Universidad Europea

TRABAJO DE FIN DE GRADO

Grado en Odontología

BACTERIOPHAGES IN DENTISTRY.

ADVANTAGES AND CHALLENGES OF USING PHAGE THERAPY IN ORAL BACTERIAL INFECTIONS.

Madrid, curso 2020/2021

Número identificativo 253

AGRADECIMIENTOS:

To my thesis tutor, who was able to accompany and guide me throughout my work without ever abandoning me, thank you for your advices and your kindness.

A mes parents, sans qui tout cela n'aurait jamais pu être possible, merci d'être un exemple de combativité et d'ambition, merci papa d'avoir toujours été emballé par tout ce que je faisais, merci maman de ne m'avoir jamais laisser tomber et toujours poussé à être la meilleure version de moi-même, tout ça c'est grâce à vous.

A ma sœur, l'amour de ma vie, pour qui tout ce que je fais est merveilleux, merci d'être toujours ma fan number one et ma définition du bonheur.

A mes nièces, qui me voient comme leur super-héroïne et qui trouve que Tata Bo est la meilleure.

A mon beau-frère sans qui cette thèse ne serait pas aussi belle, merci de t'être investi.

Gracias a Juanjo por haberme acompañado estos cinco años y por haberme apoyado en todo momento sin fallar nunca.

A mes amies, merci de m'avoir supporté toutes ces années, même si cela n'a pas toujours été facile, merci d'être rentré dans ma vie et d'avoir participé à ce gros chapitre de ma vie.

ABSTRACT:

Background: Nowadays, fighting bacterial infections has become increasingly difficult. Many treatments for infections are proving inconclusive due to the increase in antibiotic resistance. That is why scientists are currently reviewing a treatment that has been used for a century in Eastern Europe: Phage therapy. Phage therapy focuses on the fight against bacterial infections by viruses capable of destroying bacterial cells and curing or preventing the infection.

Objectives: The main objectives of this study are to understand, the mechanism of action of bacteriophages, its importance in treating bacterial infection, studying the challenges of phage therapy in the treatment of bacterial infections of the oral cavity and understanding the benefits of this therapy compared to the antibiotic therapy

Methodology: We have studied 41 articles and 15 websites to understand the benefits of this therapy and the way in which it is useful to treat bacterial infections.

Conclusion: Phage therapy is a very promising treatment against bacterial infections and has a particularly attractive advantages over antibiotic therapy since many bacteria exhibit resistance to different antibiotics. The papers we reviewed showed us that bacteriophages are very effective in fighting bacterial infection and have very few disadvantages, some of which are insignificant. Bacteriophages however do not permit the host to develop resistance and do not create an imbalance in the endogenous flora.

RESUMEN:

Introducción: Hoy en día, la lucha contra las infecciones bacterianas es cada vez más difícil. Muchos tratamientos para las infecciones están resultando inconclusos debido al aumento de la resistencia a los antibióticos. Por ello, los científicos están revisando actualmente un tratamiento que se utiliza desde hace un siglo en Europa del Este: La terapia de fagos. La fagoterapia se centra en la lucha contra las infecciones bacterianas mediante virus capaces de destruir las células bacterianas y curar o prevenir la infección.

Objetivo: Los objetivos principales de este estudio son comprender el mecanismo de acción de los bacteriófagos y estudiar los retos de la terapia fágica en el tratamiento de las infecciones bacterianas de la cavidad oral y comprender los beneficios de esta terapia en comparación con la terapia antibiótica.

Metodología: Hemos estudiado 41 artículos y 15 sitios web para entender los beneficios de esta terapia y la forma en que es útil para combatir las infecciones bacterianas.

Conclusión: La terapia con fagos es un tratamiento muy prometedor contra las infecciones bacterianas y tiene ventajas particularmente atractivas sobre la terapia con antibióticos que conoce la resistencia múltiple de las bacterias. Los artículos que estudiamos nos mostraron que los bacteriófagos eran muy eficaces para combatir una infección bacteriana y que tenían muy pocas desventajas, algunas de ellas insignificantes. Los bacteriófagos experimentan muy poca resistencia en su huésped y no desequilibra la flora endógena

INDEX:

INTRODUCTION:

Dental diseases are perhaps one of the diseases most related to infections. Biofilms are responsible for almost all infectious diseases in the oral cavity inducing caries, periodontal diseases, gingivitis, endodontic infections and periimplantitis. Nowadays antibiotics are used to treat dental diseases but the increase in antibiotic use has caused resistance which sometimes prevents the infection from being completely treated.

An alternative treatment for dental diseases considering antibiotic resistance is phage therapy. Bacteriophages are natural viruses able to infect and kill bacteria, they are present everywhere in the environment, play an important role in the natural balance of bacteria and can be effective antibacterial agents. Bacteriophages are specific to certain strains and are easy to isolate and handle. Phage therapy opens up a new perspective on the therapeutic treatment of infections and on researches. New discoveries and researches on phage therapy have been brought to light to fight infections in an era where self-medication has increased.

I. History of phage therapy

The name bacteriophage comes from the Greek word “bacteria eater” and are abundant everywhere on earth. The first suggestion of the utility of the bacteriophages was proved by Frederick Twort in 1915, an English bacteriologist, who found connection between a virus and bacterial cell lysis as a coincidence. (1)

In 1917, Félix d’Hérelle, a French microbiologist, retrieved Twort’s research and was the first to propose phage therapy in human infections. He was credited with the official discovery of bacteriophages. The process of the phage therapy was performed by mixing a bacterium free filtratrate of the patients’ fecal matter with Shigella isolated from the patient. One part of this mixture was introduced in animals and another portion was spread on agar medium to determinate the growth of the bacteria. On this agar culture, Felix d’Herelle observed plaques characterized by appearance of clear areas meaning the lysis of the bacteria. (2)

The first dose of bacteriophages was injected at the *Hôpital des enfants malades* in Paris, in a twelve years old boy with chronic dysentery and the results showed a disappearance of the symptoms with a single dose of anti dysentry phage, with a complete health recovery within twenty four hours. The results of this study were not directly published, the first report of phages to treat multiple infections, was launched in 1921, by Richard Bruynoghe and Joseph Maisin who used phage therapy to treat staphylococcal skin disease of a surgical open lesion. They observed a clear regression of the infection within 24h-48h (3). Thirty years after the first trial, the first commercialization happened, Félix d’Hérelle and his laboratory created five different strains of bacteriophages. In 1940, the pharmaceutic company Eli Lilly commercialized seven phages for humans infections such as, staphylococci, streptococci, Escherichia Coli and other bacterial pathogens, used in US for treating a range of infections,

involving in wounds, upper respiratory infection, abscesses, vaginitis and mastoiditis infections (infection of the mastoid air cells of the inner and middle ear). [2]

A few years after its first commercialization, due to the controversy existing around the use of phage therapy and the extend use of antibiotics, the production of therapeutic phage therapy ceased in the Western World but it was still used in the Eastern word and Sovietic Empire like Poland, Germany, Georgia and Russia [4]

II. Antibiotic resistance

In recent decades, a new phenomenon has emerged that is responsible for the increase in infections: antibiotic resistance, due to self-medication and overuse of antibiotics. One of the main treatments in the fight against antimicrobial resistance is phage therapy, which can be very effective in the case of bacterial infections. [5]

Consideration	Antibiotic Therapy	Phage Therapy
Specificity	Low	High
Development costs	High	Low-moderate
Side effects	Moderate-high	Usually low, but yet to be fully established
Resistance	Increasing incidence of multi-drug resistant isolates.	Can treat multi-drug-resistant isolates. Phage resistant isolates generally lack fitness.
Delivery to target	Moderate	Moderate to good. Can penetrate the blood-brain barrier.
Formulation	Fixed	Fixed or variable
Regulation	Well established	Underdeveloped
Kinetics	Single hit	Single hit or self-amplifying
Immunogenicity	Variable	Likely low, but not well established
Clinical validation	Many trial studies	Relatively few trial studies

Table 1: comparison antibiotic therapy vs phage therapy [5]

III. Bacteriophages as therapeutic treatment

1. Introduction of bacteriophages

Bacteriophages are viruses that infect bacteria. Viruses may vary in shape and can be icosahedral, head tail or filamentous. Their genetic material is enclosed in this protein capsid. Bacteriophages are widespread in their habitat and they alter and adapt to target biofilm-embedded cells. Virions can gain access to dense biofilm and migrate through the neighbouring cells that are tightly packed, undermining the entire structure. Bacteriophages are active in bacteria involved in biofilms, so they could have great potential for treatment of dental diseases. (6)

Bacteria in biofilms can, however, form anti-bacteriophage refuges, in order to coexist with bacteriophages and bacteria. In human saliva, several bacteriophages can be detected, and the most common hosts are: Firmicutes, Fusobacteria, Actinobacteria, Bacteroidetes and Proteobacteria. (7)

2. Structure of bacteriophages

The head and tail of bacteriophages is unique to them. Their genome is made up of DNA and the number of genes can range from 4 to millions.

The configuration of a head tail bacteriophage is made of: (8)

- Head: capsid containing the double stranded DNA, with the internal proteins
- Neck: connection between head-tail
- Tail: tubular structure allowing the passage of DNA when contacting bacterial surface
- Tail fibers: protein that are attached to the bacterial surface

- End plate: location of the pins allowing the penetration of the bacterial membrane to release phage DNA into the host

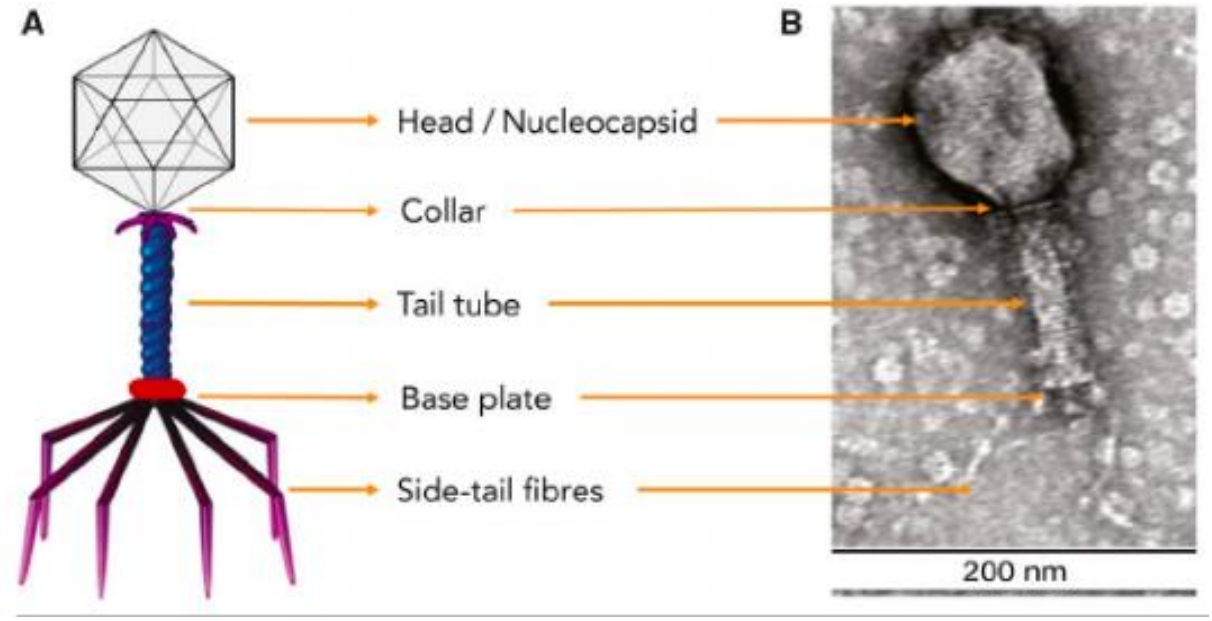


Figure 2: Structure of a bacteriophage [9]

Bacteriophages are divided in 6 groups according to their morphological components: [8]

A. Capsid and long tail with contractile sheath
B. Capsid and long tail with rigid sheath
C. Capsid and short tail
D. Grand size nucleocapsid with fibrous surface
E. Phage incorporating one nucleocapsid without tail
F. Rod-like/filamentous bacteriophages

The most interesting bacteriophages involved in oral cavity are the tailed bacteriophages which are the ones that belong to the *Caudovirales* family, viruses with double stranded DNA. The structure of their tail is unique. [8]

3. Mode of action

3.1. Chronic cycle

The chronic infection cycle is a lytic cycle without bacterial lysis. Viral particles produced at low flow rates are excreted by budding or extrusion, and infected cells continue to divide, passing the virus on to their offspring. [10]

3.2. Lytic cycle

The lytic cycle is specific to virulent bacteriophages. This cycle is defined by the infection of the bacterium by the bacteriophage leading to its complete lysis. [10-12]

The steps of the lytic cycle are:

- Attachment of the phage to the host receptor
- Entry of viral DNA into host cell
- Transcription
- Replication of phage DNA
- Assembly
- Release

3.3. Lysogenic cycle

The lysogenic cycle is specific to temperate bacteriophages. These bacteriophages reproduce into the host cell without altering or killing it but transforming its properties, thus the virus remains dormant until the host conditions deteriorate. [11,12]

The steps of lysogenic cycle are:

- Release of DNA phage into the host cell
- Integration of DNA phage into DNA host cell

- Division of the bacteria cell
- Replication of the viral DNA into bacteria cell

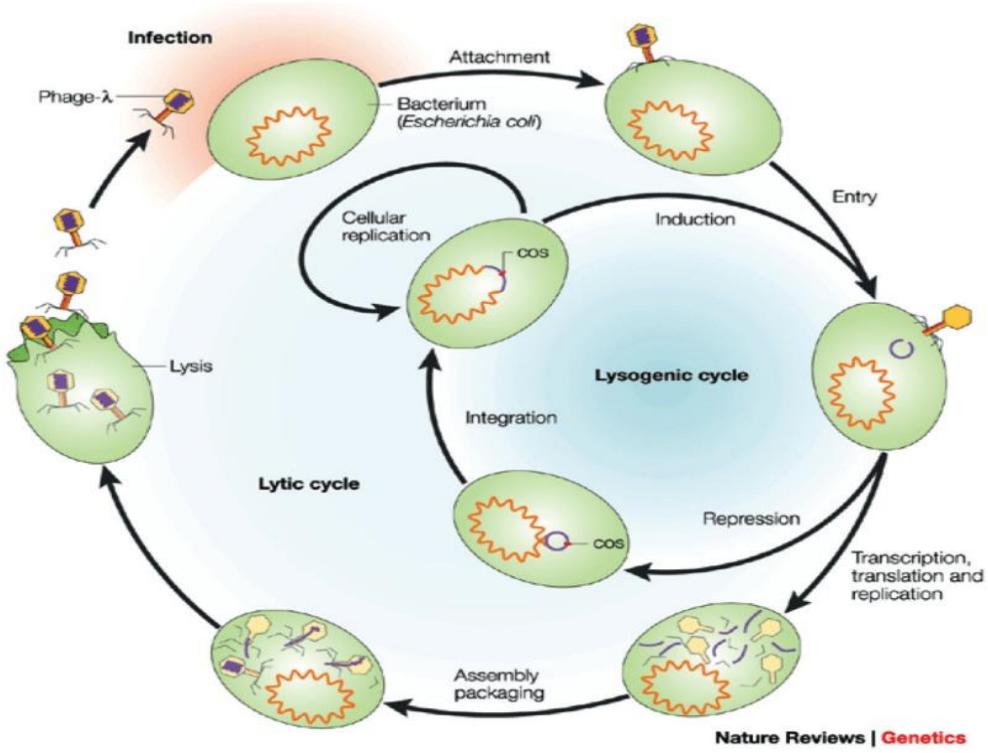


Figure 3: lytic and lysogenic life cycle of bacteriophage [13]

4. Phage preparation

In order to ensure an adequate supply of bacteriophages, they must be properly isolated and prepared before being applied for therapy. Every sample of the environment contains pathogens which may therefore contain bacteriophages that can produce the lysis and the infection of a bacteria. Due to its high concentration of microorganisms, a sewage are the best sources of bacteriophages. [14]

4.1. Isolation

Bacteriophages are isolated from an environmental sample that is sterilized, to remove cellular microorganism and is filtered through the membrane or centrifuged. The host strain

is added to the filtered sample and plating the mixture is plated using the double agar method to obtain plaques. [3]

4.2. Characterization

Phages are characterized by the following methods: [15]

- Examination of the ability to lyse others bacterial strains not used in the isolation process
- Testing of adsorption of the plaque
- Testing of the latent period, period defined by the timing of phage-induced host cell lysis
- Testing of the burst size
- Testing of the morphology

4.3. Purification

Different phage purification methods exist depending on the isolation of bacteria in culture [3]

- For the phage lysate (liquid sample of phage): low-speed centrifugation and filtration through membrane filter
- For small-scale preparations: high speed centrifugation in a cesium chloride gradient
- Extraction with organics solvents
- Anion exchange chromatography

4.4. Phage stabilization [3]

- Methods using low temperature
- Lyophilization
- Spray dying
- Encapsulation by the process of emulsification

4.5. Phage administration

Phage administration varies depending on the site of infection [16]

- Oral application
- Rectal administration
- Topical administration
- Injection into the wound
- Mouth washes
- Mouth rinses
- Toothpaste
- Tooth powder
- Slow-release implant
- Fistula an abscess rinses to fight local infections

5. Numbering of clones

This step makes it possible to know the number of active bacteriophages obtained and therefore to be certain that this quantity will be sufficient from a therapeutic point of view. The phage titer (the number of lytic phages per unit volume) in number of Range Forming Units (RFU) per milliliter is measured: it is necessary to make sure that the titer is greater than or equal to 10^7 [17]

IV. Pharmacology of phage

1. Pharmacokinetics

1.1. Adsorption

Studies have been conducted on the possibility of a passage of the gastrointestinal barrier by phages, called "phage translocation". The duration determined for this passage varies from one study to another, from a few minutes to several days. Some studies indicate that intra-rectal administration of bacteriophages generates phage action rates as high as intramuscular injections and that they are obtained more rapidly in the rectum; five minutes intrarectally versus fifteen minutes intramuscularly to obtain the same peak phage action. [7,18]

1.2. Diffusion

Some studies indicate that after oral administration of phages to laboratory animals, they are found two to four hours later in the bloodstream and ten hours later in various organs such as the liver, kidneys or spleen. It has also been proven that bacteriophages injected into the general circulation spread to the brain and that there is a multiplication of phages at the site of infection. [7,16]

1.3. Metabolism

The metabolization of phages can be their inactivation, if they interact with the immune system, or on the contrary an activation if they replicate in situ. [7,16]

1.4. Elimination

Many studies attest to the elimination of bacteriophages in urine or feces. [7,16]

2. Pharmacodynamics

2.1. Anti-infectious effect

Some studies have shown that the interaction between phages and bacteria was mainly due to a random collision, (a process common to all viruses in contact with a bacterium) and that a minimum threshold of bacterial density was necessary for the good proliferation of bacteriophages. This is why, it is necessary to have a good onset for phage therapy treatment of the disease, and not to start phage therapy too early, as too low a bacterial population at the site of infection would not allow the phage to spread and would be eliminated by the body before the infection of the bacterium has begun.[18,19]

2.2. Innate immune response and bacteriophages

2.2.1. Macrophages

The role of macrophages in the innate immune response against phages is strongly debated and studies suggest that macrophages do not play an important role in the inactivation of bacteriophages. [7]

2.2.2. Polymorphonuclear leukocytes

The polymorphonuclear leukocytes cells are incapable of being activated by bacteriophages but, however if the polynuclear cells are activated by the organism as a result of an underlying infection, these leukocytes are capable of inactivating the bacteriophages. Scientists speculate that this inactivation may be due to the acid hypochlorite that polymorphonuclear leukocytes are likely to release. [7]

2.2.3. NK cells (natural killers)

It would appear that NK cells are not responsible for the inactivation of bacteriophages. [7]

2.2.4. Dendritic cells

Some studies have shown that dendritic cells would phagocytize bacteriophages very rapidly, but oral intake of bacteriophage would reduce the activity of dendritic cell against phages. [20]

3. Adaptive immune response

3.1. Humoral immune response

Most of the antibodies observed during the administration of bacteriophages are neutralizing antibodies and can inactivate the virus only if they bind to the proteins necessary for the proper functioning of the phage cycle. These anti-phage antibodies can reduce the bactericidal properties of bacteriophages, but this immunity can be very variable from one phage to another, and some bacteriophages can have very little immunogenicity. [7]

3.2. Immune cellular response

The cellular immune response consists of the activation of T lymphocytes. Some studies suggest that T lymphocytes have no impact on the activity of bacteriophages.[7]

4. Inflammatory impact

Phages have an anti-inflammatory action on the mucous membrane of the digestive tract as well as on the gastrointestinal tract. This phenomenon is mainly due in particular to the inhibition of dendritic cells with pro-inflammatory properties.[20]

V. Dentistry application of phage therapy

1. Dental caries:

1.1. Actinomyces bacteriophages

Actinomyces bacteriophages: they potentially use surface structure that mediate the contact with streptococci as receptors. The relationship between Actinomyces and streptococci contributes to the growth of biofilms. Blocking co-aggregation with bacteriophage will reduce the formation of the biofilm without the elimination of the health associated Actinomyces which may control the development of the plaque. [5,21–23]

1.2. Streptococcus bacteriophages

Streptococci are the first cause in the formation of dental plaque, more or less fifty bacteriophages of different morphotypes can infect: *S.mitis, S.mutans, S.oralis, S.salivarius and S.sobrinus*. Their use will be helpful for fighting against periodontitis and the prevention of caries. The isolation of the *Streptococcus mutans* bacteriophages has been applied to clinical trial recently and it does not exist many study for this bacterium but scientist certifies that could be a possible effective treatment for dental caries. [8,23,24]

1.3. Lactobacillus:

Lactobacillus bacteriophages have been detected to fight against caries. [8,23]

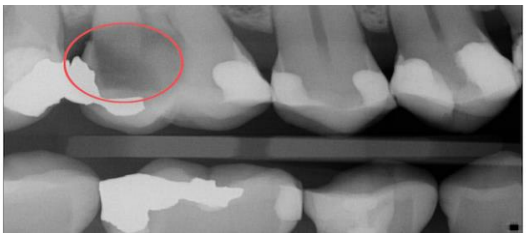


Figure 4: dental caries xrays [25]

2. Periodontal diseases:

2.1. Aggregatibacter bacteriophages

Aggregatibacter bacteriophages are involved in aggressive localized periodontal disease. Several in vitro studies show that *Aggregatibacter* bacteriophages are able to transfer antibiotic resistance cassettes and probably increase the release of leukotoxin. As we know, many periodontal diseases are caused due to aerobic and anaerobic pathogens. The variations of bacteriophage families in periodontal health and disease and the fact that a healthy individual will have greater bacteriophages communities lead us to believe that the possibility of developing therapies based on these bacteriophage communities. [23,24,26]

2.2. Lysins:

Lysins are responsible for the production of the phage enzyme that digest bacterial cell walls in order to liberate assembled phage particles. Most of the lysins are active against gram positive bacteria involved in abscess and periodontal diseases. Some lysin phage have been successfully used to fight against a wide range of streptococcus infections. [8]

2.3. Fusobacterium nucleatum bacteriophages

F.nucleatum is a pathogen that is an anaerobic gram-negative bacillus directly related to periodontal disease. Some studies have discovered the phage FnpΦ02 that attacks that bacteria and have reported that this phage was particularly useful to fight against periodontal diseases. [8,27]

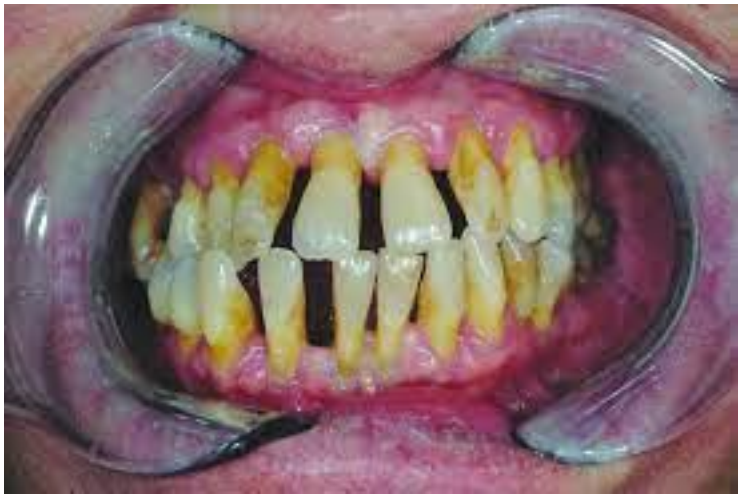


Figure 5: Periodontal disease [28]

3. Endodontic infections:

3.1. Enterococcus bacteriophages:

In *E.faecalis* strains of oral origin, lysogeny has been observed, Enterococci, sometimes responsible for oral infections, can be controlled with a wide range of bacteriophages which may be helpful, especially in patients with persistent endodontic lesions. *E.faecalis* is the main bacteria responsible for failed canal treatment because it has many survival mechanisms to live in unfavourable conditions; it can withstand an environment with:

- Very low oxygen level
- Very high pH
- Temperatures ranging from 10° to 60°
- At high salinity
- In a poorly nutrient environment

The environment of the root canal treatment is favourable for the growth of this bacteria, which is present in primary endodontic infections. *E.faecalis* phages can be used to reduce the chances of recurrent infections. [8,23,29]

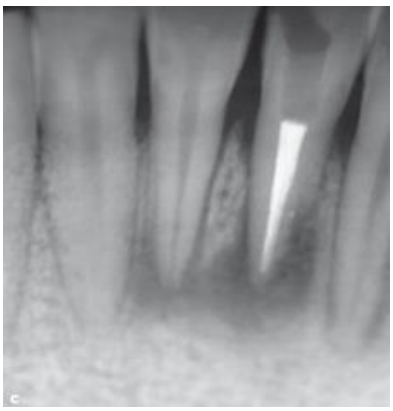


Figure 6: Apical periodontitis caused by endodontic infection [30]

4. Periimplantitis:

Periimplantitis is a pathological disease, occurring in tissues surrounding dental implants. It is characterizing by the inflammation of the connective tissues around the dental implants and a progressive loss of the supporting bone. Studies demonstrate the presence of bacteriophage on the surface of zirconia, that suggest the existence of bacteria that may be responsible of periimplantitis. The presence of bacteriophages may be useful, disturbing formation of biofilm involved in periimplantitis. [8,23]

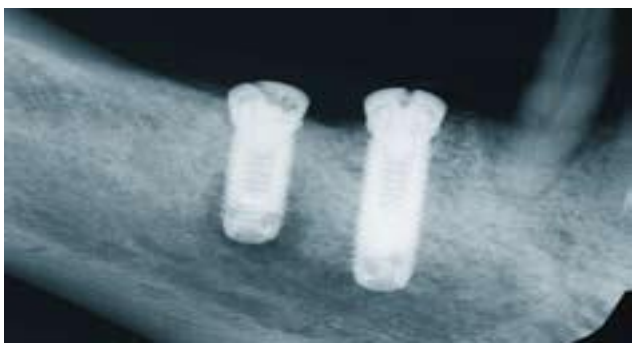


Figure 7: periimplantitis radiograph

5. Oral mucosa infections:

5.1. Bacteriophage peptides

Bacteriophage peptides can ensure the proliferation of epithelial cells in the oral human mucosa without any tumoral transformation this may lead to the improvement of tissue healing by creating wound closure. Phage therapy for skin wounds (cutaneous membrane) has already been extensively studied and performed with very satisfactory results. Additionally, phage therapy for oral wounds (mucous membranes) shows great promise. [31]

5.2. Pseudomonas aeruginosa phages

P.aeruginosa are bacteria involved in oral infections such as abscesses, apical periodontitis, pulpitis and maxillary and mandibular alveolitis. Several scientists have found an effective phage cocktail to fight the bacterium. [32]

Figure 8: Severe oral mucosa infection by *Pseudomonas aeruginosa*

VI. Potential of phages

1. Advantages

1.1. Bactericidal agent

The advantages of phage therapy in addition to being a good alternative to chemical antibiotics, is a natural therapy and has a bactericidal power, which allows the infected cell to not be able to regain its viability. Because unlike some antibiotics such as tetracycline which are only bacteriostatic, meaning it only prevents the proliferation of the bacterium and not complete death of the organism. [33]

1.2. Auto-dosing

Phages are also self-dosing, which means that during their process of destroying the bacterium, they are also able to increase their number specifically where the host is located through the lytic cycle and self-replication. Phages themselves are able to establish the dose needed to fight the host. [33-35]

1.3. Inherent toxicity

Phages also have low inherent toxicity. It is also imperative that certain protocols for the preparation of certain phages should be strictly controlled and obtain an extremely purified form of the phages to be administered in order to avoid anaphylactic responses from components of the bacteria in the mixture since bacteriophages can release components of the bacteria during the killing process. [33-35]

1.4. Minimal disruption of the normal flora

Due to the high specificity that bacteriophages have for their hosts, ranging from the ability to infect a few strains of a specific bacterium to the ability to infect more than one relatively close bacterial genus, bacteriophages have only a minimal impact on the health of the bacterial flora. In contrast to many chemical antibiotics that have a broad spectrum of activity that induce superinfections, which is the condition of reinfection or second infection by the same contagious agent. [34,35]

1.5. Narrow potential of inducing resistance and lack of cross resistance with antibiotics

The relatively narrow bacterial infection range of bacteriophages limits the phage resistance mechanism. The host cell does not have any resistance for bacteriophages, the mechanism of infection of bacteriophages differs from that of antibiotics. Antibiotic resistance mechanisms do not translate into a bacteriophage resistance mechanism, which allows bacteriophages to be used against antibiotic resistant infections and multi-drug resistance. [34,35]

1.6. Capacity for low dosage use

In situ bacteriophages are able to increase their density, which reduces the cost of treatment by reducing the dose required to achieve the expected effectiveness. The application of phages in small doses also increases the safety of the product avoiding the sides effects, phages will only increase their density whilst killing bacteria and will not persist in the body due to their cycle. [34,35]

1.7. Low environmental impact

Bacteriophages contain nucleic acids and proteins and hence their composition is much safer compared to chemical antibiotics. Phages are generally adapted to natural degradation, such as sunlight, extreme temperatures etc…[34,35]

1.8. Low cost

Typically, phage production consists of host growth and purification. The price for host growth varies according to the species studied and the price of purification is decreasing with new technologies. The price of phage isolation and characterization is particularly low in contrast to antibiotics production prices. [34,35]

2. Disadvantages

The naming of bacteriophages as a virus has a social impact and some patients are refractory to this treatment due to its name. Phages are also live microorganisms which can have consequences, they can replicate causing an overreaction of the immune system and leading to an imbalance, in the same way as vaccines or antibiotics and producing auto-immune disease [33]. Another disadvantage of the phage therapy is the lack of bacteriophages species to treat all the bacterial infections.

OBJECTIVES:

The main objectives of this research are:

1. To understand the mechanism of action of bacteriophages
2. To understand why it is useful to treat dental diseases

The secondary objectives are:

1. To compare the benefits of the phage therapy to antibiotic therapy
2. To understand the challenges of phage therapy

METHODOLOGY:

This study was conducted through 41 articles and 15 websites.

These articles were found from databases as “PubMed”, “Medline”, “Research Gate”, “Cochrane” and Google scholar using the following keywords: phage therapy, bacteriophages, alternatives to antibiotic resistance, dental treatment by phage therapy, temperate bacteriophages, virulent bacteriophages, safety of bacteriophages, bacteriophages and oral cavity

We have included articles from scientific literatures and published the last fifty years.

We have excluded the articles with irrelevant contain or just focus on antibiotic treatment and the ones that was not fully available. Articles written in another language than English or French and just with abstract was also excluded.

Table of comparison of bacteriophages used for dental caries:

N° Reference	Authors/publishing date	Name of article	Sample’s origin	Evaluation and tool
(26)	**Tylenda, 1985**	**Isolation of *Actinomyces* bacteriophage from human dental plaque.**	***in vitro,* researches of virulent phages in sample of dental plaque**	**9 out of 10 of phages have been isolated from A.viscosus MG1**
(36)	**Delisle, 1978**	**Isolation of a bacteriophage for *Actinomyces viscosus***	**Test of lyse on different strains of *A.naeslundii and A.viscosus***	**Lytic phage only produced by Av-1 on its host *A.viscosus***

(37)	**Delisle, 1995**	**Relationship among *A.naeslundii* bacteriophages isolated from sewage and oral cavity**	**Purification of phages from sewage and dental plaques**	**Existence of several type of bacteriophages useful to fight against *Actinomyces.spp***
(38)	**Dalmasso, 2015**	**Isolation of a novel phage with activity against *Streptococcus mutans* biofilms**	**Isolation of phages from 85 samples of saliva with healthy oral condition**	**Bacteriostatic or bactericidal action of bacteriophages against *S.mutans***
(39)	**Maal, 2002**	**Identification of *S.salivarius* bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy**	**Samples of dental plaque from healthy volunteers**	**Success for *E.faecalis* bacteriophages, fail for *Streptococcus* bacteriophages**

Table of comparison of bacteriophages used for periodontal disease:

(40)	**Castillo-Ruiz, 2011**	**Isolation of a novel *Aggregatibacter actinomycetemcomitans* serotype b bacteriophage capable of lysing bacteria within a biofilm**	***In vitro,* evaluation of phage's activity against *A.actinomycetemcomitans***	**Improvement of cellular lysis with decrease of DO**

(41)	**Li, 2018**	**Effects of chimeric lysins against planktonic and sessile *Streptococcus faecalis* hint at potential application in endodontic therapy**	***E.faecalis in vitro* cells treated with ClyR** ***Ex vivo teeth with one canal*** **Biofilm of *E.faecalis* treated with ClyR**	**Diminution and degradation of *E.faecalis* bacteria**
(42)	**Xu, 2018**	**Activity of the chimeric lysin ClyR against common Gram- positive oral microbes and its anticaries efficacy in rat models.**	**Effect of modified lysin ClyR on *Streptococcus***	**Reduction of dentinal lesion with lytic activity**
(43)	**Yang, 2016**	**Antibiofilm activities of a novel chimeolysin against *S.mutans,* under physiolofical and cariogenic conditions**	**Action of modified lysin ClyR on *S.mutans***	**ClyR active against all the *S.mutans* strains tested**
(44)	**Kabwe, 2019**	**Genomic, morphological and functional characterization of novel bacteriophages FNU1 capable of disruptiong *F.nucleatum* biofilms**	**Bacteriophages isolation from mouthwashes samples**	**Breaking down of *F.nucleatum biofilms* + lysis of the bacterial cells**
(27)	**Machuca, 2010**	**Isolation of novel bacteriophage specific for periodontal pathogen *F.nucleatum***	**25 samples of saliva of healthy patients + 85 saliva samples of periodontal patients**	**Total lysis of *F.nucleatum* only, no lysis or slow lysis of the other subspecies**

Table of comparison of bacteriophages used for endodontic infections:

(45)	**Tinoco, 2019**	**Effect of genetically engineered bacteriophages on *E.faecalis* biofilms**	**Tempered phages isolated and genetically modified**	**Reduction of *E.faecalis* biofilms after treatment with phages**
(46)	**Paisano, 2004**	***In vitro,* antimicrobial effect of bacteriophages on human dentin infected with *E.faecalis***	**20 humans teeth infected with *E.faecali***	**Correlation between the decrease of the bacterial biomass and the increase of MOI***
(47)	**Tinoco, 2017**	**Antibacterial effect of genetically-engineered bacteriophage ϕEf11/ϕFL1C(Δ36)P^{nisA} on dentin infected with antibiotic-resistant *Enterococcus faecali***	***In vitro,* 40 humans uniradicular teeth with dentin infection**	**Reduction of the infection caused by *E.faecalis***
(48)	**Biswas, 2002**	**Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*.**	**Isolates of vancomycin resistant *E.faecalis* from patient**	**At MOI* 0.3 and 3.0 = 100% survival rate When decrease of the dose, increase of the disease.**

*MOI: Multiplicity of infection

Table of comparison of bacteriophages used for oral mucosa infection:

(49)	**Phee, 2013**	**Efficacy of bacteriophages treatment on *P.aeruginosa* biofilms**	***Pseudomonas Aeruginosa PA-14* infected by phages**	**Reduction of the bacterial biomass. No change with combination of bacteriophages**
(50)	**Pires 2011**	**Use of newly isolated phage for control of *P.aeruginosa PAO1* and ATTC 10145 biofolms**	**35 strains of *P.aeruginosa* infected by 17 different strains of phages**	**Lytic activity of bacteriophages on *P.aeruginosa***

DISCUSSION OF RESULTS:

Different cycles of bacteriophages:

Bacteriophages are classified into two families, virulent or lytic-cycling bacteriophages and temperate or lysogenic-cycling bacteriophages. According to the following articles, virulent bacteriophages are capable of killing bacteria by lysis and temperate bacteriophages are integrated into the genome of their host as a prophage, capable of excising and producing a lytic cycle for the host bacterial cell.

Articles of Ravat (2015) Dufour (2016) and Clokie (2011) explain the mechanism of the lysogenic cycle of bacteriophages. Temperate phages have particular enzymatic activities that allow the insertion of their genome into bacterial DNA. When a temperate phage enters the bacterial cell, a lysogenic cycle occurs, allowing the phage genome or prophage to insert itself into the bacterial genome and become an integral part of the bacterial DNA. This allows the bacterial DNA to pass on viral DNA as it divides. [34,51,52]

In article of Clokie (2011) the author explains how the lytic cycle occurs. Virulent bacteriophages have the capacity to multiply themselves and not only during bacterial division, which makes it possible to create several dozen identical viruses. According to this article, bacterial replication would generate two daughter bacteria every half hour, which would not resist lysis by a dozen bacteriophages. [52]

Dental caries:

Actinomyces:

Articles of Tylenda (1985), Delisle (1978) and Delisle (1995) discuss about *Actinomyces* bacteriophages, isolated from different media, respectively; dental plaque, raw domestic use

and from sewage and dental plaque. According to article of Delisle (1978), bacteriophages have a lytic action only on its host and do not produce any growth inhibition on other organisms (36). Articles of Tylenda (1995) and Delisle (1995) found the existence of different distinct types of bacteriophages useful against *Actinomyces*. These articles confirm the presence of *Actinomyces* bacteriophages directly present in the oral cavity and can be a good alternative against the development of caries and the formation of dental plaque. [26,37]

Streptococcus:

The Article of Dalmasso (2015) and Maal (2002) concern the activity of bacteriophages on *Streptococcus*. In Dalmasso's paper, it is noted that the ability of the bacteriophage to reduce the infection or stop the proliferation of the bacteria is effective, even at a very low MOI and within hours. This study shows that even despite the narrow host range of bacteriophages, it remains a good antimicrobial agent against caries caused by streptococcal infection. In the article Maal (2002), the authors consider existence of bacteriophages capable of combating the streptococci responsible for caries and plaque, but that these have been isolated from the waters of the Persian Gulf and not directly from human saliva, which makes the fight against streptococci more complicated, but possible and very promising for phage therapy in dentistry. [38,39]

Periodontal diseases:

Aggregatibacter bacteriophages:

In the article of Castillo-Ruiz (2011), the authors present us the benefits of phage therapy against the *Aggregatibacter* responsible for the periodontal diseases. This study shows us that the isolated phages were able to infect the bacterial cell and to penetrate the biofilm to cause its lysis and in consequence to stop the periodontal infection caused by *Aggregatibacter*. [40]

Lysin ClyR:

In articles of Li (2018), Xu (2018) and Yang (2015) the authors worked on the modified lysine ClyR. The ClyR lysine has a wide host range and a strong activity against streptococci. Article of Li (2018), the author highlights the efficacy of lysine against *E.faecalis* responsible for endodontic infections. In article of Xu (2018) and Yang (2015), it is found that lysin is very effective against caries caused by streptococcus. The bacteriophage clyR is a promising agent for the prevention and treatment of caries-related oral diseases. Article of Xu (2018), the author and his collaborators defined endolysins as the antibacterial agents with the highest potential in the fight against infections. However, in article of Yang (2015), the author suggests that we should be careful with the administration of lysine, as it can cause immunological side effects due to its pharmacokinetics, so it is preferable to use a topical or oral infection. [41–43]

Fusobacterium nucleatum bacteriophages:

In the articles of Kabwe (2019) and Machuca (2010) the authors talk about Fusobacterium bacteriophages capable of fighting oral biofilms and periodontal diseases. Machuca's study

defined that bacteriophages are useful to treat periodontal disease caused by *F.nucleatum*. The host range of this phage is described as not harmful for the oral flora of the mouth, suggesting that a treatment by bacteriophages for periodontal disease can be consider, moreover in this article, scientists isolated the bacteriophage from a healthy individual, which proved that bacteriophages could be isolated from both healthy and a diseased individual. They also determined that the bacteriophage was a lytic or virulent phage and concluded that this bacteriophage was a specific phage that attacked the *F.nucleatum* bacterium. In paper Machuca and Kabwe articles, scientists demonstrate the ability of the bacteriophage to invade and destroy the *F. nucleatum* bacterium involved in periodontal disease. [27,44]

Endodontic infections:

E.faecalis:

Article of Tinoco (2019), Khalifa (2015) and Paisano (2004), emphasize the use of bacteriophages to fight against endodontic infection. All these articles present a consensus on the responsibility of *E.faecalis* in the infection of dental canals, it is explained that *E.faecalis* penetrates the dentinal tubules and can survive within the dental canals. These articles confirms that *E.faecalis* is resistant to certain endodontic disinfections due to its properties explained in the introduction and also prove the effectiveness of bacteriophages as an endodontic treatment which, contrary to basic treatments, can present resistance and superinfection. [45,46,53]

The articles of Tinoco (2017) and Biswas (2015) speak about the antibacterial effects of phage therapy, on antibiotic resistance *of E.faecalis* infection. In Tinoco's article it can be seen that the biomass of E. faecalis biofilms is significantly reduced after infection of the bacteria with

the bacteriophage. In the Biwas paper it is found that bacteriophages are beneficial to the survival of vancomycin-resistant E. faecalis infected mice. It is found that depending on the MOI of the phages inoculated into the body of the mice, up to 100% survival of mice and only minimal signs of disease can occur [41,42]. The use of bacteriophages in combination with antibiotic treatment or as monotherapy for the elimination of antibiotic-resistant bacteria has proven to be an excellent alternative. The articles of Khalifa (2015), Tinoco (2019), speak about effects of bacteriophages on *E.faecalis* biofilms. In Khalifa's article it is proven that there is a reduction in bacterial biomass during infection by E. faecalis bacteriophages responsible for the contamination of dental canals. In Tinoco's paper, a 10 to 100 folds decrease in the number of viable cells in E. faecalis biofilms was observed after treatment with bacteriophages [45,53]. Articles of Tinoco (2016), Zhang (2013) and Tinoco (2017) concentrate on the genetic modification of temperate bacteriophages into virulent bacteriophages to facilitate control of E. faecalis infection in endodontic infection. All of them obtained encouraging results and proved a better efficiency of virulent bacteriophages. [54–56]

Oral mucosa infections:

Pseudomonas Aeruginosa:

In articles of Pires (2011) and Phee (2013) the authors talk about *Pseudomonas aeruginosa.* Some difficulties to the phage therapy and its action against the bacteria can be observed. Article of Pires shows the failure of certain bacteriophages to infect their hosts, which proves that their effectiveness does not depend on their broad activity. This article also shows that phage therapy is more effective in planktonic cultures than in biofilms. In article of Phee it would seem that some bacteriophages were not effective and could not penetrate the

biofilms, which could be due to an inhibitory action of the dentin to the phage or due to the thickness of the cell walls or the reduction of adsorption sites. [49,50]

Advantages of phage therapy:

In articles Khalifa (2015) and Szafrań ski (2017) the authors highlight the advantages of phage therapy by demonstrating the ability of phages to fight against bacteria that have developed antibiotic resistance, by associating phages either with antibiotic treatment or monotherapy, these results show us a better eradication of the bacterium with a bactericidal rather than bacteriostatic effect (31,53). Articles of Paisano (2004) and Cornelissen (2011), the authors differentiate between two types of bacteriophages, virulent and temperate bacteriophages. In these articles it is noted that virulent bacteriophages have greater advantages, notably the ability to replicate at the site of infection. The authors explain that due to this advantage, virulent bacteriophages are able to control the infection with a single application. [17,46]

Disadvantages of phage therapy:

In articles Biswas (2002) and Dalmasso (2015) the authors talk about host spectra, one of the disadvantages of bacteriophages is their narrow spectra, but these authors propose a new solution by proving that it is possible to isolate phages with a larger spectrum within the same species. [32,42]

Safety:

In the article of Szafrań ski (2017), Cornelissen (2011) we can see the safety of bacteriophages on the organism it invades, these two articles show us that bacteriophages have the capacity

to destroy the host cell without damaging the bacterial flora and leaving it intact. These articles also demonstrate the self-replication power of bacteriophage allowing a unique application and control of bacterial infection. [25,51]

Article of Biswas (2002) also shows us the advantages of the safety of bacteriophages and that the waste released during bacterial lysis is in no way harmful to the bacterial flora and does not induce any anaphylactic or immune responses [42]

CONCLUSION:

Phage therapy is a therapy that was discovered a very long time ago but has declined in the face of the discovery of antibiotics, but due to many problems caused by antibiotics, phage therapy is being revived to fight bacterial diseases.

Throughout this study we have been able to analyse and study the mode of infection of bacteriophages, which mainly involves two cycles: the lytic cycle and the lysogenic cycle, i.e. being self-replicating or self-limiting, which makes them a major asset in the fight against bacterial infections.

We have observed that bacteriophages have a strong bactericidal power without altering the endogenous flora and it is only very rarely that resistance is observed, unlike with antibiotics, which is why this therapy, little known to the general public, has a very promising future.

However, bacteriophages must be subjected to a very careful biological and genetic analysis before being used clinically. Nowadays, thanks to medical, biological and engineering advances, it is now easy to produce proteins from bacteriophages. Bacteriophages are proving increasingly useful against a wide range of hosts such as gram+, gram- or mycobacteria.

The possibility of adding phage therapy to other therapeutic treatments, such as antibiotic therapy, promises great success.

Responsibility:

Bacteriophages are present by the thousands in our environment, both in man-made and natural environments such as the human body or water and are mainly made up of proteins and nucleic acid, making these viruses harmless to the environment.

The cost of preparing phage therapy is relatively low, depending on the species involved, which gives it an advantage over antibiotic therapy.

Due to the low environmental impact and the almost zero risk of side effects of phage therapy, this treatment is accessible to all types of patients, both thanks to its price and its safety

Bibliography:

1. Sulakvelidze A, Alavidze Z, Morris J. Bacteriophage therapy. Antimicrob Agents Chemother. 2001;45(3):649–59.
2. Summers WC. lix Hubert d ' Herelle (1873 – 1949): History of a scienti fi c mind F e. 2016;6(4).
3. Düzgüneş N, Sessevmez M, Yildirim M. Bacteriophage therapy of bacterial infections: The rediscovered frontier. Pharmaceuticals. 2021;14(1):1–16.
4. Phagotherapie-une-alternative-a-l-antibiotherapie @ www.revmed.ch [Internet]. Available from: https://www.revmed.ch/RMS/2016/RMS-N-533/Phagotherapie-une-alternative-a-l-antibiotherapie
5. Braitmaier H. Bacteriophages as alternatives to antibiotics. Antibiotics. 2017;1–5.
6. Gordillo Altamirano FL, Barr JJ. Phage therapy in the postantibiotic era. Clin Microbiol Rev. 2019;32(2):1–25.
7. Górski A, Miedzybrodzki R, Borysowski J. Phage therapy: A practical approach. Phage Therapy: A Practical Approach. 2019. 1–379 p.
8. Steier L, De Oliveira SD, De Figueiredo JAP. Bacteriophages in dentistry-state of the art and perspectives. Dent J. 2019;7(1).
9. mja2 @ onlinelibrary.wiley.com [Internet]. Available from: https://onlinelibrary.wiley.com/doi/full/10.5694/mja2.50355
10. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB. Lysogeny in nature: Mechanisms, impact and ecology of temperate phages. ISME J [Internet]. 2017;11(7):1511–20. Available from: http://dx.doi.org/10.1038/ismej.2017.16

11. Jamal M, Bukhari SMAUS, Andleeb S, Ali M, Raza S, Nawaz MA, et al. Bacteriophages: an overview of the control strategies against multiple bacterial infections in different fields. J Basic Microbiol. 2019;59(2):123–33.

12. Çelik A, Yaman H, Turan S, Kara A, Kara F, Zhu B, et al. No 主観的健康感を中心とした在宅高齢者における 健康関連指標に関する共分散構造分析Title. J Mater Process Technol [Internet]. 2018;1(1):1–8. Available from: http://dx.doi.org/10.1016/j.cirp.2016.06.001%0Ahttp://dx.doi.org/10.1016/j.powtec.2016.12.055%0Ahttps://doi.org/10.1016/j.ijfatigue.2019.02.006%0Ahttps://doi.org/10.1016/j.matlet.2019.04.024%0Ahttps://doi.org/10.1016/j.matlet.2019.127252%0Ahttp://dx.doi.o

13. bacteriophage @ www2.le.ac.uk [Internet]. Available from: https://www2.le.ac.uk/projects/vgec/highereducation/topics/microbial-genetics-1/bacteriophage

14. Bhetwal A, Maharjan A, Shakya S, Satyal D, Ghimire S, Khanal PR, et al. Isolation of Potential Phages against Multidrug-Resistant Bacterial Isolates: Promising Agents in the Rivers of Kathmandu, Nepal. Biomed Res Int. 2017;2017.

15. Naidu M, Robles-Sikisaka R, Abeles SR, Boehm TK, Pride DT. Characterization of bacteriophage communities and CRISPR profiles from dental plaque. BMC Microbiol. 2014;14(1):1–13.

16. Ryan EM, Gorman SP, Donnelly RF, Gilmore BF. Recent advances in bacteriophage therapy: How delivery routes, formulation, concentration and timing influence the success of phage therapy. J Pharm Pharmacol. 2011;63(10):1253–64.

17. Cornelissen A, Ceyssens PJ, T'Syen J, van Praet H, Noben JP, Shaburova O V., et al. The

t7-related pseudomonas putida phage ϕ15 displays virion-associated biofilm degradation properties. PLoS One. 2011;6(4).

18. Nilsson AS. Pharmacological limitations of phage therapy. Ups J Med Sci [Internet]. 2019;124(4):218–27. Available from: https://doi.org/10.1080/03009734.2019.1688433

19. Kasman LM, Kasman A, Westwater C, Dolan J, Schmidt MG, Norris JS. Overcoming the Phage Replication Threshold: a Mathematical Model with Implications for Phage Therapy. J Virol. 2002;76(11):5557–64.

20. Barfoot R, Denham S, Gyure LA, Hall JG, Hobbs SM, Jackson LE, et al. Some properties of dendritic macrophages from peripheral lymph. Immunology [Internet]. 1989;68(2):233–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2807381%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1385423

21. Díaz-Zúñiga J, Melgar-Rodríguez S, Yáñez J, Álvarez C, Rojas C, Benítez A, et al. Variabilidad de la síntesis de citoquinas por células dendríticas humanas estimuladas con los distintos serotipos de Aggregatibacter actinomycetecomitans. Rev clínica periodoncia, Implantol y Rehabil oral. 2013;6(2):57–62.

22. Johansson A, Kalfas S. Virulence Mechanisms of Leukotoxin from Aggregatibacter actinomycetemcomitans. Oral Heal Care - Prosthodont Periodontol Biol Res Syst Cond. 2012;165–92.

23. dental-applications-of-phage-therapy-using-viruses-to-fight-oral-diseases @ www.rdhmag.com [Internet]. Available from: https://www.rdhmag.com/pathology/article/14179560/dental-applications-of-phage-

therapy-using-viruses-to-fight-oral-diseases

24. Hitch G, Pratten J, Taylor PW. Isolation of bacteriophages from the oral cavity. Lett Appl Microbiol. 2004;39(2):215–9.

25. une-visite-chez-le-dentiste-avant-vos-vacances_3538729 @ www.francetvinfo.fr [Internet]. Available from: https://www.francetvinfo.fr/sante/soigner/une-visite-chez-le-dentiste-avant-vos-vacances_3538729.html

26. Tylenda CA, Calvert C, Kolenbrander PE, Tylenda A. Isolation of Actinomyces bacteriophage from human dental plaque. Infect Immun. 1985;49(1):1–6.

27. Machuca P, Daille L, Vinés E, Berrocal L, Bittner M. Isolation of a novel bacteriophage specific for the periodontal pathogen Fusobacterium nucleatum. Appl Environ Microbiol. 2010;76(21):7243–50.

28. 65bcbb8f88b2b00903dd66d90aed767da38f766e @ pocketdentistry.com [Internet]. Available from: https://pocketdentistry.com/4-periodontal-disease/

29. Khalifa L, Shlezinger M, Beyth S, Houri-Haddad Y, Coppenhagen-Glazer S, Beyth N, et al. Phage therapy against Enterococcus faecalis in dental root canals. J Oral Microbiol. 2016;8(1).

30. Bonaccorso A, Tripi TR. Key words bacteria, endo-perio lesions, root canal therapy Endo-perio lesion: Diagnosis, prognosis and decision-making. ENDO (l Engl). 2014;8(2):105–27.

31. Szafrański SP, Winkel A, Stiesch M. The use of bacteriophages to biocontrol oral biofilms. J Biotechnol. 2017;250:29–44.

32. Furfaro LL, Payne MS, Chang BJ. Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles. Front Cell Infect Microbiol. 2018;8(October):376.

33. Loc-Carrillo C, Abedon ST. Pros and cons of phage therapy. Bacteriophage. 2011;1(2):111–4.

34. Ravat F, Jault P, Gabard J. Bactériophages et phagothérapie : Utilisation de Virus Naturels pour traiter les infections bactériennes. Ann Burns Fire Disasters. 2015;28(1):13–20.

35. Point MAU. La phagothérapie. 2015;

36. Pootjes C. Isolation of a bacteriophage for Hydrogenomonas facilis. J Bacteriol. 1964;87(5):1259.

37. Delisle AL, Donkersloot JA. Relationships among actinomyces naeslundii (A. Viscosus) bacteriophages isolated from sewage and the oral cavity. Microb Ecol Health Dis. 1995;8(3):121–7.

38. Dalmasso M, De Haas E, Neve H, Strain R, Cousin FJ, Stockdale SR, et al. Isolation of a novel phage with activity against streptococcus mutans biofilms. PLoS One. 2015;10(9):1–18.

39. Maal KB, Bouzari M, Zavareh FA. Identification of Streptococcus salivarius bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy. African J Microbiol Res. 2010;4(20):2127–32.

40. Castillo-Ruiz M, Vinés ED, Montt C, Fernández J, Delgado JM, Hormazábal JC, et al. Isolation of a novel Aggregatibacter actinomycetemcomitans serotype b bacteriophage capable of lysing bacteria within a biofilm. Appl Environ Microbiol. 2011;77(9):3157–9.

41. Li W, Yang H, Gong Y, Wang S, Li Y, Wei H. Effects of a chimeric lysin against planktonic and sessile enterococcus faecalis hint at potential application in endodontic therapy.

Viruses. 2018;10(6):1–13.

42. Xu J, Yang H, Bi Y, Li W, Wei H, Li Y. Activity of the chimeric lysin ClyR against common gram-positive oral microbes and its anticaries efficacy in rat models. Viruses. 2018;10(7).

43. Yang H, Bi Y, Shang X, Wang M, Linden SB, Li Y, et al. Antibiofilm activities of a novel chimeolysin against Streptococcus mutans under physiological and cariogenic conditions. Antimicrob Agents Chemother. 2016;60(12):7436–43.

44. Kabwe M, Brown TL, Dashper S, Speirs L, Ku H, Petrovski S, et al. Genomic, morphological and functional characterisation of novel bacteriophage FNU1 capable of disrupting Fusobacterium nucleatum biofilms. Sci Rep [Internet]. 2019;9(1):1–12. Available from: http://dx.doi.org/10.1038/s41598-019-45549-6

45. Ren, Hong and Gray WM. 乳鼠心肌提取 HHS Public Access. Physiol Behav. 2019;176(3):139–48.

46. Paisano AF, Spira B, Cai S, Bombana AC. In vitro antimicrobial effect of bacteriophages on human dentin infected with Enterococcus faecalis ATCC 29212. Oral Microbiol Immunol. 2004;19(5):327–30.

47. 1c9841deac6b054ee7e0bb90680ad03274a8f126 @ pubmed.ncbi.nlm.nih.gov [Internet]. Available from: https://pubmed.ncbi.nlm.nih.gov/28646693/

48. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, et al. Erratum: Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant Enterococcus faecium (Infection and Immunity (2002) 70:1 (204-210)). Infect Immun. 2002;70(3):1664.

49. Phee A, Bondy-Denomy J, Kishen A, Basrani B, Azarpazhooh A, Maxwell K. Efficacy of

bacteriophage treatment on Pseudomonas aeruginosa biofilms. J Endod. 2013;39(3):364–9.

50. Pires D, Sillankorva S, Faustino A, Azeredo J. Use of newly isolated phages for control of Pseudomonas aeruginosa PAO1 and ATCC 10145 biofilms. Res Microbiol [Internet]. 2011;162(8):798–806. Available from: http://dx.doi.org/10.1016/j.resmic.2011.06.010

51. Dufour N, Chevallereau A, Debarbieux L. Les bactériophages: Comment ces virus alliés fonctionnent-ils? Biofutur. 2016;(373):31–4.

52. Clokie MRJ, Millard AD, Letarov A V., Heaphy S. Phages in nature. Bacteriophage. 2011;1(1):31–45.

53. Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradosu-Cohen R, et al. Targeting Enterococcus faecalis biofilms with phage therapy. Appl Environ Microbiol. 2015;81(8):2696–705.

54. Ashutosh Tripathi, Aaron H. Nile and VAB. 乳鼠心肌提取 HHS Public Access. Physiol Behav [Internet]. 2017;176(10):139–48. Available from: file:///C:/Users/Carla Carolina/Desktop/Artigos para acrescentar na qualificação/The impact of birth weight on cardiovascular disease risk in the.pdf

55. Zhang H, Fouts DE, DePew J, Stevens RH. Genetic modifications to temperate Enterococcus faecalis phage ϕEf11 that abolish the establishment of lysogeny and sensitivity to repressor, and increase host range and productivity of lytic infection. Microbiol (United Kingdom). 2013;159(PART 6):1023–35.

56. S0003996917301838 @ www.sciencedirect.com [Internet]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0003996917301838?via%3Dihub

ANNEXES:

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2001, p. 649–659 Vol. 45, No. 3
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.3.649–659.2001

MINIREVIEW

Bacteriophage Therapy

ALEXANDER SULAKVELIDZE,[1]* ZEMPHIRA ALAVIDZE,[1,2] AND J. GLENN MORRIS, JR.[1]

Division of Molecular Epidemiology, Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201,[1] and Eliava Institute of Bacteriophage, Microbiology, and Virology, Georgian Academy of Sciences, Tbilisi, Georgia 380060[2]

The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immunosuppressed patients. The concern that humankind is reentering the "preantibiotics" era has become very real, and the development of alternative antiinfection modalities has become one of the highest priorities of modern medicine and biotechnology.

Prior to the discovery and widespread use of antibiotics, it was suggested that bacterial infections could be prevented and/or treated by the administration of bacteriophages. Although the early clinical studies with bacteriophages were not vigorously pursued in the United States and Western Europe, phages continued to be utilized in the former Soviet Union and Eastern Europe. The results of these studies were extensively published in non-English (primarily Russian, Georgian, and Polish) journals and, therefore, were not readily available to the western scientific community. In this minireview, we briefly describe the history of bacteriophage discovery and the early clinical studies with phages and we review the recent literature emphasizing research conducted in Poland and the former Soviet Union. We also discuss the reasons that the clinical use of bacteriophages failed to take root in the West, and we share our thoughts about future prospects for phage therapy research.

DISCOVERY OF BACTERIOPHAGES AND EARLY PHAGE THERAPY RESEARCH

Discovery of bacteriophages. Bacteriophages or phages are bacterial viruses that invade bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. The history of bacteriophage discovery has been the subject of lengthy debates, including a controversy over claims for priority. Ernest Hankin, a British bacteriologist, reported in 1896 (21) on the presence of marked antibacterial activity (against *Vibrio cholerae*) which he observed in the waters of the Ganges and Jumna rivers in India, and he suggested that an unidentified substance (which passed through fine porcelain filters and was heat labile) was responsible for this phenomenon and for limiting the spread of cholera epidemics. Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis* (48), and the observations of several other investigators are also thought to have been related to the bacteriophage phenomenon (72). However, none of these investigators further explored their findings until Frederick Twort, a medically trained bacteriologist from England, reintroduced the subject almost 20 years after Hankin's observation by reporting a similar phenomenon and advancing the hypothesis that it may have been due to, among other possibilities, a virus (70). However, for various reasons—including financial difficulties (68, 70)—Twort did not pursue this finding, and it was another 2 years before bacteriophages were "officially" discovered by Felix d'Herelle, a French-Canadian microbiologist at the Institut Pasteur in Paris.

The discovery or rediscovery of bacteriophages by d'Herelle is frequently associated with an outbreak of severe hemorrhagic dysentery among French troops stationed at Maisons-Laffitte (on the outskirts of Paris) in July-August 1915, although d'Herelle apparently first observed the bacteriophage phenomenon in 1910 while studying microbiologic means of controlling an epizootic of locusts in Mexico. Several soldiers were hospitalized, and d'Herelle was assigned to conduct an investigation of the outbreak. During these studies, he made bacterium-free filtrates of the patients' fecal samples and mixed and incubated them with *Shigella* strains isolated from the patients. A portion of the mixtures was inoculated into experimental animals (as part of d'Herelle's studies on developing a vaccine against bacterial dysentery), and a portion was spread on agar medium in order to observe the growth of the bacteria. It was on these agar cultures that d'Herelle observed the appearance of small, clear areas, which he initially called *taches*, then *taches vierges*, and, later, *plaques* (68). D'Herelle's findings were presented during the September 1917 meeting of the Academy of Sciences, and they were subsequently published (18) in the meeting's proceedings. In contrast to Hankin and Twort, d'Herelle had little doubt about the nature of the phenomenon, and he proposed that it was caused by a virus capable of parasitizing bacteria. The name "bacteriophage" was also proposed by d'Herelle, who, according to his recollections (68), decided on this name together with his wife Marie on 18 October 1916—the day before their youngest daughter's birthday (d'Herelle apparently first isolated bacteriophages in the summer of 1916, approximately 1 year after the Maisons-Laffitte outbreak). The name was formed from

* Corresponding author. Mailing address: Division of Molecular Epidemiology, Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, MSTF Bldg., Room 9-34, 10 South Pine St., Baltimore, MD 21201. Phone: (410) 706-4587. Fax: (410) 706-4581. E-mail: asulakve@epi.umaryland.edu.

BACTERIOPHAGE
2016, VOL. 6, NO. 4, e1270090 (4 pages)
http://dx.doi.org/10.1080/21597081.2016.1270090

Taylor & Francis
Taylor & Francis Group

LIFE IN SCIENCE

Félix Hubert d'Herelle (1873–1949): History of a scientific mind

William C. Summers

Program in History of Science and Medicine, Yale University, New Haven, CT, USA

ABSTRACT
The discovery of bacteriophage one century ago by the French-Canadian Félix d'Herelle set off controversies as to the nature of bacteriophage as well as over the priority and credit for this discovery. The background and life of d'Herelle reveals a complex, self-taught outsider in science who was strongly influenced by his admiration of Louis Pasteur, but also his attachment to the philosophical positions of early 17th century philosophers, especially Francis Bacon. D'Herelle left substantial unpublished writings on his philosophical musings toward the end of his life.

ARTICLE HISTORY
Received 1 November 2016
Revised 4 December 2016
Accepted 5 December 2016

KEYWORDS
biography; d'Herelle; discovery; history; philosophy

Just 100 years ago, 10 September 1917, Félix Hubert d'Herelle, age 44, published a brief note in the prestigious *Comptes rendus de l'Academie des sciences* in which he described a new kind of microbe, in his words, "an obligate intracellular parasite" of bacteria.[1] This discovery would catapult this struggling, almost middle-aged, high school dropout microbiologist to international fame, honorary degrees, candidacy for a Noble Prize, and a century-long controversial reputation (Figure 1).

D'Herelle's short note in the *Comptes rendus* in 1917 truly represents what can be recognized as the discovery of bacteriophage. While an earlier report in 1915 by F.W. Twort[2] certainly described a phenomenon, called "glassy transformation" (of bacterial colonies on agar) and "transmissible lysis," that was caused by bacteriophage in his cultures, Twort failed to interpret his observations in a way that encompassed the concept of virus, of intracellular parasitism, or of serial reproduction of an infectious agent, all of which d'Herelle proposed with clarity and experimental support in his short first note of 1917. If, as Thomas Kuhn has noted, discovery involves more than an observation of a fact of nature,[3] the credit for the discovery of phage must certainly be awarded to Félix d'Herelle.

In addition to his published work, mostly on bacteriophage, he left behind 2 remarkable unpublished works that provide insights into the life and mind of this remarkable individual. One manuscript of over 700 typed pages is a personal memoir, *Les pérégrinations d'un microbiologiste* or "Wanderings of a Microbiologist,"[4] an account of his life as it relates to his world-wide travels in search of adventure, experience, and opportunities to study microbes in their natural habitat. The second work is a 3 volume manuscript setting out d'Herelle's philosophical views of science (and many other topics as well). He gave it the title *La Valeur de l'Expérience: Essai de l'Expérimentalisme* or "The value of experience: an essay on experimentalism."[5] It is a bit more than an essay in that it, too, runs to over 700 typed pages. Recent scholarship,[6,7,8,9] has shed light on the scientific work and professional growth of this unusual outsider whose insights and technical acumen initiated a century of remarkable research on phages, but less attention has been given to his deep philosophical and intellectual commitments that influenced many if not all of his successes and failures.

In his early years, d'Herelle seemed to have been thrown onto his own resources and must have developed the habits of self-education, independence and, at times, over-confidence, that characterized his mature scientific life. Documentary history suggests that even his place of birth seemed to be uncertain: in his memoir, *Les Peregrinations*, and in official government documents such as his passports and war-time identity cards, he claimed Canada as his native land. However, a recent birth certificate uncovered by Alain Dublancet indicates that he was born in Paris.[10] This

CONTACT William C. Summers william.summers@yale.edu Box 208114, New Haven, CT 06520, USA

pharmaceuticals

MDPI

Review

Bacteriophage Therapy of Bacterial Infections: The Rediscovered Frontier

Nejat Düzgüneş [1,*], Melike Sessevmez [2] and Metin Yildirim [3]

[1] Department of Biomedical Sciences, Arthur A. Dugoni School of Dentistry, University of the Pacific, San Francisco, CA 94103, USA
[2] Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, Istanbul 34116, Turkey; melikesessevmez@gmail.com
[3] Department of Pharmacy Services, Vocational School of Health Services, Tarsus University, Mersin 33400, Turkey; metinyildirim4@gmail.com
* Correspondence: nduzgunes@pacific.edu

Abstract: Antibiotic-resistant infections present a serious health concern worldwide. It is estimated that there are 2.8 million antibiotic-resistant infections and 35,000 deaths in the United States every year. Such microorganisms include *Acinetobacter*, Enterobacterioceae, *Pseudomonas*, *Staphylococcus* and *Mycobacterium*. Alternative treatment methods are, thus, necessary to treat such infections. Bacteriophages are viruses of bacteria. In a lytic infection, the newly formed phage particles lyse the bacterium and continue to infect other bacteria. In the early 20th century, d'Herelle, Bruynoghe and Maisin used bacterium-specific phages to treat bacterial infections. Bacteriophages are being identified, purified and developed as pharmaceutically acceptable macromolecular "drugs," undergoing strict quality control. Phages can be applied topically or delivered by inhalation, orally or parenterally. Some of the major drug-resistant infections that are potential targets of pharmaceutically prepared phages are *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and *Acinetobacter baumannii*.

Keywords: lytic infection; antibiotic-resistance; *Mycobacterium tuberculosis*; *Acinetobacter baumannii*; *Pseudomonas aeruginosa*; phage production; magistral phage; pulmonary delivery; oral administration; topical delivery

Citation: Düzgüneş, N.; Sessevmez, M.; Yildirim, M. Bacteriophage Therapy of Bacterial Infections: The Rediscovered Frontier. *Pharmaceuticals* **2021**, *14*, 34. https://doi.org/10.3390/ph14010034

Received: 22 November 2020
Accepted: 29 December 2020
Published: 5 January 2021

1. Introduction: Bacteriophage Treatment of a Serious Infection

A 68-year-old man with diabetes developed necrotizing pancreatitis that was complicated by a pancreatic pseudocyst infected with a multi-drug-resistant strain of *Acinetobacter baumannii* [1]. *A. baumannii* is a Gram-negative nosocomial pathogen involved in bacteremia, meningitis and pulmonary infections with a high mortality rate. It is one of the "ESKAPE" microorganisms that are grouped together because of the common occurrence of multi-drug-resistance in the group. These microorganisms include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species. The condition of the patient was deteriorating rapidly despite antibiotic treatment, to which he was obviously not responding. Bacteriophage therapy was initiated as part of an emergency investigational new drug protocol.

Bacteriophages are viruses of bacteria. Phages can cause either lytic or lysogenic infections in bacteria after attaching to a receptor or receptors on the bacterial surface and delivering their genome into the bacteria. In a lytic infection, the phage replicates and the new phage particles lyse the bacterium and continue to infect other bacteria (Figure 1). In a lysogenic infection, a DNA phage inserts its genetic material into the bacterial chromosome, and the genome is passed on to daughter cells as the bacterium divides. The integrated DNA may be activated by changes in environmental conditions to excise itself from the chromosome, producing phage particles that become lytic [2–4].

PLOS ONE

RESEARCH ARTICLE

Restriction-modification mediated barriers to exogenous DNA uptake and incorporation employed by *Prevotella intermedia*

Christopher D. Johnston[1,2]*, Chelsey A. Skeete[1], Alexey Fomenkov[3], Richard J. Roberts[3], Susan R. Rittling[1,2]

1 Department of Immunology and Infectious Disease, Forsyth Institute, Cambridge, United States of America, **2** Harvard School of Dental Medicine, Boston, MA, United States of America, **3** New England Biolabs, Ipswich, MA, United States of America

* cjohnston@forsyth.org

OPEN ACCESS

Citation: Johnston CD, Skeete CA, Fomenkov A, Roberts RJ, Rittling SR (2017) Restriction-modification mediated barriers to exogenous DNA uptake and incorporation employed by *Prevotella intermedia*. PLoS ONE 12(9): e0185234. https://doi.org/10.1371/journal.pone.0185234

Editor: Albert Jeltsch, Universität Stuttgart, GERMANY

Received: May 26, 2017

Accepted: September 9, 2017

Published: September 21, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Complete genome sequences and annotations have been submitted to NCBI under Bioproject ID: PRJNA313956 (P. intermedia ATCC25611F: Biosample SAMN04529094, Accessions CP019300 and CP019301 for Chromosome I and Chromosome II respectively. P. intermedia 17F: Biosample SAMN04529095, Accessions CP019302 and CP019303 for Chromosome I and Chromosome II respectively).

Abstract

Prevotella intermedia, a major periodontal pathogen, is increasingly implicated in human respiratory tract and cystic fibrosis lung infections. Nevertheless, the specific mechanisms employed by this pathogen remain only partially characterized and poorly understood, largely due to its total lack of genetic accessibility. Here, using Single Molecule, Real-Time (SMRT) genome and methylome sequencing, bisulfite sequencing, in addition to cloning and restriction analysis, we define the specific genetic barriers to exogenous DNA present in two of the most widespread laboratory strains, *P. intermedia* ATCC 25611 and *P. intermedia* Strain 17. We identified and characterized multiple restriction-modification (R-M) systems, some of which are considerably divergent between the two strains. We propose that these R-M systems are the root cause of the *P. intermedia* transformation barrier. Additionally, we note the presence of conserved Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) systems in both strains, which could provide a further barrier to exogenous DNA uptake and incorporation. This work will provide a valuable resource during the development of a genetic system for *P. intermedia*, which will be required for fundamental investigation of this organism's physiology, metabolism, and pathogenesis in human disease.

1. Introduction

Prevotella intermedia is a major oral pathogen associated with endodontic infections, where it is often the most frequently identified species[1], and periodontal infections, where it is a member of the orange complex [2], supporting destructive inflammatory disease. In addition, *P. intermedia* is increasingly implicated in extra-oral disease including nasopharyngeal and intra-abdominal infections and has been shown to colonize the respiratory tract in association with chronic bronchitis [3], severe bacteremic pneumococcal pneumonia [4] and cystic fibrosis lung infections [5]. Nevertheless, the virulence mechanisms employed by *P. intermedia* during human infections remain ill-defined and poorly characterized, largely due to this pathogens complete

antibiotics

MDPI

Review

Bacteriophages as Alternatives to Antibiotics in Clinical Care

Danitza Romero-Calle [1], Raquel Guimarães Benevides [1], Aristóteles Góes-Neto [1] and Craig Billington [2,*]

[1] Postgraduate Program in Biotechnology, State University of Feira de Santana (UEFS), Av. Transnordestina S/N, Feira de Santana-BA 44036-900, Brazil
[2] Health & Environment Group, Institute of Environmental Sciences and Research, PO Box 29-181, Christchurch 8540, New Zealand
* Correspondence: craig.billington@esr.cri.nz

Received: 2 August 2019; Accepted: 3 September 2019; Published: 4 September 2019

check for updates

Abstract: Antimicrobial resistance is increasing despite new treatments being employed. With a decrease in the discovery rate of novel antibiotics, this threatens to take humankind back to a "pre-antibiotic era" of clinical care. Bacteriophages (phages) are one of the most promising alternatives to antibiotics for clinical use. Although more than a century of mostly ad-hoc phage therapy has involved substantial clinical experimentation, a lack of both regulatory guidance standards and effective execution of clinical trials has meant that therapy for infectious bacterial diseases has yet to be widely adopted. However, several recent case studies and clinical trials show promise in addressing these concerns. With the antibiotic resistance crisis and urgent search for alternative clinical treatments for bacterial infections, phage therapy may soon fulfill its long-held promise. This review reports on the applications of phage therapy for various infectious diseases, phage pharmacology, immunological responses to phages, legal concerns, and the potential benefits and disadvantages of this novel treatment.

Keywords: bacteriophages; clinical trials; antibiotic resistance; infectious disease; phage therapy

1. Introduction

There are approximately 10^{30-31} bacteriophages (phages) in the biosphere [1,2], which is estimated to be 10-fold higher than the total number of bacterial cells [3]. Phages are also an inherent part of the human microbiome, and so are usually well-tolerated when used in phage therapy [4–6]. Phages are one of the most promising alternatives to antibiotics, which can be used for medicine, agriculture, and related fields [7]. The evolution of multidrug-resistant and pan-drug-resistant bacteria poses a real threat to the control of infectious diseases globally, so it is urgent to have new therapeutic tools available. The United States National Institutes of Health have stated that phages are promising tools for combatting microbial resistance [8].

A post-antibiotic era in which minor injuries and common infections can kill because of the lack of drugs or their ineffectiveness is nowadays not an apocalyptic fantasy, but a real 21st-century threat. For example, ESKAPE organisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) are extremely resistant to multiple antimicrobial agents [9] and are a serious challenge in medicine today. On the other hand, there historically has been no fit for purpose regulatory framework to deal with novel flexible and sustainable therapeutic approaches such as phages. For phages, this includes oversight of the setup and approval of adequate clinical trials, so as a result, there is no standard protocol for phage therapy.

In this review, we summarize the phage therapy clinical trials that have shown promising results in patients. We cover several diseases, immunological responses to phages, phage pharmacology, legal

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

REVIEW

Phage Therapy in the Postantibiotic Era

Fernando L. Gordillo Altamirano,[a] Jeremy J. Barr[a]

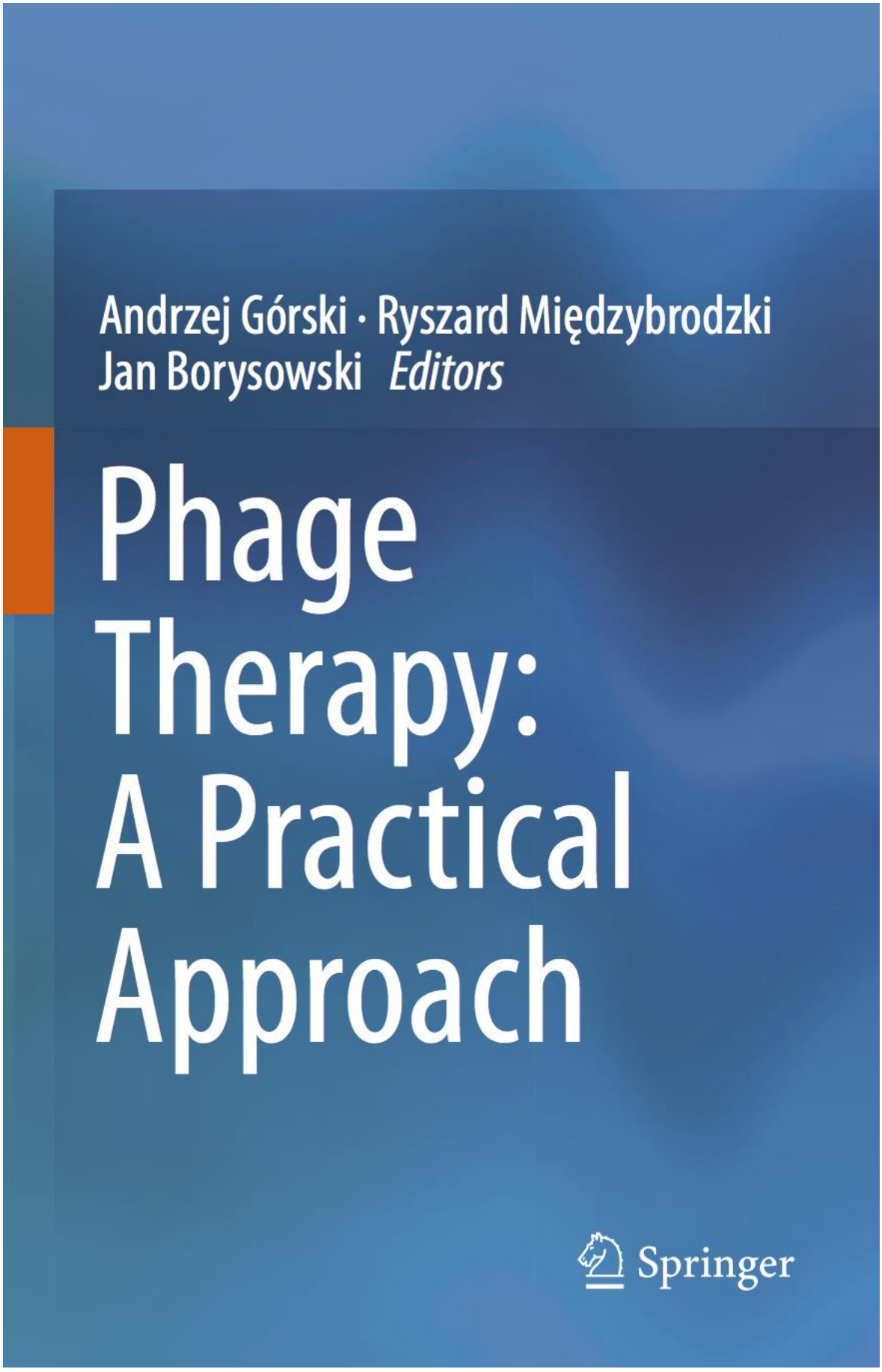
[a]School of Biological Sciences, Monash University, Clayton, Victoria, Australia

SUMMARY Antibiotic resistance is arguably the biggest current threat to global health. An increasing number of infections are becoming harder or almost impossible to treat, carrying high morbidity, mortality, and financial cost. The therapeutic use of bacteriophages, viruses that infect and kill bacteria, is well suited to be part of the multidimensional strategies to combat antibiotic resistance. Although phage therapy was first implemented almost a century ago, it was brought to a standstill after the successful introduction of antibiotics. Now, with the rise of antibiotic resistance, phage therapy is experiencing a well-deserved rebirth. Among the admittedly vast literature recently published on this topic, this review aims to provide a forward-looking perspective on phage therapy and its role in modern society. We cover the key points of the antibiotic resistance crisis and then explain the biological and evolutionary principles that support the use of phages, their interaction with the immune system, and a comparison with antibiotic therapy. By going through up-to-date reports and, whenever possible, human clinical trials, we examine the versatility of phage therapy. We discuss conventional approaches as well as novel strategies, including the use of phage-antibiotic combinations, phage-derived enzymes, exploitation of phage resistance mechanisms, and phage bioengineering. Finally, we discuss the benefits of phage therapy beyond the clinical perspective, including opportunities for scientific outreach and effective education, interdisciplinary collaboration, cultural and economic growth, and even innovative use of social media, making the case that phage therapy is more than just an alternative to antibiotics.

Citation Gordillo Altamirano FL, Barr JJ. 2019. Phage therapy in the postantibiotic era. Clin Microbiol Rev 32:e00066-18. https://doi.org/10.1128/CMR.00066-18.

Address correspondence to Fernando L. Gordillo Altamirano, fernando.gordilloaltamirano@monash.edu, or Jeremy J. Barr, jeremy.barr@monash.edu.

Published 16 January 2019


Andrzej Górski · Ryszard Międzybrodzki
Jan Borysowski Editors
Phage Therapy: A Practical Approach
Springer

dentistry journal

MDPI

Review

Bacteriophages in Dentistry—State of the Art and Perspectives

Liviu Steier [1], Silvia Dias de Oliveira [2] and José Antonio Poli de Figueiredo [3,*]

1 Visiting Professor, Post-Graduate Program in Dentistry, Federal University of Rio Grande do Sul—UFRGS, Porto Alegre 90035-003, Brazil; lsteier@gmail.com
2 Department of Biodiversity and Ecology, Pontifical Catholic University of Rio Grande do Sul—PUCRS, Porto Alegre 90619-900, Brazil; silviadias@pucrs.br
3 Department of Morphological Sciences, Federal University of Rio Grande do Sul—UFRGS, Porto Alegre 90040-060, Brazil
* Correspondence: poli.figueiredo@outlook.com

Received: 31 October 2018; Accepted: 7 January 2019; Published: 9 January 2019

Abstract: Bacteriophages, viruses capable of killing bacteria, were discovered in 1915, but the interest in their study has been limited since the advent of antibiotics. Their use in dentistry is still very limited. The authors reviewed studies about bacteriophage structure, mode of action, uses in oral health, and possible future uses in dentistry associated with their possible action over biofilm, as well as the advantages and limitations of phage therapy.

Keywords: bacteriophages; dentistry; biofilm

1. Introduction

The term bacteriophage refers to viruses that are capable of destroying bacteria, or "bacteria eaters". They are the most common biological entities on earth, at an estimated number of 10^{31} bacteriophages in the biosphere. Twort [1] and d'Hérelle [2] were the first to describe them, but it was d'Herelle who applied the term to a bacteriolytic substance that he isolated from feces. This finding leads to several studies and the creation of the "phage group", of which Max Delbrück, James Watson, and Francis Crick were the most notable scientists [3].

Delbrück, in 1939, discovered a one-step process to grow bacteriophages, which, after a one-hour latent period, would multiply to produce several hundred thousands of progeny. Together with Luria, in 1943, they found a bacterium that underwent spontaneous mutations after infection by a bacteriophage until it became immune to the phage. In 1969, Delbrück, Hershey and Luria were awarded the Nobel Prize in Physiology or Medicine for their work on bacteriophages.

The discovery of antibiotics, as well as the indiscriminate use of bacteriophages to treat all types of infections, even when they were not specific to the disease, is probably the cause for the abandonment of phage therapy [4]. However, phage therapy continued to be widely practiced in the Soviet Union due to the collaboration between Felix d'Herelle and his Georgian colleagues, especially George Eliava. As a result of their studies of bacteriophages, the Institute of Vaccine and Sera in Tbilisi produced the first commercial anticholera phage preparation, which reduced the mortality due to cholera in India to 10%. D'Herelle and Eliava spent altogether 18 months in 1933 and 1934 collaborating with other scientists in Georgia [5]. D'Herelle intended to move to Tbilisi permanently, but in 1937, Eliava and his wife were killed by the Soviet regime.

The Oswaldo Cruz Institute in Rio de Janeiro, Brazil, started the production of anti-dysentery bacteriophages in 1924 to combat dysentery in Latin American countries. Within a year, the institute had produced 10,000 vials of phages, which were sent to hospitals all over Brazil [6].

Bacteriophages: an overview of the control strategies against multiple bacterial infections in different fields

Article *in* Journal of Basic Microbiology · November 2018

DOI: 10.1002/jobm.201800412

CITATIONS
28

READS
6,235

9 authors, including:

Muhsin Jamal
Abdul Wali Khan University,
57 PUBLICATIONS **550** CITATIONS
SEE PROFILE

Sayed Muhammad Ata Ullah Shah Bukhari
Quaid-i-Azam University
5 PUBLICATIONS **28** CITATIONS
SEE PROFILE

Saadia Andleeb
National University of Sciences and Technology
81 PUBLICATIONS **1,344** CITATIONS
SEE PROFILE

Muhammad Ali
Rutgers, The State University of New Jersey
30 PUBLICATIONS **61** CITATIONS
SEE PROFILE

Hindawi
BioMed Research International
Volume 2017, Article ID 3723254, 10 pages
https://doi.org/10.1155/2017/3723254

Research Article

Isolation of Potential Phages against Multidrug-Resistant Bacterial Isolates: Promising Agents in the Rivers of Kathmandu, Nepal

Anjeela Bhetwal,[1] Anjila Maharjan,[1] Shreena Shakya,[1] Deepa Satyal,[1] Sumitra Ghimire,[1] Puspa Raj Khanal,[1] and Narayan Prasad Parajuli[1,2]

[1]*Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, Kathmandu, Nepal*
[2]*Department of Clinical Laboratory Services, Manmohan Memorial Medical College and Teaching Hospital, Kathmandu, Nepal*

Correspondence should be addressed to Narayan Prasad Parajuli; narayan.parajuli@iom.edu.np

Received 2 August 2017; Revised 28 October 2017; Accepted 2 November 2017; Published 22 November 2017

Academic Editor: Florian M. Wagenlehner

Bacteriophages are being the subject of interest for alternative antimicrobial therapy for infectious diseases in recent years. Therapeutic effectiveness regarding phage therapy is a matter of concern since it is the most promising biological treatment of this era. Hence, the present study was aimed to isolate the potential bacteriophages present in river water samples and to analyze their host range among clinical strains of bacteria. Ten different locations of Kathmandu valley were selected for the collection of river water for the detection of probable phages. Bacteriophages were isolated from water samples using the double agar overlay method. Isolated phages were purified by diluting in the SM-buffer and filtering through 0.22 μm filter. Purified lysate was further processed for analyzing its host range by using spot method. Their host range was characterized against 20 bacterial strains, including multidrug-resistant. Total 67 different phages were isolated against 8 different host organisms. Out of them, forty-seven phages were selected for analyzing its host range. Among them, *Serratia* phages (ΦSER) had the broad host range infecting 17 different bacterial strains including multidrug-resistant harboring ESBL and MBL genotypes. However, *Klebsiella* phages (ΦKP) had narrow host range in comparison to other phages. Isolated phages had the potential effect against clinical strains of bacteria along with their broader host spectrum. Most importantly, promising effect against MDR pathogens in this study has raised the probable chances of the utility of these phages for biological control of bacterial infection including MBL and ESBL strains.

1. Background

Globally, dissemination of multidrug resistance among bacterial strains has posed a significant threat to public health confronting the routine treatment of infectious diseases [1, 2]. Despite the global surge of such resistant bugs, development of new antibiotics has been decelerated since last few decades [3]. Therefore, it necessitates the incessant endeavors to develop a promising alternative for treating infectious diseases and reducing the emergence and dissemination of antibiotic resistance among pathogens [4, 5]. Recently, bacteriophages are gaining new ground as an alternative regime for the therapeutic application as they impose antibacterial properties and self-replicate during infection [1, 6]. Hence, there is renaissance in the use of bacteriophages to counteract the resistant pathogens [7].

Bacteriophages ("phages" for short) possess novel mode of action compared to that of antibacterial regimens, as they selectively infect pathogenic bacteria including multidrug-resistant pathogens (in vivo and in vitro) [8]. Furthermore, they are ecologically safe and effective in lower doses and do not show adverse reactions on their application in human body [1, 9]. To these assets, phages have garnered increasing attention in the therapeutic application in recent years. Several studies, available to date, have revealed the lytic efficacy of phages against various pathogenic organisms including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Vibrio cholerae, Salmonella species, Staphylococcus aureus, Enterococcus* spp., and *Serratia* spp. [10–15]. In addition, ability of lytic phages against multidrug-resistant bacteria producing hydrolytic enzymes including extended spectrum

Naidu *et al. BMC Microbiology* 2014, **14**:175
http://www.biomedcentral.com/1471-2180/14/175

BMC Microbiology

RESEARCH ARTICLE Open Access

Characterization of bacteriophage communities and CRISPR profiles from dental plaque

Mayuri Naidu[1], Refugio Robles-Sikisaka[1], Shira R Abeles[2], Tobias K Boehm[3] and David T Pride[1,2*]

Abstract

Background: Dental plaque is home to a diverse and complex community of bacteria, but has generally been believed to be inhabited by relatively few viruses. We sampled the saliva and dental plaque from 4 healthy human subjects to determine whether plaque was populated by viral communities, and whether there were differences in viral communities specific to subject or sample type.

Results: We found that the plaque was inhabited by a community of bacteriophage whose membership was mostly subject-specific. There was a significant proportion of viral homologues shared between plaque and salivary viromes within each subject, suggesting that some oral viruses were present in both sites. We also characterized Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) in oral streptococci, as their profiles provide clues to the viruses that oral bacteria may be able to counteract. While there were some CRISPR spacers specific to each sample type, many more were shared across sites and were highly subject specific. Many CRISPR spacers matched viruses present in plaque, suggesting that the evolution of CRISPR loci may have been specific to plaque-derived viruses.

Conclusions: Our findings of subject specificity to both plaque-derived viruses and CRISPR profiles suggest that human viral ecology may be highly personalized.

Keywords: Oral biofilm, Virus, Virome, Microbiome, Dental plaque, CRISPR

Background

Much of the study of the human microbiome has concentrated on those indigenous bacterial communities inhabiting different body surfaces [1-4], but relatively little effort has been focused on viruses [5-9]. Recent studies have identified communities of viruses inhabiting the human oral cavity [10,11], the respiratory tract [8], skin [12], and the intestinal tract [5,7,13]. While the role of viruses in these communities has yet to be thoroughly examined, a common feature shared among these body surfaces has been that most of the viruses identified have been bacteriophage [5-7,11,14]. Because bacteria generally outnumber human cells in these environments, bacteriophage might also be expected to outnumber eukaryotic viruses. Many of the viruses present in these communities have been predicted to have primarily lysogenic lifestyles, carrying gene function that might facilitate the pathogenic functions of their host bacteria [6,7].

Biofilms contain complex aggregates of microorganisms growing on self-produced solid surfaces, whose constituents and cellular activity may differ substantially from planktonic communities [15]. The oral biofilm is known to be inhabited by numerous species of bacteria and archaea [1,16-18], but has not been shown to be inhabited by communities of viruses. Because of the potential difficulty in traversing solid surface biofilms, dental plaque has been hypothesized to be relatively devoid of viruses [6], however, some viruses have previously been identified in dental plaque [19-21]. Given the abundance of bacteria residing within plaque, we hypothesize that dental plaque may have an indigenous viral community.

The human oral cavity contains many microenvironments in which the microbiota are known to differ [17]. There are characteristic differences in the relative abundances of bacteria in subgingival plaque, supragingival plaque, saliva, buccal mucosa and on the tongue. There also are shifts in oral bacteria that can be traced to diet

* Correspondence: dpride@ucsd.edu
[1]Department of Pathology, University of California, San Diego, CA, USA
[2]Department of Medicine, University of California, San Diego, CA, USA
Full list of author information is available at the end of the article

JPP Journal of Pharmacy and Pharmacology

ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1253–1264

Received January 5, 2011
Accepted May 30, 2011
DOI
10.1111/j.2042-7158.2011.01324.x
ISSN 0022-3573

Review

Recent advances in bacteriophage therapy: how delivery routes, formulation, concentration and timing influence the success of phage therapy

Elizabeth M. Ryan, Sean P. Gorman, Ryan F. Donnelly and Brendan F. Gilmore

School of Pharmacy, Queens University Belfast, Belfast, UK

Abstract

Objectives Bacteriophages are bacteria-specific viruses that infect and, in the case of obligately lytic phages, destroy their host bacteria. Phage therapy has been used therapeutically to combat bacterial infections since their discovery. This paper reviewed recent in-vivo phage therapy studies, with a distinct focus on the effect of delivery routes, phage concentration and timing of administration on the success of the therapy.

Key findings It was found that the most successful route of administration for the treatment of systemic infections was via the parenteral route. Oral delivery is mainly used to treat gastrointestinal infections. However, in some cases phages can also reach the systemic circulation. Local delivery (skin, ears, teeth) has proved extremely successful in the treatment of topical infections, as has the inhalation of phages for the treatment of lung infections. The ability of phages to prevent biofilm formation on medical devices has received much attention, mainly in the area of catheter coatings. This review also highlights areas in which phage therapy needs substantial development. Many papers were lacking in formulation details, with crude phage stocks being used in most cases. No phage stability data were included in any of the papers.

Summary The review concluded that although phage therapy is an excellent alternative for the treatment of bacterial infections, optimisation of formulations and long-term stability data is required before it can be widely used within a clinical setting.

Keywords phage therapy; delivery routes; formulation

Introduction

The application of bacteriophages (phages) as antibacterial agents first began in the early 1920s, following their discovery by English bacteriologist Fredrick Twort in 1915 and also by French Canadian scientist Felix D'Herelle in 1917. D'Herelle recorded the discovery of a microbe that was antagonistic to bacteria and resulted in lysis and bacterial cell death. Two years earlier, Fredrick Twort had recorded a similar discovery, but he never considered phage therapy. D'Herelle devoted the rest of his scientific life to the study of bacteriophages.[1,2] Phages have been used in clinical applications ever since.[3,4] The discovery of penicillin hailed the beginning of the antibiotic era and phage therapy was largely supplanted across the developed world, with the exception of a number of Eastern bloc countries. Recently, the increasing incidence of antibiotic-resistant bacterial strains has stimulated a resurgence in interest into these bacteria-specific viruses.[3,5,6]

Multi-drug-resistant bacteria pose a major threat to human health and the long-term usefulness of conventional antibiotics.[7,8] In the European Union alone, infections caused by these bacteria cause around 25 000 deaths per year. Two-thirds of these deaths are due to infection with Gram-negative bacteria, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacteriaceae, including *Escherichia coli* and *Klebsiella pneumonia*.[9] A recent report from the European Centre for Disease Prevention and Control and the European Medicines Agency states that only two new antibiotic drugs are under development, both in the early stages.[10] There are a multitude of obstacles to pharmaceutical companies investing in the development of new antibiotics. Firstly, there are many generic antibiotics that are still effective in the treatment of bacterial infections. Secondly, antibiotics are less profitable than many drugs because they are curative treatments and the duration of antibiotic regimens is limited. Thirdly, the rapid growth of resistance could shorten their

Correspondence: Brendan F. Gilmore, School of Pharmacy, Queens University Belfast, 97 Lisburn Rd, Belfast, BT9 7BL, UK. E-mail: b.gilmore@qub.ac.uk

OPEN ACCESS Freely available online

PLoS one

The T7-Related *Pseudomonas putida* Phage φ15 Displays Virion-Associated Biofilm Degradation Properties

Anneleen Cornelissen[1], Pieter-Jan Ceyssens[1], Jeroen T'Syen[1], Helena Van Praet[1], Jean-Paul Noben[2], Olga V. Shaburova[3], Victor N. Krylov[3], Guido Volckaert[1], Rob Lavigne[1]*

1 Laboratory of Gene Technology, Katholieke Universiteit Leuven, Leuven, Belgium, **2** School of Life Sciences, Biomedical Research Institute and Transnational University Limburg, Hasselt University, Diepenbeek, Belgium, **3** State Institute for Genetics of Industrial Microorganisms, Moscow, Russia

Abstract

Formation of a protected biofilm environment is recognized as one of the major causes of the increasing antibiotic resistance development and emphasizes the need to develop alternative antibacterial strategies, like phage therapy. This study investigates the in vitro degradation of single-species *Pseudomonas putida* biofilms, PpG1 and RD5PR2, by the novel phage φ15, a 'T7-like virus' with a virion-associated exopolysaccharide (EPS) depolymerase. Phage φ15 forms plaques surrounded by growing opaque halo zones, indicative for EPS degradation, on seven out of 53 *P. putida* strains. The absence of haloes on infection resistant strains suggests that the EPS probably act as a primary bacterial receptor for phage infection. Independent of bacterial strain or biofilm age, a time and dose dependent response of φ15-mediated biofilm degradation was observed with generally a maximum biofilm degradation 8 h after addition of the higher phage doses (10^4 and 10^6 pfu) and resistance development after 24 h. Biofilm age, an in vivo very variable parameter, reduced markedly phage-mediated degradation of PpG1 biofilms, while degradation of RD5PR2 biofilms and φ15 amplification were unaffected. Killing of the planktonic culture occurred in parallel with but was always more pronounced than biofilm degradation, accentuating the need for evaluating phages for therapeutic purposes in biofilm conditions. EPS degrading activity of recombinantly expressed viral tail spike was confirmed by capsule staining. These data suggests that the addition of high initial titers of specifically selected phages with a proper EPS depolymerase are crucial criteria in the development of phage therapy.

Citation: Cornelissen A, Ceyssens P-J, T'Syen J, Van Praet H, Noben J-P, et al. (2011) The T7-Related *Pseudomonas putida* Phage φ15 Displays Virion-Associated Biofilm Degradation Properties. PLoS ONE 6(4): e18597. doi:10.1371/journal.pone.0018597

Editor: Mark Alexander Webber, University of Birmingham, United Kingdom

Received December 23, 2010; **Accepted** March 5, 2011; **Published** April 19, 2011

Funding: This research was partially supported by the K.U. Leuven (STRT1/10/021TBA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding received for this study.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: rob.lavigne@biw.kuleuven.be

Introduction

Biofilms are surface-associated complex bacterial communities encased in a hydrated extracellular polymer matrix of exopolysaccharides (EPS), proteins, nucleic acids and lipids. They are formed spontaneously on both inert and living systems in various natural and man-made environments, from food processing to industrial (*e.g.* water pipes) and hospital settings (*e.g.* burn wounds, endocarditis and catheters). They represent an essential bacterial survival strategy since biofilm-associated bacteria can reach a thousand fold increased protection against antimicrobial agents compared to their planktonic counterparts [1,2]. Very often the antibiotic concentration to eradicate the biofilm is above the peak serum concentration, rendering it ineffective in treating biofilm infections [3]. As they also confer protection for host defense mechanisms, biofilms are a leading cause of latent as wells as of recurrent infections [4,5].

Pseudomonas putida belongs to the fluorescent group of the *Pseudomonas* species, a group of opportunistic pathogens that primarily cause nosocomial infections. In contrast to *Pseudomonas aeruginosa*, the most prevalent pathogen, infections caused by *P. putida* are rare and mostly reported in immunocompromised patients, such as newborns [6], neutropenic and cancer patients [7–9]. The ability to adhere to materials and to promote the formation of biofilms appears the most important feature of the pathogenicity of *P. putida* [10]. Despite a high susceptibility for anti-pseudomonal β-lactams, multidrug-resistant *P. putida* isolates to β-lactams, including carbapenems, have already been reported [11–15].

The widespread emergence of resistance to antibiotics among pathogenic bacteria emphasizes the need to explore new classes of antibacterial agents, ones that cannot be resisted by the same antibiotic resistant genes. (Bacterio)phage therapy (use of bacterial viruses) may represent such a new class, with additional advantages like self-replication at the site of infection and host specificity, leaving the normal bacterial flora undisturbed [16]. To penetrate EPS layers, some phages carry EPS depolymerases as tail spikes or tail fibers, as part of the viral particle, to enable them to reach the bacterial cell wall [17]. Consequently, phages cause biofilm and capsule disruption by cell infection and lysis, as well as by EPS degradation. The principle of EPS depolymerization as biofilm destabilizing agent was first employed in the pre-antibiotic era: potentially lethal pneumococcal infections in rabbits and monkeys were controlled through administration of partially purified depolymerase [18,19].

Despite biomedical and industrial interest for phages encoding these enzymes, recent more in-depth research is limited and mainly focused on the capsulated neuroinvasive *Escherichia coli* K1

JOURNAL OF VIROLOGY, June 2002, p. 5557–5564 Vol. 76, No. 11
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.11.5557–5564.2002

Overcoming the Phage Replication Threshold: a Mathematical Model with Implications for Phage Therapy

Laura M. Kasman,[1]* Alex Kasman,[2] Caroline Westwater,[1] Joseph Dolan,[1] Michael G. Schmidt,[1] and James S. Norris[1]

Department of Microbiology and Immunology, Medical University of South Carolina,[1] and Department of Mathematics, College of Charleston,[2] Charleston, South Carolina

Received 2 November 2001/Accepted 17 February 2002

Prior observations of phage-host systems in vitro have led to the conclusion that susceptible host cell populations must reach a critical density before phage replication can occur. Such a replication threshold density would have broad implications for the therapeutic use of phage. In this report, we demonstrate experimentally that no such replication threshold exists and explain the previous data used to support the existence of the threshold in terms of a classical model of the kinetics of colloidal particle interactions in solution. This result leads us to conclude that the frequently used measure of multiplicity of infection (MOI), computed as the ratio of the number of phage to the number of cells, is generally inappropriate for situations in which cell concentrations are less than 10^7/ml. In its place, we propose an alternative measure, MOI_{actual}, that takes into account the cell concentration and adsorption time. Properties of this function are elucidated that explain the demonstrated usefulness of MOI at high cell densities, as well as some unexpected consequences at low concentrations. In addition, the concept of MOI_{actual} allows us to write simple formulas for computing practical quantities, such as the number of phage sufficient to infect 99.99% of host cells at arbitrary concentrations.

It has long been observed that when bacteriophage are mixed with susceptible host bacteria, the number of phage in the culture supernatant does not increase until after an eclipse period of, generally, 30 to 40 min at 37°C has passed. This period of time is explained as the time the phage requires to inject its genome into the host, express its genes, and assemble progeny phage and release them into the environment. Additionally, when host cell densities are very low, it has been observed that there is a longer delay before phage numbers increase over the numbers of input phage. This period has been explained as the time needed for the host cells to reach a "replication threshold" (16) or "proliferation threshold" (7, 8) density. This density has been reported to be approximately 10^4 cells per ml for the multiple phage-host combinations tested (16) and has been said to have broad implications for the propagation of phages in natural environments and in terms of their use as antimicrobial therapies (7, 8). The mechanism of this delay in phage replication has not been widely investigated or discussed.

One explanation for the apparent threshold density would be a requirement on the part of the phage for the host cell to be in a particular metabolic state and that this state is only reached when the cell density is 10^4 CFU/ml or more. Small molecules called autoinducers or quorum factors are known to be secreted into the environment by bacteria and, by their accumulation as the number of cells increases, to allow the bacteria to monitor their local population density (3). These soluble signaling molecules alter the expression of dozens of genes and thereby regulate the metabolic state when the sensing bacteria are exposed to them at a sufficient concentration. Quorum factors could therefore explain the dependence of phage replication on cell density if, for example, molecules that serve as phage receptors are expressed in response to quorum factors. However, the data to be presented below demonstrate no detectable quorum factor effect on the ability of phage to infect bacteria.

Here we propose an alternative explanation for the phenomenon that has been interpreted as a replication threshold density that can be extracted from the mathematical model of Schlesinger (12) and Stent (13). This model makes the assumption that phage rely entirely on chance encounters with their hosts, and so, in liquid culture at least, their ability to infect and reproduce can be entirely predicted by the equations that describe the movements and coagulation (irreversible binding) of inert colloidal particles under the influence of Brownian motion (12). In this model, one finds that

$$\ln \frac{P}{P_o} = -kCt, \text{ alternatively written as } \frac{P}{P_o} = e^{-kCt} \quad (1)$$

where P/P_o is the fraction of phage that remains unbound at time t (in minutes); C is the concentration of host cells per cubic centimeter, which remains constant over time t; and k is an adsorption rate constant (in cubic centimeters per minute) that can be determined experimentally for a given phage-host combination. Variations between phage-host systems in the number of phage binding sites per cell, the diffusion rate constant of the virus, and the efficiency with which collisions between cells and phage result in infection are accounted for by empirical determination of the adsoption rate constant, k, for each system (see references 12 and 13 for a description of the

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Medical University of South Carolina, BSB-Rm. 201, P.O. Box 250504, 173 Ashley Ave., Charleston, SC 29403. Phone: (843) 792-2074. Fax: (843) 792-2464. E-mail: KASMANL@musc.edu.

Trabajo de Investigación

Variabilidad de la síntesis de citoquinas por células dendríticas humanas estimuladas con los distintos serotipos de *Aggregatibacter actinomycetecomitans*

Variability in the cytokine synthesis by human dendritic cells in response to different *Aggregatibacter actinomycetemcomitans* serotypes

Díaz-Zúñiga J[1], Melgar-Rodríguez S[1] y Yáñez JP[1], Álvarez C[1], Rojas C[1], Benítez A[1], Ciuchi P[1], Monasterio G[1], Vernal R[1]

RESUMEN

Objetivo: Sobre la base de la antigenicidad del polisacárido O del LPS, en *A. actinomycetemcomitans* se describen distintos serotipos bacterianos y entre ellos se ha especulado una patogenicidad e inmunogenicidad diferente. El objetivo de este trabajo es analizar las diferencias en la síntesis de citoquinas producidas por células dendríticas cuando son estimuladas con los distintos serotipos de *A. actinomycetemcomitans*. **Metodología:** Células dendríticas diferenciadas a partir de monocitos circulantes periféricos humanos fueron estimuladas a MOIs=10^{-1}-10^{-2} con los serotipos a, b y c de *A. Actinomycetemcomitans*. Mediante PCR y ELISA se evaluaron los niveles de expresión y secreción de citoquinas. **Resultados:** En las células dendríticas, la producción de citoquinas fue diferente ante los distintos serotipos de *A. actinomycetemcomitans*, con mayores niveles de secreción de IL-1β, IL-6, IL-12, IL-23, IFN-γ y TNF-α cuando el microorganismo estimulante fue la cepa ATCC® 43718™ (serotipo b). **Conclusión:** El serotipo b de *A. actinomycetemcomitans* posee un mayor potencial inmuno-estimulador de células dendríticas comparado con los otros serotipos bacterianos y potencialmente contribuiría a inducir un patrón de respuesta inmune tipo Th1 y/o Th17 durante las periodontitis.
Rev. Clin. Periodoncia Implantol. Rehabil. Oral Vol. 6(2); 57-62, 2013.

Palabras clave: Células dendríticas, citoquinas, *Aggregatibacter actinomycetemcomitans*.

ABSTRACT

Objective: *A. actinomycetemcomitans* expresses a number of virulence factors that contribute to direct tissue damage and, based on the antigenicity of LPS O-polysaccharide, distinct serotypes have been described. The aim of this study was to determine the pattern of cytokine expression and secretion on dendritic cells stimulated with *A. actinomycetemcomitans* serotypes a, b and c. **Methods:** Using different multiplicity of infections of the serotypes a, b, and c of *A. actinomycetemcomitans*, the mRNA expression and secretion levels for cytokines IL-1β, IL-5, IL-6, IL-10, IL-12, IL-23, TNF-α, and IFN-γ were determined in stimulated dendritic cells using PCR and ELISA. **Results:** A dose-dependent increase in the secretion levels for IL-1β, IL-5, IL-6, IL-10, IL-12, IL-23, TNF-α, and IFN-γ was elicited on dendritic cells following stimulation with each of the serotypes of *A. actinomycetemcomitans*. In addition, *A. actinomycetemcomitans* serotype b (ATCC® 43718™) induced higher levels of IL-1β, IL-6, IL-12, IL-23, IFN-γ y TNF-α compared with the other strains. **Conclusion:** These data demonstrate that the distinct *A. actinomycetemcomitans* LPS O-polysaccharide serotypes induce both quantitative and qualitative differences in the dendritic cell response. Furthermore, the observed dendritic cell response to *A. actinomycetemcomitans* b serotype was characteristic of a Th1 and Th17 pattern of cytokine expression.
Rev. Clin. Periodoncia Implantol. Rehabil. Oral Vol. 6(2); 57-62, 2013.

Key words: Dendritic cells, citokines, *Agregagibacter actinomycetemcomitans*.

INTRODUCCIÓN

Las periodontitis son un conjunto de enfermedades de naturaleza inflamatoria y etiología infecciosa, cuya causa principal son las bacterias que residen en el biofilm patogénico subgingival[1,2]. Aunque las bacterias pueden causar dañar a los tejidos, es la respuesta inmune del hospedero la principal causa de la destrucción del aparato de soporte periodontal[3,4]. Estas interacciones (bacteria-hospedero) inducen la síntesis de citoquinas y quimioquinas, las que pueden determinar la destrucción del tejido conectivo y óseo característico de las periodontitis y, eventualmente, pueden llevar a la pérdida de los dientes[5,8].

En las periodontitis, las células dendríticas reconocen las bacterias patógenas del biofilm subgingival, las fagocitan y destruyen, y luego procesan y conjugan sus antígenos a moléculas del complejo mayor de histocompatibilidad (MHC) para presentarlos a los linfocitos TCD4$^+$ y así, iniciar la respuesta inmune adaptativa[9-12]. Las células dendríticas maduras secretan interleuquina (IL)-1β, IL-5, IL-6, IL-10, IL-12, IL-23, interferon (IFN)-γ, factor de necrosis tumoral (TNF)-α y TNF-β[13], las que durante la presentación antigénica cumplen un rol fundamental en la activación y diferenciación de los linfocitos TCD4$^+$ a alguno de los fenotipos efectores: T-*helper* (Th)-1, Th2, Th17 o T reguladores (Treg) inducidos[14]. Los linfocitos cumplen un rol central en la inmunidad durante la infección periodontal y sus distintos fenotipos efectores determinan las características clínicas de la enfermedad[12,15,16].

El biofilm patogénico subgingival, constituido principalmente por bacterias anaerobias Gram-negativo, es el factor etiológico responsable del inicio y progresión de las periodontitis[2,17-19]. Las bacterias periodonto-patógenas que componen este biofilm pueden causar daño directo a los tejidos periodontales; sin embargo, es la respuesta inmuno-inflamatoria inducida en el hospedero ante ellas la principal responsable de la destrucción de los tejidos de soporte de los dientes: ligamento periodontal, cemento radicular y hueso alveolar[5].

1. Laboratorio de Biología Periodontal. Departamento de Odontología Conservadora. Facultad de Odontología, Universidad de Chile. Chile.

***Correspondencia autor:** Jaime Díaz. zaidemiaj@gmail.com / Rolando Vernal. rvernal@uchile.cl.* *Trabajo recibido el 06/09/2012. Aprobado para su publicación el 06/03/2013.*

12

Virulence Mechanisms of Leukotoxin from *Aggregatibacter actinomycetemcomitans*

Anders Johansson[1] and Sotos Kalfas[2]
[1]*Umeå University,*
[2]*Aristotle University of Thessaloniki*
[1]*Sweden*
[2]*Greece*

1. Introduction

Aggregatibacter actinomycetemcomitans is a gram-negative bacterium that is present in the oral cavity of a large proportion of the human population (Zambon et al., 1983; Henderson et al., 2010) The bacterium is acquired through transmission from infected individuals and thought to initially colonize oral mucosa as a facultative intracellular pathogen (Fine et al., 2006). When the bacteria translocate to a site in the subgingival crevices, a persistent colonization may lead to periodontal destruction and development of periodontitis in susceptible individuals (Fig. 1) (Philstrom et al., 2005; Darveau 2010). The prevalence of this bacterium shows a great variation depending on geographic origin, age and life stile of the examined population (Kinane et al., 2008; Habek 2010). *A. actinomycetemcomitans* is a part of the normal flora in many healthy individuals but it is also a major agent in some aggressive forms of periodontitis (Fine et al., 2006). Periodontitis is a chronic infection characterized by the destruction of tooth-supporting structures (Darveau 2010). The number and composition of bacteria in the subgingival dental plaque, as well as life stile and genetic predisposition are factors that determine the outcome of the disease activity (Philstrom et al., 2005; Darveau 2010). The genetic diversity among different isolates of *A. actinomycetemcomitans* is great and its ability to express and release virulence factors varies (Henderson et al., 2010). The different adhesins and fimbriae expressed by this bacterium have been shown to be important factors that promote colonization at the various ecological niches of the human oral cavity (Fine et al., 2006).

A. actinomyctemcomitans expresses two exotoxins, a cytolethal distending toxin (Cdt) and a leukotoxin. Cdt´s are expressed by a number of gram-negative bacteria and causes death of the host cells by blocking their proliferation (Belibasakis et al., 2004). The leukotoxin selectively affects human cells of hematopoetic origin by binding to the lymphocyte function associated receptor 1 (LFA-1) and cause disruption of the membrane integrity (Lally et al., 1999). Leukotoxin belongs to the Repeat in Toxin family (RTX) and shares genomic organization and molecular structures with RTX proteins produced by a number of other gram-negative bacteria (Linhortavá et al., 2010). The expression of leukotoxin and Cdt varies among different *A. actinomycetemcomitans* isolates and high leukotoxin expression has been shown to correlate with disease while the role of Cdt still is more unclear (Henderson et al.,

www.intechopen.com

Letters in Applied Microbiology 2004, **39**, 215–219 doi:10.1111/j.1472-765X.2004.01565.x

Isolation of bacteriophages from the oral cavity

G. Hitch[1], J. Pratten[2] and P.W. Taylor[1]

[1]Microbiology Group, School of Pharmacy, University of London, London, and [2]Division of Infection and Immunity, Eastman Dental Institute, University College London, London, UK

2004/0270: received 10 March 2004, revised 28 April 2004 and accepted 20 May 2004

ABSTRACT

G. HITCH, J. PRATTEN AND P.W. TAYLOR. 2004.

Aims: To isolate bacteriophages lytic for oral pathogens from human saliva, dental plaque and mature biofilms constituted from saliva-derived bacteria.

Methods and Results: Saliva and dental plaque samples from healthy volunteers and from patients with gingivitis and periodontitis were examined for the presence of lytic bacteriophage using a panel of oral pathogens and bacteria isolated from the samples. Samples were also enriched for bacteriophage using static culture techniques and mature biofilms. A limited number of samples contained bacteriophage particles that were visualized using electron microscopy. Cultures yielded phage infecting non-oral bacteria (*Proteus mirabilis*) but no bacteriophage specific for recognized oral pathogens were found. Some micro-organisms from the oral microflora elaborated antibacterial substances that inhibited growth of other residents of the oral cavity.

Conclusions: Unlike other ecosystems, the composition of the oral cavity does not appear to be heavily influenced by interactions between bacteriophages and their hosts.

Significance and Impact of the Study: Bacteriophage for control of oral infections may need to be obtained from other sources. Antibacterial substances derived from some members of the oral microflora warrant investigation as potential antibiotics.

Keywords: bacteriophage, biofilms, gingivitis, oral cavity, periodontitis, streptococci.

INTRODUCTION

Extracellular polysaccharide formation plays a key role in the pathogenesis of infections in the oral cavity. *Streptococcus mutans*, the causative agent of dental caries, typically produces a highly adhesive dextran that enables it to colonize tooth surfaces (Schilling and Bowen 1992). Bacteria implicated in the accumulation of dental plaque, the precursor of gingivitis and destructive periodontitis, are embedded in a matrix of bacterially derived exopolysaccharide that largely determines the structural integrity and diffusion properties of the plaque biofilm (Palmer *et al.* 2003). We proposed that capsule-depolymerizing enzymes, that enable bacteriophages to penetrate the protective capsule of their bacterial host, could be used to modify the course of infections caused by encapsulated bacteria by removing the virulence determinant from the cell surface (Taylor *et al.* 2002). We recently established the validity of this concept in a neonatal rat model of *Escherichia coli* K1 bacteraemia (Mushtaq *et al.* 2004).

There are a limited number of reports in the literature of the isolation of bacteriophages from the oral cavity. A bacteriophage infecting *Lactobacillus casei* has been obtained from oral material (Meyer *et al.* 1958) and a range of bacteriophages specific for species of *Veillonella* spp. were isolated by Hiroki *et al.* (1976). A small percentage of dental plaque samples yielded morphologically distinct bacteriophages lytic for *Actinomyces* spp. (Tylenda *et al.* 1985) and viruses specific for *Actinobacillus actinomycetemcomitans* have been described (Olsen *et al.* 1993). Delisle and Rotkowski (1993) have described bacteriophage lytic for *Strep. mutans*.

Correspondence to: Peter Taylor, Department of Pharmaceutics, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK (e-mail: peter.taylor@ulsop.ac.uk).

INFECTION AND IMMUNITY, July 1985, p. 1–6
0019-9567/85/070001-06$02.00/0

Vol. 49, No. 1

Isolation of *Actinomyces* Bacteriophage from Human Dental Plaque

CAROLYN A. TYLENDA,[1]* CHRISTI CALVERT,[1] PAUL E. KOLENBRANDER,[1] AND ANTHONY TYLENDA[2]

Microbiology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, Bethesda, Maryland 20205,[1] and Department of Fixed Prosthodontics, School of Dentistry, Georgetown University, Washington, D.C. 20007[2]

Received 11 January 1985/Accepted 19 March 1985

Human dental plaque samples were screened for the presence of bacteriophage for *Actinomyces viscosus* and *Streptococcus sanguis*. None of the 336 samples yielded phage for *S. sanguis*, but 10 contained virulent actinomyces phage. A high host cell specificity was observed in that one phage isolate infected only *A. viscosus* T14V, eight phage isolates infected only *A. viscosus* MG-1, and one infected both strains. None was capable of productively infecting various other actinomyces strains that represented the six actinomyces coaggregation groups. Because phage-containing samples occurred randomly in this survey, no correlation between the individual collecting the samples, dental clinic, or type of patient and the presence of phage in the sample was noted. Examination of one of the samples that yielded phage for the presence of a natural host strain for that particular phage resulted in the isolation of two strains which were identified as *A. viscosus* serotype II and *Actinomyces naeslundii* serotype I. This is the first report of an *A. naeslundii* host strain and actinomyces bacteriophage of human dental plaque origin. The finding of both phage and host strains in the same dental plaque sample along with the observation of high host cell specificity by these phage provide indicators that support an active role for actinomyces bacteriophage in oral microbial ecology. The use of these freshly isolated phage as probes to study actinomyces coaggregation properties is discussed.

Bacteriophage for actinomyces were found previously only in sewage (5). Although many actinomyces have been tested as potential hosts, the four available phage productively infect a single strain, *Actinomyces viscosus* MG-1, a human oral isolate (19). In that same study, we found that these phage bind irreversibly to several other actinomyces. In fact, their ability to bind to certain reagent actinomyces strains was used to probe the surface for structures that mediate coaggregation. Bacteriophage-resistant mutants of *A. viscosus* MG-1 were isolated and shown to be altered in cell-to-cell recognition patterns with streptococcal coaggregation partners (19). The simultaneous loss of both the ability to bind phage and the ability to mediate certain coaggregations suggested that a common surface structure participated in both functions.

The potential utility of this approach prompted a search for new and different actinomyces phage. Human dental plaque was chosen as the source, and *A. viscosus* MG-1 was used as the indicator for the presence of phage. We report here the isolation of 10 phage and discuss their use in probing the actinomyces cell surface. Our studies with phage probes were conducted in an effort to uncover critical surface adhesins that are required for cell-cell or cell-solid surface interactions and that are involved integrally in the maturation of periodontal plaque.

MATERIALS AND METHODS

Bacterial strains and bacteriophage. *A. viscosus* strains MG-1 (5), T14V (4), PK455 (10), PK455-2 (obtained from John Cisar of the National Institute of Dental Research), PK1603, PK1643, PK1610, PK1623, PK1632, PK1648, PK1657, and PK1662 (19), *Actinomyces naeslundii* strains ATCC 12104, I, PK947, PK606, PK954, and PK990, *Actinomyces* sp. strain WVa-963 VPI D33C-25 (PK1259), and *Streptococcus sanguis* DL1 (Challis) (4, 13–15) were grown in TYNP medium as described earlier (19). Phage AV-1, AV-2, AV-3, and 1281 are of nonoral (sewage) origin and have been described previously (5, 19, 20). Bacteriophage AV-1 was propagated in *A. viscosus* MG-1. Phage titers were determined by the soft-agar overlay method (1).

Reconstruction experiments. To test the efficiency of our selection procedure for isolating new actinomyces phage, we examined a reconstruction of the experimental design by using phage AV-1. Of particular importance was stability of phage in transport medium (Trypticase soy broth [BBL Microbiology Systems, Cockeysville, Md.] containing 0.05% Tween-20 [Sigma Chemical Co., St. Louis, Mo.] and 0.2% gelatin [Nutritional Biochemicals Corp., Cleveland, Ohio]), efficiency of detection of phage in diluted samples of phage stocks in transport medium, effect of particulate and biological materials in human dental plaque on phage viability, and ability to detect phage after membrane filtration of phage-dental plaque suspensions. Phage stability was tested by incubating diluted (1 to 50 phage per 5 μl of transport medium) phage suspension at room temperature for 24 h and at 4°C for 7 days, conditions that approximated those used in the dental plaque collection regimen (see below). Phage viability was determined by spotting a portion of phage suspension onto a top-agar overlay plate containing *A. viscosus* MG-1, incubating it at 33°C for 24 h, and examining the spotted area for lysis of the indicator bacterium. Testing the effect of dental plaque material on phage viability was done by incubating pooled dental plaque from all surfaces of teeth in all quadrants of two individuals along with 200 AV-1 phage in 100 μl of transport medium. After 1 h at room temperature, the mixture was centrifuged at 6,500 × *g* for 10 min, and the phage titer in the supernatant was determined. The effect of membrane filtration was monitored with a 0.45-μm-pore-size Durapore filter (Millipore Corp., Bedford, Mass.).

Collection and processing of human dental plaque samples. A 250-μl volume of transport medium in 500-μl polypropylene microfuge tubes (Beckman Instruments, Inc., Palo Alto, Calif.) was sterilized by autoclaving. The tubes

* Corresponding author.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2010, p. 7243–7250 Vol. 76, No. 21
0099-2240/10/$12.00 doi:10.1128/AEM.01135-10

Isolation of a Novel Bacteriophage Specific for the Periodontal Pathogen *Fusobacterium nucleatum*▽

Pamela Machuca,[1] Leslie Daille,[1] Enrique Vinés,[1] Liliana Berrocal,[1] and Mauricio Bittner[1,2]*

Laboratorio de Microbiologia y Biotecnologia Oral, Departamento de Ciencias Biologicas, Facultad de Ciencias Biologicas, Universidad Andres Bello, Santiago, Chile,[1] and Facultad de Odontologia, Universidad Andres Bello, Santiago, Chile[2]

Received 11 May 2010/Accepted 4 September 2010

***Fusobacterium nucleatum* is a periodontal pathogen that has been directly associated with the development and progression of periodontal disease, a widespread pathology that affects the support tissues of the tooth. We isolated a new bacteriophage (FnpΦ02) that specifically infects this bacterium. Transmission electron microscopy showed that the virion is composed of an icosahedral head and a segmented tail. The size of the phage genome was estimated to be approximately 59 kbp of double-stranded DNA. The morphological features and the genetic characteristics suggest that FnpΦ02 is part of the *Siphoviridae* family. Using one-step growth and adsorption experiments, the latent period, burst size, and adsorption rate were estimated to be 15 h, 100 infectious units per cell, and 7.5×10^{-10} ml min^{-1}, respectively. A small fragment of phage DNA was cloned and sequenced, showing 93% nucleotide identity with the phage PA6 of *Propionibacterium acnes* and amino acid identity with fragments of two proteins (Gp3 and Gp4) of this phage. To our knowledge, FnpΦ02 is the first phage described to infect *Fusobacterium nucleatum* and provides the base for future exploration of phages in the control of periodontal disease.**

The term "periodontal disease" refers to a wide set of pathological alterations of the periodontal tissue. The most common clinical manifestations are known as gingivitis and periodontitis, and both are widely distributed around the world (18). Periodontitis is a multifactorial inflammatory-based infection of the supporting tissues of the tooth. It is essentially characterized by the progressive destruction of the periodontal ligament and the alveolar bone, leading to the loss of the affected tooth (2). Periodontitis is caused by bacteria or bacterial groups embedded in a biofilm or dental plaque that protects them against antimicrobial agents (18). The bacterial species involved in periodontal disease are predominantly Gram-negative anaerobes, and although they are usually isolated from affected patients, they are also isolated from healthy individuals, but in a lesser proportion and frequency (26).

Fusobacterium nucleatum is an anaerobic, Gram-negative, long bacillus and a member of the microflora in the oral cavity. *F. nucleatum* is considered a periodontal pathogen because it is frequently isolated from lesions, produces a high number of tissue irritants, and has the ability to form coaggregates with other periodontal pathogens, acting as a bridge between early and late colonizers in the surface of the enamel (4, 11). Three different subspecies of *F. nucleatum* have been related to the pathology of periodontal disease, *F. nucleatum* subsp. *nucleatum*, subsp. *polymorphum*, and subsp. *vincentii*, all of which have been associated with lesions of periodontitis but also have been isolated in high numbers from successfully treated patients (9).

Bacteriophages are viruses that can infect and kill only bacteria and have been used for many years as powerful tools for the study of bacterial genetics and, given their specificity, used in the identification and characterization of microorganisms (phage typing). Nevertheless, phages were originally described as therapeutic elements to treat human and animal infections (34). This application, known as phage therapy, has regained interest in the past years, particularly in an era when antibiotic resistance and biofilm-based infections are permanent issues (25). Bacteriophages are denominated "temperate" when their genetic material is integrated within the bacterial genome with no immediate lysis of the bacterium until, under certain conditions, the expression of the viral genome is induced and the production of new virus particles lyses the host cell; they are called "lytic" or "virulent" when, immediately after the infection, they redirect the bacterial metabolism to the production of new phages, which are released during the bacterial lysis (22, 36). There are many examples of the use of bacteriophages at a clinical (14, 32) and commercial level (20). Specifically in the dentistry area, several bacteriophages that infect diverse oral bacteria have been isolated from saliva and dental plaque (12, 13, 23, 37).

Although *F. nucleatum* is an important periodontal pathogen, reports of bacteriophages for this microorganism do not exist. In this work, we isolated and characterized a new bacteriophage for *F. nucleatum* from a saliva sample, designated FnpΦ02, and to our knowledge this is the first bacteriophage for this bacterium.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. The *F. nucleatum* subsp. *polymorphum* clinical isolate strain used as the host for the isolation, dilution, and propagation of the FnpΦ02 phage was called Fnp. *F. nucleatum* strains were cultured anaerobically in brain heart infusion (BHI) broth (Merck) or BHI agar at 37°C for 3 days. All bacteria from the oral flora were incubated under the same conditions except black-pigmented bacteria, which were cultivated in BHI agar with sheep blood (5%) supple-

* Corresponding author. Mailing address: Laboratorio de Microbiologia y Biotecnologia Oral, Departamento de Ciencias Biologicas, Universidad Andres Bello, Echaurren 237, Santiago, Chile. Phone: (562) 770-3027. Fax: (562) 464-1630. E-mail: mbittner@unab.cl.

▽ Published ahead of print on 17 September 2010.

Journal of Oral Microbiology

CO-action PUBLISHING

REVIEW ARTICLE

Phage therapy against *Enterococcus faecalis* in dental root canals

Leron Khalifa[1], Mor Shlezinger[2], Shaul Beyth[3], Yael Houri-Haddad[2], Shunit Coppenhagen-Glazer[1], Nurit Beyth[2†] and Ronen Hazan[1*†]

[1]Institute of Dental Science, The Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel; [2]Department of Prosthodontics, The Hebrew University Hadassah School of Dental Medicine, Jerusalem, Israel; [3]Orthopedic Surgery Complex, Hadassah University Hospital, Jerusalem, Israel

Antibiotic resistance is an ever-growing problem faced by all major sectors of health care, including dentistry. Recurrent infections related to multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae,* and vancomycin-resistant enterococci (VRE) in hospitals are untreatable and question the effectiveness of notable drugs. Two major reasons for these recurrent infections are acquired antibiotic resistance genes and biofilm formation. None of the traditionally known effective techniques have been able to efficiently resolve these issues. Hence, development of a highly effective antibacterial practice has become inevitable. One example of a hard-to-eradicate pathogen in dentistry is *Enterococcus faecalis*, which is one of the most common threats observed in recurrent root canal treatment failures, of which the most problematic to treat are its biofilm-forming VRE strains. An effective response against such infections could be the use of bacteriophages (phages). Phage therapy was found to be highly effective against biofilm and multidrug-resistant bacteria and has other advantages like ease of isolation and possibilities for genetic manipulations. The potential of phage therapy in dentistry, in particular against *E. faecalis* biofilms in root canals, is almost unexplored. Here we review the efforts to develop phage therapy against biofilms. We also focus on the phages isolated against *E. faecalis* and discuss the possibility of using phages against *E. faecalis* biofilm in root canals.

Keywords: *phage therapy*; *dental biofilm*; *E. faecalis*

*Correspondence to: Ronen Hazan, Institute of Dental Sciences and School of Dental Medicine, Hebrew University, Hadassah Campus Jerusalem P.O.B 12272, Israel 91120, Email: ronenh@ekmd.huji.ac.il

Received: 5 May 2016; Revised: 13 July 2016; Accepted: 27 July 2016; Published: 16 September 2016

Antibiotics, 'the magic bullets', have proved to be one of the most revolutionary discoveries of the twentieth century (1, 2). However, their overuse and misuse in various cases, including viral and fungal infections, and patient failure to follow the prescribed course have led to a rise in antibiotic-resistant strains, the 'post antibiotic era' (3). Consequently, many resistant pathogens like MRSA (methicillin-resistant *Staphylococcus aureus*), CRE (carbapenem-resistant *Enterobacteriaceae*), VRE (vancomycin-resistant enterococci) (4, 5), multidrug-resistance *Pseudomonas* and *Acinetobacter* have developed into major threats. For instance, VRE exhibit resistance to vancomycin, which is considered 'the last resort' drug for Gram-positive bacteria, making their elimination almost impossible (6, 7). The rate of acquired antibiotic resistance is also alarming. For example, *Pseudomonas aeruginosa* was shown to rapidly develop resistance against five relevant antibiotics upon exposure to stepwise increased concentrations (8). Apart from being life threating, these antibiotic-resistant strains also lead to elevated health care costs (9). Moreover, failure in surgeries and other medical procedures related to untreatable infections is expected to increase. Having said that, should we be alarmed that we are about to face an era similar to the one prior to the discovery of antibiotics, in which mortality will be caused by common infections?

Today, it is accepted that yet another reason for the failure of antibiotics is the formation of bacterial biofilms (10). Biofilms are defined as dense aggregates of surface-adherent microorganisms that are embedded in a self-produced polymer matrix consisting of polysaccharide, protein, and extracellular DNA (11, 12). Biofilms are

†These authors have equally contributed to this work.

Citation: Journal of Oral Microbiology 2016, **8**: 32157 - http://dx.doi.org/10.3402/jom.v8.32157
(page number not for citation purpose)

Antonio Bonaccorso, Teresa Roberta Tripi

Endo-perio lesion: Diagnosis, prognosis and decision-making

Antonio Bonaccorso, MD
Private Practice, Mass Dental Center, Messina, Italy

Teresa Roberta Tripi, MD, PhD
Professor, Department of Conservative Dentistry, University of Catania, Catania, Italy

Correspondence to:
Dr Antonio Bonaccorso,
Via Luis Zuegg n. 42,
98012, Messina,
Italy
Tel: 00-39-9538892
Fax: 00-39-9538892
Email: endomaterials@

Key words *bacteria, endo-perio lesions, root canal therapy*

Presence of a tooth with simultaneous lesions of endodontic and periodontal origin is a challenge to the clinician as far as diagnosis, prognosis and decision-making are concerned. Such infections are typically polimicrobial, and therefore interactions, both antagonistic and synergistic, between different strains and species would be expected. Treatment and prognosis of endodontic-periodontal lesion depend on the cause and the correct diagnosis of each specifying condition. In particular it is critically important to determine whether the lesion is primarily periodontal or primarily endodontic in origin, because the accuracy of diagnosis will determine whether or not the appropriate treatment plan is instigated.

Introduction

When a pulpal lesion presents itself to the periodontum via the apical foramina, lateral canals or in furcation areas, progresses coronally and eventually joins with an infected marginal periodontal pocket, which progresses apically, it is defined as an 'endo-perio lesion' (EP) or 'true combined endo-perio disease'[1] (Fig 1). Harrington and Steiner defined an EP as a non-vital tooth that shows destruction of periodontal attachment reaching the whole way to the root apex or a lateral canal, for which both root canal treatment and periodontal therapy are required[2]. Infections of periodontal or endodontic origin may result in: increased periodontal probing depths; localised gingival inflammation or swelling; bleeding on probing; suppuration; fistula formation; tenderness to percussion; increased tooth mobility; angular bone loss and pain[3].

EP are difficult to classify because they may remain symptom-free for long periods and they are rarely diagnosed until the disease starts manifesting itself in the form of acute symptoms of inflammation and/or increased pain. Sometimes the lesions are detected accidentally during a general check-up with radiographs[4]. Once symptoms occur, they tend to be so severe, and the periodontal aspect can seem so dominant, that dentists tend to settle for strictly symptomatic periodontal therapy whilst overlooking the endodontic aspect.

The term EP is often abused because it lacks the characteristic manifestations of strictly endodontic or typical periodontal lesions[5]. It is difficult to distinguish by hindsight which parts of the lesion are endodontic (primary endodontic disease) and which parts are periodontal in origin (primary periodontal disease). Primary endodontic lesions are inflammatory processes located in periodontal tissues and caused by intracanal microorganisms (Fig 2)[6]. The term 'root canal infection' implicates infection (bacteria in planctonic and biofilm organisation, virus and fung) of the main root canal, lateral canals, apical delta and radicular dentine infection (Fig 3). Primary periodontal lesions are inflammatory processes located in periodontal tissues caused by bacteria that start on the external surface of the root[7] (Fig 4).

Journal of Biotechnology 250 (2017) 29–44

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

The use of bacteriophages to biocontrol oral biofilms

Szymon P. Szafrański*, Andreas Winkel, Meike Stiesch*

Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Stadtfelddamm 34, D-30625 Hannover, GermanyDepartment of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School (MHH), Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 7 October 2016
Received in revised form 9 January 2017
Accepted 10 January 2017
Available online 17 January 2017

Keywords:
Bacteriophage
Biofilm
Lysin
Oral cavity
Periodontal disease
Phage therapy

ABSTRACT

Infections induced by oral biofilms include caries, as well as periodontal, and peri-implant disease, and may influence quality of life, systemic health, and expenditure. As bacterial biofilms are highly resistant and resilient to conventional antibacterial therapy, it has been difficult to combat these infections. An innovative alternative to the biocontrol of oral biofilms could be to use bacteriophages or phages, the viruses of bacteria, which are specific, non-toxic, self-proliferating, and can penetrate into biofilms. Phages for *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Lactobacillus* spp., *Neisseria* spp., *Streptococcus* spp., and *Veillonella* spp. have been isolated and characterised. Recombinant phage enzymes (lysins) have been shown to lyse *A. naeslundii* and *Streptococcus* spp. However, only a tiny fraction of available phages and their lysins have been explored so far. The unique properties of phages and their lysins make them promising but challenging antimicrobials. The genetics and biology of phages have to be further explored in order to determine the most effective way of applying them. Studying the effect of phages and lysins on multispecies biofilms should pave the way for microbiota engineering and microbiota-based therapy.

1. Introduction

In most habitats, including the human body, microorganisms reside in biofilms, *i.e.* surface-attached aggregates embedded in a matrix of extracellular polymeric substance. The biofilm matrix consists of polysaccharides, structural proteins, enzymes, DNA, lipids, and water. The biofilm protects its inhabitants from environmental challenges, *e.g.* phagocytosis, and allows long-term colonisation, and spatial organisation (Flemming et al., 2016). Physical and chemical gradients provide diverse niches for microorganisms. Biofilms are shelters for a dynamic community of interacting microbes. Members of mixed biofilms profit from synergistic interactions such as co-aggregation, and allow colonisation, sharing of extracellular enzymes, cross-feeding, and cross-protection. Competition between community members controls ecological succession and triggers segregation. The close distance between cells in the biofilm facilitates microbial communication (quorum sensing), *i.e.* synchronised, population-wide response to a changing environment (Sztajer et al., 2014). Interspecies interactions shape the overall activity of the biofilm and can positively or negatively impact human health (Peters et al., 2012).

There is usually ***homeostasis*** between the host and associated biofilms, *e.g.* in the oral cavity, gastrointestinal tract, or vagina. Commensal flora are beneficial, since they hinder colonisation of pathogens (at all body sites), provide nutrients to the host, and positively influence the immune system and developmental processes (mainly in the gut) (He et al., 2014; Ma et al., 2012; Sekirov et al., 2010). Certain environmental or genetic factors can however induce ***dysbiosis*** – microbial imbalance that harms the host body. Dysbiosis can develop gradually or rapidly and often leads to chronic destructive inflammation (Lamont and Hajishengallis, 2015). Opportunistic pathogens dysregulate the host immune defence and elevate the virulence of the whole community. As a result, the host tissue is damaged by autoimmunity and synergistic activities of microorganisms. Dysbiotic biofilms benefit from impaired host defence and nutrients released by damaged tissue. Health-associated commensals are outcompeted. The process escalates, since inflammation/tissue damage and dysbiosis reinforce each other.

Biofilm infections are persistent and therefore hard to prevent and cure (Donlan and Costerton, 2002). Biofilm matrix reduces the penetration of antimicrobials. Sessile cells grow more slowly and consequently are less susceptible to antibiotics that target metabolic processes. Localised gradients in biofilms provide

* Corresponding authors.
E-mail addresses: Szafranski.Szymon@mh-hannover.de (S.P. Szafrański), Stiesch.Meike@mh-hannover.de (M. Stiesch).

http://dx.doi.org/10.1016/j.jbiotec.2017.01.002

frontiers in Cellular and Infection Microbiology

MINI REVIEW
published: 23 October 2018
doi: 10.3389/fcimb.2018.00376

Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles

Lucy L. Furfaro[1]*, *Matthew S. Payne*[1] *and Barbara J. Chang*[2]

[1] *Division of Obstetrics and Gynecology, School of Medicine, The University of Western Australia, Crawley, WA, Australia,*
[2] *The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Sciences, The University of Western Australia, Crawley, WA, Australia*

Increasing reports of antimicrobial resistance and limited new antibiotic discoveries and development have fuelled innovation in other research fields and led to a revitalization of bacteriophage (phage) studies in the Western world. Phage therapy mainly utilizes obligately lytic phages to kill their respective bacterial hosts, while leaving human cells intact and reducing the broader impact on commensal bacteria that often results from antibiotic use. Phage therapy is rapidly evolving and has resulted in cases of life-saving therapeutic use and multiple clinical trials. However, one of the biggest challenges this antibiotic alternative faces relates to regulations and policy surrounding clinical use and implementation beyond compassionate cases. This review discusses the multi-drug resistant Gram-negative pathogens of highest critical priority and summarizes the current state-of-the-art in phage therapy targeting these organisms. It also examines phage therapy in humans in general and the approaches different countries have taken to introduce it into clinical practice and policy. We aim to highlight the rapidly advancing field of phage therapy and the challenges that lie ahead as the world shifts away from complete reliance on antibiotics.

Keywords: bacteriophage, phage therapy, regulations, clinical trials, antimicrobial resistance, alternative treatments

OPEN ACCESS

Edited by:
Maria Tomas,
Complexo Hospitalario Universitario A Coruña, Spain

Reviewed by:
Katrine L. Whiteson,
University of California, Irvine, United States
Jose Ramos-Vivas,
Instituto de Investigación Marques de Valdecilla (IDIVAL), Spain

***Correspondence:**
Lucy L. Furfaro
lucy.furfaro@uwa.edu.au

Received: *11 August 2018*
Accepted: *05 October 2018*
Published: *23 October 2018*

Citation:
Furfaro LL, Payne MS and Chang BJ (2018) Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles. Front. Cell. Infect. Microbiol. 8:376. doi: 10.3389/fcimb.2018.00376

THE CHALLENGE OF MULTI-DRUG RESISTANT BACTERIA

In 2017 the World Health Organization published a list of global priority pathogens comprising 12 species of bacteria categorized into critical, high and medium priority based on their level of resistance and available therapeutics (Tacconelli et al., 2018). The current rate of resistance development far exceeds the level of antibiotic discovery and development and represents a global public health challenge. Estimates have suggested that upwards of 10 million people could die each year due to antimicrobial resistance by 2050 (O'neill, 2014). While this is a contentious figure (De Kraker et al., 2016), it nonetheless highlights the serious problem we face regarding therapeutic options for multi-drug resistant (MDR) bacterial infections (Bassetti et al., 2017). The natural predators of bacteria are the bacterial viruses known as bacteriophages or phages. Found ubiquitously, these organisms are estimated to be present at numbers equivalent to a trillion per grain of sand on Earth (Keen, 2015). Evolving in parallel with bacteria, phages are potential antibacterial therapeutic agents against such MDR pathogens (Burrowes et al., 2011). Here we focus on three critical priority pathogens, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and members of the *Enterobacteriaceae* (Tacconelli et al., 2018) and the current advances in phage therapy research to target these organisms, as well as exploring more general issues of clinical trials and regulatory complexities of phage therapy.

VIEWS AND COMMENTARIES

Bacteriophage 1:2, 111-114; March/April 2011; © 2011 Landes Bioscience

Pros and cons of phage therapy

Catherine Loc-Carrillo[1,2] and Stephen T. Abedon[3,*]

[1]Department of Orthopaedics; The University of Utah; [2]Department of Veterans Affairs; Health Care System; Salt Lake City, UT USA;
[3]Department of Microbiology; The Ohio State University; Mansfield, OH USA

Many publications list advantages and disadvantages associated with phage therapy, which is the use of bacterial viruses to combat populations of nuisance or pathogenic bacteria. The goal of this commentary is to discuss many of those issues in a single location. In terms of "Pros," for example, phages can be bactericidal, can increase in number over the course of treatment, tend to only minimally disrupt normal flora, are equally effective against antibiotic-sensitive and antibiotic-resistant bacteria, often are easily discovered, seem to be capable of disrupting bacterial biofilms, and can have low inherent toxicities. In addition to these assets, we consider aspects of phage therapy that can contribute to its safety, economics, or convenience, but in ways that are perhaps less essential to the phage potential to combat bacteria. For example, autonomous phage transfer between animals during veterinary application could provide convenience or economic advantages by decreasing the need for repeated phage application, but is not necessarily crucial to therapeutic success. We also consider possible disadvantages to phage use as antibacterial agents. These "Cons," however, tend to be relatively minor.

Key words: alternative medicine, antibiotics, antimicrobial drugs, biocontrol, phage therapy

Submitted: 11/08/10

Revised: 12/17/10

Accepted: 12/20/10

DOI: 10.4161/bact.1.2.14590

*Correspondence to: Stephen T. Abedon; Email: abedon.1@osu.edu

Introduction

Introduced in the early 1900s,[1] phage therapy is the application of bacteria-specific viruses (phages) to combat uncontrolled and undesired bacteria such as those associated with infectious disease.[2] In reviews of phage therapy[3] authors commonly list advantages of employing phages as antibacterials (for example, see ref. 4). These lists can be used as talking points of why, in this age of epidemic antibiotic resistance, phage therapy should not be overlooked. As lists vary from author to author, it is useful to condense them into a coherent whole. Here we highlight the strengths and weaknesses of individual assertions. We also consider possible limitations to phage use as antibacterials. A more comprehensive review of phage therapy is presented in this same issue while this commentary focuses expressly on the pros and cons of phage use as antibacterials.

Major Advantages of Phage Therapy

Advantages of phage therapy over the use of chemical antibiotics can be framed in terms of phage properties. In this section we consider those properties that, in our opinion, can contribute substantially to phage therapy utility.

Bactericidal agents. Bacteria that have been successfully infected by obligately lytic phages are unable to regain their viability. By contrast, certain antibiotics are bacteriostatic, such as tetracycline, and as a consequence may more readily permit bacterial evolution towards resistance.[5,6]

Auto "dosing". Phages during the bacterial-killing process are capable of increasing in number specifically where hosts are located,[5] though with some limitations such as dependence on relatively high bacterial densities.[3,7,8] We call this auto "dosing" because the phages themselves contribute to establishing the phage dose.[3]

Low inherent toxicity. Since phages consist mostly of nucleic acids and

Annals of Burns and Fire Disasters - vol. XXVIII - n. 1 - March 2015

BACTÉRIOPHAGES ET PHAGOTHÉRAPIE : UTILISATION DE VIRUS NATURELS POUR TRAITER LES INFECTIONS BACTÉRIENNES

USING NATURAL VIRUSES TO TREAT BACTERIAL INFECTIONS

Ravat F.,[1✉] Jault P.,[2] Gabard J.[3]

[1] Centre des Brulés, Centre Hospitalier Saint Joseph et Saint Luc, Lyon, France
[2] Centre des Brulés, Hôpital d'Instruction des Armées Percy, Clamart, France
[3] Pherecydes Pharma, Romainville, France

RÉSUMÉ. L'utilisation des bactériophages, prédateurs naturels des bactéries, est une technique pionnière efficace de lutte contre les infections bactériennes. Tombée dans l'oubli depuis un demi-siècle du coté occidental de l'ex-rideau de fer, elle fait toujours partie de l'arsenal thérapeutique des pays de l'ex-Europe de l'Est, au point de constituer une arme de choix dans la politique de santé publique de ces pays. L'émergence de bactéries multirésistantes et le risque de revenir à l'ère pré-antibiotique ont fait ressortir la phagothérapie de l'oubli injuste auquel elle avait été confinée. La biologie et la place du bactériophage dans la nature sont décrites ici. Les tenants et les aboutissants de la phagothérapie et les conditions de son retour sur le devant de la scène sont explicitées.

Mots-clés : bacteriophage, phagotherapie, infection, brûlure, résistances bactériennes

SUMMARY. *The use of bacteriophages, natural predators of bacteria, is an effective technique in the fight against bacterial infections. Long since forgotten in the western world, it is still practised in parts of Eastern Europe as the primary weapon of choice against bacterial infections in public health policy. The global emergence of multidrug-resistant bacteria, or « superbugs », and the associated risk of returning to the pre-antibiotic era have brought the benefits of phagotherapy back to the fore. The purpose of this paper is to highlight the biology and place of bacteriophages in their natural context and explain why and how phagotherapy can be an effective solution to treat bacterial infections.*

Keywords*: bacteriophage, phagotherapy, infection, bacterial resistance, burn, thermal injury*

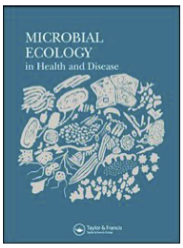
Introduction

L'émergence de bactéries résistantes aux antibiotiques et le manque de moyens thérapeutiques ont ramené sur le devant de la scène une thérapeutique ancienne que l'occident avait oubliée : la phagothérapie. Cette méthode consiste à mettre à profit les armes que l'évolution a créé pour lutter contre les bactéries. Ces armes sont des virus : les bactériophages.

Historique

C'est en observant des « plages claires » au sein d'une culture de bactéries sur gélose en 1917 que le franco-canadien Félix D'Hérelle a formulé l'hypothèse d'une part que ces plages claires correspondaient à une lyse bactérienne et d'autre part que cette lyse pouvait être provoquée par un agent inconnu qu'il a appelé « microbe filtrant ».[1] Pour être exact, une constatation similaire avait été effectuée deux ans plus tôt par l'anglais Frederick Twort mais celui a semble-t-il interprété le phénomène de lyse comme étant d'origine chimique, employant d'ailleurs, à ce sujet, le terme de diastase.[2] Poursuivant son hypothèse d'un « microbe tueur de microbes », qu'il appellera bactériophage, D'Hérelle isole en quelques mois des phages actifs contre plusieurs espèces bactériennes.[3] Dès 1918, il

✉ Auteur correspondant : Francois Ravat, Centre des Brulés, Centre Hospitalier Saint Joseph et Saint Luc, 20 quai Claude Bernard 69007 Lyon. Email : fravat@lucbrul.fr
Manuscrit : soumis le 15/12/2014, accepté le 15/12/2014.



Taylor & Francis

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: https://www.tandfonline.com/loi/zmeh20

Relationships among *Actinomyces naeslundii (A. viscosus)* Bacteriophages Isolated from Sewage and the Oral Cavity

Allan L. Delisle & Jacob A. Donkersloot

To cite this article: Allan L. Delisle & Jacob A. Donkersloot (1995) Relationships among *Actinomyces naeslundii (A. viscosus)* Bacteriophages Isolated from Sewage and the Oral Cavity, Microbial Ecology in Health and Disease, 8:3, 121-127, DOI: 10.3109/08910609509140089

To link to this article: https://doi.org/10.3109/08910609509140089

PLOS | ONE

RESEARCH ARTICLE

Isolation of a Novel Phage with Activity against *Streptococcus mutans* Biofilms

Marion Dalmasso[1,2], Eric de Haas[1,2], Horst Neve[3], Ronan Strain[1,2], Fabien J. Cousin[1,2], Stephen R. Stockdale[2,4], R. Paul Ross[1,2], Colin Hill[1,2]*

1 School of Microbiology, University College Cork, Cork, Ireland, **2** APC Microbiome Institute, University College Cork, Cork, Ireland, **3** Department of Microbiology and Biotechnology, Max Rubner-Institut, Kiel, Germany, **4** Teagasc Biotechnology Centre, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland

* c.hill@ucc.ie

Abstract

Streptococcus mutans is one of the principal agents of caries formation mainly, because of its ability to form biofilms at the tooth surface. Bacteriophages (phages) are promising antimicrobial agents that could be used to prevent or treat caries formation by *S. mutans*. The aim of this study was to isolate new *S. mutans* phages and to characterize their antimicrobial properties. A new phage, ϕAPCM01, was isolated from a human saliva sample. Its genome was closely related to the only two other available *S. mutans* phage genomes, M102 and M102AD. ϕAPCM01 inhibited the growth of *S. mutans* strain DPC6143 within hours in broth and in artificial saliva at multiplicity of infections as low as $2.5x10^{-5}$. In the presence of phage ϕAPCM01 the metabolic activity of a *S. mutans* biofilm was reduced after 24 h of contact and did not increased again after 48 h, and the live cells in the biofilm decreased by at least 5 log cfu/ml. Despite its narrow host range, this newly isolated *S. mutans* phage exhibits promising antimicrobial properties.

Citation: Dalmasso M, de Haas E, Neve H, Strain R, Cousin FJ, Stockdale SR, et al. (2015) Isolation of a Novel Phage with Activity against *Streptococcus mutans* Biofilms. PLoS ONE 10(9): e0138651. doi:10.1371/journal.pone.0138651

Editor: Indranil Biswas, University of Kansas Medical Center, UNITED STATES

Received: July 21, 2015

Accepted: September 2, 2015

Published: September 23, 2015

Data Availability Statement: All sequence files are available from the GenBank database (accession number KR153145).

Funding: This work was supported by the Science Foundation Ireland SFI/12/RC/2273. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Introduction

The microbiome of the human oral cavity is composed of numerous and diverse bacteria, archaea, eukaryotes and viruses [1–3]. Dental caries arise as a result of an ecological imbalance of metabolic activities in the stable oral microbiome. Dental caries is one of the most prevailing and persistent disease in the human population, despite the availability of various prophylactic options. *Streptococcus mutans* is a Gram-positive, coccus-shaped, non-motile and facultative anaerobic bacterium which is naturally present in the human mouth. It is an opportunistic pathogen and the principal etiological agent of dental decay in humans. *S. mutans* is able to adhere to the tooth surface in biofilm communities that contribute to dental plaque and favour the progression of dental disease [4, 5]. Within the dental plaque, *S. mutans* contributes greatly to the composition of the biofilm matrix, especially by producing abundant exopolysaccharides (EPS) [6]. *S. mutans* pathogenicity also results from its acidogenicity in the presence of dietary sucrose and its concomitant acid tolerance, both of which support changes in the ecology of the dental plaque by selecting for a cariogenic flora, increasing the probability of enamel demineralization and eventually caries formation [7]. When established as a biofilm, microbial

African Journal of Microbiology Research Vol. 4(20) pp. 2127-2132, 18 October, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Identification of *Streptococcus salivarius* bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy

Keivan Beheshti Maal[1], Majid Bouzari[1]* and Farahnaz Arbabzadeh Zavareh[2]

[1]Department of Biology, Faculty of Science, University of Isfahan, Isfahan 81746-73441, Iran.
[2]Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan 81746-73461, Iran.

Accepted 13 August, 2010

The aim of this research was to detect oral *Streprtococci* bacteriophages from Persian Gulf. Dental plaque samples were collected using sterile explorer and cultured in brain heart infusion (BHI) Broth. The oral *Streptococci* were isolated in culture media. The Persian Gulf water sample was gathered using a sterile bottle from the depth of 50 cm under the inframarine surface at Boushehr Port, Boushehr state, Iran. The Persian Gulf water was centrifuged and its supernatant was filtered through a 0.45 micrometers membrane filter and with a sterile Millipore filtration system. The filtrates were added to activate oral *Streptococci* at their logarithmic phase and cultured in (BHI) Agar using overlay method. Bacteriophage plaque forming assay in (BHI) Agar and clearance of (BHI) Broth suggested the presence of specific bacteriophages in sample. Transmission electron microscopy revealed that the capsid of the isolated bacteriophage was hexagonal (diameter: ~ 83.33 nm) most probably related to *Cystoviridae* family. This is the first report of isolation and identification of oral *Streptococci* bacteriophages from Persian Gulf located in South of Iran. The applications of these lytic phages as a potential for phage therapy of dental plaque could be considered as the significance and impact of the present study.

Key words: Persian Gulf, *Streptococcus salivarius*, bacteriophages, phage therapy, dental plaque, pharmaceutical and medical biotechnology.

INTRODUCTION

The resident microorganisms of oral cavity especially those inhabit on tooth surfaces are responsible for dental plaque formation and conversion of dietary saccharides to organic acids. These acids decalcify the tooth enamel and lead to destruction of tooth hard tissue and consequently tooth decay (Loesch et al., 1986; Hitch et al., 2004; van der Ploeg, 2007). More than 500 species from 30 deferent genera reside in oral cavity (Schaechter et al., 2004). The most important species that play key roles in dental plaque formation are oral *Streptococci* (Tanzer et al., 2001). According to Bergey's mannual of systematic bacteriology, oral *Streptococci* are formed from 12 species including *Streptococcus salivarius, Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus parasanguis*, *Streptococcus pneumonia*, *Streptococcus sanguis* and *Streptococcus sobrinus* (Holt et al., 1994; Schaechter et al., 2004). These species are the first bacteria that attach to salivary glycoproteins on tooth surfaces through their specific surface capsular polymers such as glucan and fructan (Freedman et al., 1974; Tanzer et al., 1974; Tanzer et al., 2001). *S. salivarius* as well as mutans *Streptococci* and nonmutans *Streptococci* or sanguinis *Streptococci* are present at high levels in tooth and mucosal surfaces some of which are highly acidogenic and few are acid tolerant (Nyvad et al., 1990; Tanzer et al., 2001). *S. salivarius* along with *S. sanguis*, *S. oralis* and *S. gordonii* are the first tooth colonizers however, *S. sobrinus* and *S. mutans* are more dealt with dental

*Corresponding author. E-mail: bouzari@sci.ui.ac.ir. Tel: +98 311 7932459. Fax: +98 311 7932456.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 2011, p. 3157–3159 Vol. 77, No. 9
0099-2240/11/$12.00 doi:10.1128/AEM.02115-10

Isolation of a Novel *Aggregatibacter actinomycetemcomitans* Serotype b Bacteriophage Capable of Lysing Bacteria within a Biofilm▽†

Mario Castillo-Ruiz,[1] Enrique D. Vinés,[1] Camilo Montt,[1] Jorge Fernández,[2] José Manuel Delgado,[3] Juan Carlos Hormazábal,[4] and Mauricio Bittner[1,5]*

Laboratorio de Microbiología y Biotecnología Oral, Facultad de Ciencias Biológicas, Universidad Andres Bello, Santiago, Chile[1]; Departamento de Genética Molecular, Instituto de Salud Pública de Chile, Santiago, Chile[2]; Facultad de Medicina, Universidad Andres Bello, Santiago, Chile[3]; Departamento de Microbiología, Instituto de Salud Pública de Chile, Santiago, Chile[4]; and Facultad de Odontología, Universidad Andres Bello, Santiago, Chile[5]

Received 7 September 2010/Accepted 27 January 2011

A bacteriophage specific for *Aggregatibacter actinomycetemcomitans* serotype b, able to kill the bacterium within a biofilm, was isolated. Random mutagenesis of this phage rendered a bacteriophage able to kill 99% of the bacteria within a biofilm. This is the first report of a biocontrol experiment against *A. actinomycetemcomitans*.

Periodontitis is an infection of the supporting tissues of the tooth caused by bacteria or bacterial groups embedded in a biofilm (14). *Aggregatibacter actinomycetemcomitans* (formerly, *Actinobacillus actinomycetemcomitans*) is a capnophilic, nonmotile Gram-negative bacterium (11, 22) related to the aggressive form of periodontitis (6–10, 18, 23); its isolates are classified into seven serotypes, a to g (12, 24), with serotype b frequently associated with disease (2, 13, 20) and serotype c with oral health (19). Periodontitis caused by *A. actinomycetemcomitans* often requires antibiotic therapy besides mechanical treatment due to the bacterium's ability to form a biofilm in the periodontal pocket and on all mucous membrane surfaces in the oral cavity (6). Biofilms are organized in highly efficient and stable ecosystems (15), and it has been proposed that bacterial susceptibility to antibiotics is reduced within this structure (4), making it virtually impossible to completely remove bacteria from biofilms with antibiotics only (6). These features make periodontitis a complex disease and motivate the search for novel antimicrobial therapies such as oral microbiota modification and phage therapy (1, 16). Phage therapy has been attempted for systemic diseases (1) and to control infections in the oral cavity (17). Our aim was to isolate a bacteriophage for *A. actinomycetemcomitans* and evaluate its effec-

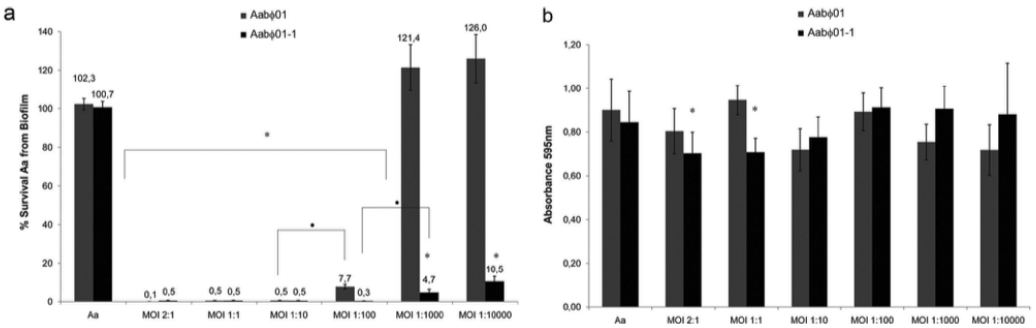


FIG. 1. Infection of an *A. actinomycetemcomitans* biofilm with phages Aabϕ01 and Aabϕ01-1. A biofilm of *A. actinomycetemcomitans* cultured during 24 h under capnophilic conditions was infected with Aabϕ01 or Aabϕ01-1 at different MOIs. The results are expressed as percentages of CFU/ml recovered 24 h postinfection compared with the CFU/ml recovered before infection (a) or from quantified biofilm at 595 nm (b). As a control, we used a biofilm of *A. actinomycetemcomitans* without infection (Aa). Bars depict the average values from three independent experiments. An asterisk represents significance in relation to the control ($P < 0.05$).

* Corresponding author. Mailing address: Laboratorio de Microbiología y Biotecnología Oral, Universidad Andres Bello, Echaurren 237, Santiago, Chile. Phone: (562) 770-3027. Fax: (562) 464-1630. E-mail: mbittner@unab.cl.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 4 March 2011.

viruses

MDPI

Article

Effects of a Chimeric Lysin against Planktonic and Sessile *Enterococcus faecalis* Hint at Potential Application in Endodontic Therapy

Wuyou Li [1,†], Hang Yang [2,*,†], Yujing Gong [3], Shujuan Wang [2], Yuhong Li [1,*] and Hongping Wei [2,*]

1 The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine, Ministry of Education, School of Stomatology, Wuhan University, Wuhan 430079, China; 2016203040006@whu.edu.cn

2 Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China; wangsj@wh.iov.cn

3 State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China; hejin@mail.hzau.edu.cn

* Correspondence: yangh@wh.iov.cn (H.Y.); 1004809372@whu.edu.cn (Y.L.); hpwei@wh.iov.cn (H.W.); Tel.: +86-27-5186-1078 (H.Y.); +86-27-8764-7443 (Y.L.); +86-27-5186-1076 (H.W.); Fax: +86-27-8719-9492 (H.Y.); +86-27-8764-7443 (Y.L.); +86-27-8719-9492 (H.W.)

† These authors contributed equally to this work.

Received: 29 April 2018; Accepted: 23 May 2018; Published: 29 May 2018

check for updates

Abstract: *Enterococcus faecalis* is a commensal opportunistic pathogen found in the intestine, mouth, and vaginal tract of humans. As an invasive pathogen in the oral cavity, *E. faecalis* is one of the leading causes of periapical endodontic lesions. However, due to the strong biofilm-forming capacity and tolerance of *E. faecalis* to conventional antibiotics and treatments, limited therapeutic options are available. In the present study, we investigated the activity of ClyR, a chimeric lysin with extended streptococcal lytic spectrum, against planktonic and sessile *E. faecalis* cells in vitro and in an ex vivo dental model. Our results showed that ClyR has robust and rapid lytic activity against multiple *E. faecalis* strains, killing >90% planktonic cells within 1 min at a concentration of 50 μg/mL. The biochemical experiments combined with microscopy analysis revealed that ClyR degrades *E. faecalis* biofilm with high efficacy in a dose-dependent manner, reducing the survival rate to <40% within biofilms after treatment with 50 μg/mL ClyR for 1 h. In the ex vivo dental model, ClyR showed a significant biofilm removal efficacy, killing >90% viable bacteria within biofilms at a low dose of 50 μg/mL, which is much better than ampicillin and similar to calcium hydroxide, the extensively used routine intracanal medicament in the treatment of endodontics and dental traumatology. The robust activity of ClyR against both planktonic and sessile *E. faecalis* suggests the potential of ClyR in treating endodontic infections caused by *E. faecalis*.

Keywords: bacteriophage lysin; *Enterococcus faecalis*; bacterial biofilm; endodontic infection; calcium hydroxide

1. Introduction

Enterococcus faecalis, an opportunistic Gram-positive pathogen, is a member of the normal microorganisms of the oral cavity, intestines, and vaginal tract of human beings and animals [1]. As a common community of oral microbiota, *E. faecalis* has found to be involved in the microflora of the root canal and the periodontal pocket that do not respond well to conventional root canal therapy [2]. In most instances, *E. faecalis* can survive as biofilm in the surface of the dentinal tubule or

viruses

MDPI

Article

Activity of the Chimeric Lysin ClyR against Common Gram-Positive Oral Microbes and Its Anticaries Efficacy in Rat Models

Jingjing Xu [1,†], Hang Yang [2,†], Yongli Bi [1], Wuyou Li [1], Hongping Wei [2] and Yuhong Li [1,*]

[1] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine, Ministry of Education, School of Stomatology, Wuhan University, Wuhan 430079, China; 2015203040023@whu.edu.cn (J.X.); 2014203040013@whu.edu.cn (Y.B.); 2016203040006@whu.edu.cn (W.L.)

[2] Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China; yangh@wh.iov.cn (H.Y.); hpwei@wh.iov.cn (H.W.)

* Correspondence: 1004809372@whu.edu.cn; Tel./Fax: +86-27-8764-7443

† These authors contributed equally to this work.

Received: 26 April 2018; Accepted: 17 July 2018; Published: 20 July 2018

check for updates

Abstract: Dental caries is a common disease caused by oral bacteria. *Streptococcus mutans* and *Streptococcus sobrinus* are the primary cariogenic microbes that often survive as biofilms on teeth. In this study, we evaluated the activity of ClyR, a well-known chimeric lysin with extended streptococcal host range, against common Gram-positive oral microbes and its anticaries efficacy in rat models. ClyR demonstrated high lytic activity against *S. mutans* MT8148 and *S. sobrinus* ATCC6715, with minor activity against *Streptococcus sanguinis*, *Streptococcus oralis*, and *Streptococcus salivarius*, which are considered as harmless commensal oral bacteria. Confocal laser scanning microscopy showed that the number of viable cells in 72-h aged *S. mutans* and *S. sobrinus* biofilms are significantly ($p < 0.05$) decreased after treatment with 50 μg/mL ClyR for 5 min. Furthermore, continuous administration of ClyR for 40 days (5 μg/day) significantly ($p < 0.05$) reduced the severity of caries in rat models infected with a single or a mixed bacteria of *S. mutans* and *S. sobrinus*. Therefore, ClyR could be a promising agent or additive for the prevention and treatment of dental caries.

Keywords: dental caries; bacteriophage lysin; biofilm; antibacterial; anticaries

1. Introduction

Dental caries remains a significant problem all over the world despite improved oral hygiene awareness [1]. For more than a century, it has been known that caries is initiated by demineralization of the enamel as a result of fermentation and acidogenesis by oral bacteria in biofilm [2]. Among cariogenic microbes, *Streptococcus mutans* is identified as the primary cariogenic pathogen because of its unique acid-producing and aciduric ability [3,4]. Glucan produced by *S. mutans* plays a fundamental role in biofilm formation, providing binding sites for bacteria colonization on enamel surface [5]. *Streptococcus sobrinus*, another important cariogenic microorganism, is less frequently detected than *S. mutans* in the oral cavity but is considered more virulent than *S. mutans* due to its high acidogenicity and acid tolerance [6]. Previous studies have observed that co-existence of both species is associated with higher incidence of dental caries and higher scores in the DMFT (decayed missing and filled teeth) index in children with early childhood caries (ECC) [7,8]. The amounts of oral *S. mutans* and *S. sobrinus* are used as risk indicators for dental caries [9].

There are presently a few ways to reduce dental caries, where fluoride therapy is the most common method to reduce the risk of the disease [10]. However, fluoride toothpaste (with concentrations of

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Antibiofilm Activities of a Novel Chimeolysin against *Streptococcus mutans* under Physiological and Cariogenic Conditions

Hang Yang,[a] Yongli Bi,[b] Xiaoran Shang,[c] Mengyue Wang,[a] Sara B. Linden,[c] Yunpeng Li,[a] Yuhong Li,[b] Daniel C. Nelson,[c,d] Hongping Wei[a]

Key Laboratory of Special Pathogens and Biosafety, Center for Emerging Infectious Diseases, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China[a]; The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine, Ministry of Education, School and Hospital of Stomatology, Wuhan University, Wuhan, China[b]; Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, Maryland, USA[c]; Department of Veterinary Medicine, University of Maryland, College Park, Maryland, USA[d]

Streptococcus mutans **often survives as a biofilm on the tooth surface and contributes to the development of dental caries. We investigated the efficacy of ClyR, an engineered chimeolysin, against *S. mutans* biofilms under physiological and cariogenic conditions. Susceptibility tests showed that ClyR was active against all clinical *S. mutans* isolates tested as well as *S. mutans* biofilms that displayed resistance to penicillin. The *S. mutans* biofilms that formed on hydroxyapatite discs under physiological sugar conditions and cariogenic conditions were reduced ~2 logs and 3 logs after treatment with 100 μg/ml ClyR, respectively. In comparison, only a 1-log reduction was observed in the chlorhexidine gluconate (ChX)-treated group, and no killing effect was observed in the NaF-treated group. A mouse dental colonization model showed that repeated use of ClyR for 3 weeks (5 μg/day) reduced the number of colonized *S. mutans* cells in the dental plaques significantly ($P < 0.05$) and had no harmful effects on the mice. Furthermore, toxicity was not noted at concentrations exceeding those used for the *in vitro* and *in vivo* studies, and ClyR-specific antibodies could not be detected in mouse saliva after repeated use of ClyR in the oral cavity. Our data collectively demonstrate that ClyR is active against *S. mutans* biofilms both *in vitro* and *in vivo*, thus representing a preventative or therapeutic agent for use against dental caries.**

Streptococcus mutans, as an initiator of dental caries, possesses unique virulence factors that play an important role in caries formation (1, 2). The ability of *S. mutans* to form biofilms, also known as dental plaque, on tooth surfaces allows the subsequent coaggregation of more fastidious organisms (3). The acidogenic and aciduric properties of *S. mutans* allow it to metabolize sucrose to lactic acid and to grow at low pH values (4). The acid formation leads to the dissolution of calcium and phosphate in tooth enamel, causing tooth decay, and further promotes adhesion of additional bacteria (5, 6). Thus, the biofilm-forming bacterium *S. mutans* has been reported to be the primary etiological agent of human dental caries (7).

Most current dental therapies include mechanical removal or broad-spectrum antimicrobial treatments that are focused on eradicating the dental plaque (8). Sodium fluoride (NaF) at 0.05% and chlorhexidine gluconate (ChX) at 0.12% are two different types of antimicrobials used clinically in toothpaste and mouthwashes to reduce plaques and prevent caries (9–11). However, the ability to rapidly form biofilms enhances the virulence of *S. mutans* and protects the bacteria from the activities of the antimicrobial agents (12). Vaccine strategies have been proposed to be a way to protect from *S. mutans*, but to date, none have been successful and streptococcal antigens may cause other health issues (13). Therefore, improved approaches are needed to prevent and remove the biofilms of *S. mutans*.

Bacteriophage-encoded lysins are peptidoglycan hydrolases that digest the bacterial cell wall, leading to the rapid lysis of susceptible Gram-positive bacteria when applied externally (14). In the last decade, natural and engineered lysins have been demonstrated extensively to be therapeutic agents with activity against various Gram-positive pathogens both *in vitro* and *in vivo* (15–17). Some investigations have also shown the activities of several lysins against staphylococcal biofilms (16, 18) and streptococcal biofilms (19–21), indicating the advantages of lysins over traditional antibiotics in removing biofilms. However, no studies to date have reported on lysins effective against *S. mutans* biofilms. ClyR is a chimeolysin engineered from two parental streptococcal lysins and is the first lysin reported to be active against planktonic *S. mutans* cells (22). In the present study, we report the efficacy of ClyR in preventing and removing biofilms formed by *S. mutans* under both physiological and cariogenic conditions.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains (see Table S1 in the supplemental material) were grown at 37°C. Planktonic cells of the *S. mutans* strains were grown in Todd-Hewitt broth supplemented with 2% yeast extract (THY) medium (Becton, Dickinson and Co., USA). For production of biofilms, THY medium was supplemented with 0.1 mM glucose to mimic physiological conditions or 1% glucose (56 mM) or 5% sucrose (146 mM) to mimic cariogenic conditions. *S. mutans*-containing clinical samples were isolated from the dental plaques of children, each of whom had a decayed-

Received 29 August 2016 **Accepted** 2 October 2016

Accepted manuscript posted online 10 October 2016

Citation Yang H, Bi Y, Shang X, Wang M, Linden SB, Li Y, Li Y, Nelson DC, Wei H. 2016. Antibiofilm activities of a novel chimeolysin against *Streptococcus mutans* under physiological and cariogenic conditions. Antimicrob Agents Chemother 60:7436–7443. doi:10.1128/AAC.01872-16.

Address correspondence to Yuhong Li, 1004809372@whu.edu.cn, Daniel C. Nelson, nelsond@umd.edu, or Hongping Wei, hpwei@wh.iov.cn.

H.Y. and Y.B. contributed equally to this article.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01872-16.

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Genomic, morphological and functional characterisation of novel bacteriophage FNU1 capable of disrupting *Fusobacterium nucleatum* biofilms

Received: 7 March 2019
Accepted: 7 June 2019
Published online: 24 June 2019

Mwila Kabwe[1], Teagan L. Brown[1], Stuart Dashper[2], Lachlan Speirs[1], Heng Ku[1], Steve Petrovski[3], Hiu Tat Chan[4], Peter Lock[5] & Joseph Tucci[1]

Fusobacterium nucleatum **is an important oral bacterium that has been linked to the development of chronic diseases such as periodontitis and colorectal cancer. In periodontal disease, *F. nucleatum* forms the backbone of the polymicrobial biofilm and in colorectal cancer is implicated in aetiology, metastasis and chemotherapy resistance. The control of this bacteria may be important in assisting treatment of these diseases. With increased rates of antibiotic resistance globally, there is need for development of alternatives such as bacteriophages, which may complement existing therapies. Here we describe the morphology, genomics and functional characteristics of FNU1, a novel bacteriophage lytic against *F. nucleatum*. Transmission electron microscopy revealed FNU1 to be a large *Siphoviridae* virus with capsid diameter of 88 nm and tail of approximately 310 nm in length. Its genome was 130914 bp, with six tRNAs, and 8% of its ORFs encoding putative defence genes. FNU1 was able to kill cells within and significantly reduce *F. nucleatum* biofilm mass. The identification and characterisation of this bacteriophage will enable new possibilities for the treatment and prevention of *F. nucleatum* associated diseases to be explored.**

Fusobacterium nucleatum is a Gram-negative facultative anaerobic bacillus that is a normal component of the oral microbiome. It has been associated with periodontal diseases[1] as well as malignancies of the oral cavity, head and neck, oesophagus, cervix, stomach and colon[2–4]. This association with a range of malignancies has led to its referral as an "oncobacterium"[4]. In these diseases, *F. nucleatum* biofilms have been demonstrated to play a critical role.

Chronic periodontitis results from a dysbiosis in subgingival plaque biofilm communities that leads to the emergence of pathogenic species that dysregulate the host immune response leading to sustained and uncontrolled inflammation[5,6]. In chronic periodontitis, *F. nucleatum* has been shown to act as a backbone for pathogenic subgingival polymicrobial biofilms by forming a bridge between the more commensal early colonisers and the more pathogenic late colonisers[1,7,8]. This microbial biofilm is therefore responsible for the initiation and progression of chronic periodontitis[9,10]. Apart from their role in periodontitis, bacterial biofilms and microbiota organisation have also been associated with gut tumours[11]. In colorectal cancer, *Fusobacterium* has been demonstrated to be intimately involved in modulating the tumour immune microenvironment and recruiting myeloid cells that assist in tumorigenesis, tumour cell proliferation and metastasis[12], modulating autophagy and resistance to chemotherapies[13].

Current treatment of periodontal disease is mechanical debridement with antibiotics and antiseptics as adjuncts. However, this approach is not without controversy[14], with the development of antibiotic resistance being a major caveat[15], along with dysbiosis of oral microbiota contributing to inflammation and disease recurrence[16,17].

[1]Department of Pharmacy and Biomedical Sciences, La Trobe Institute for Molecular Science, La Trobe University, Victoria, Australia. [2]Melbourne Dental School, University of Melbourne, Victoria, Australia. [3]Department of Physiology, Anatomy and Microbiology, La Trobe University, Victoria, Australia. [4]Department of Microbiology, Royal Melbourne Hospital, Victoria, Australia. [5]La Trobe Institute for Molecular Science, La Trobe University, Victoria, Australia. Correspondence and requests for materials should be addressed to J.T. (email: J.Tucci@latrobe.edu.au)

Oral Microbiology Immunology 2004: 19: 327–330
Printed in Denmark. All rights reserved

Oral Microbiology and Immunology

In vitro antimicrobial effect of bacteriophages on human dentin infected with *Enterococcus faecalis* ATCC 29212

A. F. Paisano[1], B. Spira[2], S. Cai[2], A. C. Bombana[1]
[1]Department of Endodontics/University of São Paulo, [2]Department of Microbiology/Institute of Biomedical Sciences/University of São Paulo, São Paulo, Brazil

Paisano AF, Spira B, Cai S, Bombana AC. In vitro *antimicrobial effect of bacteriophages on human dentin infected with* Enterococcus faecalis *ATCC 29212.*
Oral Microbiol Immunol 2004: 19: 327–330 © Blackwell Munksgaard, 2004.

This study assessed the effect of bacteriophages on the viability of *Enterococcus faecalis*. Human dental roots were inoculated with a suspension of *E. faecalis* at three different multiplicities of infection – 0.1, 1.0 and 10.0. The phage lysate was able to significantly inhibit bacteria growth when incubated at the multiplicities of infection of 1.0, 10.0 and 0.1. The dental roots were also inoculated with bacteria for 6 days to allow bacterial penetration into the teeth tubules. Addition of the phage lysate to the roots following the 6-day incubation period led to a substantial reduction in bacteria viability. Phage therapy may be an important alternative for the treatment of root canal infections refractory to conventional endodontic therapy.

Key words: bacteriophage; endodontic infection; *Enterococcus faecalis*, phage; phage therapy

Adriana Fernandes Paisano, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, Avenue. Prof Lineu Prestes 1374, room 128, São Paulo-SP, 05508–900 Brazil
Tel.: +55 11 3091 7202;
fax: +55 11 3091 7354;
e-mail: silvanac@icb.usp.br
Accepted for publication June 3, 2004

One of the purposes of endodontic treatment is the disinfection of the root canal system. Leftovers of viable microorganisms in association with favorable growth conditions prevent the repair process and may cause treatment failure (13, 15, 17). Microorganisms that survive chemical–surgical proceedings may rapidly increase in number between treatment sessions, making the use of antimicrobial medications obligatory, especially in clinical situations refractory to habitual procedures or in endodontic retreatment (17).

Enterococcus faecalis has been recovered from several oral sites and exhibits a high level of resistance to a wide variety of antimicrobial agents (18, 27). In endodontic infections, *E. faecalis* can survive in the root canal as a single organism, without the support of other species (9, 15), and represents a problem in conservative root canal therapy (4, 12, 19).

In the last decades, the resistance of microorganisms to chemotherapeutic agents has been increasing. This situation makes the introduction of new therapeutic techniques compulsory and, in this context, a renewed interest in phage therapy has arisen.

The use of phages for the control of infection has some advantages. They can be administered in a single dose because they reproduce within the target bacteria and remain in the region while infection persists. In contrast, antibiotics require multiple doses with days or weeks of treatment (5). On the other hand, phages are highly specific for a bacteria species, and sometimes even for a bacteria strain, narrowing their spectrum of application.

The aim of the present study was to assess *in vitro* the antimicrobial effect of bacteriophages specific for *E. faecalis* ATCC 29212 from the root canals and from the dentinal tubules of human teeth.

Material and methods

Phage isolation

Bacteriophages were isolated by the phage enrichment method of Smith & Huggins (25). A sample was collected from the Pirajussara stream in the city of São Paulo, Brazil, and carried to the laboratory packed in ice. A volume of 100 ml of LB medium (Luria Bertani broth) and 100 ml of TB medium (Terrific broth) was added to 300 ml of the stream water. After 1 h of incubation at 37°C, 3 ml of an *E. faecalis* ATCC 29212 culture was added and grown for 16 h at 37°C without aeration. Then 500 µl of chloroform was added to 10 ml of culture and the mixture centrifuged for 10 min at 5,300 ×g. The supernatant was supplemented with 100 µl chloroform and stored at 4°C (crude lysate). Dilutions of the crude lysate were added to a lawn of *E. faecalis* on LB plates and incubated overnight at 37°C. A phage

INFECTION AND IMMUNITY, Jan. 2002, p. 204–210 Vol. 70, No. 1
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.1.204–210.2002

Bacteriophage Therapy Rescues Mice Bacteremic from a Clinical Isolate of Vancomycin-Resistant *Enterococcus faecium*

Biswajit Biswas,[1,2] Sankar Adhya,[1] Paul Washart,[1,2] Brian Paul,[1] Andrei N. Trostel,[2] Bradford Powell,[1,2] Richard Carlton,[2] and Carl R. Merril[1]*

National Institutes of Health, Bethesda, Maryland 20892,[1] and Exponential Biotherapies, Inc., Pt. Washington, New York 11050[2]

Received 30 August 2001/Returned for modification 26 September 2001/Accepted 18 October 2001

Colonization of the gastrointestinal tract with vancomycin-resistant *Enterococcus faecium* (VRE) has become endemic in many hospitals and nursing homes in the United States. Such colonization predisposes the individual to VRE bacteremia and/or endocarditis, and immunocompromised patients are at particular risk for these conditions. The emergence of antibiotic-resistant bacterial strains requires the exploration of alternative antibacterial therapies, which led our group to study the ability of bacterial viruses (bacteriophages, or phages) to rescue mice with VRE bacteremia. The phage strain used in this study has lytic activity against a wide range of clinical isolates of VRE. One of these VRE strains was used to induce bacteremia in mice by intraperitoneal (i.p.) injection of 10^9 CFU. The resulting bacteremia was fatal within 48 h. A single i.p. injection of 3×10^8 PFU of the phage strain, administered 45 min after the bacterial challenge, was sufficient to rescue 100% of the animals. Even when treatment was delayed to the point where all animals were moribund, approximately 50% of them were rescued by a single injection of this phage preparation. The ability of this phage to rescue bacteremic mice was demonstrated to be due to the functional capabilities of the phage and not to a nonspecific immune effect. The rescue of bacteremic mice could be effected only by phage strains able to grow in vitro on the bacterial host used to infect the animals, and when such strains are heat inactivated they lose their ability to rescue the infected mice.

Isolates of vancomycin-resistant *Enterococcus faecium* (VRE) from patients in the United States, France, and England were first reported in 1989 (9, 19). By 1998, the U.S. National Nosocomial Infections Surveillance System had reported that 20% of nosocomial isolates of enterococci were resistant to vancomycin (12). Individuals with compromised immune systems, such as AIDS patients, cancer patients undergoing chemotherapy, postsurgical patients, transplant recipients, and the elderly in general, are particularly prone to develop VRE infections. While the antibiotic quinupristin-dalfopristin (Synercid; Rhone-Poulenc Rorer, Collegeville, Pa.) has recently been licensed for clinical use, its efficacy for VRE infections may be limited because (i) it is bacteriostatic, and (ii) one of its two components is an analog of virginiamycin, which has been used as an additive in hog and poultry feed for the past 2 decades. Quinupristin-dalfopristin-resistant bacteria have been isolated from turkeys fed virginiamycin, suggesting that the use of virginiamycin has created a reservoir of enterococci resistant to the analog in quinupristin-dalfopristin (5). Linezolid (Zyvox; Pharmacia and Upjohn), another recently introduced antibiotic, is also described as bacteriostatic for VRE, and resistance to it appeared during clinical trials even though it is the first member of a new class of agents (the oxazolodinones).

Early applications of antibacterial phage therapy (1920s to 1950s) were impeded by a number of factors. One factor was the use of phage strains whose host range was too narrow, a problem we have largely overcome by selecting enterococcal phages able to infect the vast majority of clinical isolates of VRE. Another factor was the large load of endo- and exotoxins in the bacterial debris present in the filter-sterilized but otherwise unpurified phage lysates that past investigators administered parenterally as well as orally, a problem we have overcome with cesium chloride density centrifugation and other modern techniques of phage purification. A third factor was sterilizing phage preparations by heat and/or by the addition of mercurials and oxidizing agents, procedures that are now known to inactivate phages and that we avoid. Finally, the pharmacokinetics of phage therapy were essentially ignored in clinical applications of phage therapy, the assumption being that oral and parenteral administration would achieve concentrations at the sites of infection sufficient to induce a cure. A pharmacokinetic study performed in 1973 demonstrated the rapid clearance of phage from the bloodstream and accumulation of phage particles in the spleen and other filtering organs of the reticuloendothelial system, where they remain viable for at least a week (7). This passive capture by and sequestration in the filtering organs would prevent the vast bulk of administered phage particles from reaching the infecting bacteria, and it would add variability in therapeutic applications. To address this problem, serial passage methods have been developed to select and enrich for phage strains whose rate of clearance by the reticuloendothelial system is reduced. The long-circulating phage strains produced have been shown to be superior to the wild types from which they were derived for rescuing animals from otherwise fatal bacteremias due to *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (11). Following these studies, we chose to work with VRE

* Corresponding author. Mailing address: NIMH/NIH, 9000 Rockville Pike, Bldg 10, Room 2D54, Bethesda, MD 20892. Phone: (301) 435-3583. Fax: (301) 480-9862. E-mail: merrilc@helix.nih.gov.

Efficacy of Bacteriophage Treatment on *Pseudomonas aeruginosa* Biofilms

Alysen Phee, BSc, DMD, Joe Bondy-Denomy, BSc,† Anil Kishen, BDS, MDS, PhD,* Bettina Basrani, DDS, MSc, PhD,* Amir Azarpazhooh, DDS, MSc, PhD,* and Karen Maxwell, BSc, MSc, PhD†*

Abstract

Introduction: Bacterial viruses (phages) have been used successfully in the treatment of animal and human bacterial infections. This study examined the potential use of phage therapy against *Pseudomonas aeruginosa* strain PA14 biofilms in a root canal model. **Methods:** Part 1: The 24-hour and 96-hour PA14 biofilms grown in microplates were treated with phages identified as possessing potential biofilm-degrading activities, and the post-treatment bacterial biomass was quantified by using crystal violet staining. Part 2: The 24-hour and 96-hour PA14 biofilms grown in prepared root canals of extracted human mandibular incisors were treated with phages identified with potential biofilm-degrading activities. Post-treatment intracanal samples were taken by using paper points and round burs to assess phage and bacterial counts. **Results:** Part 1: We identified 2 phages (JBD4 and JBD44a) with putative biofilm-degrading activities. Treatment of PA14 biofilms with these phages produced a significant reduction in the mean percentage of biomass in 24-hour ($P < .05$) and 96-hour ($P = .08$) biofilms. Part 2: In 24-hour and 96-hour PA14 biofilms in a root canal model, no significant difference was found in the number of colony-forming units after phage treatment ($P > .05$). **Conclusions:** Phage application significantly reduced the biomass of 24-hour and 96-hour PA14 biofilms grown on microplates but did not produce significant reduction of 24-hour or 96-hour PA14 biofilms grown in the extracted tooth model. *(J Endod 2013;39:364–369)*

Key Words

Apical periodontitis, bacteriophage therapy, *Pseudomonas aeruginosa*, root canal

Primary apical periodontitis is an inflammatory disease of the periodontal ligament and surrounding bone caused by infection present in the root canal system (1). Successful treatment of root canal infection requires physical reduction in the number of root canal bacteria and an alteration in the environment that discourages bacterial recolonization and survival (2). Outcome studies that assess the efficacy of current treatment methods used in the management of root canal infections report a favorable healing rate of 68%–85% (3). Persistent endodontic disease occurs when endopathic bacteria are not adequately controlled or when new bacteria or other factors that promote disease are introduced into the root canal and periapical tissues during or after initial treatment (4). Teeth with persistent infections can be treated nonsurgically and/or surgically and have an expected healing rate of 77% (5) and 74% (6), respectively. A study of the prevalence of endodontic biofilm reported its presence in the apical third of the root canal system in 80% of untreated teeth with apical periodontitis and in 74% of those with root fillings (7). This is consistent with the criteria necessary to classify apical periodontitis as a "biofilm-induced disease."

Pseudomonas aeruginosa, a gram-negative, facultative, rod-shaped bacterium that belongs to the Gammaproteobacteria class of bacteria, is frequently found in periodontal infections (8). It has been recovered from primary and persistent endodontic infections (9–16) and in 1 study represented 6.8% of the bacterial isolates recovered from persistent apical infections (9). Notably, in that study *P. aeruginosa* was found in 4 of 5 teeth with a draining sinus, 2 of which proved to be a monospecies infection. *P. aeruginosa* forms biofilms that are resistant to high concentrations of salts, dyes, weak antiseptics including chlorhexidine gluconate (17, 18), and many commonly used antibiotics (19). Its persistence after conventional endodontic treatment (9–14) also indicates that it may be resistant to commonly used endodontic disinfection protocols. Irrigation with 5.25% sodium hypochlorite was not able to routinely eliminate *P. aeruginosa* from the root canal (20), and growth was not inhibited by frequently used root canal sealers (21). A 48-hour intracanal dressing of calcium hydroxide (22) and photodynamic therapy (23) have also been shown to be ineffective in eliminating its presence. These findings, coupled with the ability of *P. aeruginosa* to form biofilms, are sufficient to designate *P. aeruginosa* as a possible cause of persistent endodontic disease.

A potent anti-biofilm strategy that is passive to host tissue is necessary in endodontics to compensate for the limitations of mechanical bacterial debridement and antiseptic properties of current root canal sealers. Bacteriophages (phages) are viruses that can infect and kill bacteria that have been used as a treatment alternative in the

From the *Discipline of Endodontics, Faculty of Dentistry, and †Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada.

Supported by grants from the American Association of Endodontists Foundation and the Canadian Academy of Endodontics Endowment Fund. This work was supported in part by an Operating Grant from the Canadian Institutes for Health Research to K.L.M. (Fund No. MOP-6279).

Address requests for reprints to Dr Anil Kishen, Department of Endodontics, Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, Ontario M5G 1G6, Canada. E-mail address: anil.kishen@dentistry.utoronto.ca

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2012.10.023

ELSEVIER

INSTITUT PASTEUR

Research in Microbiology 162 (2011) 798–806

www.elsevier.com/locate/resmic

Use of newly isolated phages for control of *Pseudomonas aeruginosa* PAO1 and ATCC 10145 biofilms

Diana Pires [a], Sanna Sillankorva [a], Alberta Faustino [b], Joana Azeredo [a,*]

[a] *IBB – Institute of Biotechnology and Bioengineering, Center of Biological Engineering, University of Minho, Campus de Gualtar 4710-057, Braga, Portugal*
[b] *São Marcos Hospital, P.O. box 2242, 4701-965 Braga, Portugal*

Received 25 June 2010; accepted 9 May 2011
Available online 5 July 2011

Abstract

Pseudomonas aeruginosa is a relevant opportunistic pathogen involved in nosocomial infections that frequently shows low antibiotic susceptibility. One of its virulence factors is associated with the ability to adhere to surfaces and form virulent biofilms. This work describes the isolation and characterization of lytic phages capable of infecting antibiotic-resistant *P. aeruginosa* strains. In addition, characterization of *P. aeruginosa* biofilms and the potential of newly isolated phages for planktonic and biofilm control was accessed. According to the results, the isolated phages showed different spectra of activity and efficiency of lysis. Four broad lytic phages were selected for infection of planktonic cells; however, despite their broad range of activity, two of the selected phages failed to efficiently control planktonic cultures. Therefore, only two phages (phiIBB-PAA2 and phiIBB-PAP21), highly capable of causing strong biomass reduction of planktonic cells, were tested against 24 h biofilms using a m.o.i. of 1. Both phages reduced approximately 1–2 log the biofilm population after 2 h of infection and reduction was further enhanced after 6 h of biofilm infection. However, biofilm cells of *P. aeruginosa* PAO1 acquired resistance to phiIBB-PAP21; consequently, an increase in the number of cells after 24 h of treatment was observed. Conversely, phage phiIB-PAA2 for *P. aeruginosa* ATCC10145 continued to destroy biofilm cells, even after 24 h of infection. In these biofilms, phages caused a 3 log reduction in the number of viable counts of biofilm cells.

Keywords: *Pseudomonas aeruginosa*; Bacteriophages; Biofilms; Control

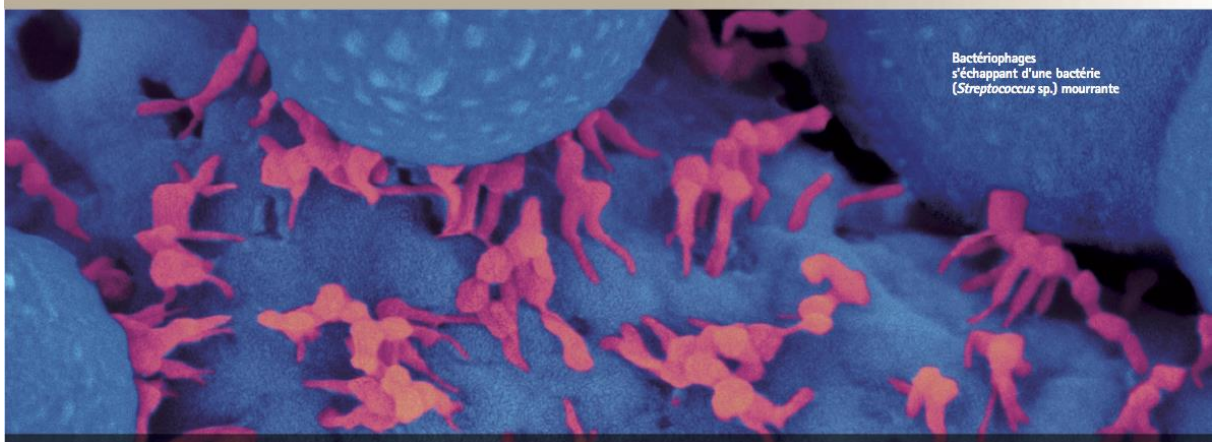
1. Introduction

Pseudomonas aeruginosa is an ubiquitous organism which has emerged as a major threat in the hospital environment. This bacterium is the most frequently isolated Gram-negative organism in bloodstream and wound infections, pneumonia and intra-abdominal and urogenital sepsis, and is a serious problem, infecting immunocompromised patients with cystic fibrosis (CF), severe burns, cancer, AIDS, etc. (Driscoll et al., 2007; Page and Heim, 2009). One of the most worrying characteristics of this bacterium is its low antibiotic susceptibility, which can be attributed to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes (Lambert, 2002).

Overuse of antibiotics has also significantly increased the emergence of antimicrobial multiresistant bacteria; consequently, treatment of most chronic *P. aeruginosa* infections with antibiotics is notoriously difficult (Cunha, 2002; Lambert, 2002). Additionally, *P. aeruginosa* has an innate ability to adhere to surfaces and form virulent biofilms particularly difficult to eradicate (Drenkard, 2003; Mah et al., 2003; Stewart and Costerton, 2001). Biofilm formation is an important bacterial survival strategy and, in humans, biofilms are responsible for numerous pathologies usually associated with use of medical devices (Azeredo and Sutherland, 2008; Donlan, 2002; O'Toole et al., 2000). Thus, new alternative

* Corresponding author.
E-mail addresses: priscilapires@deb.uminho.pt (D. Pires), s.sillankorva@deb.uminho.pt (S. Sillankorva), afaustino@bluebottle.com (A. Faustino), jazeredo@deb.uminho.pt (J. Azeredo).

0923-2508/$ - see front matter © 2011 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.resmic.2011.06.010



Bactériophages s'échappant d'une bactérie (*Streptococcus* sp.) mourrante

Les bactériophages : comment ces virus alliés fonctionnent-ils ?

Les bactériophages sont des virus qui infectent spécifiquement les bactéries. Le dicton « *les ennemis de nos ennemis sont nos amis* » pourrait dès lors s'appliquer à l'utilisation thérapeutique des bactériophages pour combattre les bactéries résistantes aux antibiotiques. En ligne de mire, l'éradication de maladies bactériennes à l'origine d'épidémies ou orphelines de traitement.

les auteurs

Nicolas Dufour[1,2], Anne Chevallereau[1,3] et Laurent Debarbieux[1]

1 Institut Pasteur, Unité de Biologie moléculaire du Gène chez les extrêmophiles, Département de microbiologie, Paris

2 AP-HP, Hôpital Louis Mourier, Service de Réanimation médico-chirurgicale, Colombes

3 Université Paris Diderot, Sorbonne Paris Cité, Cellule Pasteur, Paris

Les bactériophages sont omniprésents dans les écosystèmes microbiens, des plus vastes – les océans – aux plus réduits – le tube digestif d'un insecte microscopique, par exemple. Nous-mêmes en hébergeons des quantités impressionnantes, aussi bien à la surface de notre peau que dans notre appareil digestif. Les bactériophages sont ainsi les entités les plus abondantes et les plus diversifiées sur la Terre. La quantité estimée de 10^{30} bactériophages est un chiffre qui donne le vertige lorsqu'on le compare à celui de toutes les cellules constituant les quelque 7 milliards d'humains, soit « seulement » 10^{22} cellules ! En termes de diversité, plus difficile à appréhender, on estime qu'il y aurait au moins 100 millions de génomes de bactériophages dans la nature (1). Cette diversité est à mettre en parallèle avec la très grande spécificité des bactériophages, qui font d'eux des virus capables d'infecter seulement un nombre restreint de bactéries au sein d'une même espèce, à quelques exceptions près. À titre de comparaison, le spectre d'hôte d'un bactériophage est beaucoup plus étroit que celui d'un antibiotique à spectre étroit comme la pénicilline. Cette étroitesse de spectre bactérien peut, dans une approche thérapeutique, être compensée par l'association de plusieurs bactériophages en cocktail, chacun possédant des spectres complémentaires et non redondants.

CLASSIFICATION

Suite aux premières observations microscopiques effectuées dans les années 1940, la classification des bactériophages s'est d'abord basée sur la morphologie des virus puis sur le type d'acide nucléique qu'ils renferment (ADN ou ARN, simple ou double brin) et la présence ou non d'une enveloppe (figure p. 32). Il est à noter que 96 % des bactériophages appartiennent à l'ordre des *Caudovirales*, des virus à ADN double brin non enveloppés. D'une taille variable – de 25 à 800 nanomètres sur l'axe tête-queue –, leur organisation générale comporte : une tête (capside) à symétrie icosaédrique, à l'intérieur de laquelle est protégé le génome – taille variant de 17 à 498 kilobases – ; une queue de longueur variable – réduite chez les *Podoviridæ*, flexible chez les *Siphoviridæ* et contractile chez les *Myoviridæ* –, issue de la polymérisation de protéines. Elle est organisée de façon tubulaire ce qui permet le transit de l'ADN viral lors de son éjection vers le cytoplasme de l'hôte infecté ; enfin, des fibres de queue, fixées à une plateforme basale, permettent aux bactériophages d'interagir avec des récepteurs spécifiques présents à la surface de l'hôte qui reconnaissent précisément le type de bactérie dont ils sont les prédateurs.

Les principaux bactériophages modèles, dont l'étude a permis l'éclosion et l'essor de la biologie moléculaire, appartiennent à l'ordre des Caudovirales (figure p. 33). Les *Podoviridæ*, dont le bactériophage T7 est le représentant, sont les moins représentés au sein des *Caudovirales* (10 %). Les *Myoviridæ* représentent environ 25 % des phages et l'on

REVIEW

Bacteriophage 1:1, 31-45; January/February 2011; © 2011 Landes Bioscience

Phages in nature

Martha R.J. Clokie,[1,*] Andrew D. Millard,[2] Andrey V. Letarov[3] and Shaun Heaphy[1]

[1]Department of Infection, Immunity and Inflammation; Medical Sciences Building; University of Leicester; Leicester, UK; [2]School of Life Sciences; University of Warwick; Coventry, UK; [3]Winogradsky Institute of Microbiology RAS; Moscow, Russia

Key words: bacteriophage, ecology, cyanophages, archaeal viruses, animal microbiome

Bacteriophages or phages are the most abundant organisms in the biosphere and they are a ubiquitous feature of prokaryotic existence. A bacteriophage is a virus which infects a bacterium. Archaea are also infected by viruses, whether these should be referred to as 'phages' is debatable, but they are included as such in the scope this article. Phages have been of interest to scientists as tools to understand fundamental molecular biology, as vectors of horizontal gene transfer and drivers of bacterial evolution, as sources of diagnostic and genetic tools and as novel therapeutic agents. Unraveling the biology of phages and their relationship with their hosts is key to understanding microbial systems and their exploitation. In this article we describe the roles of phages in different host systems and show how modeling, microscopy, isolation, genomic and metagenomic based approaches have come together to provide unparalleled insights into these small but vital constituents of the microbial world.

Introduction

We live in a microbial driven world that only exists because Bacteria and Archaea tempered the previously hostile environment on early Earth to create atmospheric conditions that allow eukaryotic life forms to flourish. Bacterial and archaeal encoded enzymes catalyze all the major processes involved in global biogeochemical cycling, playing key roles in the carbon and nitrogen cycles, and producing approximately half of the oxygen in the Earth's atmosphere.[1] In macro-organisms (animals) prokaryotic cells generally outnumber eukaryotic cells, where they assist in important aspects of survival such as nutrition and defense. So what roles are phages playing in this microbial mix? Once ignored, it is now becoming increasingly accepted that phages play key roles in the biology of microbes, which themselves impact environments at large.[2-4] Many previous excellent reviews have highlighted the importance of bacteriophages in specific environments for example.[5-7] In this article we present three case studies to illustrate how an appreciation of the roles of the viruses is pertinent to understanding microbial physiology, population dynamics and evolution. We show how our microbial driven world is tempered by bacteriophages. To contextualize the case studies we summarize the history of phage research and give an introduction to the biology of bacteriophages. We review their distribution and describe how they are enumerated and characterized. Finally we discuss the ways in which phages may influence their host's evolution and population dynamics.

*Correspondence to: Martha R.J. Clokie; Email: mrjc1@le.ac.uk
Submitted: 10/28/10; Revised: 01/17/11; Accepted: 01/18/11
DOI:

Brief history of bacteriophage discovery and research. Bacteriophages were first discovered in 1915 by William Twort, and in 1917 by Felix d'Herelle realized that they had the potential to kill bacteria. After a pre-antibiotic era heyday they were then essentially disregarded as significant therapeutic agents in the West, primarily due to the comparative ease by which antibiotics could be administered. Research and the practice of using bacteriophages did continue in some countries such as Georgia (as part of the former USSR), where they were, and continue to be routinely isolated and used to treat a large number of diseases.[8] Bacteriophage research then focused on a number of model phages which primarily infected *E. coli*. These studies provided the back-bone of modern molecular biology, for example phages were used to identify the basis of genetic material, and that 3 nucleotides code for an amino acid.[9] They also allowed the identification of restriction enzymes.[9] For several decades, only a handful of phages were studied in great detail. The recent renaissance seen in phage biology has been triggered due to a growing awareness of the number of phages in all bacterial dominated environments (as revealed by epiflourescent and electron microscopy, and from molecular studies), and indeed in the genomes of bacteria following whole genome sequencing projects. This checkered history has resulted in a patchy knowledge of phage biology but with enough observations for scientists to realize that phages are dictating many aspects of Bacterial/Archaeal biology. These observations have invigorated an invigorated interested in bacteriophages, and are part of the stimulation for this journal *Bacteriophage*, in which this article is written to illustrate the roles that bacteriophages play in the natural world.

Phage life cycles. In order to appreciate the roles of phages in nature, an understanding of their possible interactions with their hosts is necessary. Phages have various possible life cycles which, along with interaction with their physical environment, dictate their role in bacterial/archaeal biology. The lytic life cycle is where phages infect and rapidly kill their infected host cells, thereby shaping bacterial population dynamics and occasionally assisting in their long term evolution via generalized transduction.[2-4] The lysogenic life cycle in contrast, is where phages instead of directly killing their hosts, integrate into their host genome, or exist as plasmids within their host cell.[10] This lysogenic life cycle can be stable for thousands of generations and the bacteriophage may

AEM Journals.ASM.org

Targeting *Enterococcus faecalis* Biofilms with Phage Therapy

Leron Khalifa,[a,b] Yair Brosh,[a,b] Daniel Gelman,[a] Shunit Coppenhagen-Glazer,[a] Shaul Beyth,[c] Ronit Poradosu-Cohen,[d] Yok-Ai Que,[e] Nurit Beyth,[b] Ronen Hazan[a,f]

Faculty of Dental Sciences[a] and Department of Prosthodontics,[b] Hebrew University-Hadassah School of Dental Medicine, Jerusalem, Israel; Department of Orthopedic Surgery, Hebrew University-Hadassah Medical Center, Jerusalem, Israel[c]; Department of Infectious Diseases of Sourasky Medical Center and Tel-Aviv University, Tel-Aviv, Israel[d]; Department of Intensive Care Medicine, Lausanne University Hospital, Lausanne, Switzerland[e]; The Israeli Institute for Advanced Research, Rehovot, Israel[f]

Phage therapy has been proven to be more effective, in some cases, than conventional antibiotics, especially regarding multidrug-resistant biofilm infections. The objective here was to isolate an anti-*Enterococcus faecalis* bacteriophage and to evaluate its efficacy against planktonic and biofilm cultures. *E. faecalis* is an important pathogen found in many infections, including endocarditis and persistent infections associated with root canal treatment failure. The difficulty in *E. faecalis* treatment has been attributed to the lack of anti-infective strategies to eradicate its biofilm and to the frequent emergence of multidrug-resistant strains. To this end, an anti-*E. faecalis* and *E. faecium* phage, termed EFDG1, was isolated from sewage effluents. The phage was visualized by electron microscopy. EFDG1 coding sequences and phylogeny were determined by whole genome sequencing (GenBank accession number KP339049), revealing it belongs to the *Spounavirinae* subfamily of the *Myoviridae* phages, which includes promising candidates for therapy against Gram-positive pathogens. This analysis also showed that the EFDG1 genome does not contain apparent harmful genes. EFDG1 antibacterial efficacy was evaluated *in vitro* against planktonic and biofilm cultures, showing effective lytic activity against various *E. faecalis* and *E. faecium* isolates, regardless of their antibiotic resistance profile. In addition, EFDG1 efficiently prevented *ex vivo E. faecalis* root canal infection. These findings suggest that phage therapy using EFDG1 might be efficacious to prevent *E. faecalis* infection after root canal treatment.

Enterococcus faecalis is a commensal Gram-positive microorganism inhabiting the gastrointestinal tract. Nonetheless, it can cause life-threatening infections such as endocarditis (1), bacteremia (2), urinary tract infection, and meningitis (3), and it appears especially in hospitals where resistance to antibiotics is developed (4). In addition, *E. faecalis* is frequently recovered from secondary persistent infections associated with root canal treatment failures (5, 6) that can result in invasion to the tissues surrounding the tip of the tooth-root (periradicular tissue) with subsequent development of abscesses and diffused infections (cellulitis) (7). Moreover, despite meticulous mechanical preparation during root canal treatment, infection may persist in 20 to 33% of the root canals (8). The frustrating rates of posttreatment disease are mainly attributed to the limitations of the present technologies, which offer no tools to combat intracanal *E. faecalis* biofilm infection (5, 6).

Biofilms may pose a severe health threat, since at this phase bacteria not only become inaccessible to antibacterial agents and the body's immune system but also provide a reservoir of bacteria for chronic infections throughout the body (9). Most biofilm-associated infections, such as implant-related infections (10), oral infections (11), device-related infections, and chronic infections (such as lung infections in cystic fibrosis patients) (12) are treated today using antibiotics, for lack of a better alternative. The extensive use or misuse of antibiotics has led to an alarming emergence of virulent, antibiotic-resistant pathogenic bacteria (13). Moreover, it is well established that attacking mature biofilms with conventional antibiotics works poorly, requiring much higher drug doses than usual (9). The penetration failure may be associated with various factors, including the extracellular sheath, multidrug resistance development of bacteria within the biofilm (14, 15), cell cluster mode of action (16, 17), and "bet-hedging" strategies in bacterial cultures such as programmed-cell-death that provide nutrients for the community and DNA for the biofilm matrix (18). This challenge calls for different measures of antimicrobial protection: one that delivers an antimicrobial agent to incapacitate biofilm-forming bacteria and one that prevents the proliferation of bacteria in biofilms. Consequently, the development of new antimicrobial agents has become paramount (14).

One alternative recently regaining interest is bacteriophage (phage) therapy (19, 20), which was first introduced by Felix d'Herelle at the beginning of the 20th century. Historically, it was successfully used in western countries (21, 22) and abandoned with the emergence of antibiotics. Nonetheless, it is in use until today in eastern European countries (21, 23). The key benefits of phage therapy (24) are as follows: (i) their relative specificity, which is less likely to impact the commensal flora; (ii) their ability to multiply at the infection site and disappear together with the pathogen; (iii) their efficacy against biofilms; and (iv) being natural products, they are likely to be devoid of apparent toxicity. Ironically, because antibiotics were considered to be wonder

Received 12 January 2015 **Accepted** 30 January 2015
Accepted manuscript posted online 6 February 2015
Citation Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradosu-Cohen R, Que Y-A, Beyth N, Hazan R. 2015. Targeting *Enterococcus faecalis* biofilms with phage therapy. Appl Environ Microbiol 81:2696–2705. doi:10.1128/AEM.00096-15.
Editor: H. L. Drake
Address correspondence to Ronen Hazan, ronenh@ekmd.huji.ac.il.
N.B. and R.H. contributed equally to this article.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.00096-15.

doi:10.1128/AEM.00096-15

Microbiology (2013), **159**, 1023–1035 DOI 10.1099/mic.0.067116-0

Genetic modifications to temperate *Enterococcus faecalis* phage ϕEf11 that abolish the establishment of lysogeny and sensitivity to repressor, and increase host range and productivity of lytic infection

H. Zhang,[1,2] D. E. Fouts,[3] J. DePew[3] and R. H. Stevens[1,2]

Correspondence
R. H. Stevens
rstevens@dental.temple.edu

[1]Department of Endodontology, Temple University Kornberg School of Dentistry, Philadelphia, PA 19140, USA

[2]Laboratory of Oral Infectious Diseases, Temple University Kornberg School of Dentistry, Philadelphia, PA 19140, USA

[3]Genomic Medicine, J. Craig Venter Institute, Rockville, MD 20850, USA

Received 15 February 2013
Accepted 3 April 2013

ϕEf11 is a temperate bacteriophage originally isolated by induction from a lysogenic *Enterococcus faecalis* strain recovered from an infected root canal, and the ϕEf11 prophage is widely disseminated among strains of *E. faecalis*. Because *E. faecalis* has emerged as a significant opportunistic human pathogen, we were interested in examining the genes and regulatory sequences predicted to be critical in the establishment/maintenance of lysogeny by ϕEf11 as a first step in the construction of the genome of a virulent, highly lytic phage that could be used in treating serious *E. faecalis* infections. Passage of ϕEf11 in *E. faecalis* JH2-2 yielded a variant that produced large, extensively spreading plaques in lawns of indicator cells, and elevated phage titres in broth cultures. Genetic analysis of the cloned virus producing the large plaques revealed that the variant was a recombinant between ϕEf11 and a defective ϕFL1C-like prophage located in the *E. faecalis* JH2-2 chromosome. The recombinant possessed five ORFs of the defective ϕFL1C-like prophage in place of six ORFs of the ϕEf11 genome. Deletion of the putative lysogeny gene module (ORFs 31–36) and replacement of the putative *cro* promoter from the recombinant phage genome with a nisin-inducible promoter resulted in no loss of virus infectivity. The genetic construct incorporating all the aforementioned ϕEf11 genomic modifications resulted in the generation of a variant that was incapable of lysogeny and insensitive to repressor, rendering it virulent and highly lytic, with a notably extended host range.

INTRODUCTION

ϕEf11 is a temperate bacteriophage that was induced from a lysogenic root canal isolate of *Enterococcus faecalis* (Stevens *et al.*, 2009). It is a member of the family *Siphoviridae*, with a long (130 nm) non-contractile tail and a small (41 nm diameter) spherical/icosohedral head. The phage produces small, turbid plaques in lawns of *E. faecalis* JH2-2. The ϕEf11 DNA has been sequenced and annotated, disclosing a genome of 42 822 bp encoding 65 ORFs (Stevens *et al.*, 2011). Furthermore, our previous studies disclosed that the ϕEf11 DNA restriction pattern produced with certain restriction enzymes, such as *Nsi*I, produced several fragments in submolar amounts (Stevens *et al.* 2009). This would be expected to occur in the case of a circularly permuted genome due to headful packaging of a concatemeric phage DNA during viral maturation.

The ϕEf11 host species, *E. faecalis*, and closely related species, such as *Enterococcus faecium*, have emerged as significant human pathogens, being major aetiological agents of infectious endocarditis, nosocomial infections, burn infections, urinary tract infections, meningitis and surgical wound infections (Lewis & Zervos, 1990; Moellering, 1992; Megran, 1992; Emori & Gaynes, 1993; Jett *et al.*, 1994; Edgeworth *et al.*, 1999; Richards *et al.*, 2000; National Nosocomial Infections Surveillance System, 2004; Biedenbach *et al.*, 2004; Linden, 2007). In terms of oral disease, *E. faecalis* is the most commonly isolated species from infected root canals of teeth that fail to heal following root canal therapy (Sundqvist *et al.*, 1998; Peciuliene *et al.*, 2000; Pinheiro *et al.*, 2003; Siqueira & Rôças, 2004; Stuart *et al.*, 2006; Zoletti *et al.*, 2006).

One supplementary table and five supplementary figures are available with the online version of this paper.