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RESEARCH ARTICLE

Bacteriophages Importance and Biotechnological Applications in Different Fields: A Review

Zakia F. S. AL-Balawy[1], Magda M. Aly[2*], Ehab H. Mattar[3], Talal F. Al-Qarhi[4]

[1,2,3,4] Department of Biological Science, Faculty of Science, King Abdulaziz University, Saudi Arabia,

[2] Botany and Microbiology Department, Faculty of Science, Kafrelsheikh University, Egypt.

[2] Princess Dr. Najla Bent Saud Al-Saud Center for Excellence Research in Biotechnology

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***Corresponding Author:**

mmmohammad@kau.edu.sa

ABSTRACT

Bacteriophages are viruses that replicate and infect bacteria within their cells; they have been investigated for use in a number of different fields. One of the most promising applications of bacteriophages is in the field of healthcare, the use of bacteriophages in various fields, such as food safety, agriculture, environmental remediation, and biotechnology, is covered in detail in this review. Bacteriophages have been used to eradicate bacterial pathogens from soil and water, reduce bacterial contamination in crops, and regulate bacterial growth in food. They have been discussed as a possible antibiotic substitute in the field of human and animal health, particularly for the treatment of bacterial infections that are resistant to antibiotics. Moreover, they have also been used in biotechnology to create new vaccines, diagnostic tools, and gene therapies. This review also drew attention to the drawbacks and restrictions associated with using bacteriophages, including their limited host range, the potential for the emergence of phage-resistant bacteria, and the requirement for standardized procedures for phage isolation, characterization, and regulatory approval. In conclusion, bacteriophages offer a promising new strategy for treating bacterial infections. They have shown great potential for a variety of applications, and their further research and development could result in significant advancements in a number of fields as well as the creation of future therapies that are more effective and targeted.

1. INTRODUCTION

Bacteriophages infect bacteria that are found in virtually every environment, including soil, water, and the human body and they have been studied for over a century for their potential applications in various fields. The previous phages have been found to have a wide range of uses, from controlling bacterial infections to food preservation (Figure 1). One of the most promising applications of phages in the field of medicine is their role as an effective treatment for bacterial infections, including those caused by antibiotic-resistant bacteria. Recent studies have explored the use of phages in treating infections such as urinary tract infections, wound infections, and sepsis (Vikram et al., 2021, Wang et al., 2022). For example, successful treatment of a multidrug-resistant *Acinetobacter baumannii* infection is demonstrated in a patient using a combination of phages and antibiotics. The pathogen was cleared up by the treatment, and the patient's lung function showed a clinical improvement (Tan et al., 2021). Phages also have potential applications in the agricultural industry (Schwarz et al., 2022). They can be used to control bacterial diseases in crops and to reduce bacterial contamination in animal products as a potential alternative to antibiotics in animal agriculture, to prevent and treat bacterial infections in livestock (Jamal et al., 2019). Another potential application of phages is in the food industry where they can be used as natural preservatives to control bacterial contamination in food products and control the growth of pathogenic bacteria to improve food safety (Zbikowska et al., 2020). In addition to these applications, phages are also being studied for their potential use in environmental monitoring and remediation to detect and track bacterial pathogens in water and soil samples (Liang et al., 2020). They have also been used in biotechnology and bioremediation, to degrade pollutants and remove harmful bacteria from contaminated sites (Abril et al., 2022). They have also been used in nanotechnology (Kaur et al., 2021). This review aims to discuss the potential applications of bacteriophages in different fields, explain the many exciting developments and discoveries being made, and detect the uncover new ways in which they can be used to benefit human health and the environment.

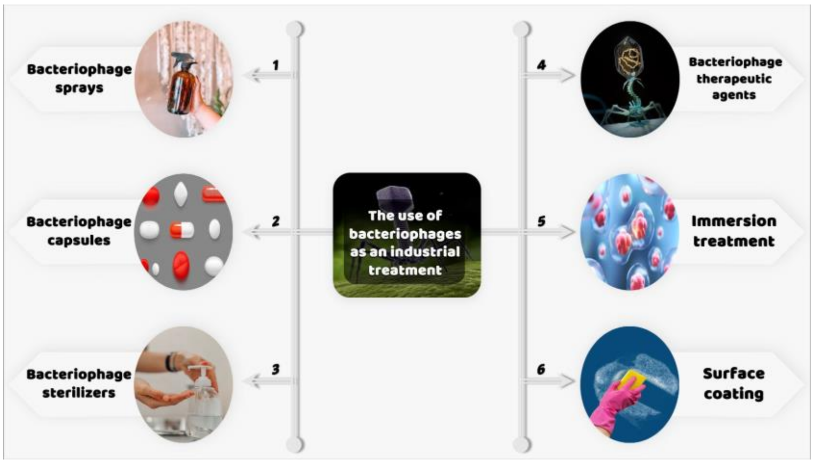

Figure 1: The different uses of bacteriophages in different fields

2. Bacteriophages applications and importance

2.1 The use of bacteriophages in medicine for treating bacterial infections:

Bacteriophages are an important part of the human and animal microbiome, and they play a role in maintaining a healthy balance of bacteria in the body. Also, they are the most abundant biological entities on the earth and play several important roles in our lives, and therapy using bacteriophages is a promising alternative to antibiotics for treating bacterial infections in humans, animals, and plants, particularly those caused by antibiotic-resistant bacteria. They selectively target and infect bacteria both in vitro and in vivo without harming beneficial bacteria or human cells. They have several advantages over antibiotics, including their specificity, their ability to evolve alongside bacterial populations, and their low toxicity to human cells (Gutiérrez and Domingo-Calap, 2020).

However, the use of phages as therapeutics has been hindered by challenges such as the narrow host range of individual phages and the development of phage resistance by bacteria. Numerous studies have been conducted in recent years to evaluate the efficacy of bacteriophage therapy for various bacterial infections by antibiotic-resistant bacteria. in the wound, urinary tract, and respiratory infections. This approach has shown promising results in animal models and clinical trials. As more and more bacteria become resistant to antibiotics, the use of bacteriophage therapy has emerged as a potential alternative treatment option. The FDA approval of bacteriophage therapy for wound infections marks a significant step towards making this treatment more widely available for patients in need. Many studies evaluate the safety and efficacy of bacteriophage therapy in humans (Sacher and Zheng, 2021). Overall, the growing body of evidence suggests that bacteriophage therapy has the potential to be an effective and safe alternative to antibiotics for the treatment of bacterial infections. However, further research is needed to fully understand the mechanisms of action and optimize the use of bacteriophages in clinical settings.

The potential use of bacteriophages as a therapeutic option for multidrug-resistant bacterial infections needs to be discussed. Sharma et al. (2021) studied the isolation and characterization of a lytic bacteriophage of *Pseudomonas aeruginosa* and they highlighted the efficacy of using bacteriophages as a treatment option for nosocomial infections caused by *P. aeruginosa* (Sharma et al., 2021). In addition, phage therapy was used to treat patients with chronic *P. aeruginosa* infections in the lungs of cystic fibrosis patients and the use of phages led to a reduction in bacterial load and improved lung function (Law et al., 2019). Furthermore, Chegini et al. (2020) highlight the efficacy of using bacteriophages as a treatment option for infections caused by bacteria (Chegini et al., 2020).

Furthermore, phage therapy was used to treat a patient with a multidrug-resistant *A. baumannii* infection and the use of phages resulted in a reduction in bacterial load and clinical improvement in the patient's health (Wu et al.,2021). In addition, the successful use of a bacteriophage cocktail to treat a patient with multidrug-resistant *A. baumannii* and *Staphylococcus aureus* infection was reported (Wu et al., 2019). Recently, phage therapy was used to treat chronic wound infections with various underlying conditions and the use of phages resulted in wound healing and reduced bacterial load in all patients (Duplessis and Biswas, 2020). In addition, the role of bacteriophages in shaping the mammalian gut microbiota was studied providing insight into how bacteriophages play a critical role in regulating the gut microbiota (Bichet et al., 2020).

2.2. Bacteriophage applications in food, wastewater, and industry:

The use of bacteriophages in the food industry is an active area of research and they have the potential to play an important role in various industries, including production and improving food safety (Figure 2). In 2006, the FDA issued the first approval for the use of bacteriophage preparation in the food supply for a *Listeria monocytogenes*-specific cocktail, called ListShield™, which was used as a food additive (Perera et al., 2015). In addition, the bacteriophage cocktail was effective in reducing the levels of *Listeria monocytogenes* in ready-to-eat meat products (Truchado et al., 2020). Later, the bacteriophages gained a lot of attention in various industries due to their abundance in nature and their specificity towards bacteria that can cause spoilage and diseases. Phages can be used to target specific bacterial pathogens and control bacterial contamination in food products, such as meat, dairy, vegetables, and fresh products. In addition, they have been used as a natural alternative to chemical preservatives to control the growth of bacterial pathogens (Zia and Alkheraije, 2023; Imran et al., 2023). For instance, the application of a phage cocktail made up of the LPSTLL, LPST94, and LPST153 phages significantly reduced the levels of *Salmonella*, a common foodborne pathogen in raw chicken, chicken breast, and in milk at either 25°C or 4°C (Islam et al., 2019). They are also used as natural preservatives to eliminate pathogenic bacteria, such as *Salmonella*, *E. coli,* and *Listeria*, and improve food safety and reduce bacterial contamination in food. In addition, bacteriophages were used to control the growth of *L. monocytogenes* in food products and on contaminated surfaces (Kawacka et al., 2020).

Recently, phages have been used in the biotechnological industries like the production of recombinant proteins and vaccines. In particular, phages can serve as vectors for the display of foreign proteins on their surfaces, which allows for the selection and screening of novel proteins with desired properties. For example, a study showed that a phage display library was used to identify a high-affinity antibody fragment against *Ebola* virus (Roth et al., 2021).

Sharma and others reported the use of phages in the development of novel antibiotics and bactericidal agents against methicillin-resistant *S. aureus* (MRSA), a common antibiotic-resistant pathogen (Sharma et al., 2018). The bacteriophages have also been used as biocontrol agents in chickens. livestock and aquaculture (Korzeniowski et al., 2022). Bacteriophages have been studied as a means of controlling bacterial contamination in food products of animal origin. For example, a recent study showed that treating beef carcasses with a bacteriophage cocktail reduced the prevalence of *E. coli* (Pinto et al., 2020). In addition, they are used for monitoring bacterial pathogens in food products. For instance, a study demonstrated the use of bacteriophages as a biosensor for detecting bacterial pathogens in food samples (Wang et al., 2022). Overall, the use of phages in the industry has the potential to provide a safe, effective, and environmentally friendly means of controlling bacterial pathogens and producing novel biotechnological products (Abril et al., 2022). However, more research is needed to fully understand their potential and limitations and develop effective strategies for their use in different industrial applications, and optimize their use in food safety programs. The addition of a phage cocktail significantly reduced the levels of *E. coli* and other bacterial pathogens in wastewater (Jassim et al., 2016).

In summary, bacteriophages have shown promise as a tool for controlling bacterial pathogens in the food industry. These examples demonstrate the potential efficacy of bacteriophages in reducing contamination of common foodborne pathogens like *L. monocytogenes*, *Salmonella*, and *E. coli*. After highlighting the potential uses of bacteriophages in the food industry, developing targeted phage applications for foodborne pathogens is recommended to improve food safety.

2.3. Bacteriophage applications in agriculture:

In recent years, research has focused on the potential use of bacteriophages as a tool in agriculture, particularly as a means of controlling bacterial pathogens that can cause crop diseases and against plant pathogens overall. One of the main advantages of using bacteriophages for plant protection is their specificity (Figure 2). Bacteriophages only infect and kill specific strains of bacteria, leaving beneficial bacteria and other organisms unharmed. This specificity can help to reduce the use of broad-spectrum antibiotics and other chemical treatments that can have negative impacts on the environment and human health. On the other hand, the potential applications of bacteriophages in agriculture are promising, and further research is needed to optimize their use and ensure their safety and efficacy. However, the use of bacteriophages in crops also raises important regulatory and safety considerations, which must be carefully addressed.

Recent studies have investigated the use of bacteriophages for controlling bacterial diseases in various crops, including fruits, vegetables, and cereal crops. The effect of bacteriophages in controlling fire blight a bacterial disease that affects apple and pear trees (Ye et al.,2022; Park et al., 2022). And reducing bacterial spot disease in tomato plants (Balogh et al.,2023). On the other hand, the study conducted by Wang and others in 2022, where examined the potential of bacteriophages as biocontrol agents against *Ralstonia solanacearum*, a soil-borne pathogen that causes bacterial wilt in many crops. They isolated lytic phages from contaminated soil samples and tested them on different *Ralstonia solanacearum* strains. The results showed that the phages could effectively control the growth of the bacteria, and could even be used in combination with other biocontrol agents for enhanced efficacy (Wang et al., 2022).

In addition, bacteriophages were used as a biosensor for detecting *Xanthomonas citri*, the bacterial pathogen that causes citrus canker disease (Rajwade et al., 2020). Another study was conducted by Kim et al in 2022, where they investigated the virulence of different strains of *Pectobacterium car otovorum* subsp. carotovorum (Pcc), a Gram-negative bacterium that causes soft rot disease in various plants. They found that using a cocktail of lytic bacteriophages could effectively control the growth of Pcc and reduce disease severity (Kim et al., 2022). According to Jungkum Park et al. (2022), Control of Fire Blight (Erwinia amylovora) on Apple Shoots with Bacteriophages in the Growth Chamber and Greenhouse. This study examined the efficacy of using nine bacteriophages against Erwinia amylovora, the cause of fire blight in apple trees, in both a controlled environment and a greenhouse setting. Bacteriophages have been considered as a biological control agent for preventing bacterial infections in plants (Park et al., 2022).

Overall, the potential applications of bacteriophages in agriculture for controlling plant pathogens and enhancing plant growth were demonstrated. Thus, the use of bacteriophages in agriculture has the potential to provide a safe, effective, and environmentally friendly means of controlling bacterial pathogens. However, more research is needed to fully understand their potential and limitations and to develop effective strategies for their use in different agricultural systems and focused strategies for eradicating bacterial plant pathogens. However, further research is needed to fully understand the potential of bacteriophages and to optimize their use in agricultural practices.

2.4. Bacteriophage applications in veterinary medicine:

The use of bacteriophages in veterinary medicine has gained increasing attention in recent years due to the emergence of antibiotic-resistant bacteria and the need for alternative therapies. Studies have shown that bacteriophages can be used to control and prevent bacterial infections in various animal species, including cattle, poultry, and fish. For example, bacteriophages have been used to control infections caused by Salmonella, *Escherichia coli*, and *Staphylococcus aureus* in livestock and poultry. Bacteriophages have also been used to treat infections in companion animals, such as dogs and cats (Jamal et al., 2019; Ferriol-González and Domingo-Calap, 2021).

In addition to their therapeutic potential, bacteriophages have also been studied for their use as prophylactic agents, diagnostic tools, and as a means of improving animal health and productivity. For example, bacteriophages have been used to prevent infections in aquaculture and to improve the growth and feed efficiency of livestock (Schulz et al., 2022).

Bacteriophages have been used to treat various bacterial infections in animals, including mastitis in dairy cows, *salmonellosis* in chickens, and respiratory infections in pigs. For example, a recent study showed that a combination of bacteriophages (a cocktail consisting of two bacteriophages) was effective in treating *Staphylococcus aureus* infections in Horses. (Marshall et al., 2023).

Bacteriophages have also been investigated for their potential to prevent bacterial infections in animals. Administering a bacteriophage cocktail to calves reduced the incidence of diarrhea caused by enterotoxigenic *Escherichia coli* (Montso et al., 2021).

Bacteriophages have been studied as a means of controlling bacterial contamination in food products of animal origin. For example, a recent study showed that treating beef carcasses with a bacteriophage cocktail reduced the prevalence of *Escherichia coli* (Witte et al., 2022).

Bacteriophages have been investigated for their potential to decontaminate animal production environments, such as barns and feedlots. A recent study showed that spraying a bacteriophage cocktail on surfaces in a barn reduced the prevalence of antibiotic-resistant bacteria (Gao et al., 2023).

New research including the development of new phage isolation and characterization methods and the use of phage cocktails, have shown promise in overcoming these challenges and may lead to the wider adoption of bacteriophages in veterinary medicine. Despite the potential benefits, the use of bacteriophages in veterinary medicine still faces challenges, such as regulatory issues, lack of standardized protocols, and limited knowledge of the safety and efficacy of bacteriophages in different animal species and production systems.

2.5. Bacteriophage applications in environmental monitoring:

Bacteriophages are as valuable tools for environmental monitoring due to their specificity target and sensitivity in detecting bacterial pathogens. The use of bacteriophages in environmental monitoring is effective in detecting and quantifying the presence of various bacterial pathogens in environmental samples, including water, soil, and food (Oliveira et al., 2023).

Bacteriophages can be used in environmental monitoring by either directly detecting the presence of bacterial pathogens or indirectly by detecting the presence of bacterial indicators, such as fecal coliforms, which are commonly used as indicators of water quality. On the other hand, bacteriophages can also be used to monitor the effectiveness of disinfection processes, such as UV irradiation and chlorination, in reducing bacterial pathogen levels in water and other environmental samples (Motlagh and Yang, 2019; McKee and Cruz, 2021). The use of bacteriophages in environmental monitoring has several advantages over traditional methods, including their ability to detect viable but non-culturable bacteria, their rapid detection times, and their potential for high-throughput screening. However, the use of bacteriophages in environmental monitoring also faces challenges, such as the need for standardized protocols and the potential for interference from environmental factors, such as pH and temperature.

A recent study used a bacteriophage-based assay to detect and quantify *Escherichia coli* in riverwater samples (Alonzo et al.,2022). On the other hand, have been studied for their potential use in soil quality monitoring. For example, a recent study used a bacteriophage-based assay to detect and quantify *Bacillus thuringiensis* in soil samples from a field trial (Hassan et al.,2023).

In conclusion, bacteriophages are used in environmental monitoring and to improve the accuracy and efficiency of bacterial pathogen detection to improve public health outcomes.

In addition, bacteriophages play an important role in the balance of the planet, particularly in maintaining the balance of microbial ecosystems. They help to regulate bacterial populations and influence bacterial diversity in various ecosystems (Gagliardi et al., 2018).

By controlling bacterial populations, bacteriophages help to maintain the balance of nutrients and energy flow in the ecosystem. Their interactions with bacterial communities have important implications for ecosystem functioning and stability. Overall, the role of bacteriophages in ecological homeostasis is an active area of research, and recent studies have highlighted their importance in maintaining the health and balance of various ecosystems. For example, Bacteriophages can control bacterial populations in various environments, such as soil, water, and the human gut. They can infect and lyse bacterial cells, reducing their numbers and preventing their overgrowth.

This helps to maintain a balance between different bacterial species and prevent the dominance of harmful bacteria. For example, phages have been shown to regulate bacterial populations in marine ecosystems, such as coral reefs and deep-sea sediments (Pascelli et al., 2018).

On the other hand, Bacteriophages can also influence bacterial diversity by selecting certain bacterial species and promoting their survival. They can also transfer genes between bacterial cells, such as antibiotic resistance genes, and contribute to the evolution of bacterial communities. This can have important implications for ecosystem functioning and stability. For example, phages have been shown to influence bacterial diversity in soil and sediment environments (Barr, 2017). Another study published in the journal PLOS ONE in 2017 showed that bacteriophages can help to control the spread of antibiotic-resistant bacteria in wastewater treatment plants, which can have important implications for public health (Hiltunen and Laine, 2017).

In addition, bacteriophages can impact nutrient cycling by lysing bacterial cells and releasing nutrients into the environment. This can stimulate the growth of other microorganisms, such as algae and protozoa, and contribute to the cycling of carbon, nitrogen, and other nutrients. For example, phages have been shown to impact nutrient cycling in marine ecosystems, such as the Arctic Ocean (Yau and Seth-Pasricha, 2019).

Another study published in the journal Nature Microbiology in 2020 found that bacteriophages play a key role in regulating bacterial populations in the ocean, which in turn affects nutrient cycling and carbon sequestration (Naureenet al., 2020).

2.6. Bacteriophage applications in biotechnology, engineering, and bioremediation:

Bacteriophages are important in bioremediation because they can specifically target and kill bacteria that are harmful to the environment or human health (Cristobal-Cueto et al., 2021). This can be particularly useful in the cleanup of contaminated sites or neutralization, such as those contaminated with oil or heavy metals that potentially reduce the use of chemicals and other harsh methods in bioremediation (Cristobal-Cueto et al., 2021). The use of bacteriophages in biotechnology and bioremediation encompasses a wide range of applications, including the production of recombinant proteins, the control of bacterial infections in industrial processes, and the remediation of contaminated environments (Rao and Zhu, 2022).

In addition to their effectiveness in bioremediation, bacteriophages have several advantages over traditional remediation methods. For example, they are highly specific to their target bacteria, which means that they do not harm beneficial bacteria in the environment. They are also self-replicating, which means that they can target, and kill bacteria even after initial application (Laforet et al., 2023).

Bacteriophages can be used in biotechnology for the production of recombinant proteins, such as enzymes and antibodies, by using phage display technology. Phage display technology involves genetically engineering bacteriophages to display foreign proteins on their surface, which can be used for screening and selecting proteins with desired properties (Pierzynowska et al., 2023).

In addition, bacteriophages have been studied for their potential use in bioremediation, particularly in the remediation of soil and water contaminated with bacterial pathogens. Bacteriophages can be used to selectively target and kill bacterial pathogens in contaminated environments, without harming other microorganisms and without introducing harmful chemicals into the environment (Łobocka et al.,2021). Overall, bacteriophages have great potential in bioremediation due to their specificity, effectiveness, sustainability, and eco-friendly process.

Despite the potential benefits, the use of bacteriophages in biotechnology and bioremediation still faces challenges, such as the need for standardized protocols and the potential for bacterial resistance to develop. However, recent advancements in bacteriophage research, including the development of new phage isolation and characterization methods, and the use of phage cocktails, have shown promise in overcoming these challenges and may lead to the wider adoption of bacteriophages in biotechnology and bioremediation.

Bacteriophages have been extensively studied for their potential applications in biotechnology and bioremediation.

Phages can be used to control bacterial populations in contaminated environments, such as soil and water that decrease the bacterial populations in soil contaminated with heavy metals. Bacteriophages have been proposed as a potential tool for the bioremediation of contaminated environments, whereas the biodegradation of Chromium (Cr), by a phage isolated from a contaminated soil sample (Huang et al., 2021). Another study demonstrated the biodegradation of phenanthrene, a polycyclic aromatic hydrocarbon, by a phage isolated from a contaminated soil sample (Cristobal-Cueto et al., 2021). The previous results may be due to the transfer of genes between bacteria by bacteriophages, making them useful tools for genetic engineering and synthetic biology. A study demonstrated the transfer of a synthetic gene circuit from constructed gut-engineered probiotics as live therapeutics using a phage vector (Huang et al., 2022). Another study demonstrated the use of a phage vector to deliver a system for therapeutic genes and medications in the treatment of cancer (Petrov et al., 2022).

Researchers may use genetic engineering techniques to modify the phage genome and improve its properties for therapeutic use. This can include modifications to enhance stability, host range, or specificity (Hussain et al., 2023). The bacteriophages were effective in reducing the levels of antibiotic-resistant bacteria in wastewater (Hamid and Mir, 2022). According to Sharma and Sharma, (2020), the potential uses of bacteriophages in bioremediation settings, such as helping to remove pollutants from aquatic ecosystems and controlling antibiotic-resistant bacteria in wastewater (Sharma and Sharma, 2020). The use of bacteriophages to remove *Pseudomonas aeruginosa* from soil by up to 99% (Mkwata et al., 2021).

These are just a few examples of the potential applications of bacteriophages in biotechnology and bioremediation. As research in this field continues, we can expect to see more innovative uses of phages in various industries and these studies demonstrate the diverse range of applications for bacteriophages in biotechnology and bioremediation and suggest that phages have considerable potential for future research and development.

2.7. Bacteriophage applications in research and development:

Bacteriophages have been studied and utilized for a variety of applications in research and development.

One area of research where bacteriophages have shown promise is in the development of alternative antibacterial strategies. With the rise of antibiotic-resistant bacteria, there is a need for new approaches to combat bacterial infections. Bacteriophages can be used to specifically target and kill pathogenic bacteria, while leaving the surrounding microbiota unaffected. Several studies have investigated the potential of phages as antibacterial agents, including a recent review published in the journal Antibiotics in 2023 (Unterer et al., 2023).

In addition to their use as antibacterial agents, bacteriophages have also been used as tools for genetic engineering. They can be used to introduce foreign DNA into bacterial cells, a process known as transduction. This technique has been used in a variety of applications, such as the production of recombinant proteins and the creation of genetically modified bacteria for bioremediation (Hussain et al., 2023; Elois et al., 2023).

Finally, bacteriophages have also been used as diagnostic and detection tools. One approach is to use phages that specifically target and infect certain bacteria as a means of detecting the presence of those bacteria in a sample. For example, a study published in 2022 demonstrated the use of phages as the recognition element in a biosensor for the detection of Salmonella bacteria (Wang et al., 2022).

Overall, bacteriophages have shown great potential for a variety of applications in research and development, and continued research in this area is likely to lead to further advances in the field.

2.8. The bacteriophages, biological weapons, and toxins:

Another area in which bacteriophages may be of great importance is in the treatment of biological weapons.

Biological weapons are intentionally created or modified microorganisms that are used to cause disease in humans, animals, or plants. These weapons can be used in a variety of ways, such as through the spraying of aerosols, contamination of food or water sources, or use in explosives (Gelman et al., 2018).

Many of these biological agents are bacterial pathogens, such as anthrax or plague, which can cause severe illness and death if not treated properly (Tao et al., 2018).

The use of bacteriophages in the treatment of biological weapons has several advantages over traditional antibiotics. Bacteriophages are specific to certain types of bacteria, reducing the risk of harming beneficial bacteria in the body. They also can rapidly reproduce, allowing for a quick response to a biological weapon attack (Gelman et al., 2018; Sharma et al., 2021).

In recent years, there has been increasing interest in the use of bacteriophages for the elimination of biological weapons and toxins. These studies highlight the potential of bacteriophages as a versatile tool for the detection, prevention, and treatment of biological weapons and toxins (Jassim et al., 2017).

A study published in 2015 by Sharp and others. investigated the use of bacteriophages to rapidly detect Bacillus anthracis spores, which can be used as a biological weapon, The extent of the contamination (Bacillus anthracis spores) of the postal facilities could be determined by the authors within 24 to 48 hours of environmental sample collection using a bacteriophage they developed (Sharp et al., 2015).

Another area of research has focused on the use of bacteriophages to decontaminate surfaces contaminated with bacterial toxins. A study published in 2021 by Stachler et al. investigated the use of bacteriophages (P1 or JG004) to eliminate the bacterial toxin *P. aeruginosa* from surfaces. The authors developed a bacteriophage-based decontamination system that was effective in reducing *P. aeruginosa* levels on surfaces (Stachler et al., 2021).

2.9. Bacteriophage-based biosensors.

Bacteriophage-based biosensors are a type of biosensor that uses bacteriophages, or viruses that infect bacteria, as a recognition element for detecting specific bacteria or bacterial products. Bacteriophages have high selectivity and sensitivity for their bacterial hosts, making them ideal candidates for use in biosensors. The use of bacteriophages in biosensors has been gaining attention in recent years due to their potential applications in various fields, such as food safety, environmental monitoring, and medical diagnostics. Bacteriophage-based biosensors have been developed for the detection of a wide range of bacterial pathogens, including Salmonella, Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus, among others (Al-Hindi et al., 2022; El-Moghazy et al.,2022).

Bacteriophage-based biosensors can be designed to detect bacterial cells or specific bacterial products, such as toxins or enzymes. The sensing mechanism of these biosensors typically involves the binding of the bacteriophage to the target bacteria or bacterial product, which results in a detectable signal, such as a change in electrical conductivity or fluorescence (Filik et al., 2022).

Several studies have demonstrated the potential of bacteriophage-based biosensors for rapid and sensitive detection of bacterial pathogens. For example, a recent study described a bacteriophage-based biosensor for the detection of *Escherichia coli* in water samples with a detection limit of 1 CFU/mL (Quintela and Wu, 2020).

Another study published in 2021 in the journal Biosensors and Bioelectronics demonstrated the use of a phage-based biosensor to detect Salmonella in food samples (Aliakbar Ahovan, et al., 2020).

Overall, bacteriophage-based biosensors have the potential to provide rapid and sensitive detection of bacterial pathogens in various applications. Further research and development are needed to optimize the performance of these biosensors and to explore their potential in real-world settings.

2.10. Bacteriophage-based nanoparticles and delivery systems.

Bacteriophage-based nanoparticles and delivery systems are emerging as promising tools for biomedical applications. These systems take advantage of the unique properties of bacteriophages, such as their ability to target specific bacteria and their structural stability, to develop novel drug delivery platforms. Recently, bacteriophages have been engineered to create nanoparticles and delivery systems that can be used for drug delivery, gene therapy, and imaging (Jamaledin et al., 2023).

Bacteriophage-based nanoparticles can be synthesized by modifying the surface of the bacteriophage with various molecules, such as peptides, proteins, and polymers. These modifications can enhance the stability, targeting, and cell penetration of the nanoparticles, making them more effective for biomedical applications. Bacteriophage-based nanoparticles can also be engineered to carry cargo, such as drugs, genes, or imaging agents, to specific cells or tissues (Bayat et al., 2021).

Bacteriophage-based delivery systems have also been developed for targeted drug delivery. These delivery systems utilize the high specificity of bacteriophages for their bacterial hosts to target specific cells or tissues. Bacteriophage-based delivery systems can be designed to release drugs in response to specific stimuli, such as changes in pH or temperature, or the presence of specific enzymes (Chung et al., 2020).

Several recent studies have reported the development of bacteriophage-based nanoparticles and delivery systems for various biomedical applications. For example, a study reported the development of bacteriophage-based nanoparticles for the targeted delivery of anticancer drugs to cancer cells. The resulting nanoparticles showed improved tumor targeting and efficacy (Ragothaman and Yoo, 2023).

Bacteriophages can also be used as delivery systems for vaccines. A study published demonstrated the use of bacteriophage Qβ as a carrier for a vaccine against the respiratory syncytial virus. The researchers engineered the Qβ bacteriophage to display a fragment of the virus, which elicited a strong immune response in mice (Gitlin et al, 2014).

Bacteriophages can also be used as imaging agents for diagnostic purposes. A study published in 2022 demonstrated the use of bacteriophage-based nanoparticles as magnetic resonance imaging (MRI) contrast agents. The researchers engineered the nanoparticles to display a bacteriophage targeting Staphylococcus aureus bacteria, which allowed for the specific detection of the bacteria in an MRI scan (Borg et al., 2022).

These are just a few examples of the potential applications of bacteriophage-based nanoparticles and delivery systems. As research in this field continues, we can expect to see more innovative uses of bacteriophages in various biomedical applications. Overall, bacteriophage-based nanoparticles and delivery systems have great potential for biomedical applications due to their high specificity, low toxicity, and ease of modification. Further research and development are needed to optimize the performance of these systems and to explore their potential in various biomedical applications.

2.11. Bacteriophages and artificial intelligence:

Recent advances in artificial intelligence (AI) and machine learning (ML), a branch of artificial intelligence, techniques have the potential to address some of these challenges and accelerate the development of phage-based therapies. Specifically, AI and ML can be used to analyze large datasets of bacterial genomes and identify phages that are specific to particular bacterial strains, as well as predict the likelihood of phage resistance developing in response to treatment (Jin et al., 2023).

In a study, the advantages and disadvantages of the machine learning techniques used in recent years to predict the proteins of phage virion were briefly discussed (Yang et al., 2020). In another study, the predicting bacteriophage sequences using deep Learning was described using a deep learning model that can predict bacteriophage sequences from raw DNA reads faster and more accurately than existing methods. The authors trained their model on a large dataset of bacteriophage sequences and achieved high accuracy in predicting both complete genomes and partial sequences (Lie et al., 2020). In 2020, a platform that combines distributed ledger technology and artificial intelligence (AI) is used to manage a supply chain for instant synthetic phages that are both ethical and sustainable (Pirnay, 2020).

On the other hand, machine learning was applied for the development of microbiome-targeted therapeutics, followed by a guide on where to find reliable microbiome big data (McCoubreyet et al., 2021). Thung et al. (2021) established a computer program called STEP3 to take advantage of the "evolutionary features" that can be identified in the genome sequences of various phages when compared to other prediction tools and benchmarked using phages from various sources, achieved a stable and robust prediction performance. On the other hand, a machine-learning model was used to predict the resistance of focus on predicting bacterial hosts from the ESKAPE group, *Escherichia coli*, *Salmonella enterica*, and *Clostridium difficile* to bacteriophages. The authors trained their model on a large dataset of bacterial and bacteriophage genomes and achieved high accuracy in predicting the resistance strains (Boeckaerts et al., 2021).

Overall, the integration of AI and phage therapy has the potential to revolutionize the treatment of bacterial infections and overcome some of the challenges associated with traditional antibiotic therapies. However, further research is needed to fully realize the potential of this approach.

2.12. The use of bacteriophages as an industrial treatment:

Bacteriophages have been used in various life applications, including sprays, capsules, sterilizers, and therapeutic agents (Figure 2). Bacteriophage sprays have been used as a biocontrol measure in agriculture to control bacterial infections in plant pathogens and crops (Malik, 2021; Zhang et al., 2021, Gibb et al., 2021).

The phage sprays were used to control bacterial spot disease in tomato plants (Khan et al., 2019). The use of phage sprays to control bacterial wilt disease in cucumber plants was also recorded (Li et al., 2020). On the other hand, Bacteriophages can be sprayed onto surfaces, such as produce, meat, and food processing equipment, to control bacterial contamination (Moye et al., 2018). In addition, bacteriophage capsules are a delivery method for phages that protect them from environmental stresses, such as heat, pH, and proteases. They have been used in animal feed to control bacterial infections in livestock and *Salmonella* infection in chickens (Clavijo et al., 2019).

Also, bacteriophage sterilizers are used to control bacterial contamination in various settings, including hospitals and food processing plants (Żbikowska et al., 2020), and control *Listeria monocytogenes* contamination in a food processing plant (Kawacka et al., 2020), also control *Acinetobacter baumannii* contamination in a hospital setting (Weinberg et al., 2020). The use of phage therapy to treat urinary tract bacterial infections in humans and animals. They can be administered orally, topically, the eye and ear drops, intravenously, or through a wound dressing (Cristobal-Cueto et al., 2021; Xu et al., 2022).

Also, food products can be immersed in a bacteriophage solution to reduce bacterial contamination (Holtappels et al., 2021) or bacteriophages can be incorporated into coatings or films that are applied to food packaging materials or other surfaces to control bacterial contamination (Stachler et al., 2021; Cristobal-Cueto et al., 2021). A study published in the journal Clinical Microbiology in 2022 investigated the use of Bacteriophage cocktails to remove Staphylococcus bacteria from surfaces in neonatal intensive care units (Chavignon et al., 2022). Overall, bacteriophages have shown great potential for a variety of life applications, and continued research in this area is likely to lead to further advances in the field.

3. CONCLUSIONS:

In conclusion, bacteriophages have been studied for their potential use in bioremediation particularly in the remediation of soil and water contaminated with bacterial pathogens, killing bacterial pathogens in contaminated environments, and due to their specificity, effectiveness, sustainability, and eco-friendly process. Despite the potential benefits, the use of bacteriophages in biotechnology and bioremediation still faces challenges. Overall, bacteriophages have shown great potential for a variety of applications in research and development, and continued research in this area is likely to lead to further advances in the field.

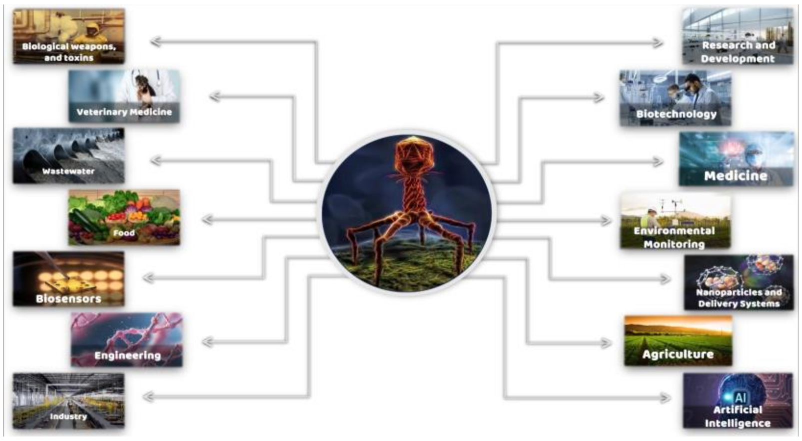

Figure 2: Bacteriophages applications and importance in different fields

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ZB, MA, EM and TA read and approved the final manuscript.

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