



## USE OF PHAGES FOR ORAL BIOFILM DISEASES.

## Medical Microbiology

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## ABSTRACT

Oral biofilm is formed by many bacteria and contributes to various oral diseases. The excessive formation of oral biofilm results in intraoral diseases such as dental caries, gingivitis and periodontitis. To maintain oral health and prevent intraoral diseases a balance between the human host and the oral microbiome is essential. The ability of pathogenic bacteria to form a biofilm increase their resistance to antibiotics. Bacteriophages are known to cure antibiotic resistant bacterial infections. Phages for *Actinomyces* species, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Lactobacillus* species, *Streptococcus* species have been isolated and characterized. Bacteriophages could be considered as potential therapeutic tools for elimination of caries, periodontitis and other diseases of the oral cavity.

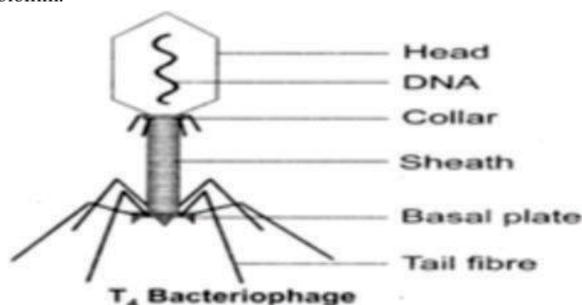
## KEYWORDS

Oral biofilm, Antibiotic- resistance, Bacteriophages, Oral diseases.

## INTRODUCTION

The oral cavity of a healthy human adult comprises of more than 700 different microbial species constituting bacteria, viruses and fungi existing as a stable ecosystem. The major genera found in the oral cavity include *Streptococcus*, *Fusobacterium*, *Gemmella*, *Veillonella*, *Granulicatella*, *Bacteroides*, *Pastuerella*, *Prevotella*, *Neisseria*, *Corynebacteria*, *Capnocytophaga*, *Rothia*, *Haemophilus*, *Eikenella*, *Leptotrichia*, *Peptostreptococcus*, *Staphylococcus*, *Eubacterium* and *Propionibacterium* (Huse *et al*2012)[1]. A large population of viruses that infect specific bacterial species present as free phages or dormant prophages called oral phageome is also a part of the oral microbiome (Szafranski *et al* 2021)[2]. The oral microbes are present on the teeth, buccal mucosa, tongue, soft and hard palates, gingival, supragingival and subgingival margins and coexist by forming polymicrobial communities called the biofilms. Any alteration in this symbiosis of the oral microflora initiates oral dysbiosis where there is a loss of community balance or diversity in the biofilm making a single or few species predominating. This oral dysbiosis in the biofilms manifests in the form of periodontal diseases, oral cancers, caries, periapical endodontic lesions (Mark Welch *et al*2016)[3]. Oral dysbiosis has been also linked to systemic diseases like cardiovascular diseases, diabetes mellitus, respiratory disease, rheumatoid arthritis, inflammatory bowel diseases (IBD), HIV infection, liver cirrhosis, colon cancer, primary sclerosing cholangitis, gastroesophageal reflux disease and Alzheimer's disease (Olsen2021)[4].

As antibiotic resistance now has become a global threat with the World Health Organization predicting that it could kill at least 50 million people every year by 2050(WHO) [5] researchers are now looking for alternative ways to manage multi-drug resistant bacterial infections. Bacteriophages are viruses that attack bacteria which can disrupt pathogenic process including exopolysaccharide formation in the biofilm.



The head tail structure is unique to bacteriophages(Fig: 1).

Phages multiply intracellularly inside the bacterial host cell. The life cycle of a bacteriophage can be lytic (causes host cell death) or lysogenic (phage DNA becomes integrated into the host cell chromosome without causing host cell death). Most of the oral phages are lytic in nature.

## Oral phages:

**Actinomyces phages:** *Actinomyces* are found in healthy human mouth but in various opportunistic conditions *actinomyces* species along with streptococci initiates the biofilm development and dental plaque formation causing intra oral diseases and oral-facial actinomycosis. *Actinomyces* phage AV-1 studied initially had a narrow host range on indicator *actinomyces* strains however, when a combination of *actinomyces* phages was used they lysed most of the indicator *actinomyces* strains (Yeung and Kozelsky1997)[6]. The *actinomyces* phages reported belong to families Siphoviridae (61%) and Podoviridae (11%) (Konoen and Wade2015)[7]. A prophage Xhp1 infecting a human oral isolate *Actinomyces odontolyticus* has also been characterized (Shen *et al*2018)[8]. This prophage has been found to promote biofilm assembly of host bacteria by release of extracellular DNA (e-DNA) which could be beneficial to human health. The genomes of *actinomyces* phages AV-1 and xhp1 have been described in National Center for Biotechnology Information (NCBI).

**Aggregatibacter phages:** *Aggregatibacter actinomycetemcomitans* causes localized aggressive periodontitis. Aa17 and Aa23, the most extensively studied aggregatibacter phages have a relatively broad host range. However, a drawback of the aggregatibacter phages is they are mainly lysogenic as they can transfer antibiotic resistance genes and induce serotype conversion (Stevens *et al*2013)[9]. Aggregatibacter phages are temperate phages that have now been engineered to release biofilm degrading enzymes like dispersin B (Szafranski *et al* 2017)[10]. A metagenomics analysis has shown that aggregatibacter phages preferably lysogenize specific phylogenetic lineages not correlating with specific clinical conditions (Szafranski *et al*2019)[11]. Aggregatibacter phages Aa23, S1249 have been characterized and their genomes are present in NCBI database.

**Enterococcus phages:** *Enterococcus faecalis* is now known to be implicated in periodontitis, tooth root infections, implantitis which are biofilm mediated diseases. Most strains of *E.faecalis* isolated from root canal and periodontitis infections show a high level of resistance to antibiotics vancomycin, tetracycline and erythromycin. The early bacteriophages isolated against *E.faecalis* strains of oral origin include phage IME-EF1, EF24 C. Both these phages protected the mice model from lethal challenges of *E.faecalis* (Khalifa *et al*2015)[12]. Another phage EFDG1 tested on *E.faecalis* biofilms of post treated root canal infections showed dead bacteria in phage treated teeth versus the control group (Uchiyama *et al*2008)[13]. A novel phage HEF13 has demonstrated high lytic activity against clinical isolates from recurrent or refractory apical periodontitis related to *E.faecalis* infection (Lee D *et al*,2019)[14]. The efficacy of oral phages formulated in thermo-sustained release system against *E.faecalis* has been studied in vivo using rat model. The study showed that per-apical inflammation of the tooth was improved after phage treatment (Shlezinger *et al*2019)[15]. In our study a novel *E.faecalis* phage was found effective in reducing biofilms formed by drug resistant clinical isolates of *E.faecalis* from periodontitis patients (Bhardwaj *et al*2020)[16].

**Streptococcus phages:** Oral Streptococci play an important role in dental plaque and biofilm formation. Early studies reported the isolation of *S.mutans* lytic bacteriophages from human saliva. A

diverse group of around 50 bacteriophages which infect *S.mitis*, *S.mutans*, *S.oralis*, *S.salivarius*, *S.sobrinus* have been identified and reported (Delisle,2004)[17]. These phages are mostly temperate phages unlike the *S.mutans* phages which are lytic in nature. SM1 and B6 prophages have been isolated and sequenced against *S.mitis* B6. Virulent pneumophages DP-1 and CP-1 have been found to infect *S.mitis* (Queannene *et al* 2015)[18]. These virulent streptococcal phages and their enzymes are now being used to control oral infections. A new phage targeting biofilm of *S.mutans* has shown efficiency in caries prevention both in vitro and in vivo caries mice model (Wolfviz-Zilberman *et al*2021)[19].

Oral anaerobe phages: *Fusobacterium nucleatum* phages Siphovirus Fnp01 and phage Fnp02 were isolated from saliva samples( Machua *et al*2010) [20]. This study showed Fnp01 and phage Fnp01 could target three subspecies of *F.nucleatum*, *F.vincentii* and *F.polymorphum*. Fnp02 was rapidly absorbed on cell surface but slow lysis was observed. Recently a novel lytic phage FNU1 against *F.nucleatum* has been isolated which can breakdown biofilms (Mwila *et al*2019)[21]. Prevotella phages have been detected in vivo (Pride *et al* 2012)[22]. Treponema phage isolation has been reported in a single study (Mitchel *et al*2010)[23]. In this study from the biofilm culture of *Treponema denticola* phage td1 belonging to myoviridae family was isolated Application of phages in oral biofilm related infections.

Phages have now been applied to oral biofilms in several ways including in vitro, ex vivo and in vivo animal infection model (Table-1).

<i>S.mutans</i> and <i>E.faecalis</i> single phages	Oral infection	In vitro	Polystyrene plate	Szafranski and Stich,2017.
<i>E.faecalis</i> single phage.	Oral infection	In vitro, ex vivo	Polystyrene plate, ex vivo root canal infection.	Khalifa <i>et al</i> ,2015.
<i>E.faecalis</i> single phage.	Oral infection	In vivo	Rat model	Shlezinger <i>et al</i> ,2019.
<i>S.mutans</i> single phage.	Oral infection	In vitro, in vivo	Murine caries model.	Wolfviz-Ziberman <i>et al</i> ,2021.

The research pertaining to application of phage therapy is still in the preliminary stages, many oral phages have been isolated and identified but have not been put in actual practice yet.

Limitations of oral phages: The effectiveness of oral phages has been mainly seen by reduction in count of viable bacteria in the oral biofilm. However, the phages were not able to reduce the amount of extracellular matrix in the biofilms. Another factor while using phages is the phage therapy will be partially effective particularly if the biofilm is old.

## CONCLUSION:

Bacteriophages are proving useful candidates for oral biofilm mediated diseases. As antibiotics have a limited effect on the biofilms and with antibiotic resistance becoming a matter of global concern phages can be used to reduce the impact of oral infections. Moreover, phages are cost effective and easy to isolate. Phages have a great potential to be used in prevention and control of oral biofilm related infections but only few oral phages have been isolated and characterized. Henceforth oral phage research essentially needs to be promoted.

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