first described in 1985 by Smith, it has seen numerous applications in the identification of receptor and ligand interactions of infectious diseases and cancers, with these developments allowing for drug discovery and vaccine design. Phage display is now allowing the modification of phages into vehicles for chemotherapeutic drug delivery by the attachment of a drug to the phage surface and presentation of peptides on the surface of that phage with specificity to a ligand of interest. Such constructs have even been designed to target non-host bacteria, including mammalian cells. These phages, displaying therapeutic peptides, can even be designed to pass the blood–brain barrier, and such constructs could thus have potential in the treatment of diseases such as Alzheimer’s and Parkinson’s. Phages with an affinity to specific cell receptors, such as those overexpressed in cancer cells, may be exploited beyond drug delivery to allow for simultaneous target detection by displaying diagnostic reporter molecules or by detection of bound phage DNA by real-time PCR.

Empty phage capsids are also being employed as carriers, with studies demonstrating the potential to encapsulate RNA molecules, peptides, and therapeutic compounds. Phage capsids or virus-like particles have also been modified to present ligands on their surface to allow the delivery of encapsulated RNA-guided endonucleases to specific cell types for genome editing. When phages are used as nanocarriers to deliver chemotherapeutic drugs for cancer treatment, drug half-life is extended and toxicity is focused only on the site of interest, lessening damage to body tissues. Capsid-based carriers have also been developed by fusing drug-loaded liposomes to capsid proteins displaying peptides with binding specificity to a particular target.

Biotechnology

Genetically modified filamentous phages have been used in material synthesis to construct nanowires and films for semiconductor applications, piezoelectric energy generation, and photo-response properties. These materials have been used to create devices such as ion batteries and catalysts, with phage M13-based nanowires also being constructed into scaffolding to allow guided cell growth for human tissue formation.

Phage-derived enzymes, which have formed part of a core toolbox in traditional molecular biology, are now being applied to novel concepts. Phage RNA polymerase and ribonuclease H are being used to create in vitro genetic circuits that have potential future applications in nanodevices and the regulation of processes within artificial cells. Recombinases are seeing use in these constructions by extending memory capabilities to these circuits. These enzymes are also being used to create novel tools for bacterial genome editing and accelerated evolution. It is noteworthy that many past phage-dedicated reviews have not satisfactorily encompassed the recent advances of phage applications in nanomedicine; a recent excellent article comments in a comprehensive way on the many roles and opportunities of phages in nanomedicine; a recent excellent article comments in a comprehensive way on the many roles and opportunities of phages in nanotherapeutics, bioimaging probes, biomimetic biomaterials, tissue regenerative scaffolds, matrices for directing stem cell fate, and probes for detecting disease biomarkers, among numerous others.

Summary

This commentary provides a snapshot of the increasing diversity of phage research in recent years and shows that it is advancing rapidly and that new applications are being reported frequently. Since the discovery of phages a century ago, their research focus has diversified from applying these agents to simply treat bacterial infections to a broad range of useful functions including biocontrol, diagnostics, drug discovery, and drug delivery as well as several applications in nanomedicine.

Competing interests

The authors declare that they have no competing interests.

Grant information

L. O’Sullivan is supported by a Teagasc Walsh Fellowship Ref. 2013003.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Wittebole X, De Roock S, Opal SM: A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virol. 2014; 8(1): 226–35. Published Abstract | Publisher Full Text | Free Full Text
2. Davies J, Davies D: Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010; 74(3): 417–53. Published Abstract | Publisher Full Text | Free Full Text
3. Abedon ST, Kuhl SJ, Blasdel BG, et al: Phage treatment of human infections. Bacteriophage. 2011; 1(2): 66–85. Published Abstract | Publisher Full Text | Free Full Text
4. Microsol: LISTEX: Nature’s solution for Listeria. 2007. Reference Source
5. Loeffler JM, Nelson D, Fischetti VA: Rapid killing of Streptococcus pneumoniae with a bacteriophage cell wall hydrolase. Science. 2001; 294(5549): 2170–2172. Published Abstract | Publisher Full Text
6. Dong Q, Wang J, Yang H, et al: Construction of a chimeric lysin Ply187N-V12C with extended lytic activity against staphylococci and streptococci. Microb Biotechnol. 2015; 8(2): 210–20. Published Abstract | Publisher Full Text | Free Full Text
7. Briers V, Walmagh M, van Puyvelde V, et al: Engineered endolysin-based...
“Artlysins” to combat multidrug-resistant gram-negative pathogens. MBio. 2014; 5(4): e01379–14. Published Abstract | Publisher Full Text | Free Full Text

8. Shen Y, Barros M, Vinenmann T, et al.: A bacteriophage endolysin that eliminates intracellular streptococci. eLife. 2016; 5: p. e13152. Published Abstract | Publisher Full Text | Free Full Text

9. Bikard D, Euler CW, Jiang W, et al.: Exploiting CRISPR-Cas nucleases to produce sequence-specific anti-microbials. Nat Biotechnol. 2014; 32(11): 1146–50. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

10. Singueev KV, He Y, Borschel RH, et al.: Rapid and sensitive detection of Yersinia pestis using amplification of plague diagnostic bacteriophages monitored by real-time PCR. PLoS One. 2010; 5: e11337. Published Abstract | Publisher Full Text | Free Full Text

11. Ho SL, Li Y, Varanasi JK, et al.: Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazocyanurazine substrate. ACS Chem. Biol. 2012; 7(11): 1848–57. Published Abstract | Publisher Full Text | Free Full Text

12. Zhang D, Coronel-Aguilera CP, Romero PL, et al.: The Use of a Novel NanoLuc-Based Reporter Phage for the Detection of Escherichia coli O157:H7. Sci Rep. 2010; 6: 32325. Published Abstract | Publisher Full Text | Free Full Text

13. Jain P, Hartman TE, Eisenberg N, et al.: eGFP10, a high-intensity fluorophore, enables detection and rapid drug susceptibility testing of Mycobacterium tuberculosis directly from sputum samples. J Clin Microbiol. 2012; 50(4): 1362–9. Published Abstract | Publisher Full Text | Free Full Text

14. Javed MA, Poshtiban S, Arutyunov D, et al.: Bacteriophage receptor binding protein based assays for the simultaneous detection of Campylobacter jejuni and Campylobacter coli. PLoS One. 2013; 8(7): e69770. Published Abstract | Publisher Full Text | Free Full Text

15. Singh A, Poshtiban S, Eovv S: Recent advances in bacteriophage based biosensors for food-borne pathogen detection. Sensors (Basel). 2013; 13(2): 1763–86. Published Abstract | Publisher Full Text | Free Full Text

16. Olsson AL, Wargenaus A, Tufekji N: Optimizing Bacteriophage Surface Densities for Bacterial Capture and Sensing in Quartz Crystal Microbalance with Dissipation Monitoring, ACS Appl Mater Interfaces. 2016; 8(22): 13698–708. Published Abstract | Publisher Full Text | Free Full Text

17. Hu Y, Yang H, Wang J, et al.: Comparison between a chimeric lysin ClyH and other enzymes for extracting DNA to detect melioidosis resistant Staphylococcus aureus by quantitative PCR. World J Microbiol Biotechnol. 2016; 32(1): 1. Published Abstract | Publisher Full Text

18. Lee JH, Dommale DW, Cha JN: Amplified protein detection and identification through DNA-conjugated M13 bacteriophages. ACS Nano. 2012; 6(6): 5621–6. Published Abstract | Publisher Full Text

19. Li S, Li Y, Chen H, et al.: Direct detection of Salmonella typhimurium on fresh produce using phage-based magnetoelectric biosensors. Biosens Bioelectron. 2010; 25(4): 1313–9. Published Abstract | Publisher Full Text

20. Park M, Park JW, Wilde HC, et al.: Evaluation of phage-based magnetoelectric biosensors for direct detection of Salmonella Typhimurium on spinach leaves. Sensors and Actuators B: Chemical. 2013; 176: 1134–40. Published Full Text

21. Shen W, Lakshmanan RG, Mathison LC, et al.: Phage coated magnetoelectric micro-biosensors for real-time detection of Bacillus anthracis spores. Sensors and Actuators B: Chemical. 2009; 137(2): 501–6. Published Abstract | Publisher Full Text

22. Smith GP: Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science. 1985. 228(4705): 1315–7. Published Abstract | Publisher Full Text

23. Mullen LM, Nair SP, Ward JM, et al.: Phage display in the study of infectious diseases. Trends Microbiol. 2006; 14(3): 141–7. Published Abstract | Publisher Full Text

24. Yp VL, Ward RL: Application of phage display technology to cancer research. Curr Pharm Biotechnol. 2002; 3(1): 29–43. Published Abstract | Publisher Full Text

25. Omidfar K, Daneshpour M: Advances in phage display technology for drug discovery. Expert Opin Drug Discov. 2015; 10(6): 651–69. Published Abstract | Publisher Full Text

26. Gao J, Wang Y, Liu Z, et al.: Phage display and its application in vaccine design. Ann Microbiol. 2010; 60(1): 13–9. Published Full Text

27. Vale L, Benhar I: In vivo characteristics of targeted drug-carrying filamentous bacteriophage nanomedicines. J Nanobiotechnology. 2011; 9: 58. Published Abstract | Publisher Full Text | Free Full Text

28. Koonzovskyz A, Walbridge S, Saunders RC, et al.: Convection-enhanced delivery of M13 bacteriophage to the brain. J Neurosurg. 2012; 117(2): 197–203. Published Abstract | Publisher Full Text | Free Full Text

29. Hosoya H, Dobroff AS, Driessen WH, et al.: Integrated nanotechnology platform for tumor-targeted multimodal imaging and therapeutic cargo release. Proc Natl Acad Sci U S A. 2016; 113(9): E1315–25. Published Abstract | Publisher Full Text | Free Full Text

30. Bravino M, Chi JN: Real-time fentomolar detection of cancer biomarkers from photoconjugated antibody-phage constructs. Analyst. 2016. Published Abstract | Publisher Full Text

31. Wei B, Wei Y, Zhang K, et al.: Development of an antisense RNA delivery system using conjugates of the MS2 bacteriophage capsids and HIV-1 Tat cell-penetrating peptide. Biomed Pharmacother. 2009; 63(4): 313–8. Published Abstract | Publisher Full Text

32. DePorter SM, McNaughton BR: Engineered M13 bacteriophage nanocarriers for intracellular delivery of exogenous proteins to human prostate cancer cells. Biomater Chem. 2014; 25(9): 1620–5. Published Abstract | Publisher Full Text

33. Stephanopoulos N, Tong GJ, Hsiao SC, et al.: Dual-surface modified virus capsids for targeted delivery of photodynamic agents to cancer cells. ACS Nano. 2012; 4(10): 6014–20. Published Abstract | Publisher Full Text | Free Full Text

34. Qin S, Mettineen HM, Wilkinson RA, et al.: Programmed Self-Assembly of an Active P22-Cas9 Nanocarrier System. Mol Pharm. 2016; 13(3): 1191–6. Published Abstract | Publisher Full Text | Free Full Text

35. Lu RM, Chen MS, Chang DK, et al.: Targeted drug delivery systems mediated by a novel Peptide in breast cancer therapy and imaging. PLoS One. 2013; 8(6): e66128. Published Abstract | Publisher Full Text | Free Full Text

36. Wang T, D’Souza GG, Bedi D, et al.: Enhanced binding and killing of target tumor cells by drug-loaded liposomes modified with tumor-specific phage fusion coat protein. Nanomedicine. 2010; 5: 33235. Published Abstract | Publisher Full Text | Free Full Text

37. Lee BY, Zhang J, Zueger C, et al.: Virus-based piezoelectric energy generation. Nat Nanotechnol. 2012; 7(6): 351–6. Published Abstract | Publisher Full Text

38. Murugesan M, Abbini M, Nimmo SL, et al.: Virus-based photo-responsive nanowires formed by linking site-directed mutagenesis and chemical reaction. Sci Rep. 2013; 3: 1820. Published Abstract | Publisher Full Text | Free Full Text

39. Lee Y, Kim J, Yun DS, et al.: Virus-templated Au and Au/Pt Core/shell Nanowires and Their Electrocatalytic Activities for Fuel Cell Applications. Energy Environ Sci. 2012; 5(8): 8262–74. Published Abstract | Publisher Full Text | Free Full Text

40. Nam KT, Kim D, Yoo PJ, et al.: Virus-enabled synthesis and assembly of nanowires for lithium ion battery electrodes. Science. 2006; 312(5775): 865–8. Published Abstract | Publisher Full Text

41. Yoo SY, Chung W, Kim TH, et al.: Facile patterning of genetically engineered M13 bacteriophage for directional growth of human fibroblast cells. Soft Matter. 2011; 7(2): 363–8. Published Full Text

42. Kim J, Winfree E: Synthetic in vitro transcriptional oscillators. Mol Syst Biol. 2011; 7: 485. Published Abstract | Publisher Full Text | Free Full Text

43. Siuti P, Yazbek J, Lu TK: Synthetic circuits integrating logic and memory in living cells. Nat Biotechnol. 2013; 31(5): 448–52. Published Abstract | Publisher Full Text

44. Esvelt KM, Carlson JC, Liu DR: A system for the continuous directed evolution of biomolecules. Nature. 2011; 472(7344): 499–503. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

45. Sunderland K, Yang M, Mao C: Phage-Enabled Nanomedicine: From Probes to Therapeutics in Precision Medicine. Angew Chem Int Ed Engl. 2016. Published Abstract | Publisher Full Text
Open Peer Review

Current Peer Review Status: ✔ ✔ ✔

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of Faculty Opinions. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

**Version 1**

1. **Laurent Debarbieux**  
   Department of Microbiology, Interactions Bacteriophages Bacteria in Animals, Institut Pasteur, Paris, France  
   **Competing Interests:** No competing interests were disclosed.

2. **Sam R Nugen**  
   Department of Food Science, University of Massachusetts, Amherst, MA, USA  
   **Competing Interests:** No competing interests were disclosed.

3. **Rob Lavigne**  
   Laboratory of Gene Technology, Katholieke Universiteit Leuven, Leuven, Belgium  
   **Competing Interests:** No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com