An insight Review on Bacteriophage with emphasis on its application in Veterinary Medicine

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Abstract

Bacteriophages are naturally-occurring viruses that can infect and kill bacteria. They are remarkably diverse, numerous and widespread. Each phage has a narrow host range yet a large majority of bacteria studied so far play host to bacteriophages, hence the remarkable phage diversity. Phages were discovered just over 100 years ago and they have been used for treatment of bacterial infections in humans and other animals since the 1920s. While infecting bacterial host, they promote bacteria destruction, holding therefore a highly efficient antimicrobial activity. During the past years, bacteriophages have been applied and used as valuable tool in microbiological diagnostics and basic research. Even though the potential of bacteriophages for fighting bacterial pathogens is known for a long time, the phage therapy is not used in daily routine worldwide. Due to the continuing spread of bacterial resistance to antimicrobials and an increasing awareness of the necessity to protect consumers' health, the phage therapy meanwhile has become a subject of major interest in veterinary medicine, too. This article begins with a description of bacteriophages and explains why there has recently been a strong interest in their clinical use for treatment of bacterial infections, particularly those caused by organisms resistant to multiple antimicrobial compounds.

Keywords: Application of Phages, Bacteriophages, Phage Therapy, Veterinary medicine.

INTRODUCTION

Bacteriophages are natural predators of bacteria and are ubiquitous in the environment. The use of host-specific bacteriophages is cost-effective and adaptable approach to control bacterial diseases. The unique advantage of bacteriophages over antibiotics lies on their ability to replicate only on the targeted subset of bacteria, avoiding the imbalance of commensal gut flora often caused by broad-spectrum antibiotics (Kutter et al., 2010; Sulakvelidze, 2004).

Infections caused by bacteria that are resistant to antibiotics can lead to failure of conventional treatment, longer treatments and death. Antibiotic resistance also leads to higher medical costs and endangers the success of certain treatments. It is well known that animals can harbor antibiotic resistant and zoonotic pathogens. One of the possible replacements for antibiotics is the use of bacteriophages or simply phages as antimicrobial agents (Chhibber and Kumari, 2012). As natural killers of bacteria, phages are obvious candidates for exploitation as antibacterial agents. Phages have many intrinsic characteristics which make them attractive candidates for such applications. They are highly specific in their bactericidal potential (Gazeev, 2018).

The effect of bacteriophages as feed additives on growth performance and Salmonella and E. coli colonization in broilers would be of interest to the poultry industry and be an important tool to control such infections in broilers. Previous studies had reported the inclusion of bacteriophages could successfully reduce the Salmonellaand E. coli counts in chicken internal organs (Atterbury et al., 2007; Toro et al., 2005; Wang et al., 2013). Even though the effects of bacteriophages on poultry production have been evaluated in other countries...
such trends are yet to be evaluated and implemented in Ethiopia. Thus, this review article is meant to present day fields of application for bacteriophage therapy as well as current trends of development in the field of Veterinary medicine.

History of Bacteriophages and Principles of Phage Therapy

History of phage therapy

Bacteriophages were discovered and was first reported by the Englishman Frederick Twort in 1915 (Twort, 1915) and the French-Canadian Felix d'Hérelle in 1917 (D'Herelle, 1917). D'Hérelle said that bacteriophages always appeared in the stools of Shigella dysenteriae patients shortly before they began to recover (Hausler, 2006). He "quickly learned that: bacteriophages are found anywhere bacteria thrive: in sewers, in rivers that catch waste run off from pipes, and in the stools of convalescent patients (Kuchment, 2012). Bacteriophage therapy was immediately recognized by many to be a key way forward for the abolition of pathogenic bacterial infections. Intensive studies on the therapeutic use of phages for treating infectious diseases were taken up in 1920 (Walker, 2006) and continued phage therapy trials were reported from Baylor University's College of Medicine in 1923 (Ho, 2001). Not long after their discovery, bacteriophages were successfully used to treat certain bacterial diseases, such as dysentery (McKinstry and Edgar, 2005).

Principle of Bacteriophage Therapy

Bacteriophages mean to eat bacteria, and are called so because virulent bacteriophage can cause the compete lysis of a susceptible bacteria culture (Wommack and Colwell, 2000). They are commonly referred as "phage". Bacteriophages like all viruses are obligate intracellular parasites, which have no intrinsic metabolism and require the metabolic machinery of the host cell to support their reproduction (Withey et al., 2011). Several bacteriophages are highly specific for host cell surface receptors and any slight changes in structure results in little or no interaction between the phage and its host. Therefore; many bacteriophages typing schemes for the identification of bacterial species are based on this specificity (Wernicki et al., 2017) but there are some exceptions, like Listeria A511, which can infect entire genus (Loessner et al., 1993).

Upon the presentation of modern bacteriophages diary, propelled in early 2011, Alexander Sulakvelidze characterized bacteriophages as "the most omnipresent organisms on Earth, playing an important part in maintaining microbial adjust on this planet(Sulakvelidze, 2011). Without a doubt, bacteriophages are all over where their bacterial host is present; it has been set up that the populace number of bacteriophages in oceanic framework lies inside the run of 10^6 to 10^9 virions per ml and about 10^14 virions per g within the soil (Weinbauer, 2004), with an evaluated add up to number of 10^32 bacteriophages on the planet (Gazeen, 2018; Hanlon, 2007).

At first portrayed nearly a century prior by William Twort, and freely found without further ado from that point by Félix d’Herelle considered as the originator of bacteriophages and its therapeutic suggestion. Historically, bacteriophage therapy has been practiced in France since 1919, when d’Herelle’s preparations were given to patients with dysentery at the Hospital (Marinelli et al., 2012).

Bacteriophages have been extensively used in veterinary medicine. One of the earliest animal models used in several phage therapy studies was murine salmonellosis, a systemic disease produced in susceptible mice by several serotypes of Salmonella. Bacteriophages are ubiquitous in the environment and their use in livestock is likely to provide one of the most environmentally friendly antibacterial approaches available today (Sulakvelidze, 2004). In Britain, Smith and Huggins carried out a series of excellent, well-controlled studies on the use of phages in systemic E. coli infections in mice and then in diarrheic disease in young calves and pigs. Bacteriophages might be useful for the prevention of P. aeruginosa infections in patients with burn wounds (Soothill, 1994). Phage therapy has been successfully used to remove E. coli O157:H7 from livestock. The protective effect of bacteriophage was assessed against experimental S. aureus infection in mice(Tanji et al., 2004).

Biology, Isolation and Purification of Bacteriophages

Biology of Bacteriophages

Bacteriophages can be viewed as obligate parasites for bacteria. These viruses received this functional name, since they invade bacteria and “consume” their cells, replicate within them and leave the cell with all their copied “progeny” usually by lysing the bacteria. Bacteriophages can be found in many different environments, such as soil, water, glacier, etc. The important condition being that bacteria also be found there, since bacteriophages cannot live without bacterial hosts. It has been noticed that bacteriophages effectively infect enteric bacteria, such as E. coli, Shigella, Salmonella Typhimurium, etc. Bacteriophages are unique microorganisms, they are also known as “respectable viruses”, their survival dependence on bacteria, which are sensitive to phages, including even most pathogenic and virulent strains thereof, gives rise to a distinctive treatment

Ethiopia

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technique known as phage therapy (Gazeev, 2018).

The potential magnitude of bacteriophage therapy effect can be imagined if one considers how bacteriophages replicate. It is similar to how any virus replicates: the viral genome enters the cell and the genetic information within the virus redirects the cell to produce many new copies of the virus. Subsequently, the phages are released from the lysed bacterial cell and the newly formed bacteriophages can infect a multitude of other bacteria in the vicinity. All this provides bacteriophages with an extraordinary bactericidal potential, rendering them to be powerful therapeutic agents against bacterial diseases (Kutter et al., 2010; Sulakvelidze, 2011).

Structure of Bacteriophages

Bacteriophages have a really basic structure. Their hereditary material is contained in a crystal molded head, surrounded by a protein capsid. This is typically associated to the stretched sheath some of the time called the tail by a neck. The sheath shapes a hollow tube through which the viral nucleic acids are injected into the host cell and is surrounded by protective sheath proteins. At the foot of the sheath is the base plate to which the tail strand which are six in numbers that facilitate attachment to the host cell are attached (Hodgson, 2013).

Life Cycles of Bacteriophages

Bacteriophages have the ability to interfere between two cycles lysogenic or lytic. In the lytic phage, the viral DNA exists as a separate molecule within the bacterial cell, and replicates separately from the host bacterial DNA. Each phage follows a unique pathway to control bacteria. Some of them show a lytic infection cycle upon infecting their bacterial host. In this case, they grow in high numbers in bacterial cells, leading to cellular lysies. At the end of the cycle, a release of newly formed bacteriophages particles is observed. Using the lysogenic pathway, the phage genome integrates as part of the host genome. It stays in a dormant state as a prophage for extended periods of time. Adverse environmental conditions for the host bacterium may activate the prophage, turning on the lytic cycle. At the end, the newly formed phage particles are ready to lyse the host cell (Mandal et al., 2014). The life cycles of bacteriophages are very different to their bacterial hosts and can typically, be characterized by four distinct phases: adsorption, infection, multiplication and release (Guttman et al., 2005).

Lytic Cycle

The typical lytic phage infection consists of six different stages and begins with the adhesion of viral particle to the surface of bacterial cell. The general stages of virulent bacteriophage replication are as follows

1. **Attachment.** The bacteriophage attaches or adsorbs to a specific receptor on the host cell. Common receptors include surface proteins, parts of the lipopolysaccharide, pili and flagella.

2. **Penetration.** Phage nucleic acid is “injected” into the host cell. The capsid and other protein structures remain outside. In some tailed phages, the tail sheath contracts to allow the nucleic acid to make its way into the cell.

3. **Synthesis of nucleic acid and protein.** The phage takes over host metabolic machinery to produce its own nucleic acids and proteins. The first (early) proteins to be translated are those responsible for inhibiting host systems and replicating the phage genome. The ‘late’ proteins are involved in the formation of new phage particles and lysis of the host cell.

4. **Assembly and maturation.** The nucleic acid and protein products are assembled to form mature phage particles.

5. **Release.** The host cell wall is lysed by phage encoded enzymes called lysins, releasing the newly formed phage particles into the environment (Martin and Parker, 2006).

Lysogenic cycle

The lysogenic cycle, is one of two cycles of viral reproduction. Lysogeny is characterized by integration of the **bacteriophage** nucleic acid into the host bacterium's genome or formations of a circular **replicon** in the bacterial cytoplasm. In this condition the bacterium continues to live and reproduce normally. The genetic material of the bacteriophage, called a **prophage**, can be transmitted to daughter cells at each subsequent cell division, and at later events can release it, causing proliferation of new phages via the lytic cycle. Lysogenic cycles can also occur in **eukaryotes**, although the method of DNA incorporation is not fully understood (Ash et al., 2018; Gazeev, 2018).

Isolation and Purification of Bacteriophages

The main requirement for developing “bacteriophage technology” for practical purpose is the ability to quantitate viable bacteriophages accurately and reproducibly. Plaque assay is a simple enumeration procedure, where several dilutions of the bacteriophage preparation are gently mixed with a permissive host bacterium in molten agar and dispersed evenly on the surface of the agar medium. After incubation, a clear circular area of lysed cells known as plaque was developed in a confluent lawn of host bacterium on the agar medium (Abedon, 2011). Lately, the double agar overlay or soft agar overlay methods of plaque assay are
most commonly used approach for determining bacteriophage titers. This technique has been used by researchers with *Escherichia*, *Salmonella* and others (Cormier and Janes, 2014; Gazeev, 2018).

(Mahadevan et al., 2009) used five bacterial pathogen isolates for the host specific bacteriophage isolation and found that phage of *Escherichia coli* and *Salmonella* Typhi were able to infect its original host bacterium, whereas, the phage for *Pseudomonas aeruginosa* was able to infect both *Pseudomonas* and *Escherichia coli*. (Oliveira et al., 2009) isolated five bacteriophages (PhiF78E, PhiF258E, PhiF2589E, PhiF61E, PhiF5318E) against *Escherichia coli* by double agar over lay technique and conducted phage purification from single plaque isolation from higher dilution plates. (Jamalludeen, 2012) isolated bacteriophages against O1, O2, O78 *Escherichia coli* from wastewater and fecal samples collected from poultry processing plants by using double agar over lay method and isolated 11 phages. (Bardina et al., 2012) isolated fifty-five bacteriophages out of 189 chicken cloaca and pig rectal swabs. From this collection, bacteriophages UAB-phi20 and UAB-phi87 were isolated from chicken and bacteriophage UAB-phi78 from a pig (Jamalludeen et al., 2009).

Carey-Smith et al. (Carey-Smith et al., 2006) used soft agar overlay method for the isolation of bacteriophages against *Salmonella* serovars. Higgins et al. (Higgins et al., 2007) obtained waste water from local municipal waste water treatment plant and isolated bacteriophages against *Salmonella* Enteritidis from poultry by employing double agar over lay method using tryptic soya agar. Individual plaques resulting from this were differentiated on the basis of plaque morphology. This technique yielded 71 bacteriophages from four independent waste water samples. Atterbury et al. (Atterbury et al., 2007) collected samples of poultry excreta and effluent from 26 farms, poultry processing plants and waste water treatment plants in southern England during 2004 and 2005 for the isolation of *Salmonella enterica* serotype Enteritidis P125109, Hadar 18, and *Typhimurium* 4/74 by employing double agar over lay method using nutrient agar. Single plaques were propagated in this way for a total of three times to ensure that the isolates represented a single clone.

**Application of Bacteriophage Therapy**

Bacteriophages have been extensively used to combat various bacterial infections after their discovery in the early 20th century. For example, d’Herelle had employed bacteriophages as therapeutic agents in 1929. Since bacteriophage infection typically results in the death of the host bacterial cell, their use as antimicrobial agents have long been recognized. Indeed, the first known applications of bacteriophage to treat bacterial disease are reported to date back to within five years of their discovery (Sulakvelidze et al., 2001).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Phage therapy</th>
</tr>
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<tbody>
<tr>
<td><em>Staphylococcus</em></td>
<td>Staphage, pyophage, intestiphage</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>Streptophage (liquid), pyopolyphage (tablets)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Polyvalent pyophage, coliproteal phage</td>
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<tr>
<td><em>Enterococcus</em></td>
<td>Intestiphage</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td><em>Pseudomonas aeruginosa</em> phage (liquid)</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Polyvalent pyophage (liquid, purified)</td>
</tr>
<tr>
<td><em>Proteus vulgaris et mirabilis</em></td>
<td>Proteal phage (liquid), pyopolyphage (tablets)</td>
</tr>
</tbody>
</table>

Source: (Gazeev, 2018)

Bacteriophages have many advantageous properties as antimicrobial agents; however, some of those properties can also be limiting in certain applications. The most characteristic of bacteriophages is their high specificity of disease, typically recognizing a limited range of bacterial strains. This reduces the damage caused to the normal microbial community of the host but it also requires identification of the specific target pathogen and the selection of an effective bacteriophage (Bardina et al., 2012; Vandamme, 2014), which may delay the treatment. Also, as the propagation of phages depends on their host, they replicate only at the site of infection, are self-limiting and self-dosing, and do not persist when their specific bacterial pathogen becomes absent (Gazeev, 2018; Węgrzyn and Węgrzyn, 2015).

There is a conceivable concern about phage immunog-
enicity and consequent in vivo efficacy. Phages are perceived by the immune system as invaders and can be rapidly removed from systemic circulation, making it hard to sustain an effective phage concentration. A recent study by Lusiak-Szelachowska et al. demonstrated inductions of anti-bacteriophage antibodies after bacteriophage therapy, with the activity being dependent on the route of administration and bacteriophage type. Nevertheless, Lusiak-Szelachowska and his colleagues considered that the detection of anti-phage activity during and after bacteriophage therapy does not exclude a favorable result of the treatment (Gazeev, 2018; Łusiak-Szelachowska et al., 2014; Węgrzyn and Węgrzyn, 2015).

Bacteriophages have potential advantages as unique therapeutic agents over classical antibiotics. Apart from those generalized advantages there are specific advantages such as Auto dosing. Hence, phages are capable of increasing in number specifically during the bacterial-killing process where hosts are located (Summers, 2001).

CONCLUSION AND RECOMMENDATION

Bacteriophages are the most important and numerous organisms on Earth that play key roles in bacterial gene exchange and bacterial pathogenesis and continue to provide important insights into the basic molecular workings of life. Through a combination of their antagonistic but metabolically intimate relationship with their bacterial hosts, lytic phages possess ideal properties to serve as agents of both antibacterial, bio-control and bacterial identification. Bacteriophages have played an important role in the expansion of vaccinology, molecular biology and in veterinary science. Besides, it becomes best solution for the current resistance development in antibiotics have been used as antibacterial agents.

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REFERENCES


