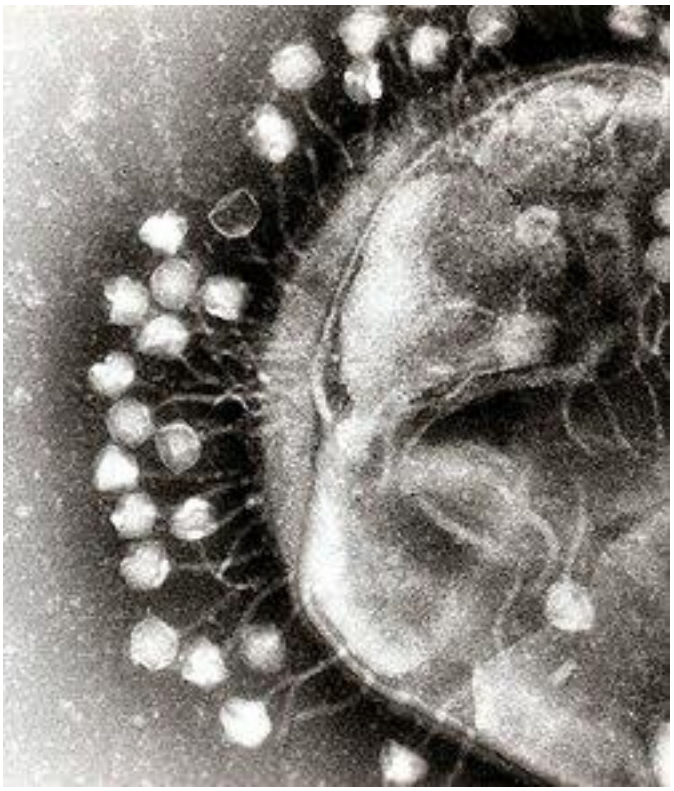
Bacterial Viruses in Biotechnology



A group of tailed bacteriophages infecting a bacterium. Source Wikipedia

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Bacterial Viruses in Biotechnology

Storyline

Viruses are often considered as dangerous entities that impact our health. One largely benign type of viruses that can be found in our environment are the viruses that infect bacteria. These viruses are called bacteriophages and provide beneficial characteristics that can be exploited in biotechnology. They infect specific bacteria strains and amplify within the host cells leading to the killing of the host. Moreover, they have a low inherent toxicity, thus making them attractive alternatives to current antibacterial methods in medicine, agriculture and food production. Furthermore, bacteriophage-derived biotechnology tools for genome editing and other purposes have been developed. However, not all bacteria are hazardous. Some show beneficial properties for the dairy industry and are used in fermentation processes. Bacteriophages can eradicate these bacteria and can thus be considered a threat to these processes. Therefore, the use of bacteriophages in medicine and industry has multiple consequences for the Sustainable Development Goals.

1. Phage-derived biotechnology tools

Alongside the application as a therapeutic and biocontrol agent, molecular biology and biotechnology tools have been developed based on bacteriophages. These tools are either based on phage-derived molecules (*e.g.* T7 RNA polymerase , T4 DNA ligase and restriction enzymes) or use the complete phage structure (*e.g.* biopanning or CRISPR-Cas technology). The latter approach will be discussed in more detail.

1.1. Enzyme production and recombinant expression

Not only the antibacterial properties of phages can be used in biotechnology. Phages can produce enzymes and express recombinant proteins via a method called 'phage display'. In this method, a peptide or protein library is fused to a phage coat protein and exposed on the surface of the viral particles. The video 'Introduction to phage M13' (Supplementary data, V8 – Phage M13) explains the build-up and infection cycle of phage M13, that is most commonly used in phage display. The video will help you to better understand this Nobel Prize-winning technique, which is used to optimize enzymes, *e.g.* enhance thermostability, activity, substrate binding, *etc.* Moreover, it can be used for the identification of protein interaction partners, selection of clinically relevant peptides and proteins, and to generate recombinant antibodies.

The first phase in phage display is the construction of a protein library. Multiple approaches can generate protein variants. Physical and chemical methods introduce random mutations in the protein of interest, while directed methods introduce random mutations in specific parts of the protein of interest. The library can also contain proteins that consist of different sequences combined via recombination. In the second phase, called biopanning, selection rounds are performed in which the variants with stronger binding to the desired target are selected. For this, a labelled target is immobilized on a matrix. The phage particles that display the protein library are added. The matrix is subsequently washed to remove non-binders. Phages bound to the target remain attached and are eluted in a next step. The binding phages are amplified via infection in the host and are resubmitted to a selection round. This process can be repeated multiple times and the strong binding phages are analyzed and identified via DNA sequencing. The phage display technique enables high throughput screening of thousands of peptides and proteins. An expert discussion of the phage display technique can be reviewed in the video 'From biology to

tool' (Supplementary data, V9 – Phage display mechanism). Applications of this technique are illustrated in the video 'Applications of M13' (Supplementary data, V10 – Applications of M13).

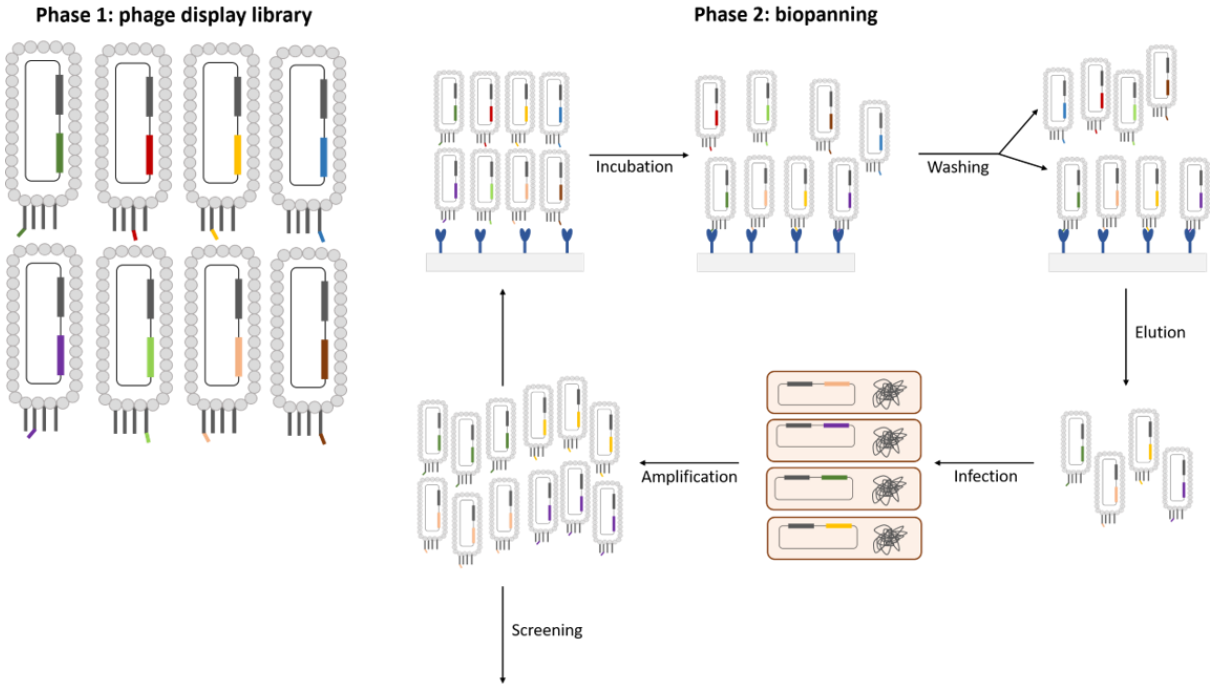

Figure 1. Schematic representation of the phage display process. A phage display library is generated in a first phase. This library is characterized by protein variants, generated by mutation inducing physical or chemical methods. In the second phase, biopanning, phages are added to a desired target immobilized on a matrix. After incubation, a washing step is performed to remove non-binding phages. Phages bound to the target are eluted and amplified via infection in the host bacteria. The resulting phages are resubmitted to a selection round. This biopanning can be repeated multiple times to increase the binding strength of the phages, and thus its specificity for the target. Finally, the strong binders are analyzed and identified via DNA sequencing in a screening step.

1.2. CRISPR genome editing

One of the mechanisms by which bacteria protect themselves from foreign DNA (*e.g.* plasmids and phage) invading the cell are the CRISPR-Cas systems. This adaptive immunity consists of a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) array, which is a cluster of short repeats separated by spacer sequences, and CRISPR associated proteins (Cas). Many variants of the system have been described, but CRISPS-Cas9 from *Streptococcus pyogenes* is the best-characterized. In the first stage of the immune response, called the adaptation or acquisition phase, the bacteriophage DNA is recognized and cleaved by a complex of Cas1 and Cas2. A small part of the viral DNA, the protospacer, is inserted in the CRISPR array as a spacer. During the expression step, the CRISPR array and *cas* genes are transcribed. A long RNA molecule, the pre-CRISPR RNA, is formed and processed into CRISPR RNAs (crRNAs) via Cas9. The crRNAs contain partial spacer sequences and partial repeats and can bind to trans-activating CRISPR RNA or tracrRNA. During the interference step, this hybrid complex of crRNA and tracrRNA guides the Cas9 endonuclease to the complementary sequence in the viral DNA, resulting in cleavage of the invading DNA. However, the targeted sequence is also present in the spacer from the CRISPR array. To prevent autoimmunity, a protospacer adjacent motif or PAM motif is present in the proximity of the protospacer in the viral DNA. This motif is not present in the CRISPR array. Cleavage of viral DNA is only possible when both a sequence matching the spacer and the PAM are present.

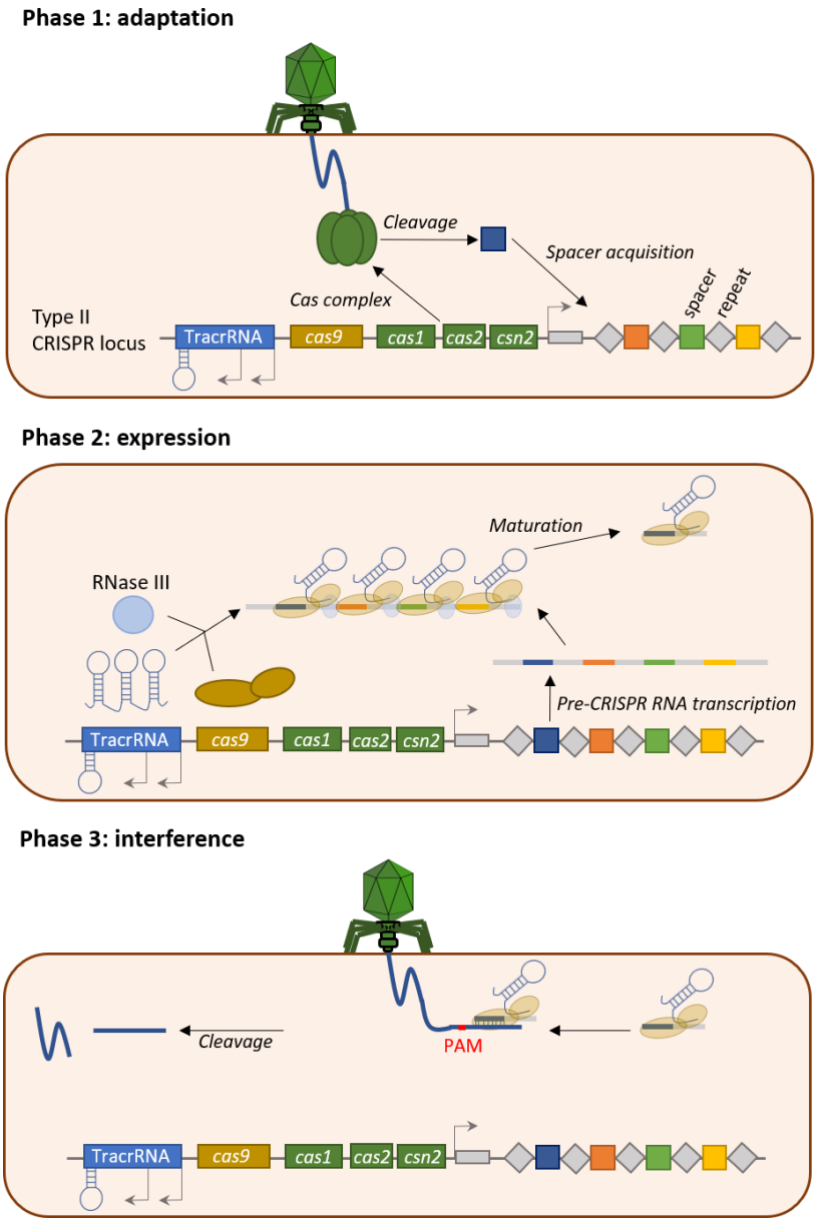

Figure 2. Schematic overview of the CRISPR-Cas9 immune response. Three phases can be distinguished. In the first phase, the adaptation or acquisition phase, bacteriophage DNA is recognized and cleaved by the Cas complex (Cas1, Cas2 and Csn2), resulting in the formation of a protospacer. The latter is inserted in the CRISPR array, which consists of short repeats separated by spacer sequences. In a next step (the expression phase), the CRISPR array and *cas* genes are transcribed and a pre-CRISPR RNA molecule is formed which is processed into CRISPR RNAs (crRNAs). These crRNAs bind to trans-activating CRISPR RNA or tracrRNA. RNase III is recruited and crRNAs mature. In the third and final phase of the CRISPR-Cas9 response, the interference phase, the mature complex of crRNA and tracrRNA guides the Cas9 endonuclease to the complementary sequence in the phage genome, resulting in the cleavage of the invading viral DNA. However, the targeted sequence is also present in the spacer from the CRISPR array. To prevent autoimmunity, cleavage is only possible if a protospacer adjacent motif (PAM) is present in the proximity of the target sequence in the viral DNA. This motif is absent in the CRISPR array.

CRISPR-Cas9 can be used in genome engineering. A guide RNA (gRNA) composed of the crRNA and tracRNA, is developed to direct the Cas9 enzyme to the DNA target. The

complementary sequence is subsequently cleaved, introducing a a double-stranded break. The latter can be repaired by non-homologous end joining or by homologous recombination. Various modifications of the DNA are possible, including deletions or insertions to respectively remove or add base pairs. Other modifications include point mutations (where one nucleotide is altered) or the introduction of a complete gene.

This genome editing technique can be used to determine the origin of diseases by understanding the function of genes. Creating knockouts of a gene via point mutations, insertions or deletions disables its functionality. The effect of this knockout can be linked to the development of certain genetic illnesses. In addition, gene therapy via CRISPR-Cas enables the treatment of diseases by replacing a mutated gene by a wildtype gene. Over the years, many studies have shown promising results with the CRISPR-Cas editing system, which did not stay unnoticed. The 2020 Nobel Prize in Chemistry was awarded to Emmanuelle Charpentier and Jennifer Doudna for the development of the CRISPR-Cas9 genetic scissors, demonstrating the great potential of this genome editing technique. Table 1 shows some important applications of the technique. The video 'Applications of the CRISPR-Cas system' demonstrate the great potential of the CRISPR-Cas technique in various field (Supplementary data, V11 – Applications of the CRISPR-Cas system).

Table 1. Milestone applications of CRISPR genome editing.

Year	Application	Reference
2012	Development of *in vitro* CRISPR-Cas9 genome editing technique	Gasiunas *et al.* Jinek *et al.*
2013	Gene editing in mammalian cells: precise cleavage of genes in human and mouse cells	Cong *et al.* Mali *et al.*
2014	Targeting of multiple genes in monkey embryos	Niu *et al.*
2016	Inactivation of cancer mutations to assess the role of these mutations	Gebler *et al.*
2016	Characterization of CRISPR-Cas9 targeting stability in living cells	Ma *et al.*
2017	Editing of mammalian RNA by CRISPR-Cas13 system	Abudayyeh *et al.*
2017	Modification of beta-globin gene mutations in human embryos	Liang *et al.*
2019	Elimination of HIV-1 in humanized mice.	Dash *et al.*
2021	Treatment of sickle cell disease in humans	Frangoul *et al.*

2. The potential of phage therapy in humans

As mentioned previously, bacteriophages were employed as therapeutic agents to treat bacterial infections in humans not long after their discovery. More than a hundred years ago, d'Hérelle already applied phages to treat purulent infections and dysentery in children. He commercially produced phage preparations that were marketed by what later became the company L'Oréal. At the time, phage therapy also showed promising results in the treatment of cholera, staphylococcal bacteremia, urinary tract infections, sepsis, and other diseases. While the Western world slowly lost interest in phage therapy due to the discovery and availability of broad-spectrum antibiotics, many countries in Eastern Europe continued to use phages to combat bacterial infections. What is now the Eliava Institute in Georgia became a major center for phage research and production. It remains one of the largest centers that develop therapeutic phage preparations, including IntestiPhage targeting close to twenty different gastrointestinal bacteria and PyoPhage targeting *Staphylococcus, Streptococcus, Pseudomonas, Proteus* and *Escherichia coli*. These phage preparations are publicly available without medical prescription in Georgia and Russia. The PyoPhage preparation has also been incorporated in commercially available polymeric wound bandages, called

PhagoBioDerm. In rare cases, these and locally produced phage cocktails are also used in other countries and for other indications, such as for implant-associated infections in trauma patients (Figure 4).

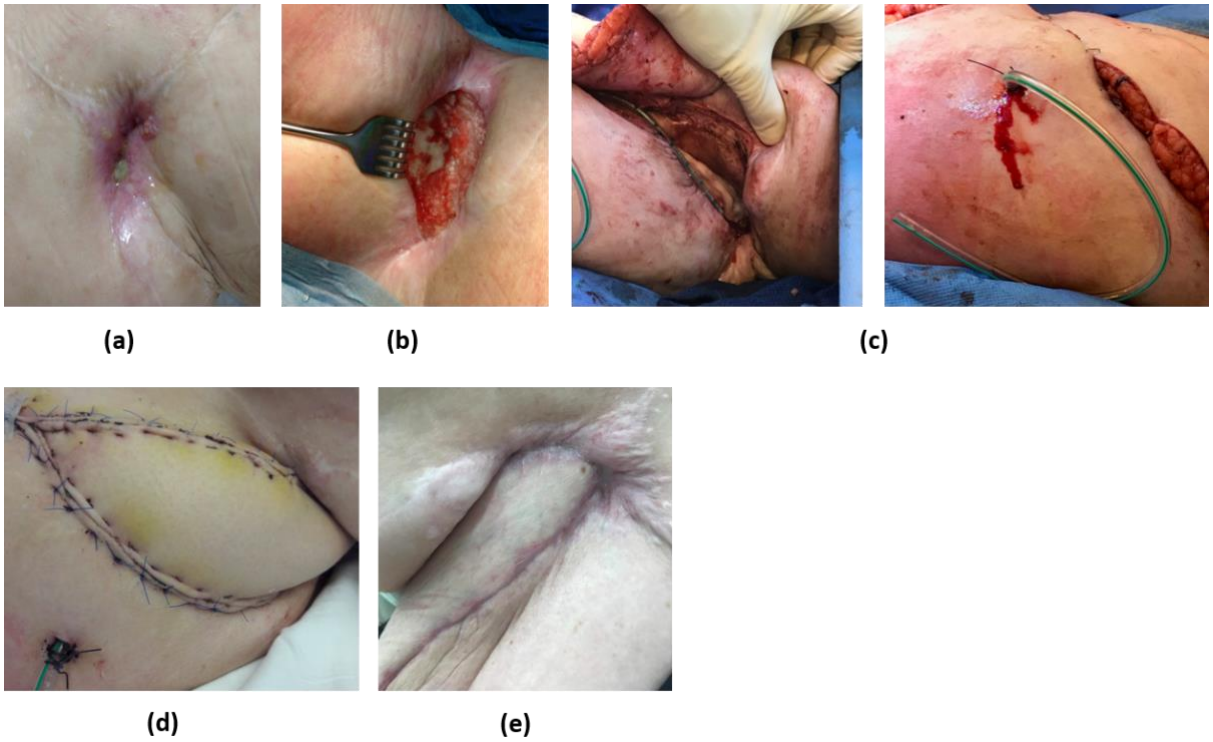

Figure 3. Bacteriophage application for a musculoskeletal infection (osteomyelitis) of the pelvis.[1] (a) Preoperative status of the patient: chronic osteomyelitis with a draining fistula; (b) Previous treatments included multiple surgical debridements and antibiotic treatment regimens. The wound was temporarily closed using negative pressure wound therapy; (c) Intraoperative image: a selected phage cocktail was applied and a draining system placed in the vicinity of the infected site, allowing postoperative rinsing of the wound with phage cocktail. The wound was closed with an anterolateral thigh flap. After wound closure, the phage solution was applied postoperatively three times a day, for 7 days. The patient received concomitant antibiotics during three months; (d-e) Postoperative status of the patient: 8 days after the operation (d) and two months after the phage therapy (e).

Phages specifically infect certain isolates of a given bacterial species, amplify within the host cells and have a low inherent toxicity. The effect on the natural microbiome is said to be minimal, and production costs can be low, although productions according to Good Manufacturing Practice (GMP) to be used in clinical trials and medicinal products receiving marketing authorization face a high cost. These characteristics make bacteriophages attractive alternatives or complements to current antibacterial therapies. However, phage therapy is not yet approved in the US or in most Western European countries. Its use is generally limited under the framework of 'last resort cases', as described in the article 37 from the Declaration of Helsinki. In this regard, phage therapy can only be applied to treat life-threatening and/or seriously debilitating diseases that are not treatable using the currently available therapies. Since 2016, Belgian and French authorities have begun to allow the use of phages as the Active Pharmaceutical Ingredients in magistral preparations, enabling the treatment of selected, individual patients, under the auspices of the medical doctor's prescription. a controlled production of the phages and formulation by hospital pharmacies. This major milestone may therefore be a critical step towards the routine implementation of phage therapy in modern (Western) medicine.

[1] Pictures with courtesy of Willem-Jan Metsemakers and Jolien Onsea, obtained via personal communication. A detailed description of the treatment course for this patient can be found in Onsea *et al.* (2019). Bacteriophage application for difficult-to-treat musculoskeletal infections: Development of a standardized multidisciplinary treatment protocol. *Viruses,* 11(10).
Jolien Onsea: https://www.researchgate.net/profile/Jolien_Onsea

Phage cocktails can be designed in two ways. In the 'prêt-à-porter' approach, a generalized cocktail is developed containing phages that target the most common bacterial strains. The 'sur-measure' approach is patient-specific. A phage cocktail is prepared to specifically target the bacterial infection affecting the patient The pros and cons, the legalization and the development of cocktails according to both approaches is further explained in the video 'Can phages be used to fight bacteria in medicine?' (Supplementary data, V5 – Phage therapy).

3. Phage-based biocontrol

Bacterial infections in some food crops are responsible for annual production losses up to 40%. Common diseases include black rot in cabbage by *Xanthomonas campestris* pv. *campestris*, bacterial blight in leek by *Pseudomonas syringae* pv. *porri* and crazy roots in hydroponically grown tomato or cucumber by rhizogenic *Agrobacterium* biovar 1 species (Figure 4). With the global population expected to reach over 9.6 billion people by 2050, food demand is projected to increase by to approximately 59-98%. Since food security is essential, addressing the bacterial pests responsible for major losses is crucial.

(a) (b) (c)

Figure 4. Pests in crops.[2] (a) Black rot in cabbage caused by a *Xanthomonas campestris* pv. *campestris* infection; (b) Bacterial blight in leek, caused by *Pseudomonas syringae* pv. *porri*; (c) Crazy roots formation in tomato, caused by rhizogenic *Agrobacterium* biovar species.

Currently, preventive measures are taken to minimize the impact of these bacterial infections, such as crop rotation and the removal of symptomatic plants from fields and greenhouses. However, the effect is limited because bacteria remain present in the soil environment and on plant residues. Under standard settings, infections are treated with chemicals. These include conventional antibiotics, copper-based chemicals, *e.g.* copper sulphate or copper oxide, but also hydrogen peroxide can be used in some cases. These chemicals affect the natural microbiota, leave residues in the soil and resistance has been reported. Alternatively, the use of antibiotics might lead to the introduction of these compounds in the food chain and promote the spread of bacterial antibiotic resistance. Consumers, supported by governments, demand healthy, natural, and sustainable biocontrol agents. With respect to this, bacteriophage biocontrol represents a promising alternative for plant protection.

Strictly lytic bacteriophages possess remarkable advantages over traditional measures in agriculture biocontrol settings. They are biodegradable, leaving no residues on food products, and they amplify within the host, resulting in significant lower application cycles. Bacteriophages selectively infect bacteria within their host range, making them harmless towards soil bacteria and minimizes their ecological impact. The use of phages as biocontrol agents is also a cost-effective alternative for farmers, because phage propagation is cheaper than the production of conventional pesticides. However, it is possible that some of the bacterial strains present on the crop are not infected by the phage, due to its limited host range. The use of bacteriophage cocktails, containing multiple and diverse phages with a different host spectrum, are

[2] Pictures from field trials or bioassays conducted by Dominique Holtappels and Kiandro Fortuna, Laboratory of Gene Technology, KU Leuven, Belgium; obtained via personal communication.
Dominique Holtappels: https://www.researchgate.net/profile/Dominique_Holtappels
Kiandro Fortuna: https://www.researchgate.net/profile/Kiandro_Fortuna

required to solve this problem. Moreover, bacteriophage cocktails might also reduce resistance development when composed rationally.

The application strategy depends on the specific infection time and route of the pathogen. Soil-borne infections are prevented and treated by applying bacteriophage cocktails to soil systems and post-harvest control is used for tuber infections. Seed treatment is a preventive measure that allows to disinfect potentially infected seed material. Pathogens on the aerial parts of the plant, the phyllosphere, are controlled via spray treatment. The bacteriophage cocktail can be diluted in irrigation water and sprayed on the plants or seedlings. This treatment has to be repeated several times a week, preferably in the evening or before dawn, because phages are UV-sensitive and will be destructed by light unless formulated appropriately

Currently, seversal phage-based biocontrol agents or biopesticides are commercially available. The AgriPhage product line, produced by the US company OmniLytics, contains four bacteriophage cocktails targeting speck and spot disease in tomato and pepper, citrus canker in citrus trees, *Clavibacter* wilt in tomato and fire blight in apple and pear trees. In Europe, Erwiphage (Enviroinvest, Hungary) specifically targets *Erwinia amylovora* and controls fire blight of apple trees. The latter can only be used locally and under strict regulations for a limited time and is not registered officially as a biopesticide in Europe. However, the post-harvest food processing aid Biolyse (APS Biocontrol, Scotland) prevents soft rot disease (caused by *Pectobacterium*) in potato tubers by spraying phages on the tubers before packaging. By classifying the product as a packaging aid rather than a plant protection product, difficulties concerning the regulation of these phages as active substances are circumvented. Indeed, intensive research precedes the marketing of biopesticides. *In vitro* studies characterize the infection properties of a phage (cocktail). Field or greenhouse trials investigate the biocontrol potential and the reduction of symptomatic plants. Cocktails that show promising results can be registered and approved as active substances of a biopesticide by the responsible authorities, such as the European Food Safety Authority (EFSA). However, to date, no phage-based biocontrol agent has been registered as such. Cost efficacy, product stability and large scale implementation capacity may be limiting factors for the widespread adoption of phages as efficient biocontrol agents.

4. Bacteriophages in the food industry

4.1. Application potential

The World Health Organization (WHO) reports that one in ten people suffer from food poisoning, with over 420,000 deaths annually. The economic burden associated with these foodborne diseases was estimated at a productivity loss of US$ 95 billion, illness treatment of US$ 15 billion and trade loss of US$ 5 billion (*anno* 2016). *Salmonella*, *Campylobacter, Listeria* and *E. coli* are the most common foodborne pathogens. Food safety is therefore another public health priority and one of the pillars of the UN Sustainable Development Goals. As mentioned in the previous section, population growth will drastically increase the food demand. With a decreasing agricultural area due to climate change, it is crucial to promote sustainable development and limit food losses.

Common decontamination methods are generally based on chemical and physical processes: chlorine, formaldehyde, ethylene oxide, hydrogen peroxide, ozone, heat treatments, irradiation by UV, high hydrostatic pressure, ultrasonication, *etc.* However, these methods can alter sensory properties and have a negative impact on the quality of the food products. Moreover, many of these chemical disinfectants pose environmental concerns or require hazardous production processes. Moreover, consumers demand minimally processed food without chemical additives, but with a long shelf-life. In this respect, there is a growing interest to use obligately lytic bacteriophage cocktails to reduce foodborne pathogens. The phage mixture can be sprayed on equipment and contact surfaces (*e.g.* slicer blade in the slaughter house), misted or washed on live animals before slaughter (hide wash), or sprayed on the product during packaging.

The potential of bacteriophages as a biocontrol agent in agriculture and in the food industry is summarized in the video 'Phage therapy in food and agriculture' (Supplementary data, V6 – Phage

biocontrol). This video also provides an overview of the phage-based products that are currently used in these sectors.

4.2. Bacteriophages targeting probiotics and fermentation bacteria

Some bacteria show beneficial properties for the dairy industry. Lactic Acid Bacteria (LAB) are commonly used in fermentation processes, and comprise various genera, including *Lactococcus*, *Lactobacillus* and *Streptococcus*. *Lactococcus* can grow in milk and convert lactose to lactic acid, which makes it an important genus in cheese and yoghurt production. LAB strains are therefore used as starter cultures to facilitate this conversion. In this context, bacteriophages represent a threat by inhibiting these starter cultures and are considered one of the main causes for fermentation failures. They can enter the fermentation process via raw milk as free virions or as prophages in wild LAB strains. Aerosols containing phages can be formed through personnel and equipment movement, raw material handling and air displacements in the proximity of contaminated surfaces. Also the recycling of ingredients lsuch as whey proteins contribute to the spread of phages in dairy manufacturing plants.

The spread of phages in the dairy plant should be contained by regular decontamination of equipment and facilities using disinfectants. The interior contact surfaces of machinery can be cleaned via clean-in place (CIP) technologies. However, it is important that the final product is not affected. Furthermore, the effectiveness of these techniques against phages should be validated. Chelating agents can be added to the starter cultures, because they inhibit or delay phage propagation. Furthermore, phage-free LAB strains are rotated to avoid recontamination by the same phage. Phage-resistant LAB strains have been developed, but concerns regarding GMOs makes application in the industry difficult. The company 'Phage Consultants' (CEO Marcin Łoś) assists companies to eradicate and prevent phage infections in production plants and provides personnel training to avoid bacteriophage contamination. Sources of bacteriophage contamination and the effect of the control measures are explained in more detail in the video 'Phages as enemies' (Supplementary data, V7 – Bacteriophages as a threat for the dairy industry).

5. Importance of viruses in education

5.1. Teaching initiatives

The need to educate students in virology is demonstrated in a recent comment in Nature Microbiology (Gomez-Lucia *et al.*, 2019). This comment provides an overview of innovative teaching initiatives that have been developed recently (Table 2) and are intended to complement current teaching methods.

Table 2. Innovative teaching initiatives.

Teaching tool	Type	Developed by	Accessible via
This Week in Virology (TWiV)	Podcast	MicrobeTV, Columbia University, New York	https://www.microbe.tv/twiv/
Cimaza virology comics	Comics	Susan Nasif and collaborators	https://virologycomics.com/
Virology course	Massive Open Online Course (MOOC)	Columbia University, New York	https://www.virology.ws/course
Animal Viruses: their transmission and the diseases they produce	MOOC	Complutense University of Madrid and l'École Nationale Vétérinaire d'Alfort	https://www.futurelearn.com/courses/animal-viruses
SEA-PHAGES	Hands-on training	US Hatfull Lab	http://www.hatfull.org/courses

Innovirology	E-book, games and video lectures	Collaboration between different labs	www.innovirology.com

5.2. Link with Sustainable Development Goals

Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture.

The use of bacteriophages as a biocontrol agent in both agriculture and food industry reduces produce and food losses by combatting crop pests and foodborne illnesses in humans. However, bacteriophages can also kill starter cultures in the dairy industry, resulting in ingredient loss, product waste, *etc.*

Goal 3: Ensure healthy lives and promote well-being for all at all ages.

Bacteriophage therapy contributes to the achievement of this goal, both by preventing foodborne illnesses and by treatment of bacterial infections in medical settings. Bacteriophages are often applied when other treatments have failed, for example due to antibiotic resistance development. The latter shows the crucial contribution of phages to ensure healthy lives.

Goal 6: Ensure availability and sustainable management of water and sanitation for all.

Waterborne infections can be controlled by the lytic activity of phages in water. In this way, *Vibrio cholerae* infections decrease. The natural biocontrol of phages in aquatic environments could especially be of importance in developing countries where access to clean water and sanitation facilities is limited.

Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.

Bacteriophage therapy can be used as an alternative or complement for antibiotics and other antibacterial agents. The production of phage-based products enables the development of novel, and sustainable infrastructures. Many phage companies originate from a university spin-off or actively collaborate with academic partners which thrives R&D, and further innovation.

Goal 10: Reduce income inequality within and among countries.

This goal is relevant in ways that Tobi Nagel has highlighted in Nagel *et al.*, 2016. Pathogenic bacteria affect more people in developing countries than in developed ones (*e.g.* fatality rate of a *Campylobacter* infection is 0.1% in developed countries compared to 8.8% in a developing country like Kenya). Moreover, many developing countries lack access to clean water and sanitation, making them less able to prevent infections. Bacteriophage therapy can play a crucial role in targeting these infections in developing countries. Bacteriophages are easy to isolate from environments that contain bacteria (*e.g.* sewage water, hospital waste) and the equipment required for isolation is already available in these countries. Moreover, bacteriophages can be formulated as a dry powder without the need for refrigeration allowing easy storage and transportation. However, each country should establish the appropriate regulatory guidelines to use bacteriophages as a therapeutic agent and inform the public to avoid misperceptions, with respect to local cultural contexts.

Goal 12: Ensure sustainable consumption and production patterns.

Consumers demand for natural and sustainable products without additives. The use of bacteriophages as a biocontrol agent is an answer to this demand, because phages are natural and have a minimal impact on the natural microbiome. They are biodegradable and do not negatively affect the environment.

Goal 14: Conserve and sustainably use the oceans, seas and marine resources for sustainable development.

Bacteriophages can be used to inactivate bacteria in fish farming plants and in aquaculture systems. These production systems provide nearly one-third of the global seafood supplies. However, they often suffer from fish pathogenic infections, resulting in major financial losses. Therefore, bacteriophages can be used

as a biocontrol agent. Moreover, population dynamics can be characterized via phage typing. This technique aids to trace the infection source and provides useful information to prevent future outbreaks.

Goal 15: Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification and halt and reverse land degradation and halt biodiversity loss. Conventional pesticides often contain copper residues that stably remain present in the soil after use on the field. Moreover, disinfectants like chlorine harm soil and aquatic organisms. The replacement of these decontaminating agents by bacteriophage-based products contributes to goal 15, because bacteriophages are natural entities that are sustainably produced. Bacteriophages do not disrupt the balance in aquatic and terrestrial ecosystems, because they do not infect non-target species and thus do not impact biodiversity.

5.3. Pupil participation

1. Class discussion of issues related with bacteriophage biotechnology
    a. The public image of bacteriophage therapy and the influence of the Covid-19 pandemic on this image.
    b. Ethical debate: genome engineering via CRISPR-Cas
2. Exercises
    a. How would you formulate a bacteriophage cocktail and produce it on a large scale?
    b. Younger children: construction of a bacteriophage structure with Lego, K'nex, etc
    c. How would you convince farmers and food producers to replace conventional methods with bacteriophages for biocontrol?

6. Further reading

6.1. Expert reading

The following references were used to compose the 'Bacterial Viruses in Biotechnology' framework.

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6.2. Recommended reading

- The online Innovirology course provides in-depth classes on bacteriophages and their biotechnological potential. The E-book, video lectures, webinars and podcasts guide you through the different topics. Available via: http://www.innovirology.com/

- Tedx Talks by Dr. Matthew Sullivan on 'The Power of Viruses, for Good'. Available via https://www.youtube.com/watch?v=4GpD8CJefL4

7. Supplementary data

The videos listed in Table 3 have been modified from the online bacteriophage course (Innovirology). Additional video's discussing related topics can be accessed via http://www.innovirology.com/media/resources/eLearning/bacteriophages/story_html5.html

Table 3. Overview of the 'Bacterial Viruses in Biotechnology' video lectures

Video title

V1 – Bacteriophage ecology
V2 – Bacteriophage genomes
V3 – Overview of the bacteriophage infection cycle
V4 – Lytic vs. lysogenic infection cycle
V5 – Phage therapy
V6 – Phage biocontrol
V7 – Bacteriophages as a threat for the dairy industry
V8 – Phage M13
V9 – Phage display mechanism
V10 – Applications of M13
V11 – Applications of the CRISPR-Cas system

8. Glossary

The words in Table 4 are used in the 'Bacterial Viruses in Biotechnology' framework and are explained in the context of this framework.

Table 4. Glossary

Word	Description
Aerosols	Suspension of liquid droplets in air
Algal blooms	Accumulation of algae in an aquatic system
Anterolateral thigh flap	A flap of skin and skin-related tissue of the lateral aspect of the anterior thigh
Antibiotics	Compounds that kill bacteria or slow down their growth
Antibodies	Proteins used by the immune system to recognize and bind to foreign material in order to remove them from the body
Antiseptic	Stopping or retarding the growth of microorganisms
Autoimmunity	Immune response of an organism against its own cells
Bacteremia	The presence of bacteria in the blood
Bacteria strains	A taxonomic rank in the classification of microorganisms, below the rank of species
Bactericidal	Killing bacteria
Bacteriologist	A person that professionally studies bacteriology (*i.e.* bacteria)
Base pairs	The building blocks of the DNA double helix structure that consist of two pairing bases: adenine (A) pairs with thymine (T) and guanine (G) with cytosine (C)
Bioassays	A method to characterize a compound (*e.g.* effective concentration) by adding it to living cells or tissue
Biocontrol	Methods to control infections based on biological processes
Biosphere	All ecosystems on the planet
Biotechnology	A technology based on biological systems (*e.g.* living organisms or parts) to develop products
Capsid	The protein shell of a virus
Carbon cycle	The flow of carbon atoms from the atmosphere to Earth and back
Cell wall	A structure surrounding cells
Chelating agents	Chemical compounds that are able to bind metal ions
Chromosome	Large DNA molecule that contains the genetic material of an organism
Clinical trials	Experiments that are performed in order to evaluate a certain drug in terms of safety and efficacy
Complementary sequence	A DNA sequence that can bind to a matching sequence via base pairing in order to form a double-stranded sequence
Crop rotation	Growing consecutively different crops on the same land
Debridement	Surgical technique that removes dead tissue in order to clean a wound
Decontamination	The removal of microorganisms
Disinfectants	Chemical compounds that inactivate or kill microorganisms

DNA ligase	An enzyme that joins the ends of two DNA strands
DNA polymerase	An enzyme involved in DNA replication: it adds bases to a DNA strand
DNA replication	A process that makes a copy of DNA based on the original DNA molecule, in order to amplify DNA
DNA sequencing	A process that determines the order of nucleotides in DNA. Multiple sequencing methods are available
Dysentery	Heavy, often bloody, diarrhea
Ecosystems	Natural systems composed of living organisms that interact with their environment
Eluted	To remove bound proteins from a matrix
Endonuclease	And enzyme that cuts within a DNA or RNA molecule
Enzymes	A protein that accelerate a chemical reaction
Fermentation	In the context of food processing: fermentation is the conversion of a substrate (*e.g.* sugar) via microorganisms in the presence of oxygen
Filamentous	A long thin cellular structure
Fistula	A tissue tunnel that develops between two hollow spaces
Flagella	Hair-like structure, used by cells to move
Food poisoning	An illness caused by eating food that is contaminated with infectious organisms
Genera	A taxonomic rank in the classification of microorganisms: a group of related species
Genes	DNA or RNA encoding a functional molecule
Genome editing	Techniques that modify the genome of an organism
Genomes	All the genetic material of an organism
GMOs	Genetically modified organisms: organisms with altered genetic material
Good Manufacturing Practice (GMP)	Guidelines recommended by authorities in order to manufacture and market products according to quality standards
Homologous recombination	In bacteria: a DNA repair process by exchanging genetic material between regions of two DNA strands with similar sequences
Hydroponically	Growing plants without soil, in a nutrient-containing aqueous solvent
Icosahedral	Geometrical shape with twenty triangular faces
Implant	Artificial object that is placed within the body
In situ	Locally, on site
In vitro	Outside a living organism, *e.g.* in a test tube
Intracellular parasitism	Phenomenon used to describe an organism that lives and reproduces in the cells of another organism
Knockouts of a gene	Modification of a gene in order to inhibit its functionality
Lysis	The dead of an organism by disruption of the cell membrane that surrounds the cell, resulting in the release of cellular components in the environment
Magistral preparations	Medicinal products that are prepared in a pharmacy by a pharmacist
Microbiologist	A person that professionally studies microorganisms
Microbiome	Microorganisms living in a specific environment and their genetic content
Microbiota	Microorganisms living in a specific environment
Molecular biology	The study of the structure, function and interaction of cellular molecules
Musculoskeletal	Related to both the muscular and skeletal system
Mutations	The alteration of a nucleotide in the DNA
Negative pressure wound therapy	A therapy in which negative pressure is applied on a wound to remove fluids. This therapy promotes the healing process. The wound is sealed with a bandage and a vacuum pump is applied
Non-homologous end-joining	DNA repair technique in which DNA ends that are non-homologous (no similar sequences) are joined

Nucleotide	Building block of DNA, consisting of a base (A, T, G or C) and a sugar- and phosphate group
Osteomyelitis	Infection in a bone
Pathogen	Microorganism that causes disease
Pathologist	A person that professionally studies pathogens
Peptides	Short chains of amino acids, which are the building blocks of proteins
Pesticides	Compounds that are used to destroy pests
Phage cocktails	A solution containing multiple and diverse phages with different host spectrum
Phage propagation	The amplification of phages by infection of a host cell
Phyllosphere	Parts of a plant that are above soil-level
Plasmids	Circular molecule carrying DNA, distinct from the chromosome
Polymeric	An organic compound that consists of consecutive repeats of similar molecules
Post-harvest	After harvesting
Preventive	In order to avoid an infection, a disease outbreak, *etc.*
Prokaryotic	Organisms that consist of one cell (*e.g.* bacteria)
Recombinant proteins	Proteins that are encoded by genes that have been modified
Recombination	Breaking DNA parts and recombining them in a different way
Reproduction	The production of new phage particles by infection of a host cell
Restriction enzymes	Enzymes that recognize a DNA sequence and subsequently cut the DNA at or near this sequence
Rhizogenic	Root-producing
Ribosomes	Complex of proteins and RNA involved in protein synthesis
RNA polymerase	Enzyme that accelerates the conversion of DNA to RNA
Sensory properties	Characteristics of food: texture, aroma, appearance and taste
Sepsis	Microorganisms in the blood or other tissue, leading to organ failure
Shelf-life	The time you can store a product
Species	Highest taxonomic rank in the classification of microorganisms: similar organisms within a genus
Symptomatic	Indications of a disease
Thermostability	Stable (*i.e.* no structural or functional changes) at high temperatures
Toxicity	Able to cause disease via toxins that damage tissue and disable the immune system
Transcription	Biological process to convert DNA to messenger RNA with the help of RNA polymerase
Transcription factors	Proteins that control certain parameters of the transcription process
Trauma patients	Patients with sudden physical injury (wound, broken bones, etc.)
UV irradiation	Electromagnetic radiation with wavelengths from 10-400 nm, present in sunlight
Virions	Virus particle
Whey	The liquid part of milk that remains after coagulation in cheese making