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Bacteriophage as a Bio Controller: A Review

Rucha V. Moharir , Krishna Khairnar

M. Tech, Environmental Engineering, Environmental Virology Cell, CSIR-NEERI, Nagpur-440020, India

Scientist & Head, Environmental Virology Cell, CSIR-NEERI, Nagpur-440020, India

ABSTRACT: Phage therapy is the application of bacteria-specific viruses with the goal of reducing or eliminating pathogenic or nuisance bacteria. While phage therapy has become a broadly relevant technology, including veterinary, agricultural and food microbiology applications, it is for the treatment or prevention of human infections that phage therapy first caught the world's imagination – see, especially, Arrowsmith by Sinclair Lewis (1925) – and which today is the primary motivator of the field. Nonetheless, though the first human phage therapy took place in the 1920s, by the 1940s the field was in steep decline despite early promise. This review is mainly focused on the advantages of phages as a bio controller. In this review we strive toward three goals: **1.** To provide an overview of the potential of phage therapy as a means of treating or preventing human diseases; **2.** To explore the phage therapy state of the art as currently practiced by physicians in various pockets of phage therapy activity around the world, including in terms of potential commercialization; and **3.** To highlight the beneficial outputs of phage therapy and useful effects of bioremediation.

KEYWORDS: Bacteriophages, antibiotics, therapy, treatments.

I. INTRODUCTION

Phages [1] first target the bacterial cell by using the cell machinery and further release from the bacterial cell and responsible for killing the bacterium. Besides, therapeutic applications of phage in human and the usage of phages in agriculture and veterinary medicine are also highly evaluated.

Viruses are considered obligate intracellular parasites which required a specific host cell for its replication [2]. Phage is virus that specifically target and reproduce within bacterial cells. In general, phages attach to the surface of their host cell by specialized structures called tail fibres. Once get attached, the phage injects their nucleic acid into the bacterium. By using the host cells replication, translation, and transcription machinery, the viral nucleic acid is replicated and incorporated into its protein capsid. The escape of mature viruses from the host cell places stress on the plasma membrane resulting in the eventual death of the bacterium [3].

By using phage in the treatment of bacterial infections gives smart alternative for the current therapies for example, antibiotics, because unlike broad-spectrum antibiotics phage specifically target a particular host and are unlikely to illicit resistance in untargeted bacterial strains.

The host specificity of viruses offer an exclusive technology for the treatment of environments contaminated with pathogenic bacteria and for fighting infections caused by bacteria. Research into potential use of viral therapy is limited, but studies have shown success using this technology to treat infections in plants, livestock, aqua-cultured fish and humans [4, 5].

II. PHAGES BIOREMEDIATION

Bacterial viruses, or bacteriophages, appear to be ubiquitous, there being examples in most bacterial species with sensitivity to one or more phages. Most of these phages have double-stranded DNA; all the known RNA viruses are

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single-stranded. Except for the filamentous phages, all of the phage groups have a polyhedral capsid which contains the phage genome. This capsid is usually joined to a tail, which is a helical protein structure required for adsorption of the virion to the bacterial cell. Bacteriophages undergo two possible life cycles. These are the lytic (or virulent) and lysogenic. Lytic phages multiply vegetatively and kill the host cell at the end of the growth cycle. Temperate phages which undergo the lysogenic cycle as well as multiplying vegetatively can also persist in a lysogenic state, whereby the phage genome can exist indefinitely by being inserted in the bacterial chromosome (known as the prophage state). The lysogenic life cycle of phage lambda, for example, ensures the replication of the integrated prophage along with the bacterial genome for many peers. When induction occurs through damage of the DNA, which signifies the imminent death of the host, the phage switches to the lytic cycle, which results in the release of new phage particles.

The consensus is that, 20 years after initial observations of unfilterable, heat labile agents with activity against Vibrio cholera [6], it was discovered by Twort in 1915, who made similar observations and who hypothesized this to be due to a virus, and independently by d'H ERELLE in 1917 [7,8]. Since it was realized that these bacterial viruses destroy their bacterial host while remaining harmless to humans, it has been the dream of researchers to use phages to treat bacterial infections. There was much early promise. Using experimental practices common at the time, d'Herelle and co-workers first showed safety by ingesting Shigella dysenteriae phage preparations. The Shiga-phage successfully treated patients with dysentery. Others soon afterwards treated staphylococcal skin disease with phages injected in the vicinity of the infection [9, 27]. The commercialization of therapeutic phage preparations to treat bacterial infection in humans was started in France by d'Herelle and in the United States in the 1940s by the pharmaceutical company Eli Lilly. However, because of controversial results [10, 11] and the promise of antibiotics in the 1940s, the commercial pursuit of therapeutic phages in the "West" ceased, although not in Eastern Europe. Recently, interest in phage therapy, the use of phages to control bacterial infections, has been rekindled [12-14]. This is mainly to overcome the urgent problem of antibiotic resistance due to multidrug-resistant bacteria. According to recent WHO figures, in the US 14,000 people each year die from drug-resistant infections acquired in hospitals, and worldwide, 60% of such infections are drug-resistant. Bacteriophages are found in all bacteria, so it is hoped to be able to develop control therapies against pathogenic bacteria such as antibiotic resistant *Streptococci, Staphylococcus aureus* and *Streptococcus pneumoniae*.

III. ADVANTAGES OF PHAGE THERAPY OVER ANTIBIOTICS

Bacterial resistance to phages, although likely to arise, should not be a major concern, certainly compared with bacterial resistance to antibiotics. This is because phages grow exponentially, essentially shadowing the bacterial growth and thereby mutating at the same rate and, furthermore, due to the plethora of phages, there will certainly be a species that can attack mutated, resistant bacteria. Studies on *E. coli* 018:K1:H17ColV +, which is pathogenic in calves, showed that when mice were infected with this strain, nine K1 coliphages were found which effectively eliminated the infection after a single dose, compared with several doses of various antibiotics [14]. Based on this study it was suggested that the reason for the greater effectivity of phages over antibiotics is that, whilst both kill bacteria, antibiotics are metabolized and excreted, whereas the phage titers actually increase [15]. Phage multiplication is indeed very rapid, a single phage producing 4×10^3 progeny within an hour, this number increasing exponentially to 4×10^6 an hour later.

Bacteriophages have high specificity for particular bacterium, thereby reducing the possibilities of secondary infections developing. Repeated administration is unlikely because as long as the target bacterium is present, the phage will be able to reproduce. Cheap to produce and to date without any observed side-effects. The receptors to which phages are targeted on the bacterial cell surface are virulence factors, so when bacteria develop phage-resistance, they are usually altered, which results in an attenuation of virulence. Finding a phage which will be active against bacteria which has developed phage resistance is rapid, taking only a matter of days. The only advantage of antibiotics is an active against wide range of bacteria, thereby avoiding the need to characterize the infective bacterium [16].

IV. PHAGE THERAPY AGAINST BACTERIAL INFECTIONS IN ANIMALS AND HUMANS

The scale of antibiotic resistance now results in over five million people dying every year from infections not responding to antibiotics. The very dangerous *Staphylococcus* bacterium is only sensitive to one antibiotic, vancomycin, but already in the year 2000 the first case of vancomycin-resistant *Staphylococcus* was found in Japan in a baby undergoing major heart surgery. A successful series of preclinical studies in animals using phage therapy focused

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Vol. 3, Issue 1 , January 2016

initially on *E. coli* infections in mice [17]. Lambs, piglets and calves treated using phages were also cured of the diarrhoea-causing *E. coli* [18, 19]. In a study from the Institute for Animal Health, UK, an *E. coli* phage isolated from sewage which was found to infect via the K1 capsular antigen, and which was able to multiply in the blood, was used to protect chickens from septicemia and a meningitis-like infection [20].

V. TREATMENT OF HUMAN INFECTIONS

Fighting Surgical and Wound Infections in the major tertiary care centres as well as wound and burn facilities in Georgia, phages generally play an important role in treatment. Priority indications for phage therapy include:

• Antibiotic penetration difficulties in the infection site, caused by poor circulation or the presence of a fibro- granulate barrier, such as in diabetic foot infections – a key area where phages are very successful when used with circulation stimulation.
• Chronic osteomyelitis.
•Wounds covering a large area, predominantly where therapeutic concentration of antibiotic is not possible to achieve during systemic introduction.

Phage therapy is the primary tool in Georgia for successful treatment of multi-resistant infections as there is no correlation between antibiotic and phage resistance. Phages are just one component of successful wound care and treatment of surgical infections. Successful phage therapy requires also a rigorous application of all of the technologies of effective wound care, including: 1. Radical necrectomy and wide opening of the wound, 2. provision of adequate drainage, 3. on-going provision of a reasonably optimal ratio of phage-to pathogens, and 4. early wound closure.
Phages infect and propagate in two possible ways, a lytic life cycle and a lysogenic life cycle. Both cycles make use of the host's DNA machinery. The lytic life cycle occurs when phages kill their hosts by replicating separately from the host's genetic information. The lysogenic life cycle, on the other hand, is generally associated with temperate phages that can grow vegetatively and can integrate their genetic material into host chromosomes. Replication of the genome is carried out within the host for many generations and eventually an environmental stimulus causes the phage progeny to lyse out of the bacteria [21].

Phages are a kingdom of viruses that infect bacteria, and are distinct from the animal and plant viruses. The lytic phages are the most suitable candidates for phage therapy, because they quickly reproduce within and lyse the bacteria in their host range, growing exponentially in number in the process. Depending on the species and conditions, each "parent" phage can produce on average approximately 200 "daughters" per lytic cycle. If each daughter infects and kills a host bacterium there will be 40 000 progeny at the end of the 2nd cycle; 8 million at the end of the 3rd cycle; 1.6 billion at the end of the 4th cycle; and so on. There are many attributes of phages (Table 1) that would tend to favour a positive outcome in therapy.

Table 1. Attributes of phages that tend to favour a therapeutic response [22-26]

The issue	Limitations of antibiotics	Advantages of phages
Fate of the "drug" molecule	Metabolic destruction of the molecule, as it works	Exponential growth in numbers, so that the "drug" makes more of itself at the site of infection, where it is needed
Concentration of the "drug"required to kill a given bacterium within the spectrum	Numerous molecules of the antibiotic are needed to kill a given bacterium. During initiation of therapy (and between doses), the sub-lethal dose that bacteria "see" affords them the opportunity to express resistance genes	"All or nothing" effect: one phage particle is sufficient to kill a given bacterium
Ability to overcome bacterial Resistance	Antibiotics are fixed, immutable chemicals that cannot adapt to a bacterial mutation and therefore	Phages are "living" organisms that undergo mutations, some of which can overcome bacterial mutations.

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	become obsolete. Bacteria that have resisted them can pass along the resistance trait within and between species	E. g., mutated phage tail fibres can allow binding to a mutant bacterial receptor, or mutated phage DNA can escape cleavage by mutant bacterial endonucleases
Spread of bacterial resistance	The antibiotics in use tend to be broad spectrum, thereby provoking resistance in several species and genera of bacteria (in addition to the one targeted)	Although there are some exceptions, phages tend not to cross species boundaries. Thus even though the targeted bacterial species may become resistant to the phage, it is unlikely that other species will

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