ZKBS
Zentrale Kommission für die Biologische Sicherheit

Ref. 08020204.0005.0003 May 2026

ZKBS position statement on the risk assessment of viruses from bacteria and archaea as donor and recipient organisms and of propagated phage particles in phage preparations in accordance with § 5 (1) GenTSV

The viruses of prokaryotes comprise the bacteriophages of bacteria ('phages' for short) and archaea viruses of archaea. They are ubiquitous and, with an estimated number of 10^{31} particles, represent the largest and most diverse group of 'organisms' on Earth [1–4]. They play an important role in the balance of prokaryotic populations and communities in the environment, in the emergence and maintenance of microbial diversity, and in the maintenance of biogeochemical cycles.

1 Phages

1.1 Structure of phages

Bacteriophages consist of a protein coat enclosing nucleic acid, which can be either double-stranded (ds) or single-stranded (ss) DNA or RNA, in a linear or circular conformation. The genome sizes of phages cultivated to date range from approx. 5 kb (e.g. phage PhiX174, 5386 bases ssDNA) up to 500 kbp (e.g. phage G of *Bacillus megaterium*, 497 kbp dsDNA), although metagenomic analyses have also identified genome sizes of up to 700 kbp dsDNA [5]. The small phages possess filamentous or polyhedral phage capsids. The morphology of the larger phages is more complex [6]. The vast majority of these phages belong to the class *Caudoviricetes* within the phylum *Duplodnaviria.* These are tailed phages whose capsid consists of an icosahedral head and a tail (with an attachment system) of varying morphology and complexity.

1.2 Reproduction of phages of the class *Caudoviricetes*

This section primarily describes the reproduction of phages of the class *Caudoviricetes*, as they are numerically dominant and their mode of reproduction is relevant to the risk assessment of phages. The replication of a phage proceeds through several steps, from infection to the release of the phage progeny [7]. First, the phage binds to a specific receptor on the host cell surface (adsorption) and the nucleic acid enters the cell, e.g., by injection or by being drawn in. This is followed by the expression of phage genes and the replication of the phage

genome, usually via multimeric intermediates (concatemers). In parallel, the capsid proteins are formed, from which heads and tails are assembled. During the packaging of the nucleic acid into the heads, the concatemers are processed into monomers. This occurs via the headful mechanism (see section 1.3.1) or through sequence-specific cleavage of the concatemers. The latter results in uniform genomes with either cohesive single-stranded ends (e.g. phage λ) or double-stranded redundancies at the ends (e.g. phage T7). The newly assembled phage particles are typically released via host cell lysis mediated by phage-specific proteins. The average number of phage progeny per infected cell (burst size) and the time span from adsorption to lysis (latency period) are characteristic of each phage.

Phages that reproduce exclusively by producing infectious offspring (lytic cycle) are known as virulent phages. A so-called temperate phage can, in rare cases, undergo a replication cycle alternative to the lytic cycle. During this process, the phage DNA integrates into the host genome at a specific site (attachment site) within the infected cell, or it persists in the host cell as a plasmid. In both cases, the DNA is passively replicated during host cell division and is then referred to as a prophage. A host carrying a prophage is termed ‘lysogenic’, as the prophage can be induced under certain stress conditions for the host cell (e.g. DNA damage caused by UV radiation) and revert to the lytic cycle.

1.3 Gene transfer via transduction

In their natural habitat, phages contribute significantly to horizontal gene transfer via transduction. During transduction, sections of the host bacterium’s genome are packaged into phage particles and transported to the next host upon infection. A distinction is made here between two mechanisms of transduction [8].

1.3.1 General transduction

In general transduction, some phage heads contain only a DNA fragment from the host bacterium that is as long as the phage genome. General transduction is restricted to phages that, with the aid of a pac-type processive terminase, recognise, bind to and cleave their concatemer replication intermediates at a specific pac site, and then package them into the heads via the headful mechanism [9, 10]. Once the head is filled with DNA – whereby the length of the packaged DNA may exceed the length of the phage genome by a few percent – the pac-type terminase cuts the DNA again without depending on a recognition sequence [10]. After the DNA has been packaged, the tail is attached to the phage head, resulting in the formation of a mature phage particle. After the head has been filled, the pac-type terminase remains bound to the concatemer and packages the next genome [10]. The genomes of the phages resulting are redundant and cyclically permuted, i.e. they begin at different positions on the genetic map. The terminases can also recognise pac-like sequences in the bacterial chromosome and thus package random chromosomal sequences upstream of such pseudo-pac sites. It can therefore be assumed that only phages with pac-type terminases are capable of general transduction. Using bioinformatic tools, it is possible to determine from phage sequencing data whether the phage genome is presumed to be packaged in the pac-type manner and whether the phage is therefore capable of general transduction [11]. Also plasmids can be transferred via transduction.

A lysate of a generally transducing phage contains only a small proportion of transducing particles carrying fragments of the genome of the previous host bacterium, e.g. 0.3% to 0.5% for phage P1 [12] and 0.7% for phage P22 [13]. The transducing particles can adsorb to the host cell and inject the DNA in the same way as infectious phages. The transduced DNA can subsequently be integrated into the host genome via homologous recombination or form a plasmid again. The transduction frequency of bacterial markers ranges e.g. from 10^{-4} to 10^{-5} per infectious phage particle for phage P1 [12]. Only for a small number of phages it has been investigated if they are capable of general transduction, but the capacity appears to be widespread. For instance, 99% of the phages induced from lysogenic *Salmonella* isolates were generally transducing [14, 15] in contrast to 69% and 24%, respectively, from *Escherichia coli* isolates [14, 15].

Only recently, a subtype of general transduction, known as lateral transduction, has been described for certain temperate *Staphylococcus* phages [16]. These phages replicate their DNA whilst still in the integrated prophage state, resulting in multiple integrated phage genomes [17]. Some prophages are excised and packaged. In other, still-integrated prophages, the pac-type processive terminase binds to the phage-specific pac sites and begins packaging whilst still in the integrated form [17]. Phage heads are then filled, according to the pac-type headful mechanism as described above, initially with phage DNA and, as the replication fork progresses and due to the processive terminase, with host chromosomal DNA. Lateral transduction is approximately 1,000 times more efficient (transductants per infectious phage particle) than general transduction [16, 17].

1.3.2 Specific transduction

The second mechanism of transduction, specific transduction, is restricted to temperate phages. Upon induction of the prophage, in some cases the excision of the prophage from the genome may proceed incorrectly, resulting in regions of the host genome adjacent to the phage insertion site (attachment site) being excised and packaged along with it. The best-studied example is the specific transduction of the λ phage [18]. Its attachment site lies between the *gal* and *bio* operons of *E. coli*. Accordingly, only genes from these operons can be transferred via specific transduction with the λ phage genome [19].

1.4 Lysogenic conversion

Some genomes of temperate phages contain, in addition to the genes required for phage reproduction, genes known as morones [20, 21] or auxiliary metabolic genes (AMG [22]). These are expressed in lysogenic host bacteria and result in altered fitness or other phenotypes (lysogenic conversion). The best-known morones are the toxin genes of some temperate phages, which can cause an apathogenic bacterium to become pathogenic. Examples of prophage-encoded toxins include botulinum toxin, diphtheria toxin, cholera toxin and shiga toxin [21]. Another example are genes for photosynthesis, encoded in the genomes of cyanophages [23]. Lysogenic conversion can also lead to increased biofilm formation, higher colonisation frequencies or the expansion of the host range of lysogenic bacteria [24–26].

1.5 Host range and stability of phages

The vast majority of phages have a so-called ‘narrow host range’, which encompasses only a few strains of a species. However, there are also phages with a ‘broad host range’, which can span species or genera [27]. For example, 23 strains of different species were tested to determine the host range of phage P1 [12]. P1 was able to adsorb to and inject DNA into 21 of these strains. Infectious phage progeny was formed in 14 species. These 14 different species originated from nine different genera in the order *Enterobacterales* and two from the Pseudomonad group. The phage Mu forms plaques on various strains of species belonging to the order *Enterobacterales*, e.g. *E. coli*, *Serratia marcescens*, *Salmonella* Typhi, *Enterobacter cloacae* and on some *Erwinia* species [28].

The stability of phages in the environment depends on the phage itself and the environmental conditions. Sunlight has a particularly detrimental effect on phage survival, mainly due to its UV component [29, 30]. Phages can often survive for long periods outside their host, e.g. weeks or months in soil, water or cell-free culture media [31, 32] or years in spray-dried formulations [33]. Some phages are known to be resistant to heat or drought [34, 35]. For example, phages of lactic acid bacteria often survive heat treatment for 15 minutes at 90°C. However, treatment at 90°C for 45 minutes inactivated all thermotolerant phages tested [36].

1.6 Applications of phages

Phages were originally used primarily for the construction of bacteria via transduction. Today, phages are used in many areas of biotechnology and food technology, human and veterinary medicine (pathogen control) and agriculture [37–40]. To this end, they are increasingly being genetically modified [41–45]. The aim is to improve the phage properties for the respective application, e.g. by broadening the host range, stabilising the particles, switching from temperate to virulent replication, preventing lysis of the host cell or enhancing biofilm dissolution. Phages also serve as donor organisms for a wide range of genes, including those for enzyme production, e.g. T4 DNA ligase and T7 DNA polymerase for molecular biology, depolymerases for capsule or biofilm lysis [46] or endolysin in the food industry (e.g. against *Listeria* [47]). Phage genomes are frequently used as vectors for the transfer of foreign genes and are optimised for this purpose through genetic modifications, e.g. based on the phages λ and M13 [48].

1.7 Safety-related properties of phages

Phages themselves do not pose a risk to humans, animals or plants. However, due to various factors they can increase the pathogenic potential of their host bacteria [49].

1.7.1 Lysogenic conversion

Temperate phages may carry their own safety-relevant genes (morones, AMG) in their genome, which can be transferred to infected cells during lysogenisation and expressed there (lysogenic conversion). In addition to known pathogenicity genes (e.g. toxin genes), it must

also be kept in mind that the function of approximately 70% of all phage genes is unknown [50]. If phages are used as donor or recipient organisms in genetic engineering operations, the risk assessment must be carried out on the basis of the nucleotide sequence of the phage genome. Through bioinformatic analyses (e.g. BLAST analyses, annotations or analyses using the *virulence factor database*), it can be ruled out with a sufficient degree of certainty that the phage genes of unknown function include any factors that increase the virulence of host bacteria.

The nucleotide sequence also indicates whether the phage is a temperate phage (based on specific genes for integration/excision of the phage DNA and regulatory genes, e.g. for repression of lytic replication).

1.7.2 Transduction

The proportion of transducing phages among all phages is high, but a phage's ability to transduce is rarely determined. Preparations of generally transducing phages contain a small proportion of infectious particles with genomic segments from the last host of replication (up to approx. 6% of the bacterial genome in jumbophages). These nucleic acid sequences are transferred to the new host and can be integrated via recombination (transduction). Such a DNA fragment may comprise pathogenic genes or gene clusters, or islands of pathogenicity, etc. For the risk assessment of the phage preparation and, where applicable, the transductants, the decisive factors are the characteristics of the last host (e.g. virulence-enhancing genes in its own genome) and whether the phage is capable of general transduction (demonstrated by experimental data or predicted on the basis of the phage's genome sequence, see section 1.3.1).

1.7.3 Host range of phages

Phages can infect either a single host (a strain or a species) or various species or genera (narrow or broad host range). For most phages, however, the host range is unknown. In studies leading to lysogenisation (see section 1.4) or transduction (see section 1.3.1) of the infected cells, it should be noted that organisms other than the intended target organisms may also be infected.

2 Archaea viruses

Compared with the current state of research and utilisation of phages, the understanding of archaea viruses is significantly more limited. The International Committee on the Taxonomy of Viruses (ICTV) currently recognises 61 families of archaea viruses. The genome of archaea viruses consists mostly of dsDNA, with the exception of two virus families whose members possess ssDNA genomes. The genomes are either linear or circular and range in size from 5 to 150 kbp [51].

The proportion of genes with unknown function in the archaea virus genome averages 75% [52].

The archaea viruses discovered to date can be classified into two distinct types. The older type of archaea virus originated in the common ancestor of today's bacteria and archaea. This type belongs to the *Caudoviricetes* and often has a phage-like morphology with a head and a tail. Later in the course of evolution, archaea-specific viruses emerged with a morphology that is unusual compared to bacteriophages (e.g. droplet-, spindle- or bottle-shaped, or with two tails).

Both lytic replication cycles and chronic infections are known to occur in archaea viruses [53]. The mechanisms of replication have been elucidated for some archaea viruses, but not yet for the majority of them [52].

It is not yet clear whether gene transfer mechanisms such as specific or general transduction also occur in archaea viruses. The transfer of foreign genetic material into viral envelopes has so far only been demonstrated for *Alphafusellovirus beppuense* (Sulfolobus spindle-shaped virus 1, SSV1) and *Alphafusellovirus reykfanense* (Sulfolobus spindle-shaped virus, SSV2), which each transfer genetic material from a so-called satellite virus (pSSVx or pSSVi) to their host bacteria (*Sulfolobus solfataricus* or *Sulfolobus islandicus*) [54].

Archaea, the host organisms of archaea viruses, are currently classified in risk group 1 in the list of donor and recipient organisms under § 5 of the GenTSV, with the only exception of *Methanobrevibacter oralis*, which is assigned to risk group 2.

3 Recommendation

Phages are assigned to **risk group 1** as donor and recipient organisms pursuant to § 5 (1) in conjunction with the criteria in Annex 1 of the GenTSV, unless it is documented that they enhance the human- or animal-pathogenic properties of a bacterium, and if their genome does not contain any genes homologous to genes for virulence-enhancing factors for human or animal pathogens.

Phages are assigned to **risk group 2** as donor and recipient organisms pursuant to § 5 (1) in conjunction with the criteria in Annex 1 of the GenTSV, if it is described that they enhance the human- or animal-pathogenic properties of a bacterium, or if their genome contains genes with homology to genes for virulence factors or virulence-enhancing factors for human or animal pathogens.

Propageted phage particles in phage preparations are assigned to **risk group 1** pursuant to § 5 (1) of the GenTSV, if they are phages that are assigned to **risk group 1** as donor and recipient organisms, and if

- they are not generally transducing, or
- they were produced by replication in non-pathogenic bacteria.

Multiplied phage particles in phage preparations are assigned to **risk group 2** pursuant to § 5 (1) in conjunction with Annex 1, No. 1 (c) and No. 2.1 (b) of the GenTSV, if the phages are assigned to **risk group 2** as donor and recipient organisms, or if genes for virulence-enhancing factors or genes with another hazard potential have been inserted into their genome, or if

- they are capable of general transduction, such that it cannot be ruled out that the phages generated are a mixture of phages and generally transducing particles, and

- they have been propagated in pathogenic bacteria, so that it cannot be ruled out that any generally transducing particles present may transfer genes for virulence-enhancing factors.

Archaea viruses (including mixtures of archaea viruses in preparations) are assigned to **risk group 1** as donor and recipient organisms pursuant to § 5 (1) in conjunction with the criteria in Annex 1 of the GenTSV.

4 Rationale

The classification of phages as donor and recipient organisms depends on whether their genome contains genes that could increase the pathogenic potential of infected or transduced bacteria (e.g. toxin or other virulence-enhancing genes). The classification of phage preparations depends on the classification of the phages used.

If the phages used are capable of general transduction (see section 1.3.1), the risk group of the bacteria used to produce the preparations must also be taken into account. Even preparations of risk group 1 phages that are capable of general transduction may contain phages with toxin or virulence-enhancing genes, if pathogenic bacteria are used for propagation. In the event of accidental inoculation, these phages could transduce the employees' endogenous bacteria and thereby increase their hazard potential.

Archaea viruses and their preparations are assigned to risk group 1. The reason for this is that, with one exception, all archaea are assigned to risk group 1 and no toxin genes or other virulence factor genes are known for archaea viruses. For this reason, it is also not to be expected that archaea viruses will transmit genes that increase the pathogenic potential of infected archaea.

Note:

For phages obtained from strain collections such as the German Collection of Microorganisms and Cell Cultures, the host bacteria used to produce the phage preparations are usually specified. If preparations of phages, whose genome is packaged into phage particles via the pac-type headful mechanism (see section 1.3.1), are obtained from collaboration partners, the bacteria used to propagate the phages are decisive for their classification for the reasons stated above. This information must therefore always be requested. If this information is not available and it is stated that bacteria assigned to risk group 2 are part of the phage host range, the aforementioned phage preparations must be assigned to risk group 2.

5 References

1. **Hendrix RW, Smith MC, Burns RN, Ford ME, Hatfull GF** (1999). Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage. *Proc Natl Acad Sci U S A* **96**(5):2192–7.
2. **Ashelford KE, Day MJ, Fry JC** (2003). Elevated abundance of bacteriophage infecting bacteria in soil. *Appl Environ Microbiol* **69**(1):285–9.
3. **Brüssow H, Kutter E** (2005). Phage ecology. *In* Kutter E, Sulakvelidze A (Eds), Bacteriophages: Biology and Application, p. 129–64. CRC Press, Boca Raton, Florida.

4. **Chibani-Chennoufi S, Bruttin A, Dillmann M-L, Brüssow H** (2004). Phage-host interaction: an ecological perspective. *J Bacteriol* **186**(12):3677–86.

5. **Buchan A, Wiedman S, Lambirth K, Bellanger-Perry M, Figueroa JL, Wright ET, Suresh PS, Zhang Q, Thomas JA, Serwer P, White RA** (2025). Unlocking the genomic repertoire of a cultivated megaphage. *Npj Viruses* **3**(1):71.

6. **Ackermann H-W** (2009). Phage classification and characterization. *In* Clokie MR, Kropinski AM (Eds), Bacteriophages. Methods in Molecular Biology, vol. 501, p. 127–40. Humana Press.

7. **Calendar R, R. Inman** (2005). Phage biology. *In* Waldor MK, Friedman DI, Adhya SL (Eds), Phages: Their Role in Bacterial Pathogenesis and Biotechnology, p. 18–36. ASM Press, Washington DC.

8. **Chiang YN, Penadés JR, Chen J** (2019). Genetic transduction by phages and chromosomal islands: The new and noncanonical. *PLoS Pathog* **15**(8):e1007878.

9. **Casjens SR, Gilcrease EB** (2009). Determining DNA packaging strategy by analysis of the termini of the chromosomes in tailed-bacteriophage virions. *Methods Mol Biol* **502**:91–111.

10. **Chiang YN, Penadés JR, Chen J** (2019). Genetic transduction by phages and chromosomal islands: The new and noncanonical. *PLoS Pathog* **15**(8):e1007878.

11. **Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M** (2017). PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci Rep* **7**(1):8292.

12. **Yarmolinsky MB, Sternberg N** (1988). Bacteriophage P1. *In* Calendar R (Ed), The bacteriophages, p. 291–438. Plenum Publishing Corporation, New York, N. Y..

13. **Schmieger H, Backhaus H** (1973). The origin of DNA in transducing particles in P22-mutants with increased transduction-frequencies (HT-mutants). *Mol Gen Genet* **120**(2):181–90.

14. **Schicklmaier P, Moser E, Wieland T, Rabsch W, Schmieger H** (1998). A comparative study on the frequency of prophages among natural isolates of *Salmonella* and *Escherichia coli* with emphasis on generalized transducers. *Antonie Van Leeuwenhoek* **73**(1):49–54.

15. **Shousha A, Awaiwanont N, Sofka D, Smulders FJM, Paulsen P, Szostak MP, Humphrey T, Hilbert F** (2015). Bacteriophages Isolated from Chicken Meat and the Horizontal Transfer of Antimicrobial Resistance Genes. *Appl Environ Microbiol* **81**(14):4600–6.

16. **Chen J, Quiles-Puchalt N, Chiang YN, Bacigalupe R, Fillol-Salom A, Chee MSJ, Fitzgerald JR, Penadés JR** (2018). Genome hypermobility by lateral transduction. *Science* **362**(6411):207–12.

17. **Humphrey S, Fillol-Salom A, Quiles-Puchalt N, Ibarra-Chávez R, Haag AF, Chen J, Penadés JR** (2021). Bacterial chromosomal mobility via lateral transduction exceeds that of classical mobile genetic elements. *Nat Commun* **12**(1):6509.

18. **Shpakovski GV, Berlin YA** (1984). Site-specificity of abnormal excision: the mechanism of formation of a specialized transducing bacteriophage lambda plac5. *Nucleic Acids Res* **12**(17):6779–95.

19. **Weisberg RA** (1996). Specialized transduction. *In* Neidhardt FC, Lin EC, Curtiss R, III, Ingraham JL, Low KB, Magasanik B, Reznikoff WS, Riley M, Schaechter M, Umbarger HE (Eds), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, p. 2442–8. ASM Press, Washington, DC..

20. **Abedon ST** (2022). Bacteriophages as Drivers of Evolution. An Evolutionary Ecological Perspective. *In* Abedon ST (Ed), Bacteriophages as Drivers of Evolution. An Evolutionary Ecological Perspective, p. 153–64. Springer, Cham.

21. **Brüssow H, Canchaya C, Hardt W-D** (2004). Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiol Mol Biol Rev* **68**(3):560-602.

22. **Breitbart M, Thompson L, Suttle C, Sullivan M** (2007). Exploring the Vast Diversity of Marine Viruses. *Oceanography (Wash D C)* **20**(2):135–9.

23. **Puxty RJ, Millard AD, Evans DJ, Scanlan DJ** (2015). Shedding new light on viral photosynthesis. *Photosynth Res* **126**(1):71–97.

24. **Gummalla VS, Zhang Y, Liao Y-T, Wu VCH** (2023). The Role of Temperate Phages in Bacterial Pathogenicity. *Microorganisms* **11**(3):541.

25. **Krylov VN** (2003). Rol' gorizontal'nogo perenosa genov bakteriofagami v vozniknovenii patogennykh bakteriĭ. (Role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria). *Genetika* **39**(5):595–620.

26. **Wagner PL, Waldor MK** (2002). Bacteriophage control of bacterial virulence. *Infect Immun* **70**(8):3985–93.

27. **Jensen EC, Schrader HS, Rieland B, Thompson TL, Lee KW, Nickerson KW, Kokjohn TA** (1998). Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **64**(2):575–80.

28. **Harshey RM** (1988). Phage Mu. *In* Calendar R (Ed), The bacteriophages, p. 193–234. Plenum Publishing Corporation, New York, N. Y..

29. **Wommack KE, Hill RT, Muller TA, Colwell RR** (1996). Effects of sunlight on bacteriophage viability and structure. *Appl Environ Microbiol* **62**(4):1336–41.

30. **Duarte J, Pereira C, Calado R, Almeida A** (2025). Phage Stability Research: A Systematic Review Bridging Methodological Gaps and Paving the Way for Industrial and Clinical Applications. *Phage (New Rochelle)* **6**(3):189–200.

31. **Paepe M de, Taddei F** (2006). Viruses' Life History: Towards a Mechanistic Basis of a Trade-Off between Survival and Reproduction among Phages. *PLoS Biol* **4**(7):e193.

32. **Pantastico-Caldas M, Duncan KE, Istock CA, Bell JA** (1992). Population Dynamics of Bacteriophage and Bacillus Subtilis in Soil. *Ecology* **73**(5):1888–902.

33. **Li M, Ke W-R, Chang RYK, Chan H-K** (2025). Long-term Storage Stability of Inhalable Phage Powder Formulations: A Four-Year Study. *AAPS J* **27**(5):128.

34. **Marsh P, Wellington E** (1994). Phage-host interactions in soil. *FEMS Microbiology Ecology* **15**(1):99–107.

35. **Silva YJ, Costa L, Pereira C, Cunha Â, Calado R, Gomes NCM, Almeida A** (2014). Influence of environmental variables in the efficiency of phage therapy in aquaculture. *Microb Biotechnol* **7**(5):401–13.

36. **Capra ML, Neve H, Sorati PC, Atamer Z, Hinrichs J, Heller KJ, Quiberoni A** (2013). Extreme thermal resistance of phages isolated from dairy samples: Updating traditional phage detection methodologies. *Int Dairy J* **30**(2):59–63.

37. **Vandamme EJ, Mortelmans K** (2019). A century of bacteriophage research and applications: impacts on biotechnology, health, ecology and the economy!. *J Chem Technol Biotechnol* **94**(2):323–42.

38. **Marks T, Sharp R** (2000). Bacteriophages and biotechnology: a review. *J Chem Technol Biotechnol* **75**(1):6–17.

39. **Jaglan AB, Anand T, Verma R, Vashisth M, Virmani N, Bera BC, Vaid RK, Tripathi BN** (2022). Tracking the phage trends: A comprehensive review of applications in therapy and food production. *Front Microbiol* **13**:993990.

40. **García-Cruz JC, Huelgas-Méndez D, Jiménez-Zúñiga JS, Rebollar-Juárez X, Hernández-Garnica M, Fernández-Presas AM, Husain FM, Alenazy R, Alqasmi M, Albalawi T, Alam P, García-Contreras R** (2023). Myriad applications of bacteriophages beyond phage therapy. *PeerJ* **11**:e15272.

41. **Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK** (2016). Genetically Engineered Phages: a Review of Advances over the Last Decade. *Microbiol Mol Biol Rev* **80**(3):523–43.

42. **Pizarro-Bauerle J, Ando H** (2020). Engineered Bacteriophages for Practical Applications. *Biol Pharm Bull* **43**(2):240–9.

43. **Gibb B, Hyman P, Schneider CL** (2021). The Many Applications of Engineered Bacteriophages-An Overview. *Pharmaceuticals (Basel)* **14**(7):364.

44. **Jo SJ, Kwon J, Kim SG, Lee S-J** (2023). The Biotechnological Application of Bacteriophages: What to Do and Where to Go in the Middle of the Post-Antibiotic Era. *Microorganisms* **11**(9):2311.

45. **Mahler M, Costa AR, van Beljouw SPB, Fineran PC, Brouns SJJ** (2023). Approaches for bacteriophage genome engineering. *Trends Biotechnol* **41**(5):669–85.

46. **Islam MM, Mahbub NU, Shin WS, Oh MH** (2024). Phage-encoded depolymerases as a strategy for combating multidrug-resistant *Acinetobacter baumannii*. *Front Cell Infect Microbiol* **14**:1462620.

47. **Lee C, Kim H, Ryu S** (2023). Bacteriophage and endolysin engineering for biocontrol of food pathogens/pathogens in the food: recent advances and future trends. *Crit Rev Food Sci Nutr* **63**(27):8919–38.

48. **Monaci P, Urbanelli L, Fontana L** (2001). Phage as gene delivery vectors. *Curr Opin Mol Ther* **3**(2):159–69.

49. **Verheust C, Pauwels K, Mahillon J, Helinski DR, Herman P** (2010). Contained use of Bacteriophages: Risk Assessment and Biosafety Recommendations. *Appl Biosaf* **15**(1):32–44.

50. **Hatfull GF** (2025). All the world's a phage. *Proc Natl Acad Sci U S A* **122**(43):e2523344122.

51. **ICTV** (2026). Virus Properties. https://ictv.global/virus-properties. Accessed 07-04-2026.

52. **Krupovic M, Cvirkaite-Krupovic V, Iranzo J, Prangishvili D, Koonin EV** (2018). Viruses of archaea: Structural, functional, environmental and evolutionary genomics. *Virus Res* **244**:181–93.

53. **Munson-McGee JH, Peng S, Dewerff S, Stepanauskas R, Whitaker RJ, Weitz JS, Young MJ** (2018). A virus or more in (nearly) every cell: ubiquitous networks of virus-host interactions in extreme environments. *ISME J* **12**(7):1706–14.

54. **Wang Y, Duan Z, Zhu H, Guo X, Wang Z, Zhou J, She Q, Huang L** (2007). A novel *Sulfolobus* non-conjugative extrachromosomal genetic element capable of integration into the host genome and spreading in the presence of a fusellovirus. *Virology* **363**(1):124–33.