Chapter

Oral Bacteriophages

Sonia Bhonchal Bhardwaj and Seema Kumari

Abstract

Bacteriophage or phage therapy involves using phages or their products as bio-agents for the treatment or prophylaxis of bacterial infections or diseases. Bacteriophages have the ability to regulate the oral microflora by lysing sensitive bacterial cells and releasing bacterial components with pro-inflammatory activity. Bacteriophages carry specific polysaccharide depolymerases that aid viral penetration and can disrupt the pathogenic process associated with biofilm and exopolysaccharide in the oral cavity. Oral diseases are mainly caused by biofilm forming microorganisms and phages are now being used for biocontrol of oral biofilms. Phages for *Actinomyces* species, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Lactobacillus* species, *Neisseria* species, *Streptococcus* species, and *Veillonella* species have been isolated and characterized. Bacteriophages could be considered as potential therapeutic tools for the elimination of caries, periodontitis, and other diseases of the oral cavity.

Keywords: oral microbiome, oral phages, oral biofilms, oral diseases, bacteriophage therapy

1. Introduction

Bacteriophages are viruses that attack bacteria. Phages are now known to cure antibiotic-resistant bacterial infections as well as decrease bacterial virulence by overcoming the barriers bacteria used to avoid them. Bacteriophages are now being explored as potential therapeutic tools for the elimination of oral bacterial pathogens. Bacteriophages can disrupt pathogenic processes associated with biofilm and exopolysaccharide formation by oral microflora. Bacteriophages are a habitat to the human oral cavity where the oral pathogenic bacteria exist. Earlier studies show the isolation of oral bacteriophage from the oral cavity when an oral bacteriophage infecting *Lactobacillus casei* was obtained by Meyer et al. [1]. Subsequently, a range of oral bacteriophages infecting *Veillonella* species was isolated by Hiroki et al. in 1976, lytic bacteriophages for Actinomyces species were isolated by Tylenda et al. in 1985, oral bacteriophages specific for *Actinobacillus actinomycetocomitans* were described by Olsen et al. in 1993, oral bacteriophages specific for *Streptococcus mutans* were isolated by Delisle and Rotkwoski in 1993, and bacteriophages specific for *Enterococcus faecalis* were by Bachrach in 2003 [2–6]. Metagenomic analysis estimates 10^8–10^{10} virus-like particles existing per ml of human saliva and per gram of dental plaque [7]. The isolation studies for oral phages have been challenging, where phages have been obtained from clinical (saliva, plaque, oral washings) and environmental samples. The bacteriophages for oral bacteria implicated in various oral diseases have been described in the following section. The phages

for the oral bacteria Actinomyces, Aggregatibacter, Fusobacterium, Parvimonas, Porphyromonas, Prevotella intermedia, *E. faecalis*, *S. mutans*, *Treponema denticola* are described here.

2. Actinomyces bacteriophages

Actinomyces species are found in healthy mouth but are also implicated in oral abcesses and oral-facial actinomycosis. Actinomyces, together with streptococci, initiates the biofilm development and formation of dental plaque [8]. Bacteriophages are used to block this co-aggregation to reduce the biofilm development without reducing health-related Actinomyces, which is part of the oral microbiome. The most commonly studied Actinomyces phage was AV-1, but it had a very narrow host range [9]. However, when the phage AV-1 was combined with AV-11, they lysed most of the indicator strains used for Actinomyces studies [10]. Actinomyces phages probably use surface structures of streptococci as receptors. These phages are from the families' Siphoviridae (61%) and Podaviridae (11%) [11, 12].

3. Aggregatibacter bacteriophages

Aggregatibacter is the causative agent of localized aggressive periodontitis. Aggregatibacter phages are mostly temperate phages and easy to isolate. Engineered Aggregatibacter bacteriophages that release biofilm degrading enzymes like dispersion B to breakdown biofilm have been used against periodontitis causing *Aggregatibacter actinomycetemcomitans* [13]. ϕAa 17 and Aaϕ23 are the most extensively studied Aggregatibacter phages [14]. These Aaϕ phages have a relatively broad host range. The limitation of these Aggregatibacter phages is that they can transfer antibiotic resistance genes, which are acquired macrolide lincosamide streptogramin B (MLS) resistance genes such as erm (A), erm (B), erm (C), erm (F), and erm (Q), and induce serotype conversion and release of leukotoxin [15]. In a recent study, it has been seen by metagenomics analysis that Aggregatibacter phages preferably lysogenize specific phylogenetic lineages not correlating with specific clinical conditions. They have either a very narrow host range or a broad host range [16]. The clinical conditions/impact in which these phages are used remains unknown.

4. Enterococcus bacteriophages

E. faecalis is one of the most frequently isolated species from nosocomial infections, endocarditis, bacteremia, urinary tract infections, meningitis, systemic infections. It has also been reported in periodontitis, which is a biofilm-mediated disease, tooth root infections, which are an example of endodontic biofilms, and also on implants. *E. faecalis* bacteriophages isolated belong to myoviridae and siphoviridae and are tailed phages. The bacteriophages isolated against *E. faecalis* strain of oral origin include phage IME-EF1 when administered intraperitoneally in a murine sepsis model protected the mice from lethal challenge around 60 to 80% mice surviving [17]. Another phage φEF 24C protected the BALB/C mouse model from the lethal challenge of *E. faecalis* [18]. Another phage EFDG1 tested on *E. faecalis* biofilms of post-treated root canal infections using an *ex vivo* two-chamber bacterial leakage model of human teeth showed dead bacteria in phage-treated teeth as compared to dentinal tubules of the control group [19]. The genetics of

three phages ϕEF11, EFDG1, and EFLK1 has been studied by genome sequencing [20]. Full-genome sequencing of the EFDG1 genome revealed that it did not contain harmful genes and also efficiently prevented *E. faecalis* infection after root canal treatment. The authors concluded that phage therapy using these phages might be efficacious to prevent *E. faecalis* infection after root canal treatment. *E. faecalis* has also been recovered from periodontal pockets in 1–51.8% of chronic periodontitis patients [21]. In our recent study, a novel *E. faecalis* bacteriophage was isolated from sewage and was found effective in reducing biofilms formed by drug-resistant clinical isolates of *E. faecalis* from chronic periodontitis patients [22]. Passage of phage ϕ EF11 through *E. faecalis* strains JH2–2 harboring a defective prophage produced a new strain with more antimicrobial efficacy [23]. The use of enterococci bacteriophages can probably control colonization of teeth surfaces by reducing the biofilm in chronic periodontitis. The application of bacteriophages as a strategy to conventional antibiotic treatment particularly in the case of biofilm and multidrug-resistant strains is promising.

5. Streptococcus bacteriophages

The most important species that play a key role in dental plaque formation are oral Streptococci. The oral streptococci mainly constitute 12 species including *Streptococcus salivarius*, *S. agnisosus*, *S. mutans*, *S. constellates*, *S. cristareus*, *Streptococcus gordonii*, *S. mitis*, *Streptococcus oralis*, *S. parasanguis*, *Streptococcus pneumoniae*, *S. sanguis*, *S. sobrinus*. The initial colonizers of the tooth are *S. salivarius*, *S. sanguis*, *S. oralis*, and *S.gordonii*; however, *S. sobrinus* and *S. mutans* are more involved in dental infections [24]. Initial studies reported the isolation of lytic bacteriophages from human saliva [25]. The complete genome sequence of *S. mutans* lytic bacteriophage M102 was revealed [26]. In 2008, Van de Ploeg reported the complete genome sequence of prophage 15 infecting *S. gordonii*, which was a lysogenic phage [24]. A diverse group of around 50 bacteriophages that infect *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius*, and *S. sobrinus* have been identified and reported [27]. Unlike the *S. mutans* phages that are seen as lytic phages, the phages for *S. mitis* have been found as temperate. These temperate phages have the property to transfer host DNA into other bacterial strains. Seven phage-related gene clusters were detected in the genome of *S. mitis* B6, SM1, and ϕB6 prophages were isolated and sequenced [28]. Virulent pneumophages DP-1 and CP-1 were able to infect *S. mitis* and are also able to infect and replicate in commensal streptococci [29]. As *S. pneumoniae* and *S. mitis* carry numerous temperate phages in their genomes they are closely related and these virulent cross-infecting streptococcal phages and their enzymes are being used to biocontrol of oral infections [30].

6. Bacteriophages for oral anaerobes

6.1 Fusobacterium bacteriophages

Fusobacterium nucleatum bacteriophages have been isolated from saliva samples [31]. Siphovirus Fnpϕ02 could target three subspecies of *F. nucleatum*, *F. vincentii*, and *F. polymorphum*. The second phage Fnpϕ02 was rapidly absorbed on the cell surface but slow lysis was observed. In another study, non-infective phages were obtained by mitomycin C treatment of *F. nucleatum* [32]. The full-genome sequence and functional characterization of a novel lytic bacteriophage FNu1 against *F. nucleatum* which can break down oral biofilms have been reported recently [33].

6.2 Porphyromonas, prevotella, and tannerella

Prevotella phages have been detected *in vivo* [34]. Phages against *Porphyromonas gingivalis* and *Tannerella forsythia* have not been isolated so far. *P. gingivalis* that is an important anaerobic periodontal pathogen-causing microbial dysbiosis may protect itself in the periodontal pockets where many bacteriophages are preset by CRISPR-CAS systems providing it adaptive immunity [35]. These CRISPR-CAS systems are the only adaptive immune system in bacteria to fight phages/viruses, plasmids, transposons, integrative conjugative elements and are also found to target undesirable bacteria in the microbiome. On invasion or exposure to foreign DNA, the spacer sequences are transcribed into small CRISPR RNAs used by Cas proteins to cleave foreign DNA thus acquiring "acquired memory" of this adaptive immune system.

6.3 Treponema

A single study has reported the isolation of Treponema phage [36]. Phage ϕtd1 that belongs to Myoviridae family was harvested from the biofilm culture of *T. denticola* and its genome was detected by polymerase chain reaction.

6.4 *Veillonella* phages

It is a non-motile gram-negative diplococci. *Veillonella* is a part of the normal flora of the mouth is also associated with oral infections. Around 25 *Veillonella* phages have been isolated from mouth wash specimens. The small plaque-forming was found to be active against *Veillonella rodentium*. The large plaque formers were active against clinical *Veillonella* spp. isolates. Virion morphology was studied only for functional phages N2, N11, and N20 [37].

6.5 Lactobacillus

Bacteriophages for the caries associated with 12 strains of Lactobacillus including *L. casei* have been isolated. They have been divided into two groups: PL-1 is a lytic phage and temperate phage phi FSW of *L. casie* ATCC27139 [38].

7. Uses of oral bacteriophages

7.1 Bacteriophages and oral biofilms

The effectiveness of oral bacteriophages has been mainly seen by the reduction in the count of viable bacteria in the oral biofilms by using them. However, the phages were not able to reduce the amount of extracellular matrix in the biofilms [39]. Another factor while using phages is the phage therapy will be partially effective if particularly if the biofilm is old. The penetration and effect of phages on multispecies oral biofilms has also not been much studied. In a study in two species of biofilm constituting of phage-resistant and phage-susceptible bacteria, it was seen that the species composition of the biofilm may modulate phage effectiveness [40]. Limited studies show the application of oral phages *in vivo* using animal models. The efficacy of oral phages formulated in thermo-sustained release system against *E. faecalis* has been studied *in vivo* using a rat model. The study showed that per-apical inflammation of the tooth was improved after phage treatment [41].

7.2 Bacteriophages in oral diseases

Bacteriophages are being isolated to bacteria causing oral infections. Bacteriophages have been isolated to both aerobic and anaerobic microorganisms associated with periodontitis. Bacteriophages also constitute the majority of periodontal viral communities [42]. This variation in bacteriophages in healthy and periodontitis patients suggests a potential for more bacteriophage exploration. The use of bacteriophages has also been done in root canal treatment but targeted mainly against *E. faecalis*. Bacteriophages have also been explored for their therapeutic role in peri-implantitis [43] and also in the healing of oral mucosal infections [44].

7.3 Bacteriophages as antibiotic adjuvants

Phages can be used as adjuvants to antibiotic therapy. Resistance developed in phages can be reduced by using a cocktail of phages or phage recombinant lysins. Now, genetically engineered phages have also been developed to tackle resistance strains [45, 46].

The use of strictly lytic phages that infect only the target bacteria without affecting the normal microflora can be used as an alternative to local or systemic antibiotic therapy. This phage-based treatment can be designed in each case favoring personalized medicine.

8. Conclusion

The oral diseases caries, periodontal diseases, periapical and endodontic lesions, perimplantitis, and oral mucosal infections are microbial in origin. Bacteriophages are useful candidates for these biofilm-mediated diseases. As antibiotic resistance has become a matter of global concern, the bacteriophages or phage therapy can be used particularly to reduce the impact of acute infections. Moreover, antibiotics have a limited effect on the biofilm and are not much useful for the treatment of oral diseases. However, few bacteriophages are not effective against degrading biofilms; therefore, enzymatic or engineered phages are being investigated. Phages are low in cost, easy to isolate, and efficient against biofilm, and are bacteria specific. Phages have a great potential to be used in the prevention, control, and therapeutics of oral infections.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Meyer CE, Walters EL, Green LB. Isolation of a bacteriophage specific for a *Lactobacillus casei* from human oral material. Journal of Dental Research. 1958;**37**:175-178

[2] Hiroki H, Shiki A, Totsuka M, Nakamura O. Isolation of bacteriophages specific for the genus *Veillonella*. Archives of Oral Biology. 1975;**27**:261-268

[3] Tylenda C, Calvert C, Kolenbrander PE, Tylenda A. Isolation of Actinomyces bacteriophage from human dental plaque. Infection and Immunity. 1985;**49**:1-6

[4] Olsen I, Namork E, Myhrvold V. Electron microscopy of phages in serotypes of *Actinobacillus actinomycetemcomitans*. Oral Microbiology and Immunology. 1993;**8**:383-385

[5] Delisle AL, Rotkowski CA. Lytic bacteriophages of *S. mutans*. Current Microbiology. 1993;**27**:163-167

[6] Bachrach G, Leizerovici-Zigmond H, Zlotkin A, Naor R, Steinberg D. Bacteriophage isolation from human saliva. Letters in Applied Microbiology. 2003;**36**:50-53

[7] Naidu M, Robbes-Sikisaka R, Abeles SR, Boehm TK, Pride DT. Characterization of bacteriophage communities and CRISPR profiles from dental plaque. BMC Microbiology. 2014;**14**:175

[8] Mark Welch JL, Rosetti BJ, Rieken CW, Dewhirst FE, Borisy GG. Biogeography of a human oral microbiome at the micron scale. Proceedings of the National Academy of Sciences of the United States of America. 2016;**113**:E791-E800

[9] Delisle A. Growth of *Actinomyces viscosus* bacteriophage AV-1 in the presence of serum, saliva and dental plaque. Microbiology Letters. 1986;**33**:107-113

[10] Yeung MK, Kozelsky CS. Transfection of Actinomyces species by genomic DNA of bacteriophages from human dental paque. Plasmid. 1997;**37**:141-153

[11] Kolenbrander PE, Palmer RJ Jr, Periaswamy S, Jakubovics NS. Oral multispecies biofilm development and the key role of cell-cell distance. Nature Reviews. Microbiology. 2010;**8**:471-480

[12] Konoen E, Wade WG. Actinomyces and related organisms in human infections. Clinical Microbiology Reviews. 2015;**28**:419-442

[13] Szafranski SP, Winkel A, Stiesch M. The use of bacteriophages to biocontrol oral biofilms. Journal of Biotechnology. 2017;**250**(5):29-44

[14] Resch G, Kuik EM, Dietrich FS, Meyer J. Complete genomic nucleotide sequence of the temperate bacteriophage Aa phi23 of *Actinobacillus actinomycetemcomitans*. Journal of Bacteriology. 2004;**186**:5523-5528

[15] Stevens RH, de Moura Martins Lobo Dos Santos C, Zuanaff D, De Accioly Mattos MB, Ferreira DF, Kaxhlany SC, et al. Prophage induction in lysogenic *Aggregatibacter actinomycetemcomitans* cells co-cultured with human gingival fibroblasts and its effect on leucotoxin release. Microbial Pathogenesis. 2013;**54**:54-59

[16] Szafranski SP, Kiliam M, Yang I, Wieden GBD, Winkel A, Hegermann J, et al. Diversity pattern of bacteriophages infecting Aggregatibacter and Haemophilus species across clades and niches. The ISME Journal. 2019;**13**:2500-2522

[17] Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradusu-Cohen R, et al. Targeting *E. faecalis* biofilms with phage therapy. Applied Environment Microbiology. 2015;**81**:2696-2705

[18] Zhang W, Miz Yin X, Fan H, An X, Zhang Z, et al. Characterization of *E. faecalis* phage IME-EF1 and its endolysin. PLoS One. 2013;**8**:e80435

[19] Uchiyama J, Rashel M, Takemura I, Wakiguch H, Matsuzaki S. In silico and in vitro evaluation of bacteriophage phiEF 24 C, a candidate for treatment of *E. faecalis* infections. Applied Environment Microbiology. 2008;**74**:4149-4163

[20] Khalifa L, Coppenhagen-Glazer S, Shlezinger M, Kolt-Gutkowski M, Adini O, Beyth N, et al. Complete genome sequence of Enterococcus bacteriophage EFLK1. Genome Announce. 2015;**3**:e01308-e01315

[21] Sun J, Song X. Assessment of antimicrobial susceptibility of *E. faecalis* isolated from chronic periodontitis biofilm vs planktonic phase. Journal of Periodontology. 2011;**82**:84

[22] Bhardwaj SB, Mehta M, Sood S, Sharma J. Isolation of novel phage and targeting biofilms of drug resistant oral enterococci. Journal of Global Infectious Diseases. 2020;**12**:11-15

[23] Zhang H, Fouts DE, De Pew J, Stevens RH. Genetic modifications to temperate *E. faecalis* phage Ef11 that abolish the establishment of lysogeny and sensitivity to repressor and increase host range and productivity of lytic infection. Microbiology. 2013;**159**:1023-1035

[24] der Ploeg V Jr. Characterization of *Streptococcus gordonii* prophage PH15: Complete genome sequence and functional analysis of phage encoded integrase and endolysin. Microbiology. 2008;**154**:2970-2978

[25] Delisle AL, Rostkowski CA. Lytic bacteriophages of *Streptococcus mutans*. Current Microbiology. 1993;**27**: 163-167

[26] der Ploeg V Jr. Genome sequence of *Streptococcus mutans* bacteriophage M102. FEMS Microbiology Letters. 2007;**275**:130-138

[27] Delisle A. Bacteriophage-encoded enzymes for the treatment and prevention of dental caries and periodontal diseases. Patent US. 2004;**0234461**:A1

[28] der Ploeg V Jr. Genome sequence of the temperate bacteriophage PH10 from *Streptococcus oralis*. Virus Genes. 2010;**41**:450-458

[29] Queannane S, Leprohon P, Moineau S. Diverse-virulent pneumophages infect *Streptococcus mitis*. PLoS One. 2015;**10**:e0118807

[30] Garcia GP, Mendez FM, Garcia LE, Duz MR, De PA, Bastamante SN. Improved bactericidal enzybiotics against Pneumococcus and other bacteria. Patent. 2014:w02014191598A1

[31] Machua P, Daille L, Vines E, Berrocal L, Bittner M. Isolation of a novel bacteriophage specific for the periodontal pathogen *Fusobacterium nucleatum*. Applied Environment Microbiology. 2010;**76**:7243-7250

[32] Cochrane K, Manson McGuire A, Priest ME, Abouelleil A, Cerqueria GC, Lo R, et al. Complete genome sequences and analysis of *Fusobacterium nucleatum* subspecies animalis 7-1 bacteriophages Funu1 and Funu2. Anaerobe. 2016;**38**:125-129

[33] Mwila K, Teagan L, Brown JT. Genomic, morphological and functional characterization of novel bacteriophage FNu1 capable of disrupting *Fusobacterium nucleatum* biofilms. Scientific Reports. 2019;**9**:9107

[34] Pride DT, Salzman J, Relman DA. Comparisons of clustered regularly interspaced short palindromic repeats and viromes in human saliva reveal bacterial adaptations to salivary viruses. Environment Microbiology. 2012;**14**:2564-2576

[35] Chen T, Olsen I. *Porphyromonas gingivalis* and its CRISPR-Cas system. Journal of Oral Microbiology. 2019;**11**(1):1638196

[36] Mitchell HL, Dashper SG, Catmull DV, Paolini RA, Cleal SM, Slakeski N, et al. *Treponema denticola* biofilm-induced expression of a bacteriophage, toxin-antitoxin systems and transposases. Microbiology. 2010;**156**:774-788

[37] Totsuka M. Studies o *Veillonella* phages isolated from washings of human oral cavity. Bullentin Tokyo Medicine Dental University. 1976;**23**:261-273

[38] Meyers CE, Walter EL, Green LB. Isolation of a bacteriophage specific for a *Lactobacillus casei* from human oral material. Journal Dental Research. 1958;**37**:175-178

[39] Castillo-Ruiz IM, Vines ED, Monlt C, Fernandez J, Delgado JM, Hormamazabal JC, et al. Isolation of a novel *Aggregatibacter actinomycetemcomitans* serotype b bacteriophage capable of lysing bacteria within a biofilm. Applied Environment Microbiology. 2011;**787**:3157-3159

[40] Gonzalez CF, Domingo-Calap P. Phages for biofilm removal. Antibiotics. 2020;**9**:268. DOI: 10.3390/ antibiotics 9050268

[41] Shlezinger M, Friedman M, Houri-haddad Y, Hazan R, Beyth N. Phages in a thermoreversible sustained release formulation targeting *E. faecalis* in vitro and in vivo. PLoS One. 2019;**14**(7):e029599. DOI: 10.1371/ journal.pone.0219599

[42] Yu Z, Shan T-L, Li F, Yu T, Chen X, Deng X-T, et al. A novel phage from periodontal pockets associated with chronic periodontitis. Virus Genes. 2019;**55**:381-393. DOI: 10.1007/ s11262-019-01658-Y

[43] Hashimoto K, Yoshinari M, Matsuzaka K, Shiba K, Inoue T. Identification of peptide motif that binds to surface of Zirconia. Dental Materials Journal. 2011;**30**:935-940

[44] Li GJ, Jiang DY, Zang X, Xu X. Keratinocyte growth factor phage model peptides can promote human oral mucosal epithelial cell proliferation. Oral Surgery Oral Medicine Oral Pathology Oral Radiology. 2013;**116**:e92-e97

[45] Santiago Rodriguez TM, Naidu M, Abeles SR, Bopehm TK, Ly M, Pride DT. Transcriptome analysis of bacteriophage communities in periodontal health and disease. BMC Genomics. 2015;**16**:549

[46] Tkhilashvili T, Wrinkler T, Muller M, Perka C, Trampuz A. Bacteriophages as adjuvant to antibiotics for the treatment of periprosthetic joint infection caused by multi-drug resistant *Pseudomonas aeruginosa*. Antimicrobial Agents Chemotherapy. 2020;**64**: e00924-e00919. DOI: 10.1128/ AAC.00924-19