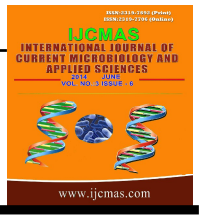
Int.J.Curr.Microbiol.App.Sci (2014) 3(6) 437-442

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 6 (2014) pp. 437-442**
http://www.ijcmas.com



Original Research Article

Bacteriophage Therapy: A possible new alternative for oral diseases

Sonia Bhonchal Bhardwaj*

Department of Microbiology, Dr Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh, India
Corresponding author

Keywords

Bacterio-phages, Phage therapy, dental plaque, caries

A B S T R A C T

Bacteriophages are viruses that attack bacteria. Reports indicate that appropriate administration of living phages can be used to treat lethal infectious diseases caused by gram- negative bacteria, such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio vulnificus and Salmonella* spp and gram positive bacteria such as *Enterococcus faecium* and *Staphylococcus aureus*. Bacteriophages specific for *Enterococcus faecalis, Streptococcal* phage lytic for *Streptococcus mutans* and recently phage to *Streptococcus salivarius* have been isolated. The isolation and identification of lytic bacteriophages to oral pathogens could be considered as approach towards phage therapy of dental plaque and caries.

Introduction

Bacteriophages are viruses that attack bacteria. Phage therapy, a method using phages for the treatment of bacterial infectious diseases was introduced by Felix'D Herelle, who co discovered phages in 1920.[1] However , with the arrival of the the antibiotic era in the 1940's it got suppressed, but the ongoing evolution of bacterial multidrug resistance, the potential of phage therapy is again being evaluated.

Advantages of using Phage Therapy

The advantages of using phage therapy (2-8) are ;

1) They are extremely specific to their target bacteria and won't affect other
2) cells. This is especially useful if patients are allergic to antibiotics because phages will not affect any other cells than the ones targeted for infection .
3) Phages are known to outnumber bacteria 10:1, they can overpower bacteria and stop them from dividing and continuing to infect the host.
4) Phages mutate at a higher rate than bacteria and are able to respond fast to possible phage- resistant bacteria.
5) The cost of developing a phage system is cheaper than that of developing a new antibiotic.
6) Phages or their products do not have any side effects.

Effect of Phages on Bacteria

The first step of phage infection is adsorption to the receptor on the bacterial surface. After adsorption, phage DNA is injected into the bacterial cytoplasm, the DNA is replicated and synthesized, multiple copies of DNAs are then taken into the capsid which is constructed de novo during the late stage of phage infection. The progeny phages are released and the descendant phages infect the neighbouring bacteria in quick succession. Even if the number of phages is less than that of bacteria, the number of phages will exceed that of bacteria after several generations, the entire bacterial population will eventually lyse.[9]

A recent study concluded by Laano *et al*,[10] shows that bacterial virulence seems to be dependent on the structure, shape and motility of the colony and phages are only able to successfully decrease the virulence when the colony is in the proper morphotype formation that is the stability of the bacterial colonies affected their abilities to resist the phage.

Phage Therapy

The effectiveness of phage therapy against bacterial infectious diseases has accumulated since 1980, when Smith *et al*[11] undertook rigorous investigations into phage therapy for pathogenic *Escherichia coli* infections in a veterinary context. Subsequently phage efficiency has been proved against experimental infections by *Escherichia coli* [12],*Pseudomonas aeruoginosa* [13], *Klebsiella Pneumoniae* [14], *Enterococcus faecium*[15], *Vibrio Vulnificus* [16] *and Salmonella spp* [17], *Staphylococcus aureus* [18] in animal models.

Isolation of Bacteriophages from oral cavity

Bacteriophages have been isolated from the oral cavity although there are limited number of reports. A bacteriophage infecting *Lactobacillus casei* has been obtained from the oral material [19],bacteriophages specific for species of Veillonella species, were isolated by Hiroki *et al*[20]. Phages lytic for actinomyces spp were isolated by Tylenda *et al*[21] from dental plaque specimens and virus specific for Actinobacillus actinomycetomcomitans have been described[22]. Delisle and Rotkowski,[23] have described bacteriophage lytic for *Streptococcus mutans*. Bahrach *et al*[24] tried to isolate bacteriophages for Gram positive oral pathogens such as *Streptococcus sobrinus, Streptococcus mutans* and *Streptococcus salivarius* from human saliva but found only bacteriophage for *Enterococcus faecalis*. Hitch *et al*[25] isolated bacteriophages from oral cavity but they obtained phages specific for non oral bacteria such as *Proteus mirabilis* but did not find any phage specific for oral pathogenic bacteria.

Nelson *et al* [26] reported the genome sequence of C1 as the first Streptococcal phage. The complete genome sequence of *Streptococcus mutans* lytic bacteriophage M102, has been revealed [27]. Temperate bacteriophages harbored by *Enterococcus faecalis* strains have been isolated from root canals of teeth in patients undergoing retreatment following unsuccessful endodontic therapy [28]. This study also demonstrated lysogenic bacterial strains and their inducible viruses in infected root canals.

A recent study[29] describes the isolation of a new phage from *Neisseria meningitides* strain (WUE 2594) which were attained from 3 healthy plaque samples. The study highlights that phages are present in dental plaques of healthy individuals and that they could potentially provide a selective bacterial therapy against their host species Neisseria[31]. A study by David T Pride *et al*[30] has identified a vast majority of human salivary viruses as viruses of bacteria, having integrase homologs suggesting a predominant role in lysogeny. They have found the viruses of Veillonella, Streptococcus and Megasphaera also suggesting they might exist as prophages in the respective hosts, supporting the presence of lysogenic viruses in the community.

Bacteriophage Therapy in Dentistry

Bacteriophages lytic for a range of oral bacteria may be considered as a potential resource of bacteriophage therapy[32] of oral infection. A study by Paisano *et al*[33] on in vitro antimicrobial effect of bacteriophages on human dentin infected with *Enterococcus faecalis* ATCC 29212 showed that addition of phage lysate to the roots following the 6 day incubation period led to a substantial reduction in bacteria viability. They concluded that phage therapy may be an important alternative for the treatment of root canal infections refractory to conventional endodontic therapy. Keivan *et al*,[34] have identified the *Streptococcus salivarius* bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy. They have isolated a lytic bacteriophage from Persian Gulf that attached specifically to *Streptococcus salivarius*, a member of dental caries producing Streptococci. The identification of new lytic phages capable to eliminate oral streptococci, starters of dental plaque formation, could be considered as a powerful approach for phage therapy of oral pathogenic bacteria. Recently the efficacy of bacteriophage treatment on *Pseudomonas aeruginosa* biofilms in a root canal model has been studied. It was seen that phage application significantly reduced the biomass of 24 hour and 96 hour PA4 biofilms grown on microplates but did not produce significant reduction of 24 hour or 96 hour PA 14 biofilms grown in the extracted tooth model [35.]

Development of Therapeutic Phages

A newly isolated phage needs to be examined in detail by 1) Genome analysis as the phage genome reflects the biological characteristics of the phage and the presence of pathogenic and lysogenic genes [36]. ii) The therapeutic effectiveness of the phage must also be tested in vivo, because phage multiplication depends on host bacterial growth (ie bactericidal effectiveness differs in vitro and in vivo.

Problems with Phage Therapy

The problems in phage therapy are the following:

1) The inactivation of administered phages or lysine by a neutralizing antibody and allergic reactions to them. Though it has not been practically seen the decrease in therapeutic effect with multiple administration.

2) Appearance of mutants resistant to phages. Resistance of bacteria to phages is often caused by changes in the phage-receptor molecules in Gram-negative bacteria. In phages of Gram-negative bacteria, host –range mutant phages, which restore the ability to adsorb to the host are easily isolated from the original phage population [37].There are very few

studies on interactions between Gram-positive bacteria and their phages , and more research is required for further development of phage therapy.

3) Another problem of phage therapy is the capture and transfer of bacterial toxin genes by phages. This problem may be overcome by selection of suitable phages that do not have natural generalized or specialized transduction abilities or by construction of genetically modified mutant phages against such phages [38].

4) Issues with quorum sensing and phage uptake in bacterial cells: A problem involved with phage therapy usage on bacterial colonies is the bacterial ability to sense its environment and change itself accordingly. Quorum sensing enables bacteria to sense local population densities and switch their patterns of gene expression appropriately. The use of quorum–sensing to regulate anti-phage activities allows bacteria to improve their defense mechanisms and avoid infection during growth in competitive conditions [39].In a system modeled using phages against an *Escherichia coli* host by Kroghsbo *et al*, it was found that *Escherichia coli* cells avoided infection by phages much more when they were grown in cultures containing AHLs (signaling molecules assosciated with quorum sensing) than when they were not grown with AHLs. The AHL treatment of bacterial cells reduced the phage adsorption rate and allowed bacterial cells to prosper , it did not have any effect on the adsorption rate of phage in the Sid A receptor mutant. The sid A receptor mutant did not allow the AHL signals to resist phage adsorption and this resulted in less free phages in the sample and more uptake of phages in the *E.coli* cells.

Phages have been proven to infect vulnerable bacterial colonies and decrease their number when administered in laboratory settings. Phages are advantageous and unique in their abundance over bacterial cells and their abilities to target specific bacteria that have receptors on their cell surfaces. Phages can overcome the barriers that bacterial cells use to resist their adsorption if the bacterial cells are genetically altered. Phages are the key to solving problems of antibiotic resistance and helping people fight disease faster. The isolation and identification of new bacteriophages capable to eliminate oral bacteria, starters of dental plaque formation could be considered as a powerful approach for phage therapy of oral pathogenic bacteria in dentistry as well as modern medical and pharmaceutical biotechnology.

References

1) Summers WC.1999. Felix d'Herelle and the origins of molecular biology. Connecticut: Yale University Press.
2) Phage therapy [editorial].1983.Lancet ;2:1287-8.
3) Carlton RM. Phage therapy : past history and future prospects. 1999.Arch Immunol Ther Exp ; 5 :267-74.
4) Pirisi A. 2000.Phage therapy-advantages over antibiotics? Lancet ;356: 1418.
5) Stone R .2002. Bacteriophage therapy. Stalin's forgotten cure. Science ; 298:728-31.
6) Thacker PD.2003. Set a microbe to kill a microbe: drug resistance renews interest in phage therapy. JAMA ;290:3183-6.

7) Bradbury J.2004. "My enemys enemy is my friend". Using phages to fight bacteria .Lancet ;363:624-5.
8) Ghannad,M and Mohammadi, A.2012. "Bacteriophage: Time to reevaluate the potential of phage therapy as a promising agent to control multidrug resistant bacteria." Iranian journal of basic medical sciences ;15,2;693-707.
9) Wang IN, Smith Dl, Young R. 2000.Hollins : the protein clocks of bacteriophage infections. Annu Rev Microbiol ;54: 799-825.
10) Laanto *et al*.2012.Phage driven loss of virulence in a fish pathogenic bacterium. Plos one7:12, 1-8.
11) Smith HW, Huggins MB. 1983.Effectiveness of phages in treating experimental *Escherichia coli* diarrhea in calves , piglets and lambs ;129:2659-75.
12) Chibani- Chennoufi S, Sidoli J, Brutten A, Kulter E, Sarker S,Burrow H.2004. In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: implications for phage therapy. Antimicrob Agents Chemother ;48: 2558-69.
13) Ahmad SI. 2002.Treatment of post burns bacterial infections by bacteriophages, specifically ubiquitous Pseudomonas spp notoriously resistant to antibiotics . Med Hypotheses ; 58:327-31.
14) Bogovazova GG, Voroshilova NN, Bondarenko VM.1991. The efficacy of *Klebsiella pneumoniae* bacteriophage in the therapy of experimental Klebsiella infection. Zh Mikrobiol Epidemiol Immunobiol :4; 5-8.
15) Biswas B, Adhya S, Washart P, Paul B, Testel AN, Powell B *et al*. 2002.Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin resistant *Enterococcus faecium*. Infect Immun ;70;204-10.
16) Cerveny KE, De Paola A, Duckworth DH, Gulig PA.2002. Phage therapy of local and systemic disease caused byy Vibrio Vulnificus in iron dextran treated mice. Infect Immun ; 70;6251-62.
17) Toro H, Price SB, McKee AS,Hoerr FJ, Krehling J, Perdue M, *et al*. 2005.Use of bacteriophages in combination with competitive exclusion to reduce Salmonella from infected Chickens. Avian Dis ;49: 118-24.
18) Noble WC.1998. Staphylococcal diseases .In : Microbiology and microbiology infections. Vol 3. New York: Oxford University Press ;231-56.
19) Meyer , C.E; Walters ,E.L and Green ,L.B.1958. Isolation of a bacteriophage specific for a *Lactobacillus casei* from human oral material. Journal of Dental Research; 37,175-178.
20) Hikoki, H; Shiki, A; Totsuka,M and Nakamura, O.1976. Isolation of bacteriophages specific for the genus Veillonella. Archives of Oral Biology ;27,261-268.
21) Tyrendra, C; Calvert, C; Kolenbrander, PE and Tylendra, A.1985. Isolation of actinomyces bacteriophage from human dental plaque; Infection and Immunity 49,1-6.
22) Olsen, I; Namork, E and My Hrvold,V.1993. Electron microscopy of phages in serotypes of *Actinobacillus actinomycetemcomitans*. Oral Microbiology and Immunology ;8,383-385.
23) Delisle, A.L and Rotkowski, C.A. 1993.Lytic bacteriophages of *Streptococcus mutans* .Current Microbiology ;27,163-167.
24) Bachrach ,G; Leizerovick Zigmund, M; Zlotkin, A; Naor, R and Steinberg,

D.2003. Bacteriophage isolation from human saliva. Letters in Applied Microbiology ;36, 50-53.

25) Hitch, G; Pratten, J and Taylor, P.W.2004. Isolation of bacteriophages from the oral cavity . Letters in Applied Microbiology;39,215-219.
26) Nelson D, Schuch R, Zhu S, T Scherne DM. 2003.Genomic sequence of C1, the first Streptococcal phage.J Bacteriol;185:3325-3332.
27) Van der Ploeg JR. 2007.Genome sequence of Streptococcus Mutans bacteriophage M102. FEMS Microbiol Lett ;275: 130-138.
28) Stevens RM,Porras OD, Delisle AL. 2009.Bacteriophages induced from lysogenic root canal isolates of Enterococcus faecalis. Oral Microbiol Immunol ; 24(4):278-84.
29) Ahmed Aljarbou N, Anthony De Luca and Mohamad Aljofan.2012.Isolation of a new Neisseria phage from the oral cavity of healthy humans ; Scientific Reports ;1:416.
30) Pride DT, Salzman J, Haynes M, Rohwer F, Long C D, White III R A, Loomar P, Armitage G C, Relman D A. 2012.Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. The ISME Journal ;6:215 - 226.
31) Al-Jarbau AN . 2011.Genomic library screening for viruses from the human dental plaque revealed pathogen specific lytic phage sequences. Curr Microbiol ;64 :1-6.
32) Chanishvili N, Chanishvili T, Tediashvili M, Barrow PA . 2001.Review: Phages and their application against drug resistant bacteria. J. Chem.Technol.Biotech ;76:689-699.
33) Phee A, Bondy- Denomy J, Kishen A, Basrani B, Azarpazhooh A, Maxwell K. 2013.Efficacy of bacteriophage treatment on Pseudomonas aeruginosa biofilms. J Endod ; 39 (3):364-9.
34) Maal K B, Bouzari M, Zavareh F A. 2010.Identification of *Streptococcus salivarius* bacteriophage isolated from Persian gulf as a potential agent for dental caries phage therapy. African J of Microbiol Research ; 4 (20): 2127-2132.
35) Phee A, Bondy- Denomy J, Kishen A, Basrani B, Azarpazooh A,Maxwell K.2013. Efficacy of bacteriophage treatment on *Pseudomonas aeruginosa* biofilms. J Endod ;39(3):364-9.
36) Merril CR, Scholl D and Adhya S . Phage therapy. 2006.The bacteriophages , 2nd edition (Calender R & Abedon ST eds);725-741.Oxford University press, New York.
37) Drexler K,Dannull J, Hindennach I, Mutscher B, Henning U.1991. Single mutations in a gene for a tail fiber component of an Esherichia Coli phage can cause an extension from a protein to a carbohydrate as a receptor. J Mol Biol ;219:655-63.
38) Schoonik GK, Summers WC ,Watson JD.2004. Phage offer a real alternative . Nat Biotechnol ;22: 505-6.
39) Hoyland Kroghsbo,N *et al*.2013. A Quorum sensing induced bacteriophage defense mechanism. M Bio;4(1),1-8.