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A review on bacteriophage mediated control of phytopathogenic bacteria for plant protection

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Abstract

Bacteriophage therapy entails the application of bacteriophage viruses for the remedy of bacterial infections, a practice that has persisted for well over a century. Despite its enduring presence, the implementation of this therapeutic approach has faced persistent challenges, even though it has garnered support for over 25 years from microbiologists and physicians who perceive it as propitious remedy in the context of the escalating threat posed by anti-microbial resistance (AMR). The underlying justifications for these ongoing challenges are intricate in nature. This article aims to delve into the impact of bacteriophages on phytopathogens in the realm of plant biology. It endeavors to elucidate how bacteriophages function as potent antimicrobial agents in the management of bacterial infections afflicting plants, highlighting their reliability in comparison to synthetic antibiotics employed to mitigate such infections. It is worth noting that synthetic antibiotics occasionally falter in their efforts to curb or eradicate disease-causing bacteria. These microorganisms often exhibit a vigorous opposition to microbicides and various antibiotic drugs. Some bacteria even display resistance to colistin, rendering them multi-drug resistant (MDR), extensively drug-resistant (XDR), or even pan-drug resistant (PDR). In the relentless battle against these formidable bacterial foes, bacteriophage therapy emerges as a beacon of hope, offering promising results in the eradication of phytopathogenic bacteria and the suppression of further bacterial infections. This innovative phage therapy represents a significant milestone in the field of microbiology.

Keywords: Bacteriophage, phage therapy, phytopathogens, bacterial diseases in plants.

1. Introduction

Bacteria are most environment and they can easily cause infection in the host cell. Most of the time antibiotics are used to kill these bacteria. But there are some strains of bacteria which exhibits high resistance towards the

antibiotics or to any other drugs. These kinds of bacteria are called as Multi-drug resistant (MDR) and extensive drugs resistant (XDR). It had become a challenge to win over these resistant bacterial strains. In this criteria bacteriophage plays a vital role to suppress the bacterial infection and to kill the disease causing bacteria [1].
Bacteriophages are he specialized virus that infects only bacterial cell neither plant nor animals cell. Phage is the word derived from a Greek word "Phagein" which means "to devour". A phage can knock down the bacterial cell by injecting the genetic material into the bacterial cell. The genetic material present in a phage can be ssRNA, dsRNA, ssDNA or dsDNA. This method of eradicating bacteria is very helpful and do not cause any harm to either host plants or to the environment. Since most of the living creatures includes humans depend on plants for food. Using chemical antibiotics or drugs on the infected plant may indirectly affect the consumer that may lead to a serious damage by increasing the toxicity and sometime can even cause life threatening disease. But when bacteriophage is used instead of synthetic antibiotics, they do not cause any damage to any other cell except the bacterial cell. This is the greatest advantage of this phage therapy. By this we can certainly assure that phage therapy can efficiently replace the phyto- antibiotics [2].
With the persistent threat posed by resistant strains of bacteria to traditional therapies for bacterial infections, there is growing interest in exploring alternative antimicrobial approaches. Many microbiologists hold the belief that bacteriophages, which exclusively infect bacteria, offer a promising avenue for the remedy of bacterial diseases.
Another advantageous aspect of phages is their capacity for exponential proliferation. Phages tend to mirror the patterns of bacterial growth and existence, multiplying in tandem with bacteria and diminishing once the bacterial host is eliminated. This natural abundance of phage species offers the potential to target even newly emerged resistant bacterial strains, providing a dynamic and adaptable approach to combating bacterial infections [3].
The emerging cohort of bacteriophage researchers has confronted the necessity to ameliorate the adverse publicity surrounding their field and to navigate complex scientific intricacies. Notably, the precision of bacteriophages in targeting specific bacterial species imposes a stringent demand for impeccable bacterial detection. The exigencies of swiftly escalating and potentially fatal bacterial infections compress the timeframe available for the identification and cultivation of a suitable bacterial strain for phage selection. A pragmatic approach involves the deployment of a consortium of bacteriophages that, in concert, comprehensively encompass the predominant, if not the entirety, of known bacterial strains within a pathogenic bacteria species [4].

2. Biology of bacteriophage: -classification, structure, mode of action and lifecycle.

2.1 Classification:

Bacteriophages, or phages for short, represent the most prevalent biological entities in existence. These minuscule life forms can be discerned across a wide spectrum of habitats, spanning from aqueous environments to terrestrial and oceanic landscapes, and even in the harshest extremities characterized by exceedingly low or exceedingly high temperatures. Remarkably, phages are not confined solely to natural settings; they infiltrate man-made environments as well, with their presence documented in hospital settings and within wastewater. They inhabit any locale where bacteria can thrive, including the cellular tissues of plants and animals. Numerous phages have been meticulously characterized within the scientific community [5]. Phages have undergone extensive categorization based on a multitude of criteria, encompassing their morphological characteristics, nucleic acid composition, preferred ecological niches, and the specific host species they are capable of targeting and destroying [6].

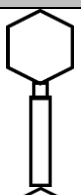
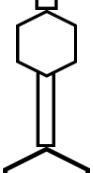
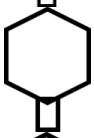
The prevalent classification scheme for phages primarily hinges on their biological life cycle, segregating them into two distinct categories: lytic (virulent) phages and lysogenic (temperate) phages. These phases of the phage life cycle revolve around their attachment to and invasion of bacterial hosts. However, it is crucial to recognize that the initiation of this binding process necessitates a match between the structural components of the phage and strain- precise receptors on the bacterial superficial surface face. Considering the frequent and recurrent mutations occurring in both phages and bacterial configuration, often facilitated by phages themselves, a singular phage can only infect a limited spectrum of bacterial strains and, in many instances, a single strain exclusively. This underscores the remarkable specificity inherent in the activity of phages [7].
Following the entry of the phage into the bacterial cell, a remarkable transformation takes place. The bacterial synthetic apparatus is commandeered, redirecting its efforts towards the synthesis of viral proteins and genetic material. Subsequently, a highly orchestrated process unfolds, culminating in the assembly and packaging of new phage particles. Eventually, the bacterial cells meet their demise through lysis, resulting in the release of

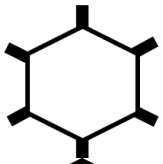
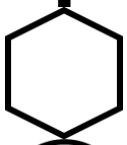
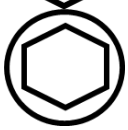
newly formed virions, ready to embark on the journey of infecting other host cells in a cyclical continuation of the phage life cycle [8].

Nonetheless, it's worth noting that the burst size, or the number of new phage particles produced per infected bacterial cell, can exhibit significant variation depending on the specific phage involved, the pathogenic target against which the phage is deployed, and the environmental conditions in which these phage-pathogen interactions occur. In stark discrepancy, the lysogenic cycle is depicited by the assimilation of viral genetic material into the host bacterium's genome. When the host cell undergoes division, this integrated viral genetic material is passed on to the daughter cells, perpetuating the presence of viral chromosomes within the bacterial population [9]. Only exceptionally can the viral genome to be segregated from the host DNA and enters to lytic stage. Due to their special infections caused by bacteria [10].

They are classified into 13 families and the parameters considered in bacteriophage for their classification are, Morphological characters, nucleic acid content, bacterial target, region where phage can predominantly be existed and lifecycle [11, 12].

Table. 1 Various families of bacteriophage based on morphology and nucleic acid content [12].

Family	Morphology	Nucleic acid content	Characteristic
Myoviridae		Linear dsDNA	Non-enveloped, contractile tail.
Siphoviridae		Linear dsDNA	Non- enveloped, long non-contractile tail
Podoviridae		Linear dsDNA	Non-enveloped, short non-contractile tail
Tectiviridae		Linear dsDNA	Non-enveloped, Isometric
Cortiviridae		Circular dsDNA	Non-enveloped, Isometric
Lipothixviridae		Linear dsDNA	Enveloped, Rod shaped
Plasmaviridae		Circular dsDNA	Enveloped, pleomorphic
Rudiviridae		Linear dsDNA	Enveloped, rod shaped
Fuselloviridae		Circular dsDNA	Non-enveloped, lemon shaped

Inoviridae		Circular ssDNA	Non-enveloped, Filamentous
Microviridae		Circular ssDNA	Non-enveloped, Isometric
Leviviridae		Linear ssDNA	Non-enveloped, Isometric
Crystoviridae		Segmented dsDNA	Enveloped, spherical

2.2 Structure of Bacteriophage

Bacteriophages exhibit a diverse array of sizes and shapes in their structural composition. Highlighting the bacteriophage T4 as an illustrative example, its fundamental structural attributes include [13]

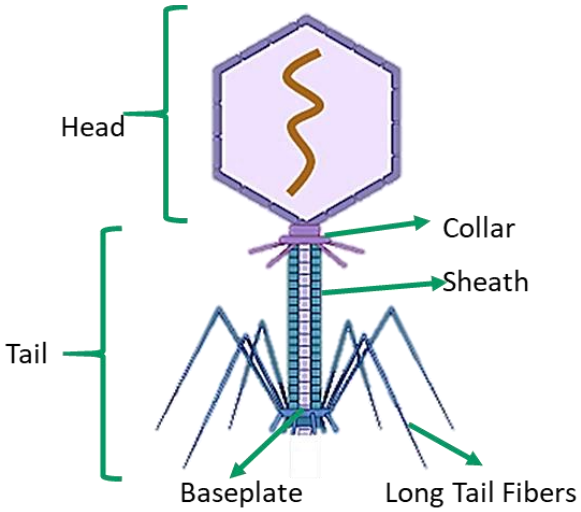


Fig.1 Structure of bacteriophage.

***Size*:** The size of bacteriophages can vary significantly, and the T4 phage stands out as one of the largest among them. Its dimensions typically range from approximately 200 nanometers in length and 80 to 100 nanometers in width. It's important to note that the size of bacteriophages can vary widely, and most bacteriophages fall within a size range of 24 to 200 nanometers in length. This diversity in size is reflective of the wide range of bacteriophage types that exist, each adapted to their unique host bacteria and infection strategies.

***Head or Capsid*:** Bacteriophages universally feature a head structure, a critical component that can manifest variability in both its size and shape. Among bacteriophages, some possess filamentous head structures, while others assume an icosahedral form distinguished by its 20-sided geometry. This head structure, alternatively known as a capsid, is assembled from a multitude of replicas of one or more unique proteins. Nestled within the confines of this head is the nucleic acid, housing the phage's genetic material, essential for its functioning [14].

***Tail*:** The size and structure of the tail can vary significantly among different bacteriophage species. In some cases, certain bacteriophages may lack a distinct tail structure altogether. More complex bacteriophages, such as the T4 phage you mentioned, often showcase a more intricate tail structure. Regarding T4, the tail is encircled by a contractile jacket that has the capability to contract during the infection process. This contraction facilitates the transmittal of the phage's genetic material into the host bacterium. The diversity in tail structures

and functions among bacteriophages underscores the incredible adaptability and specialization that these viral entities have developed over time to optimize their interactions with bacterial hosts [15]. Importantly, not all bacteriophages exhibit the presence of base plates and tail fibers. In such cases, different structural elements come into play to foster the adhesion of the bacteriophage to the bacterial host cell. The variability in these structural components highlights the remarkable diversity in strategies that bacteriophages employ to successfully engage and infect their target bacterial hosts [16].

2.3 Mode of action:

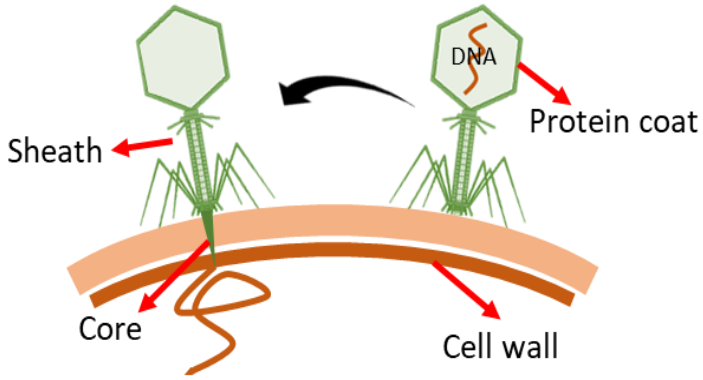


Fig. 2 Mode of action of bacteriophage.

The bacteriophage infection involves mainly four steps. They are,

Adsorption*:* The tail fibers, or analogous structures, attach specifically to receptors located on the superficial of the bacterial host cell. This receptor recognition is pivotal and defines the host specificity of the bacteriophage, meaning it determines which type(s) of bacteria the phage is capable of infecting. The diversity in tail fibers among different bacteriophages underscores their adaptability to engage a range of bacterial hosts, a trait that influences their effectiveness as agents of bacterial infection [7, 17]. The interaction in between tail fibers and host cell receptors forms the foundation for the subsequent steps of the infection process, including DNA injection and replication within the host cell. This complex interplay showcases the intricate strategies that bacteriophages have evolved to ensure successful infection and propagation within their target bacterial hosts [18]. The bacterial receptor's characteristics vary across different types of bacteria. For instance, the outer surface of the bacterium features various proteins, such as LPS, pili, and lipoproteins. While these receptors serve other purposes for the bacteria, bacteriophages have resulted to exploit these receptors for the purpose of infection [19].

Irreversible attachment or pinning: The connection between the bacteriophage and the bacterium by means of the tail fibers is delicate and can be undone, making it a reversible process. However, the firm and irreversible attachment of the bacteriophage to the bacterium is facilitated by one or more components found within the base plate. In cases where a bacteriophage lacks base plates, an alternative mechanism exists through which it can tightly bind to the bacterial cell [20].

Sheath Contraction**:** The immutable attachment of the bacteriophage to the bacterium triggers a cascade of events. It initiates the contraction of the sheath, propelling the void tail fiber via bacterial envelope [21]. Bacteriophages lacking contractile sheaths employ alternative mechanisms to breach the bacterial envelope. Certain bacteriophages utilize enzymes that are involved in digesting various components of the bacterial envelope, facilitating the passage of the phage particle into the bacterial host [22].

Nucleic acid injection**:** Once the bacteriophage has successfully traversed the bacterial envelope, the nucleic acid contained within the confines of head structure is then transferred through the void tail and gains entry into the host cell. This crucial step marks the initiation of the infection process [23]. Frequently, it's the nucleic acid of the bacteriophage that penetrates the host cell, while the other components of the phage typically remain exterior of the bacterium. However, there are exclusion and peculiarities to this general rule, as some phages may exhibit variations in their infection mechanisms, potentially involving additional components or processes during host cell invasion [24]. This contrast in the entry mechanism between phages and virus that infects animal cell likely arises from the distinct nature of bacterial and animal cells. Bacterial cells lack the ability to

engulf large materials like animal cells can, which may explain why, in the case of bacteriophages, primarily only the nucleic acid enters the host cell, while in animal cell viruses, a greater portion of virus components can penetrate the cell membrane [25].

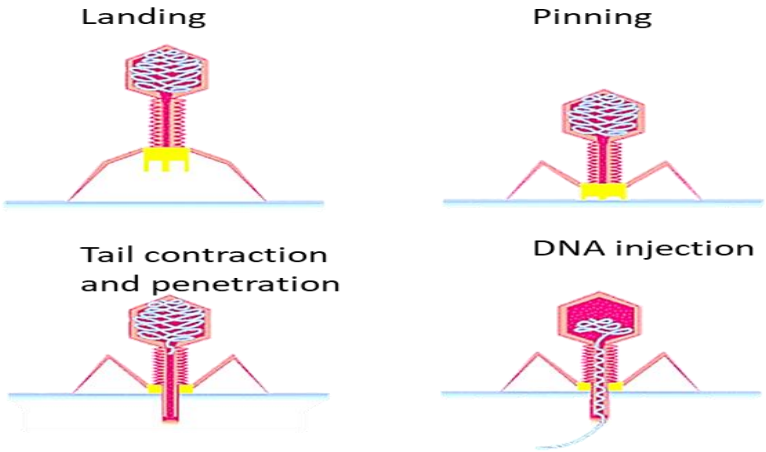


Fig. 3 Steps involved in bacteriophage infection.

3. Biological life cycle of bacteriophage:
The life cycle of bacteriophage is of two types. One is lytic (virulent) cycle and the other one is lysogenic (temperate) cycle [26, 27].

3.1 Lytic cycle
3.1.1 Definition of lytic bacteriophages:
Lytic or virulent bacteriophages are a category of phages that exclusively reproduce within bacterial hosts and culminate their life cycle by causing cell lysis, leading to the destruction of the host cell. The mechanism underlying the lytic cycle is described below [28, 29].

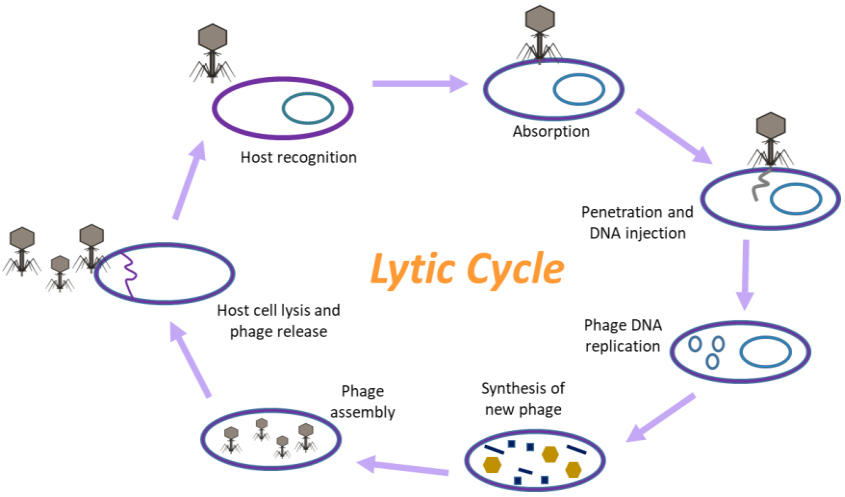


Fig. 4 Lytic cycle of bacteriophage.

***Eclipse period*:** At this specific stage, there are no observable infectious phage particles either within or outside the bacterial cell. Instead, the genetic material of the bacteriophage takes command of the host's biosynthetic machinery, initiating the synthesis of specific messenger RNA molecules and proteins as dictated by the bacteriophage's genetic instructions. This process follows a well-defined pattern of bacteriophage-induced macromolecular synthesis, resembling the highly organized gene expression seen in infections caused by animal pathogens [30]. During the initial phase, the early messenger RNA serves as a blueprint for the synthesis of crucial proteins that serve two fundamental functions. Firstly, these early proteins facilitate the synthesis of bacteriophage DNA while simultaneously putting a halt to the host cell's DNA, RNA, and protein production. In specific cases, these early proteins are involved in actively dismantling the host chromosome.
Following the completion of bacteriophage DNA synthesis, a subsequent phase involves the activation of late messenger RNA molecules. These late messenger RNAs drive the production of late proteins, which fulfill a

dual role in the bacteriophage life cycle. Firstly, they constitute the structural elements that make up the bacteriophage itself. Secondly, they encompass the proteins required for the ultimate rupture of the bacterial cell, a critical step referred to as lysis [31].

***Intracellular Accumulation Phase*:** During this stage, the nucleic acid and structural proteins that were synthesized earlier converge and undergo assembly, resulting in the accumulation of infectious bacteriophage particles within the host cell. As time passes, the bacterial cell begins to undergo lysis due to the accumulation of phage lysis proteins. This ultimately leads to the release of the intracellular phage particles into the surrounding environment.
The number of phage particles released per infected bacterial cell can be remarkably high, reaching levels of up to a thousand particles per cell. This prolific production and release of phage particle plays a crucial role in the phage's capability to rapidly propagate and infect neighboring bacterial cells, contributing to the efficient control of bacterial populations [32].

3.1.2 Plaque Assay: Lytic bacteriophages are typically assessed and quantified using a method known as a plaque assay. A plaque, in this context, refers to a distinct clear area that outcomes from the lysis or destruction of bacterial cells. Importantly, each plaque originates from the activity of a single infectious phage particle. The specific infectious particle responsible for forming a plaque is referred to as a PFU, which stands for Plaque Forming Unit. The plaque assay is a valuable tool for estimating the concentration of infectious phage particles in a sample and for studying their activity against bacterial host [33, 34].

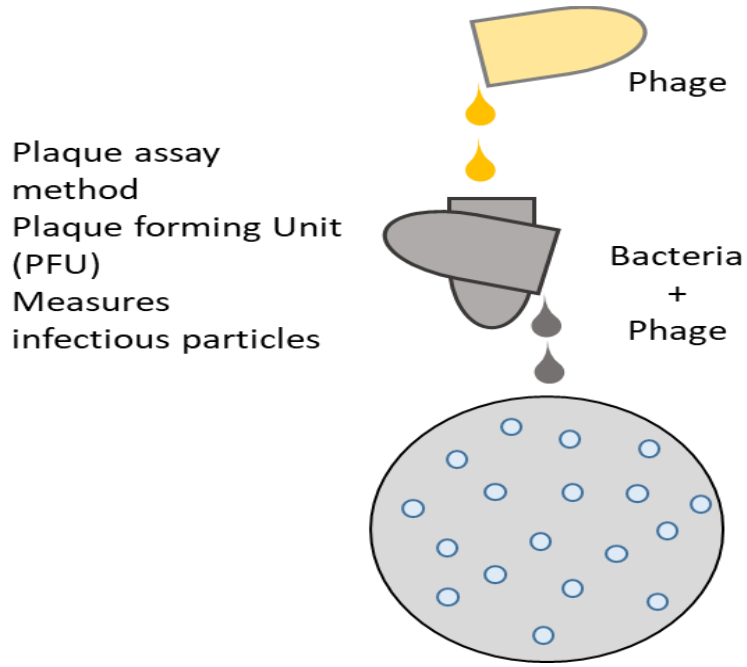


Fig. 5 Assay for lytic bacteriophage.

3.2 Lysogenic cycle
3.2.1 Definition of lysogenic bacteria: Lysogenic or temperate phages are a distinct category of phages that possess the ability to make a critical decision regarding their replication strategy once inside a host cell. These bacteriophages can either to track the lytic cycle, leading to the destruction of the host cell, or they can enter a dormant or inert state within the cell. During this dormant phase, a significant portion of the phage's genes remain untranscribed, and the phage genome assumes a repressed state. This dormancy allows the phage to coexist peacefully with the host cell for an extended period without causing immediate harm. In some cases, the phage may integrate its genetic material into the host's genome, becoming a part of the host's genetic material in a process known as lysogeny [35]. The phage DNA, while in this repressed state, is referred to as a prophage. The term "prophage" is used because, although it is not an active phage, it retains the potential to give rise to phage particles. In many instances, the prophage DNA becomes integrated into the host chromosome, becoming part of the host's genetic material. It is then replicated along with the host chromosome and is transmitted to the daughter cells during cell division.
Host cells that harbor a prophage are not unfavorably afflicted by the presence of the prophage, and the

lysogenic phase, where the prophage exists within the host, can persist indefinitely. Such host cells, which have integrated prophage DNA into their genome, are referred to as lysogens. In this state, the host cell and the prophage coexist peacefully, and the prophage remains latent until specific triggers prompt it to enter the lytic cycle and produce new phage particles [36].

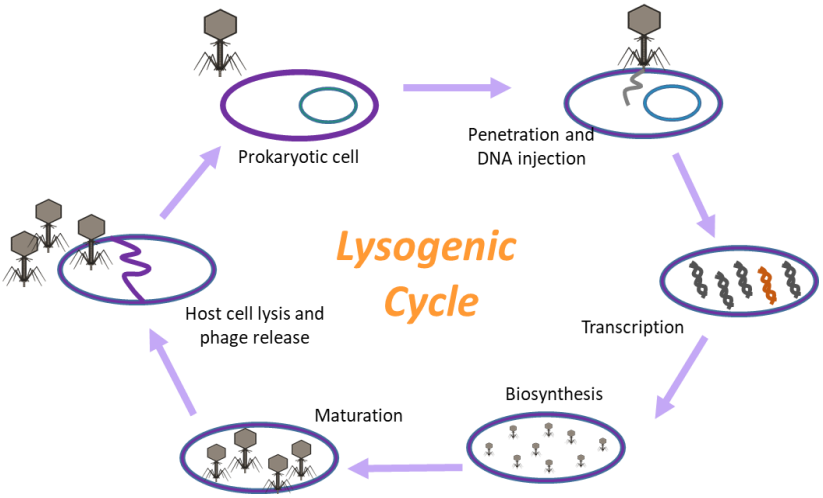


Fig. 6 Lysogenic cycle of bacteriophage.

3.2.2. Mechanism of lysogenic cycle: Prototype Phage: Lambda

Circularization of the phage chromosome: Lambda DNA is a double-stranded linear molecule with small, single-stranded regions located at the 5' ends. These single-stranded ends are complementary to each other, allowing them to pair up and create a circular molecule. Within the cell, the open terminals of this circular molecule can be chemically linked (ligated) to form a covalently enclosed ring structure. This circularized form of Lambda DNA is stable and serves as a critical component in various biological processes, including the replication and regulation of Lambda phage in bacterial hosts [37].

Site-specific recombination: A recombination process, facilitated by an enzyme encoded by the phage, takes place between a specific region on the rounded phage DNA and a corresponding region on the host chromosome. This recombination process outcome in the incorporation of the phage DNA into the host chromosome. This integration event is a key stage in the lysogenic cycle of temperate phages, as it allows the phage to become part of the host cell's genetic material and remain in a dormant or latent state until specific triggers prompt it to enter the lytic cycle and initiate phage replication [38].

Repression of the phage genome: In the context of the lysogenic cycle of temperate phages, a phage-encoded protein called a repressor is synthesized. This repressor protein binds to a specific site on the phage DNA, known as the operator, and exerts a regulatory function. Its primary role is to block or inhibit the transcription of most phage genes. However, it is important to note that the repressor gene itself is an exception, and its transcription is not halted. This regulatory mechanism ensures that the repressor protein is continuously produced and maintains control over the expression of other phage genes, effectively keeping them in a repressed state during the lysogenic phase [39]. As a result of these regulatory processes, a temperate phage genome assumes an inert and repressed state, becoming integrated into the host chromosome. Importantly, each temperate phage will exclusively suppress the transcription of its own DNA and will not interfere with the DNA of other distinct phages. This specificity ensures that the suppression of gene expression is highly targeted and distinct, allowing each temperate phage to coexist within the host cell without interference from other temperate phages. This selective repression is a key feature of the lysogenic cycle [40].

Events guiding to Termination of Lysogeny

When a lysogenic bacterium encounters unfavorable environmental conditions, it can disrupt the lysogenic phase through a process known as induction. Unfavorable conditions that trigger the termination stage of the lysogenic state include factors like desiccation, exposure to UV or ionizing radiation, and susceptibility to mutagenic chemicals.

Under these negative circumstances, the bacterium responds by synthesizing proteases, with the RecA protein being a prominent example. These proteases target and degrade the repressor protein, which is responsible for

maintaining the repression of phage genes. As a result, the repression of phage genes is lifted, leading to the activation of phage gene expression. This activation, in turn, initiates a series of events that reverse the integration process of the phage DNA into the host chromosome. The phage then progresses towards a lytic life cycle characterized by active viral replication. Ultimately, this leads to the lysis of the host cell, enabling the release of new phage particles that can go on to infect other host cells. This shift from lysogenic to lytic replication is a crucial adaptive response that allows the phage to thrive in the face of adverse conditions [41].

4. Lytic cycle vs lysogenic cycle

The lambda phage's fate, whether it follows the lytic or lysogenic cycle, is determined by the dynamic interaction between two critical components: the repressor and another bacteriophage protein called *Cro*. The *Cro* protein plays a pivotal role by interfering with the formation of the repressor-operator complex, thereby preventing the establishment of lysogeny. When environmental conditions favor the synthesis of the *Cro* protein, it tips the balance in favor of the lytic cycle. In this scenario, the *Cro* protein prevents the repressor from binding to its operator site, promoting the expression of lytic genes and ultimately leading to host cell lysis. Conversely, conditions that promote the production of the repressor protein favor the lysogenic pathway. In this case, the repressor binds to its operator site, suppressing lytic gene expression, and facilitating the integration of the phage's genetic material into the host genome. The phage remains in a dormant state until future induction signals trigger its activation, allowing it to shift back to the lytic cycle if necessary. This intricate regulatory mechanism enables the lambda phage to adapt its life cycle to the prevailing environmental conditions [35].

4.1 Significance of Lysogeny

***Lysogenic conversion*:** In cases where a cell undergoes lysogeny, there are situations where extra genes found within the phage genome can become active and are expressed within the host cell. These genes have the potential to modify the characteristics of the bacterial host cell. This phenomenon is known as lysogenic conversion or phage conversion. It involves the integration of genetic elements from the bacteriophage into the bacterial genome, leading to changes in the host cell's phenotype. Lysogenic conversion can bring about alterations in the host cell's traits, such as modifications in surface structures, increased virulence, or the acquisition of new metabolic capabilities. These changes stem from the expression of these additional phage genes, and they can have significant implications for the behavior and properties of the infected bacterial cell [38]. Indeed, lysogenic conversion is of significant clinical importance. For instance, lysogenic phages have been observed to carry genes capable of modifying crucial bacterial components, such as the Salmonella O antigen, which is a prominent target of the immune system. Additionally, certain toxins produced by bacteria, such as *Corynebacterium diphtheriae*, are controlled by genes carried by phages. In these instances, bacterial strains that have undergone lysogenic conversion can become pathogenic. These genetic alterations triggered by lysogenic phages can result in changes in bacterial virulence, toxin production, and antigenic properties. Consequently, the clinical behavior and impact of bacterial infections can be substantially influenced by these lysogenic conversion events. Understanding these mechanisms is crucial for comprehending the dynamics of bacterial infections and their clinical implications [42].

5. Isolation, Production and Preservation of bacteriophage

***Isolation*:** The host range of a bacteriophage is a critical determinant of its suitability for phage therapy. Host range refers to the range of bacterial strains or species that a specific phage can infect. This characteristic holds paramount importance as it dictates the potential effectiveness of a phage in addressing specific bacterial infections. In phage therapy, selecting a phage with a host range that matches the target bacteria is essential. A phage with a broad host range may be effective against a wide range of bacterial strains, while a phage with a narrow host range may only target a specific subset of strains. Understanding and matching the host range of the phage to the bacteria causing the infection is crucial for achieving successful outcomes in phage therapy [43].

Standard isolation protocols for bacteriophages typically do not inherently select for a specific host range. Instead, the determination of a phage's host range is typically carried out after the phage has been isolated. During the isolation process, researchers often employ a single bacterial strain as the host for the phage, but some opt to use a mixture of multiple host strains. Using a variety of host strains can be advantageous because it increases the likelihood of capturing a broader spectrum of phages, potentially including those that may be effective against various bacterial strains. Once isolated, the host range of the phage is assessed through

experiments that involve testing its infectivity against different bacterial strains or species. This post-isolation host range determination helps researchers identify the specific bacterial strains that a given phage can target effectively, providing valuable information for its potential use in phage therapy or other applications [44].
Your perspective on examining phage isolation to enhance their suitability for specific applications is absolutely valid and important. Understanding a phage's host range is a fundamental aspect of tailoring phage therapy or other applications to target specific bacterial infections effectively. Expanding a phage's host range can indeed involve selecting for phages that can infect a broader range of bacterial strains. This broadened host range can be particularly advantageous for addressing a wider array of infections caused by the same bacterial species. Moreover, characterizing a phage's host range provides valuable insights for researchers, enabling them to make informed decisions about which phages are best suited for various therapeutic or practical purposes. This knowledge ensures that the selected phages will efficiently target the intended bacterial strains while minimizing any potential impacts on beneficial bacteria or unintended targets, contributing to the success and safety of phage-based applications [45].

5.1 Production of phages

Bacteriophages are currently being utilized in significant quantities in various industries, particularly in the food and agriculture sectors. Bacteriophages are used in the food industry to control bacterial contamination and improve food safety, while in agriculture, they are employed to combat plant diseases caused by bacteria. The emergence of antibiotic-resistant bacterial strains is a pressing global issue, and as a result, the use of bacteriophages as an alternative treatment for bacterial diseases in plants has garnered increased attention. Bacteriophages offer a promising approach to address these challenges while minimizing the use of antibiotics. As a consequence, it is likely that even larger quantities of bacteriophages will be required in the future to meet the growing demand for phage-based treatments in agriculture [46]. High request incorporated with a broad range of utilization needs well organized bacteriophage production methods operating at less production costs and with great efficiency [47]. Certainly, various approaches and methods have been implemented to enhance bacteriophage production, and each approach comes with its own set of advantages and disadvantages. Here's a brief overview of some common bacteriophage production methods, including batch culture, semi-continuous culture, and continuous culture, along with their pros and cons [48]. Factors influencing bacterial physiological state can significantly impact bacteriophage formation. Understanding these factors and employing techniques to resolve phage growth limitations is crucial for efficient phage production [49].

5.2 Preservation of bacteriophages:

Ensuring the long-term and secure conservation of lytic bacteriophages is indeed of critical importance, particularly in the context of research, diagnostics, and therapeutic applications. These phages are valuable tools for targeting specific bacterial strains. It's essential to understand the impact of various external factors on phage conservation and to develop organized strategies for maximizing their longevity. In this article, its focused on the conservation of lytic bacteriophage VP3, which is used for typing Vibrio cholerae O1 biotype El Tor. To optimize the conservation of this phage, you likely considered several key aspects. Efforts to systematically evaluate and optimize the conservation of lytic bacteriophage VP3 demonstrate a commitment to preserving these valuable resources for continued research and applications related to Vibrio cholerae. Your work contributes to the broader understanding of phage preservation techniques and their importance in microbiology and biotechnology [50]. Efforts to systematically evaluate and optimize the conservation of lytic bacteriophage VP3 demonstrate a commitment to preserving these valuable resources for continued research and applications related to *Vibrio cholerae*. Your work contributes to the broader understanding of phage preservation techniques and their importance in microbiology and biotechnology [51]. The summation of cryoprotectant, glycerol (30%, w/v) or dimethyl sulfoxide (DMSO, 10%, w/v) significantly improves the survival rates of phages preserved at −20 °C. At 4 °C, −80 °C, and −196 °C, the cryoprotectant effect was only slightly positive or even harmful. The better conservation method is to directly conserve the bacteriophage stocks in Luria-Bertani medium at -80° C or −196 °C in contrast of preserving them in SM buffer or adding cryoprotectant. The results provided insights into the outer influencing constituents on bacteriophage VP3 during conservation at minimum, temperature and can be implied to the hike of bacteriophage preservation in further [52].

6. History of Phage therapy against phytopathogens: -

Bacteriophage therapy boasts a rich history that spans back hundreds of years, demonstrating its enduring relevance. At the forefront of this innovation was the French-Canadian scientist, Felix d'Herelle, who is often

attributed with the identification and nomenclature of phages. While a debate persists regarding whether d'Herelle or the British microbiologist Twort first discovered bacteriophages, d'Herelle's pivotal role in utilizing phages to combat infections is widely acknowledged. This catalyzed global endeavors, particularly prominent within the former Soviet Union, to explore the potential of bacteriophage therapy in addressing ailments ranging from typhoid fever to cholera. Early investigations yielded promising outcomes, although by contemporary standards, some experiments suffered from inadequate design—lacking elements like placebos or control groups. These studies were often documented in non-English journals, rendering them largely inaccessible to Western researchers. Nonetheless, phage therapy gained traction in the United States as well. Throughout the 1940s, multiple pharmaceutical companies in the U.S. produced bacteriophage preparations for the treatment of diverse infections, spanning from upper respiratory tract issues to abscesses [53].

In the early 20th century, Felix d'Herelle introduced the concept of utilizing bacteriophages for the treatment of bacterial disease in both humans and animals. However, this innovative approach encountered limited acceptance in Western medical circles [54]. The advent of antibiotics in the 1940s led to a shift in phage research towards more fundamental investigations. During this period, a significant development occurred in the Soviet Union where phage therapy gained substantial traction. This was largely attributed to the collaboration between Felix d'Herelle and his colleagues from Georgia. Notably, the bulk of literature on this subject stems from the 1930s and 1940s, reflecting the fervent exploration of phage therapy during that era [55].

The articles detailing these findings were predominantly published in Russian, rendering them inaccessible to Western scientists. This linguistic barrier hindered the dissemination of knowledge across geographical boundaries. Despite this, the initial Western skepticism surrounding phage therapy experienced a resurgence and reevaluation, catalyzed by the rise of drug-resistant bacterial strains. These limitations underscore the need for methodological rigor in scientific research and highlight the evolution of perspectives on phage therapy, from skepticism to renewed interest, driven by the pressing concern of antibiotic-resistant bacterial strains [56].

Although specific phage titers and assessment methods were frequently omitted in the published literature, the comparative approach with previous years' results provided insights into the efficacy of phage interventions. These records, despite their limitations, underscore the potential positive impact of phage therapy and prophylaxis. Hence, they should not be disregarded when designing forthcoming studies in this field. Recognizing the historical context and considering the insights gleaned from past clinical experiences can contribute valuably to the trajectory of future research initiatives [57].

6.1 What is phage cocktail?

Phage combinations, also known as phage cocktails, can consist of individual phages that collectively have an impact on different bacterial species, such as bacterial species 'A' and bacterial species 'B'. These types of combinations, which target multiple species, are often used strategically to address infections that involve a variety of bacterial strains. For instance, infections of the skin or soft tissues can be caused by different species of bacteria, and they can be effectively treated using phage cocktails like Pyophage. These types of phage combinations are designed to target a broad range of bacterial taxa, including Enterococcus, Escherichia, Proteus, Pseudomonas, Staphylococcus, and Streptococcus, among others. The advantage of using such phage cocktails is their versatility in combating polymicrobial infections where multiple bacterial species are involved. By including phages that can infect a spectrum of bacteria, these cocktails offer a more comprehensive approach to treating complex infections, enhancing the likelihood of successful therapy [58].

To achieve a broad spectrum of activity against different bacterial species, phage cocktails are typically composed of at least one phage that targets each specific species of interest. This strategy allows for the customization of the cocktail to match the range of bacteria causing an infection. For example, if an infection involves both species A and species B, the cocktail would ideally contain at least one phage effective against species A and one against species B. Alternatively, achieving a broad spectrum of activity can also be accomplished through polyvalent phages, as you mentioned. Polyvalent phages are phages that have the ability to infect multiple bacterial species [59]. These phages are versatile and can be particularly valuable in situations where it might be impractical to create a cocktail with multiple single-host-range phages. Polyvalent phages can provide a more streamlined approach to treating polymicrobial infections. In essence, both strategies—using multiple single-host-range phages in a cocktail and employing polyvalent phages—aim to broaden the range of bacterial strains a phage therapy can effectively target, offering flexibility and efficacy in treating complex infections. The greater the distinction between the targeted bacteria, such as Gram-positive versus Gram-negative, the lower the likelihood that versatile polyvalent phages can be effectively employed. While theoretically, combining various species might offer a wide range of applications, our primary focus

remains elsewhere [60].

6.2 Application of phage therapy against phytopathogenic bacteria

Agrobacterium tumefaciens

A. tumefaciens Conn strain B2 was introduced in contrast to strain B6-806 of the same organism, it resulted in the formation of structures referred to as plaques. Within these plaques, a notably potent bacteriophage with enhanced virulence was extracted. The isolation process was achieved using D'Herelle's method, which entails the deliberate selection of phages exhibiting virulent characteristics [61]. When grown on nutrient agar, phage PB21 displayed the development of large, transparent plaques that maintained well-defined boundaries without excessive expansion. Intriguingly, when introduced to a glutamate medium or White's plant tissue culture medium, the plaques produced by PB21 were even larger and had a three-dimensional appearance when observed within White's medium. Importantly, PB21 does not demonstrate traits typical of an oncogenic virus, indicating it lacks the capacity to initiate tumor formation [62].

On the contrary, when phage particles were introduced under precise conditions ensuring substantial lysis, it completely halted the initiation of tumors. Interestingly, even when there were fewer than 10 phage particles at the beginning of a 21-hour induction phase, they occasionally demonstrated the ability to completely inhibit tumor initiation caused by highly virulent bacteria, like strain B6 [63]. The data presented further strengthen the idea that any factor disturbing the metabolic processes associated with bacterial growth also hinders tumor induction. Nevertheless, attempts to use the phage to eliminate bacteria from crown gall tissue yielded unsuccessful results. This underscores that interfering with the bacteria's metabolic activity, closely linked to its growth, can profoundly affect the tumor induction process. Despite efforts, the application of the phage to clear bacteria from crown gall tissue did not yield the intended result [64].

Ralstonia solanacearum

The identification of three new lytic bacteriophages has proven to be remarkably efficient in controlling the pathogenic microorganism *R. solanacearum.* This pathogen is of great concern as a quarantine organism in many countries and is the primary cause of bacterial wilt, a widespread and severe vascular plant disease. The utilization of these recently discovered bacteriophages offers a promising approach to curbing the dissemination and impact of this formidable pathogen [65]. The management of bacterial wilt has yielded varied outcomes, underscoring the need for alternative treatment methods. In this study, the isolation of three lytic bacteriophages from different geographical regions in Spain's environmental water marked a significant achievement. These phages displayed an impressive ability to selectively infect a range of *R. solanacearum* strains, as well as certain closely related pathogenic environmental bacteria. Remarkably, their effectiveness remained consistent across diverse environmental conditions, including factors like water temperature, pH, salinity, and even in conditions lacking aeration typically found in storage tanks. The trio of bacteriophages exhibited potent control over *R. solanacearum,* leading to substantial reductions in bacterial populations within a matter of hours [66].

In terms of their biocontrol potential, these phages exhibited outstanding effectiveness in reducing high populations of the target pathogen in environmental water. Furthermore, their ability to reduce the incidence of bacterial wilt in plants was evident when administered individually or in various combinations. This efficacy was confirmed through extensive tests involving over 300 plants. Importantly, this represents the first successful instance of controlling *R. solanacearum* using single or combined bacteriophages delivered through irrigation water. This approach effectively replicates natural conditions [67]. Consequently, the utilization of these bacteriophages for the control and potential biocontrol of bacterial wilt disease caused by *R. solanacearum* has received formal patent recognition. This acknowledgment underscores the innovative and practical importance of using these phages as a promising strategy to combat this devastating plant ailment [5]. The formal patent recognition of these bacteriophages for controlling and potentially biocontrolling bacterial wilt disease caused by *R. solanacearum* highlights their innovative and practical significance as a promising strategy to combat this destructive plant ailment [68].

Streptomyces scabis

Potato common scab, caused by the pathogen Streptomyces scabies, poses a significant economic threat as it profoundly affects potato crops. This disease leads to the degradation of potato tuber quality, ultimately resulting in decreased market values and substantial financial losses for potato growers. As a response, a promising solution resides in the domain of biological control, specifically through the use of bacteriophages. The effectiveness of these viruses, which target the disease-causing pathogen, offers great promise [5]. This newly discovered phage possesses a characteristic structure, featuring a 55 nanometer icosahedral head and a

short 7.5 nanometer tail—typical traits of a podovirus. Its infection cycle spans approximately 90 minutes, encompassing a latent period of around 50 minutes followed by a 40-minute rise period, and culminating in a burst size of approximately 200 plaque-forming units (PFU) per infected cell [69].

The genomic composition of SscP1EGY comprises 51,751 nucleotides and encompasses 76 predicted genes. In tests, SscP1EGY effectively infected and completely lysed seven different strains of S. scabies. Importantly, its lytic activity was absent when tested against three beneficial Streptomyces species, other helpful bacterial strains, and non-target plant pathogenic bacteria. When applied to potato tubers previously exposed to S. scabies, the treatment with phage SscP1EGY resulted in significant reductions in the percentage of lesion surface, as compared to untreated tubers that had been inoculated with the pathogen. This led to a decrease in the severity of scab and the number of lesions [65]. Furthermore, scab lesions observed on the tubers treated with the phage displayed a superficial appearance, which was notably different from the pitted appearance of lesions on tubers not treated with the phage. These findings highlight the significant potential of SscP1EGY as a promising biological control agent against S. scabies. Importantly, the phage's impact on the disease is accompanied by desirable characteristics, including its ability to alter the appearance of lesions, which can be of great significance in terms of potato quality [70].
The contributions of this study go beyond the laboratory, with the genomic sequencing of SscP1EGY setting a noteworthy precedent. Remarkably, it represents the first S. scabies-infecting phage in Egypt to undergo sequencing, signifying a significant advancement in our comprehension of the phage diversity in this region and its potential for addressing agricultural challenges [71].

Erwinia amylovora

Indeed, the contributions of this study extend beyond the laboratory, as the genomic sequencing of SscP1EGY establishes an important milestone. Remarkably, it is the first S. scabies-infecting phage in Egypt to undergo sequencing, marking a significant advancement in our understanding of phage diversity in this region and its potential for addressing agricultural challenges [72]. These phages, identified as ΦFifi011, ΦFifi044, ΦFifi051, ΦFifi067, ΦFifi106, ΦFifi287, ΦFifi318, ΦFifi450, and ΦFifi451, were obtained from soil and water samples collected from seven orchards in Korea, all of which were affected by fire blight. The genetic diversity of the bacteriophage isolates was confirmed through the examination of restriction fragment length polymorphism patterns [73]. To assess their effectiveness, the nine phages were tested to determine their host range. This evaluation included 45 strains of *E. amylovora*, 14 strains of *E. pyrifoliae*, and an additional nine strains representing various bacterial species. Among these phages, ΦFifi044 and ΦFifi451 exclusively infected and lysed *E. amylovora*. In contrast, the remaining seven phages demonstrated the capability to infect both *E. amylovora* and *E. pyrifoliae*. These findings highlight the diversity among the isolated bacteriophages and their distinct characteristics. Importantly, they emphasize the efficacy of these phages in targeting and controlling *E. amylovora*. This data provides a solid foundation for the development of biological agents and the potential utilization of phage cocktails—a strategic approach that can harness the combined power of multiple phages to combat the threat posed by this devastating bacterial pathogen [74].

Pseudomonas tolaasii

Brown blotch disease, caused by the bacterium *P. tolaasii*, is a prominent and worrisome ailment in mushroom cultivation. Effectively controlling this disease remains a significant challenge in the field of mushroom farming. In response, this study aimed to isolate and evaluate pathogen-specific bacteriophages with the goal of utilizing their potential for biological control as a strategy to combat the disease [75]. Previous research efforts have provided valuable insights into *P. tolaasii*. A total of 23 different strains of this bacterium were isolated from mushrooms showing disease symptoms. These strains were classified into three distinct subtypes: Ptα, Ptβ, and Ptγ. This classification was determined through a comprehensive analysis of their 16S rRNA gene sequences and their pathogenic characteristics. These categorizations enhance our understanding of the diversity and attributes of *P. tolaasii* strains linked to the disease symptoms observed in infected mushrooms [76, 77].
In this specific study, 42 potent bacteriophages were isolated with a specific focus on targeting these pathogens. These bacteriophages were then thoroughly examined to determine their host range, which essentially defines the spectrum of pathogens they can effectively target. Some of these phages demonstrated the ability to lyse more than two different pathogens, but notably, this lytic capability was confined to pathogens within the corresponding subtype. In simpler terms, a phage capable of targeting pathogens in subtype Ptα, for example, would not be equally effective against pathogens in subtypes Ptβ or Ptγ. It's important to note that no phage was found to possess a broad host range, capable of targeting pathogens across multiple subtypes. These findings emphasize the specificity of these bacteriophages in their ability to target specific pathogens [78, 79]. To eradicate all pathogens belonging to the Ptα, Ptβ, and Ptγ

subtypes, it was necessary to use one, six, and one corresponding phages, respectively. Remarkably, these phages demonstrated the ability to completely suppress the disease, a phenomenon confirmed through on-farm cultivation experiments conducted on a field scale. These results indicate that a combined mixture of these eight specific phages is effective in controlling the disease caused by all 23 distinct *P. tolaasii* pathogens.
Importantly, the impact of these phage combinations extended beyond the initial cycle of mushroom growth. The antibacterial effect of the phage cocktail persisted even into the second cycle of mushroom cultivation on the designated bed. This sustained effectiveness highlights the potential long-term viability of this approach in mitigating the impact of brown blotch disease and presents an encouraging path for the integration of these phages as a biological control measure in mushroom farming [80].

Xanthomonas citri

Phytopathogenic bacteria are pivotal players in the economic landscape, as they severely affect crop productivity and pose a substantial threat to farmers worldwide. Among these pathogens, the genus *Xanthomonas* is of particular concern due to its involvement in various plant diseases that significantly reduce yields of crucial crops on a global level. The consequences of these bacterial diseases reverberate throughout the agricultural sector, emphasizing the pressing requirement for effective management strategies to protect agricultural production and the livelihoods of farmers worldwide [81]. The current management methods used to combat these bacterial diseases have proven to be insufficient, displaying traits of unsustainability and posing risks to natural ecosystems. In response to this challenge, the use of bacteriophages (phages) as a biocontrol method to manage plant diseases has gained significant attention. This interest in phage-based biocontrol dates back to the early nineteenth century and continues to be relevant today. The exploration of bacteriophage therapy as an alternative approach holds promise for addressing the limitations of current practices and offering a more ecologically sound solution to mitigate the impact of phytopathogenic bacteria on agricultural systems [82].

Research involving *Xanthomonas* phages for plant disease management has demonstrated ongoing potential, yielding promising results both in laboratory settings and under field conditions. A prominent player in this field is AgriPhage, which has made significant progress in developing phage-based products tailored to combat *Xanthomonas*-related plant diseases.

AgriPhage has created phage products specifically designed to target *Xanthomonas campestris pv. vesicatoria and Xanthomonas citri subsp. citri*. These bacterial strains are responsible for causing diseases such as tomato and pepper spot and speck diseases, as well as citrus canker disease. The development of these phage products represents a concrete step toward introducing effective, targeted solutions for managing these harmful plant diseases. This innovation has the potential to revolutionize disease control strategies in agriculture [83].
The idea of using phages for biocontrol is gaining momentum as a viable and promising approach. This is due to the natural presence of phages, their safety in disease control and management, and their potential as effective agents. In this context, a thorough understanding of the biological characteristics of *Xanthomonas* phages is of paramount importance in the development of successful biocontrol products [84].

Clavibactermichiganense: -

Bacteriophage CMP1 belongs to the Siphoviridae family and displays a particular affinity for infecting the plant-pathogenic bacterium *Clavibacter michiganensis* subsp. *michiganensis*. Its genetic material consists of linear double-stranded DNA with terminal redundancy, a feature that distinguishes it from some other phages. This unique DNA configuration, along with its specific targeting of the mentioned plant-pathogenic bacterium, underscores the potential significance of Bacteriophage CMP1 in the realm of biocontrol and disease management in agriculture [85].
The complete nucleotide sequence of the bacteriophage CMP1 genome spans 58,652 base pairs (bp), including the 791 bp terminal redundant ends. Interestingly, the G+C content of this phage's genome is significantly lower at 57% compared to its host's G+C content of 72.66%. Using various bioinformatic tools, a total of 74 potential open reading frames (ORFs) were identified within the genome and subsequently annotated. This complex genetic makeup, coupled with the differences in G+C content, highlights the uniqueness and potential functional significance of Bacteriophage CMP1 in its interaction with its host, *Clavibacter michiganensis subsp. Michiganensis* [86].

The genome of Bacteriophage CMP1 reveals the presence of two extensive clusters, each responsible for encoding distinct early and late functions. These clusters display divergent transcription patterns. Within these

genetic regions, there are only a limited number of conceptual gene products that exhibit conserved domains and significant similarity to sequences stored in databases.
Further practical investigation substantiates the actions of four specific gene products. These include an endonuclease, an exonuclease, a single-stranded DNA binding protein, and a thymidylate synthase. These functional assignments provide insights into the potential roles and activities of these gene products within the context of Bacteriophage CMP1's infection and interaction dynamics with its host, *C. michiganensis subsp. Michiganensis* [87].

The analysis of restricted genomic sequences from CN77, a phage that targets *C. michiganensis* subsp. nebraskensis, disclosed a remarkably analogous genome framework. Additionally, noteworthy resemblances were observed at the level of deduced amino acid sequences. Notably, both phages possess an endolysin with peptidase activity. This shared characteristic holds significant potential as a tool for managing diseases affecting tomato plants caused by *Clavibacter* infections.
The ability of the endolysin to break down bacterial cell walls could potentially be harnessed as a valuable resource in the field of disease control. The use of phages and their associated endolysins as biocontrol agents represents an intriguing approach to address agricultural challenges by specifically targeting pathogenic bacteria like Clavibacter michiganensis subsp. nebraskensis, ultimately benefiting the health and yield of tomato crops [88, 89].

6.3 Advantages and limitations of lytic phage therapy: -

***Advantages*:** Bacteriophages are effective opposed to both susceptible and antibiotic-resistant bacteria. They can be utilized lonely or in fusion with antibiotics and variant anti-bacterial drugs. Phages replicate and enhance in number throughout the course of treatment, often requiring only a single dose. They have a relatively mild impact on the normal "good" bacteria in the body. Phages are natural and readily available. They are not noxious to the body and are safe for animals, plants, and the environment [90-92].

***Limitations*:**
Phages are presently hard to implement for use in people and animals. It's anonymous to what dose or amount of phages should be utilized. It's unknown how long bacteriophage therapy may consume to work. It may be hard to discover the exact phage needed to cure an infection. Phages may provoke the immune system to overreact or cause an unevenness. Some category of phages doesn't act as well as other kinds to cure bacterial infections. There may not be adequate forms of bacteriophages to cure all bacterial infections. Some phages may turn bacteria to become resistant [93, 94].

7. Conclusion

Bacteriophages, as potent antibacterial agents, possess several qualities that make them compelling alternatives to synthetic antibiotics. While concerns related to phage therapy may arise, they can likely be effectively addressed through careful phage selection, skillful application, and a deeper clinical understanding of product use.

An intriguing aspect is that many of the considerations highlighted in this article have been explored in the past. In an era marked by the increasing prevalence of antibiotic-resistant bacterial infections, phages offer a range of advantages with relatively limited drawbacks. Importantly, modern scientific insights into bacteriophage biology, along with higher levels of medical research and exploration, position phage therapy to deserve renewed attention within Western medicine. This resurgence has the potential to reveal the full extent of its capabilities and benefits.

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