The human oral phageome

Szymon P. Szafrański | Jørgen Slots | Meike Stiesch

1Department of Prosthetic Dentistry and Biomedical Materials Science, Hannover Medical School, Hannover, Germany
2Division of Periodontology, Diagnostic Sciences and Dental Hygiene, Ostrow School of Dentistry of USC, University of Southern California, Los Angeles, California, USA

Correspondence
Szymon P. Szafrański, Lowe Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Stadtfelddamm 34, D-30625 Hannover, Germany.
Email: Szafranski.Szymon@mh-hannover.de

1 | INTRODUCTION

The human oral cavity harbors complex and diverse biofilms on tooth enamel, periodontal tissues, the tongue, buccal mucosa, hard and soft palates, and the lips. Oral bacteria may also seed to tonsils, pharynx, and esophagus. Oral microbiomes support large populations of viruses, mostly bacteriophages (phages) that infect specific bacterial species, and can appear as free phage virions (phage particles) or as dormant prophages (in bacterial lysogens).1-4 A 1 μl volume of saliva may harbor as many as 100,000 virus-like particles, most of which are phages.5,6 This article reviews the diversity of the oral phageome and the potential impact of phages on the ecology and dynamics of dental biofilms, with emphasis on the phage interaction with periodontopathic bacteria. The potential utility of phage therapy in oral health care is highlighted as well.

2 | BASIC FEATURES OF BACTERIOPHAGES

Phages comprise a significant part of virtually all human and environmental microbiomes, and they may modulate the formation and ecology of such microbionts.1,2,6,7 Phages are used for fingerprinting of bacterial strains (known as phage typing), food biopreservation, pathogen detection, and treatment of bacterial diseases of humans, animals, and plants.8-12 The continual increase in multiple-drug resistance among bacterial pathogens has created a need for new approaches to combat infectious diseases. An intriguing aspect of lytic phages is their ability to kill specific bacterial pathogens without upsetting the beneficial commensal microbiota, without causing superinfections by antibiotic-resistant microorganisms, and without inducing adverse drug reactions. Phage therapy may be particularly important for treatment of oral biofilm microorganisms, which can show resistance to common antibiotics and antiseptics. Also, phages or their enzymes may be used to edit the human microbiome toward a health-preserving state and to interact directly with human immune cells to modulate human immune responses.3,13,14 Prophages can also transfer genetic traits or cause genome excision of bacterial genes, which may confer novel properties to the bacteria.15,16 The life-threatening toxins of Vibrio cholerae, Escherichia coli, Corynebacterium diphtheriae, and Clostridium botulinum are examples of prophage-encoded virulence factors.15,16 Phage classification is based on morphology, infectious cycle and genomic content.4,20-31 Phages can have a tailed proteinaceous capsid with a double-stranded deoxyribonucleic acid (DNA) genome or a nontailed capsid with either double-stranded DNA, single-stranded DNA, or ribonucleic acid (RNA) genomes (Figure 1). Filamentous and pleomorphic phages also exist. Tailed bacteriophages (classified in the order Caudovirales) make up 96% of all phage isolates and are divided into families based on the tail morphology. Large virions (phage particles) with a long contractile tail belong to the families Myoviridae (mostly), Ackermannviridae, or Herelleviridae. Virions with a long, flexible but noncontractile tail belong to the Siphoviridae family. Small virions with a short, noncontractile tail belong to the Podoviridae family. Huge phages are grouped by genomic size as jumbo phages (200-500 kbp) and megaphages (>500 kbp).32-34 Huge phage virions contain genes involved in biochemical processes, in CRISPR-based immunity, and in phage replication.

Phages multiply intracellularly and follow the typical viral mechanism of infection (Figure 2). The tailed phage particles attach to specific receptors (cell wall proteins, lipopolysaccharides, teichoic acids, capsular polysaccharides, pili, flagella) of permissive bacteria, and...
the phage nucleic acid is subsequently injected into the bacterial cell. The intracellular phage uses the bacterial machinery for genomic replication, and, with a lytic infection, the phage genome is packed into phage particles that are released by a phage-mediated bacterial lysis. Instead of lysing bacterial cells, phages can persist in a latency/chronic prophage stage by integrating into the bacterial genome or by forming an extrachromosomal, independently replicating plasmid-like episome. Prophages may persist in the lysogenic state or initiate lysis, depending on the energetic/physiologic status of the bacterial host. Host starvation (due to lack of energy for phage replication) or nutrient-rich condition (prophage protects expending lysogen from phage attack) favor a prophage state. Unfavorable

**FIGURE 1** A. Selected phage morphotypes. ds, double-stranded; ss, single-stranded; C, circular; L, linear. DNA, deoxyribonucleic acid. B. Virion morphologies of oral phages. Siphovirus: phages FNU1, ΦAPCM01, transposable phage; Myovirus: Aaphi23-like phage; Podovirus: phage SOCP. Bar represents 100 nm. The electron micrographs (with minor formatting modifications) are licensed but permit reproduction.

**FIGURE 2** The replication cycle of a phage. A. Plaques formed by phages on a lawn of the host bacteria. B. Lytic and lysogenic pathways of phage replication. Lytic phages follow a lytic cycle; lysogenic phages can follow either a lytic or lysogenic cycle. DNA, deoxyribonucleic acid.
host conditions (e.g., suboptimal temperature or pH, ultraviolet radiation, presence of DNA-targeting antibiotics, reactive oxygen species, foreign DNA) can trigger a latent phage to enter the lytic cycle and propagate before the death of the bacterial host cell. Phages can also guide lysis-lysogeny decisions by measuring a high abundance of host cells (via quorum sensing) or via phage-encoded arbitrium, an interphage communication peptide.37,39

Phages are complex macromolecules that exhibit both virulence factors and defense mechanisms against counterattacks by the bacterial host (Figure 3). Recent studies have provided insights into the population genetics of phages, phage defensive mechanisms, and the interplay between phages and bacteria.40-48 The bacterial CRISPR-CRISPR associated (Cas) adaptive immune system aims to inhibit phage infections. Fragments of phage DNA become incorporated into CRISPR memory arrays (protospacers) on the bacterial genome, and RNA probes (spacers) transcribed from these arrays can identify the complementary invading phage DNA and guide antiphage bacterial nucleases.49 Bacteria-encoded restriction enzymes can cleave unmodified phage DNA but not the methylated bacterial DNA. In response, phages may mutate in the genomic fragments targeted by the bacterial RNA probes, form a nucleus-like compartment barrier, or elaborate proteins inhibitory to the CRISPR-Cas system.50-53 Phages may also seek protection by masking phage restriction sites with defensive proteins, by mutation/modification of the restriction sites, by altering the spatial conformation of the bacterial nucleases, or by removing essential enzymatic cofactors.54 Mutation in bacterial receptors for phage recognition or changes in the expression of bacterial surface structures constitute additional mechanisms of resistance toward phage infection, but phages may attach to another bacterial site or the phage receptor-binding proteins may mutate to fit the mutated bacterial receptor. Bacterial capsule or extracellular matrix, characteristics of sessile growth of biofilm cells, may also hamper the access of phages to receptors on the bacterial surfaces,55 but some phages can drill through such barriers by means of polymer-degrading hydrolases.56 Bacteria may release extracellular membrane vesicles that can intercept phages or may block entrance of new phage DNA by superinfection exclusion systems of already resident prophages.57 Bacterial "defense islands" can provide several additional lines of active defense against phage invasion.58 Bacteria can also undergo abortive infection to limit phage replication within a bacterial population, which again, however, phages may be able to overcome. The ability of phages to kill bacteria and the bacterial inherent defenses against phage infection and the rapid adaptation of new phage-invading mechanisms and of bacterial countermeasures may be important determinants of the oral/periodontal phageome.

Table 1 describes methods to detect, isolate, and characterize phages, including culturing, microscopic examination, serologic identification, and recent omics techniques. Each method of phage identification has advantages and disadvantages, and a combination of methods is often used in studies of phageomes.4 Metagenomics and prophage profiling have uncovered numerous previously unknown phage phylotypes. The extensive phage diversity has led to reorganization of the tailed bacteriophage taxonomy towards comparative genomics.59-61 However, a gene-based phage taxonomy is complicated by the absence of a marker gene that is universally present in all phage genomes, comparable with the 16S ribosomal RNA gene that revolutionized bacterial phylogeny.62 Moreover, "hybrid" phages have mosaic genomes that

**FIGURE 3** Selected antiphage defense systems in bacteria. A. Systems involving blocking of phage adsorption and deoxyribonucleic acid (DNA) injection. B. Systems involving degradation of phage DNA. Cas, CRISPR associated; IM, inner membrane; OM, outer membrane; OMV, outer membrane vesicles; RM, restriction modification; crRNA, CRISPR ribonucleic acid. See text for more details.
## Overview of methods to study phages

| Technique | Description | Reference(s) |
|-----------|-------------|--------------|
| Isolation and culture of phages using host propagation strain | A phage infection is traditionally identified by counting areas of clearing (known as plaques) on a lawn of the bacterial host and express the titer as "plaque-forming units" (PFU). Plaques result from successive infections and phage bursts. PFU can be determined by the "spot-titer method" (a phage sample is spotted on a small area where a future bacterial lawn will be formed) or by the "overlay-titer method" (the phage sample is mixed with a bacterial suspension together with an overlaying semisolid layer that allows the formation of plaques within the entire bacterial lawn). Phage sample can also be added to a liquid culture of bacteria, and the phage infection can be estimated as a loss of culture turbidity. | 69-71 |
| Phage enrichment and expansion | Filtration, polyethylene glycerol precipitation, ultracentrifugation, or successive co-incubation cycles with a bacterial propagator strain can be used to concentrate or amplify phages. Phage samples spiked with phage standards help to control the impact of phage enrichment on the phageome. Antibody-based "reverse genomics" technique may be used to enrich specific phages. | 72-76 |
| Phage production ("rebooting") | Functional virions can be "produced" using cell-deficient propagator cells transformed with phage genomes that are engineered/rebuilt in vitro. | 77,78 |
| Prophage detection and induction | Most bacterial cells carry functional prophages in a dormant state. Prophages can be detected by polymerase chain reaction (PCR) targeting conserved phage deoxyribonucleic acid (DNA) fragments, by Southern blot (dot blot) with phage-specific DNA probes, or by whole bacterial genome sequencing. Prophages can be activated (induced) with antibiotics that interfere with bacterial DNA integrity (mitomycin C, fluoroquinolones), ultraviolet light, or heat. The physiological state of lysogenic cells (e.g., a growth phase) can influence the efficiency of phage induction. | 79,80 |
| Phage preservation | Expended phage clones are usually stored in stabilizing buffer at 4°C or −80°C. Functional prophages can be stored in bacterial stocks. As phage titers decline over time, virion stability ought to be monitored during long-term storage of phages. | 81 |
| Efficiency of plating | Plating efficiency is expressed as the phage titer for a specific bacterial strain compared with the maximum titer for the reference phage/host combination. Low efficiency of plating indicates the phage is not fully virulent or productive, or the phage infection is partly controlled by defensive mechanism(s) of the bacterial host. | 82 |
| One-step growth curve | One-step growth curve can characterize the growth of phages in selected host strains, in terms of eclipse period, latent period, and burst size. Bacterial cells with phages and phage titer (PFU per infected cell) are monitored over time with or without added chloroform, which releases phage particles from intact cells and allows for a distinction between intracellularly functional phages and phages released during cell lysis. Number of infected cells is determined as a decrease in virion numbers due to adsorption to the host. Eclipse period is the time between phage adsorption and production of the first virions. Latent period is the time between adsorption and progeny release. Burst size is defined as the number of progeny phages released during lysis of a single bacterial cell. Low phage multiplicity rate ensures that only one phage infects each target cell. Physical and chemical parameters strongly shape phage-host interaction, and in vitro experiments may not fully mimic the physiological situation. Biofilm, organoid, animal, and ex vivo models may more properly reflect phage-host interaction. | 83,84 |
| Host range testing and host prediction | A phage host range is defined by the bacterial strains being lysed by the phage. High phage specificity is advantageous for phage therapy because it leaves the commensal flora unaffected. However, a phage that is effective against most strains of a targeted species can be broadly applied, without extensive prior activity testing. Knowledge of phage specificity is also key to understand the effect of phages on microbiome dynamics. Spot-titer or plaque testing is used to determine the host range for a given phage isolate. Host specificity for metagenomically assembled phages (i.e., genome sequences reconstructed from reads obtained by high-throughput sequencing of environmental phage DNA) can be predicted based on high DNA similarity between the phage isolate and a known host range. Degree of phage DNA sequence matching bacterial CRISPR spacers can also be used to indicate host range. Phage-host coevolution assessed by guanine-cytosine content, k-mer frequency, frequency of DNA uptake signal sequences, and codon usage may also reveal the most likely host. | 4,30,85 |
| Transmission electron microscopy | Transmission electron microscopy of negatively stained phage particles is the classic method to study size and morphology of phage particles. Phages are concentrated by ultracentrifugation, adsorbed onto a carbon film, washed, stained with uranyl acetate or phosphotungstate, attached to a copper specimen grid, and examined. | 86,87 |
are formed gradually by shuffling genomic modules between the phageome members. Extensive gene flow, observed, for example, in some *Mycobacterium* phages, might obfuscate the phylogenetic boundaries. However, since most phages show low or moderate recombination rates, future phage classification may be based on extensive population genomic data or robust network-based algorithms. It has been proposed to place two phages in the same species if they share greater than 95% nucleotide identity or, in the case of phage genomes assembled from metagenomic reads, if at least 85% of their genomes share 90% nucleotide identity. This mode of taxonomic assignment can readily be automatized. New phage taxonomy is created and approved by expert consensus according to rules by the International Committee on Taxonomy of Viruses (https://talk.ictvonline.org/information/w/ictv-information/383/ictv-code).

### TABLE 1 (Continued)

| Technique | Description | Reference(s) |
|-----------|-------------|--------------|
| Fluorescence microscopy | Fluorescent staining of viral nucleic acid in filtrates is used to estimate the size of a virion population. Fluorescence in situ hybridization can monitor phage infections at a single-cell level. Fluorescent dyes coupled with DNA probes can detect selected phage genes or host sequences. Dual color epifluorescence microscopy can mark host cells with one color and phage particles (both intracellular and extracellular) with a second color to visualize infection stages like phage adsorption, replication, and release. | 5,8,8-90 |
| PCR and phage marker gene amplicon profiling | Phages lack universal marker genes like the bacterial 16S ribosomal ribonucleic acid (RNA) gene, but targeted sequencing of small phage genome fragments can provide sufficient sequence diversity to serve as an individual-specific barcode. | 4,91,92 |
| CRISPR loci profiling | CRISPR loci profiling allows characterization of the phage population in bacteria which carry the adaptive immune system CRISPR-CRISPR associated (Cas). Phage DNA fragments become incorporated into CRISPR memory arrays (protospacers) on the bacterial genome, and RNAs transcribed from these arrays identify complementary invading phage DNA for degradation. Protospacers are transmitted vertically during genome replication and cell division and can be used to study bacterial exposure to phage elements. CRISPR loci are profiled using DNA and RNA sequencing and meta-omics techniques. | 93-96 |
| Omics and meta-omics | Genomics, transcriptomics, proteomics, and their meta-variants can provide a systemic view on phage biology and phage-host interaction in vitro and in vivo biofilm studies. Whole phage genomics can reveal presence of toxins, phage lifestyle, and the potential for phage engineering. Bacterial genomes can be screened for phage-like elements and CRISPR spacers. Transcriptomics reveals the expression of phage genes. Proteomics quantifies phage proteins, not only genes or transcripts, and is used to distinguish between phage particles and unassembled phage components. High-throughput sequencing combined with cell sorting can provide single-cell resolution. Meta-omics can characterize phageomes in complex clinical situations. | 4,30,97-103 |
| Mutagenesis of phages | Phage genetics is typically employed in functional phage gene studies. Mutagenesis targets specific phage traits, such as antimicrobial performance or phage range, and the mutants obtained can be evaluated for phenotypic changes. Ultraviolet irradiation or hydroxylamine treatment of virions (substitutes cytosine with thymine) can induce mutations. | 104 |
| Genetic engineering of phages | Genetic modification of phages aims to improve phage antimicrobial properties, change phage host range, reduce phage immunogenicity, target phages against bacteria harboring specific sequence signatures, detect bacteria, deliver drugs, or create new biomolecules. Phage engineering techniques include homologues recombination, recombination of electroporated DNA, in vivo recombination, CRISPR-Cas-mediated genome engineering, rebuilding/refactoring phage genomes in vitro, whole-genome synthesis, and assembly from synthetic oligonucleotides. | 105,106 |
| Heterologous expression of phage enzymes | Phage lysins (cell-wall hydrolases) constitute intriguing antibiotic alternatives. Phage lysins have been developed against gram-positive and, more recently, against gram-negative pathogenic bacteria and have been engineered to improve efficiency. | 107,108 |
| Antiphage sera | Polyclonal antibodies against phage virions are used in downstream immunochemical and biological studies of patient sera. The antiphage activity in sera can be assessed by a neutralization test that estimates the rate of phage inactivation at various incubation times. | 109 |

**Note:** For detailed information and more methods, see the *Bacteriophages: Methods and Protocols* compendium in four volumes edited by Martha R. J. Clokie, Andrew Kropinski, and Rob Lavigne.110-113

3 | THE ORAL PHAGEOME

Metagenomic profiling of oral biofilms has led to an increased understanding of the potential role of phages in the development, regulation, and treatment of pathogenic microbiomes of the periodontium and other oral sites. However, metagenomic profiling has yielded many new phage genomic sequences that remain to be characterized, and most phages that were phenotyped in the past lack genetic information.3 The phages reviewed here infect six bacterial
phyla, which accounts for 96% of all oral taxa: Actinobacteria (with classes Actinomycetales and Coriobacteria), Bacteroidetes (Bacteroidia and Flavobacteriia), Firmicutes (Bacilli, Clostridia, and Negativicutes), Fusobacteria (Fusobacteriia), Proteobacteria (Beta-, Delta-, Epsilon-, and Gammaproteobacteria), and Spirochaetes (Spirochaetalia).\textsuperscript{114} The oral phyla Synergistetes and Saccharibacteria (formerly known as TM7) have each been associated with a single phage. The oral cavity also hosts phages of pathogenic invaders (eg, \textit{E. coli}, \textit{Pseudomonas aeruginosa}, \textit{Staphylococcus aureus}, \textit{Enterococcus faecalis} and \textit{Lactobacillus} phages that can be found in environments other than the oral cavity.\textsuperscript{115-121} No phages of the phyla Absconditabacteria (formerly known as SR1), Chlamydiae, or Chloroflexi have been identified in the oral cavity. Figure 4 depicts the distribution of oral phages across host taxons.

Figure 5 shows the oral phage diversity at the bacterial phylum level.

3.1 | Phages infecting Actinobacteria

\textit{Actinomyces} and \textit{Rothia} (both of the order Actinomycetales) and \textit{Corynebacterium} (order Corynebacteriales) are diverse and ecologically relevant genera of the Actinobacteriia class.\textsuperscript{114} \textit{Actinomyces} spp are primary colonizers of dental plaque and are typically associated with oral health, but they can also cause oral morbidity (eg, caries, endodontic, periodontal and peri-implant diseases) and systemic diseases (eg, orocervicofacial, thoracic, and abdominal/pelvic actinomyositis).\textsuperscript{122,123} Newly described \textit{Actinomyces} species are emerging as opportunistic pathogens in several body sites.\textsuperscript{122} \textit{Rothia} spp can inhabit cariogenic biofilms and produce extracellular levan from sucrose.\textsuperscript{126} \textit{Corynebacterium} spp form long filamentous cells that may provide a scaffold for oral biofilms.\textsuperscript{127} Species of the Coriobacteriia class are less studied, but \textit{Atopobium} spp and \textit{Oslenella uli} have been linked with periodontal disease.\textsuperscript{123,126}

The Integrated Microbial Genome/Virus (IMG/VR) database suggests that 241 phages exhibiting high-quality draft genomes have an oral origin and are predicted to infect hosts of the phylum Actinobacteria.\textsuperscript{29} However, the IMG/VR database occasionally contains concatenation artefacts, and huge phages in particular need to be validated experimentally.\textsuperscript{34} The National Center for Biotechnology Information (NCBI) database describes genomes of two \textit{Actinomyces} phage isolates, Av-1 and xhp1 (Figure 4).\textsuperscript{127,128} Among phages infecting oral Actinobacteria, 126 are linked to \textit{Actinomyces} spp, 20 to \textit{Peptidiphaga} sp HMT-183 (formerly known as \textit{Actinobaculum} sp), and 53 to \textit{Rothia} spp. A total of 27 phages are linked to \textit{Corynebacterium} spp, and three phages are linked to \textit{Pseudopropionibacterium propionicum} of the order Propionibacteriales. All the Actinobacteria taxons mentioned belong to the class Actinobacteriia. Another class of the Actinobacteria phylum, Coriobacteria, is represented by the genus \textit{Atopobium}, which is linked to nine phages. Genus-specific phage clusters can be derived from the genomic content of protein conserved domains superfamilies\textsuperscript{129} (Figure 5). \textit{Actinomyces} and \textit{Rothia} phages show more conserved domain superfamily diversity than \textit{Corynebacterium} phages do. Most Actinobacteria phages carry genomes of an average size of 40 kbp, but phages with smaller (<20 kbp) and larger (>100 kbp) genomes are also observed, and a huge phage (infecting \textit{Actinobacteria}) carries CRISPR-Cas system.\textsuperscript{34} A recently described phage xhp1 of the family Siphoviridae infecting the \textit{Actinomyces odontolyticus} subsp \textit{actinonysbacter} strain XH001 produced biofilm formation, possibly because of a phage-induced extracellular release of bacterial DNA contributing to biofilm development.\textsuperscript{128}

3.2 | Phages infecting Bacteroidetes

\textit{Porphyromonas}, \textit{Tannerella}, and \textit{Prevotella} spp representing the Bacteroidia class and \textit{Capnocytophaga} spp representing the Flavobacteriia class can greatly affect oral health. \textit{Porphyromonas gingivalis} is a keystone pathogen of periodontal disease\textsuperscript{130} and has been recovered from various types of cancer.\textsuperscript{131} \textit{Porphyromonas endodontalis} and \textit{P. gingivalis} are closely associated with severe apical periodontitis.\textsuperscript{132} \textit{Tannerella forsythia} is a pathogen\textsuperscript{133} that often coexists with \textit{P. gingivalis} and \textit{Treponema denticola} in severe periodontitis lesions.\textsuperscript{134} \textit{Prevotella} and \textit{Alloprevotella} are proteolytic members of periodontal and other oral biofilms.\textsuperscript{135} Some members of the Bacteroidia class are still not cultivable, and their ecologic and pathogenic roles are poorly understood (eg, Bacteroidales [F-2] [G-2] HMT-272).\textsuperscript{136,137} \textit{Capnocytophaga} species are common inhabitants of dental plaque but exhibit relatively little periodontopathogenicity.

The IMG/VR database contains 308 phages with high-quality draft genomes that have an oral origin and which are predicted to infect hosts of the phylum Bacteroidetes (Figures 4 and 5). Of the 308 phages, 173 are putative \textit{Prevotella} phages, which are predominantly linked to \textit{Prevotella histicola}, \textit{Prevotella scopos}, unnamed \textit{Prevotella} spp, or multiple species. Forty phages are linked to commensal \textit{Porphyromonas} spp, 23 phages to \textit{Bacteroidiales} [F-2] [G-2] HMT-272, 4 phages to \textit{T. forsythia}, and a single phage to \textit{Alloprevotella tannerae} (all from the Bacteroidia class). Seventy phages are associated with \textit{Capnocytophaga} spp, most often \textit{Capnocytophaga gingivalis}, \textit{Capnocytophaga granulosa}, and \textit{Capnocytophaga} HMT-323 (all from the Flavobacteriia class). Five jumbo phages have genomes larger than 300 kbp, which are the longest genomes found in oral phages according to IMG/VR: these phages are predicted to infect either \textit{P. scopos} or \textit{Prevotella fusca}, or both. Eighteen other \textit{Prevotella} phages have genomes approaching 200 kbp. A study questioned these phages as potential artefacts and reported different phages.\textsuperscript{34} In summary, most oral phages, including the jumbo phages, of the phylum Bacteroidetes, infect commensal bacteria, and only a few are linked to classical periodontopathogens: none to \textit{P. gingivalis}, four to \textit{T. forsythia}, and 19 to \textit{Prevotella intermedia}/\textit{Prevotella nigrescens}.

3.3 | Phages infecting Firmicutes

Firmicutes are the most abundant constituents of the indigenous, health-associated microbiota in most oral sites. Firmicutes encompass Bacilli, Clostridia, and Negativicutes, which differ considerably
in physiology, ecologic role, and pathogenic potential. Oral streptococci representing the Bacilli class are central to oral ecosystems as early colonizers of biofilms and members of mature biofilms. Oral streptococci are generally considered as commensals but produce lactate and the mutans group of streptococci are key organisms of dental caries. Oral streptococci may also participate in periodontal disease and can cause abscesses and systemic infections (mostly the anginosus group of streptococci) and endocarditis (the mitis and the sanguinis groups of streptococci). "Nutritionally variant streptococci" of the Granulicatella genus and Gemella spp of the Bacilli class can also inhabit dental plaque and cause endocarditis. Strict anaerobes of the Clostridia class (e.g., Eubacterium nodatum, Parvimonas micra, and Filifactor alocis) are prevalent organisms in periodontal disease and many types of odontogenic infection. Oral members of the Negativicutes class are anaerobes that are generally considered as commensals (Veillonella spp), but some are linked to periodontitis (e.g., Dialister pneumosintes and Selenomonas sputigena).

The IMG/VR database contains 961 phages with high-quality draft genomes that have an oral origin and which are predicted to infect a host of the Firmicutes phylum (Figure 4). A total of 712 phages are linked to Bacilli, 187 to Negativicutes, and 62 to Clostridia. Streptococcus spp harbor the most numerous and diverse phage population (Figure 5), which includes Streptococcus mitis and/or Streptococcus oralis of the mitis group (154 phages), Streptococcus sanguinis, Streptococcus gordonii, and Streptococcus parasanguinis of the sanguinis group (127 phages), Streptococcus salivarius of the salivarius group of streptococci (88 phages), and Streptococcus anginosus of the anginosus group (80 phages). Several phages are linked to unnamed streptococcal species and are predicted to infect multiple species, often from different streptococcal groups. Five jumbo streptococcal phages are linked to S. mitis and one jumbo phage to S. sanguinis. Fourteen phages are linked to Granulicatella adiacens and 14 phages to Gemella sp, mostly Gemella hemolysans and less often to Gemella HMT-928. Phages infecting oral Clostridia are linked to Lachnoanaerobaculum spp (29 phages, four of which are jumbo phages) and Oribacterium spp (13 phages, 10 of which are jumbo phages). Few phages are linked to established or emerging periodontopathic species: four phages to...
$P. \, \text{micra}$ and a single phage to $F. \, \text{alocis}$. Most Negativicutes phages (180 phages, 29 of which are jumbo phages) are predicted to infect Veillonella parvula, Veillonella dispar, Veillonella atypica, Veillonella HMT-780, or unclassified Veillonella strains. The remaining phages are linked to Centipeda periodontii, Anaeroglobus geminatus, and few Selenomonas spp. The NCBI database contains the genomes of 46 Streptococcus phages, 22 of which infect Streptococcus pneumoniae, 17 Streptococcus thermophilus, 3 Streptococcus mutans, and 1 of each of S. gordonii, S. mitis, S. oralis, and S. salivarius.$^{3,141}$ Phage isolates with taxonomic classification are brussoviruses (eight phages) infecting S. thermophilus, moineauviruses (six phages) infecting either S. thermophilus or S. salivarius (these 14 phages belong to Siphoviridae), cepunaviruses (two phages belonging to Picovirinae), and saphexavirus (one phage belong to Siphoviridae); the latter three phages infect S. pneumoniae. Streptococcal phage isolates that have been characterized include four lytic siphoviruses infecting S. mutans (e10, f1, M120, ΦAPCM01), four lytic pneumophages infecting the mitis group (Cp-1, Cp-7, Dp-1, SOCP), and four temperate phages infecting the mitis or sanguinis groups (SM1, PH10, PH15). Notably, the SM1 phage genome encodes a platelet-binding factor that is implicated in $S. \, \text{mitis}$–related endocarditis.$^{142}$

A study of 1306 genomes from different streptococcal species revealed nearly 800 prophages or satellite prophages (ie, phages that relay upon a helper phage to replicate), and reported on phage diversity patterns across species and common cross-species transmission.$^{143}$ S. gordonii has yielded four putative intact prophages, but their functionality remains unknown.$^{144}$ Twenty-five Veillonella phage isolates represented two...
phage groups that differed in serology, plaque size, and virion morphology, but they are not characterized genetically.  

3.4 | Phages infecting Fusobacteria

Fusobacteria, representing the Fusobacteriia class, are highly diverse and the most abundant oral anaerobes and can serve as “bridge” organisms in oral biofilm structures. Fusobacteria contribute to periodontal and endodontic infections and possibly to adverse pregnancy outcomes, atherosclerosis, gastrointestinal disorders, and colorectal cancer. Leptotrichia spp, which also are members of the Fusobacteria class, may cause opportunistic infections in immunocompromised patients.

The IMG/VR database includes 211 phages of likely oral origin that are predicted to infect fusobacteria, and the NCBI database contains the genome of a single Fusobacterium phage isolate, FNU1 (Figure 4). A total of 147 phages, including 26 jumbo phages, are linked to Fusobacterium spp and 65 phages to Leptotrichia spp. Based on the conserved domain superfamily content (Figure 5), Fusobacterium and Leptotrichia phages are grouped together in two locations, but Fusobacterium-specific groupings also exist. Fusobacterium phages of the Siphoviridae family show poor lytic properties and have not been characterized genetically. However, a recently isolated large lytic siphovirus designated FNU1 (Figure 1) has been sequenced and reported to effectively kill biofilm cells of Fusobacterium nucleatum. The ecologic significance (a core oral bacterium) and medical relevance (role in colorectal cancer) of F. nucleatum should encourage research on Fusobacterium phages.

3.5 | Phages infecting Proteobacteria

Virtually all Proteobacteria are aerobic/facultative organisms. Gammaproteobacteria of the oral cavity belong to the families Pasteurellaceae and Cardiobacteriaceae. The Pasteurellaceae family includes Haemophilus and Aggregatibacter species, which are prevalent organisms of oral and pharyngeal mucosal surfaces. Haemophilus parainfluenzae is an abundant species across many oral niches. Haemophilus influenza causes invasive pediatric diseases and pulmonary complications in adults. Aggregatibacter actinomycetemcomitans is a potent toxin-producing species that is linked to severe periodontal disease, especially localized juvenile (aggressive) periodontitis, and to systemic diseases like endocarditis and abscesses. Other Aggregatibacter spp are oral and pharyngeal commensals that may cause systemic infections. Cardiobacterium hominis of the Cardiobacteriaceae family can cause endocarditis. Oral Betaproteobacteria of the Neisseriaceae family inhabit mostly biofilms associated with periodontal health. Eikenella spp and Kingella spp usually comprise only a tiny fraction of oral biofilms (except that eikenellas can expand in periodontal disease), but organisms of both genera can cause endocarditis. Oral Epsilonproteobacteria of the Campylobacteraceae family are implicated in periodontal disease.

Oral Deltaproteobacteria are less studied, but one member of the class (Desulfovibulb HMT-041) has been related to periodontal disease. The IMG/VR database includes 254 phages with high-quality draft genomes that have an oral origin and which are predicted to infect hosts of the Proteobacteria phylum (Figure 4). Most of the Pasteurellaceae phages are linked to the Gammaproteobacteria class, including H. parainfluenzae and/or Haemophilus haemolyticus (83 phages), A. actinomycetemcomitans, Aggregatibacter segnis, and/or Aggregatibacter HMT-458 (15 phages), or both Haemophilus and Aggregatibacter species (10 phages). Nine Haemophilus jumbo phages seem to be linked to H. haemolyticus. Pasteurellaceae phages have different conserved domain superfamily content than phages infecting other Proteobacteria (Figure 5). C. hominis of the Cardiobacteriaceae family has been associated with six phages. Oral Betaproteobacteria phages are mostly linked to Neisseria spp (76 phages) and occasionally to Kingella oris. Eikenella corrodens, or unnamed Eikenella spp. (23 phages). Twenty-three phages of the Epsilonproteobacteria class are linked to Campylobacter spp, mainly Campylobacter concisus, but occasional phages are linked to Campylobacter gracilis, Campylobacter rectus, unnamed Campylobacter spp, or multiple species.

The NCBI database contains genomes of well-characterized isolates from two Aggregatibacter phages (AaΦ23, S1249) and two Haemophilus phages (HP1, HP2). The HP1 and HP2 phages belong to the Myoviridae family and the HP1 phage to the Peduvirinae subfamily. A total of 237 potentially functional phages belong to the Proteobacteria phylum (Figure 4). Most of these phages are transposable P2-like or lambdoid phages that carry DNA uptake signaling sequences to facilitate a direct uptake of phage DNA through a dedicated protein machinery of the bacterial host. The phages have been assigned provisional phylogeny, and their diversity patterns have been reported across host clades and niches.

3.6 | Phages infecting Spirochaetes

Treponema denticola and related species represent the Spirochaetes phylum and are implicated in periodontal disease. The IMG/VR database includes two oral phages with high-quality draft genomes that are predicted to infect Treponema socranskii. A single Treponema phage has been isolated, but its functionality is unknown. Most of these phages are transposable P2-like or lambdoid phages that carry DNA uptake signaling sequences to facilitate a direct uptake of phage DNA through a dedicated protein machinery of the bacterial host.

3.7 | Phages infecting bacteria from other oral phyla

The oral cavity also harbors bacteria of less-studied phyla: Absconditabacteria(formerly known as SR1), Chlamydiae, Chloroflexi, Saccharibacteria (formerly known as TM7), and Synergistetes. Anaerolineae [G-1] bacterium HMT-439 of the Chloroflexi phylum
and Fretibacterium spp of the Synergistetes phylum have been associated with periodontal disease.123 The IMG/VR database includes two oral phages (including one jumbo phage) with high-quality draft genomes that are predicted to infect Fretibacterium fastidiosum and an unnamed species of the Saccharibacteria phylum.

4 | ORAL PHAGE ECOLOGY

4.1 | Phage diversity patterns across oral niches and time

Oral bacteria tend to be site specialists,156 and oral phages follow a similar pattern.157 By correlating phage phylotypes and oral niche presence,91 some Haemophilus and Aggregatibacter phages were found to exhibit a generalized oral distribution (eg, SuMu-like transposable phage was prevalent at tongue, buccal mucosa, and gingival), whereas other phage phylotypes showed a clear niche specificity (eg, cluster 19 and cluster 20 of MhaA1-like phages were linked to buccal mucosa and tongue, respectively).7 Phage generalists, which can infect strains of multiple subspecies or different species, possess a mode of infection that carries low fitness cost advantageously with low abundance of host cells.157 That some phages infect niche-specific subspecies of H. parainfluenzae points to a coevolutionary specialization for both phage and host. The strategic benefits and the molecular mechanisms behind phage generalists and specialists have still to be revealed.

The oral microbiomes and related phageomes may be affected by disease, aging, and different food intake. A 60-day study of salivary phage and bacterial communities found most phage populations to be stable and highly personalized.158 An earlier study by the same research group described transient salivary phage populations but may have overestimated the number of phage genotypes.5 A 30-day study of tongue phages showed a generally stable phage community, although some major phage phylotypes reappeared in cycles that appeared to be unrelated to diet and oral hygiene efforts.91 The apparent stability of major oral phage groups ensures a continuous phage effect on oral microorganisms.

4.2 | Oral phage populations in health and disease

The dental phage population is probably more characteristic of periodontal disease and dental caries than the salivary phage population is, which receives contributions from several different oral surfaces. However, information on the periodontal phageome is sparse.159 Subgingival myoviruses (ie, phages representing the Myoviridae family sensu lato) and an uncharacterized siphovirus have been related to periodontitis.159,160 Periodontal patients have shown increased transcriptional lytic activity of salivary phage genes, predominantly of siphoviruses infecting the Firmicutes phylum.161 Metagenomic and metatranscriptomic studies have reported on periodontal phage presence and activity, but with very limited phage genomic analysis.162,163 Using classical microbiological methods, dormant Aggregatibacter prophages were detected in both periodontal health and disease,4,165 but active Aggregatibacter phages were associated preferentially with advanced periodontitis.168,169 Finally, oral lysogenic bacteria may be at increased risk of causing systemic infections; for example, an A. actinomycetemcomitans producer strain of Aaphi23-like phages was recovered from an actinomycotic lung lesion.5 The Streptococcus SM1 prophage of S. mitis encodes a platelet-binding factor that can promote platelet activation and aggregation with risk of causing endocarditis.170 A streptococcal satellite prophage carrying the vapE gene was related to S. pneumoniae sepsis in a murine model.143

4.3 | Reservoir and transmission of oral phages

A phage reservoir comprises an external environment, a host, and an infectious phage. Phages may infect oral permissive bacteria (susceptible to either lytic or prophage infection) of the mouth or the gastrointestinal and pulmonary tracts containing swallowed saliva or inhaled air.174 Oral phages may also cross the oral epithelial barrier and disseminate to extraoral sites as prophages of invasive lysogenic bacteria or as a cargo of phagocytic cells. Colorectal tumor or cystic fibrosis lesions can harbor microbial communities that resemble oral assemblages.174,175 Some species, like veillonellas, colonize both the mouth and the gastrointestinal tract, but it is unclear whether strains in different niches diverge from each other or whether both infected sites contain the same phage population, or how often transmission may occur in any direction. In addition to systemic transmission of phage-infected oral bacteria, enterococci, staphylococci, pseudomonads, and other atypical oral bacteria may colonize the mouth and introduce novel phage populations.176,178

Transmission of oral phages can take place between mother and children, family members, couples with intimate contact and people in the same household.91,179 A study of couples demonstrated common features in the tongue phage population.91 People who are sharing or have shared households in the past also harbor many phages of same sequences.179 Neither a genetic relationship nor a spousal relationship seem to be required to share oral phages, and even relatively brief contact may lead to transmission.180 However, transmission of oral phages between mother and infant have been rarely observed, perhaps due to methodological study limitations.181 Direct contact, like kissing,182 or indirect transfer by deposited oral material (eg, food, utensils) or severe coughing are potential routes of phage transmission. Bidirectional transmission of oral phages between people and pets has not been described, but it may occur in the case of close contact or sharing of utensils. Person-to-person exchange of prophage-carrying oral bacteria may comprise a major route of phage transmission.183,185

4.4 | Phage interactions within microbiomes

Phage-induced bacterial lysis may remove pathogenic bacteria and create a niche for previously suppressed low-pathogenic species or may release bacterial components that can contribute to the
formation of a protective biofilm matrix. Phages can quickly adapt to environmental changes and apply strong pressure on bacterial populations, especially on bacterial lineages that show high fitness and abundance. In in vitro and animal studies, phage-induced lysis of host species has resulted in major changes in the abundance and diversity of bacterial communities. As bacteria must adapt to the phage attack to avoid decimation, an oscillatory dynamics may develop between phage and host. Lysogeny protects the host from new phage infection and phage predation. Bacteria may also avoid phage-mediated predation by surface remodeling but face the risk of losing receptors for important biofilm bacterial taxa. Actinomycetes strains exposed to phages yielded two different receptor mutants that both lost the ability to coaggregate with streptococci. Phage infection has reduced the expression of Enterococcus genes involved in interspecies interactions. However, phage-induced transfer of antibiotic resistance genes among A. actinomycetemcomitans strains may increase the adaptability of oral biofilms, as implied in an in vitro study. A study on murine fecal phage populations found that antibiotic treatment led to an enrichment of phage-encoded resistance genes and a microbiota with increased resistance. A long-term antibiotic study in humans detected an expansion of genes involved in resistance to numerous antibiotics in fecal phageomes but not in oral phageomes. However, phages can also interfere with transformation and conjugation, and thus negatively affect gene transfer.

Aquatic environmental studies provide another perspective on phage-bacterium interactions. Phages and bacteria share niches in aquatic ecosystems, and prophages may protect their host from phagocytosis or from reactive oxygen species during oxidative bursts of the host’s phagolysosomes. A similar mechanism may be relevant for oral microbiomes that harbor phages with genes mitigating the oxidative stress effect (Szafranski et al, unpublished).

Virus-bacterium synergy is a well-known pathogenetic paradigm in various serious diseases. Herpesviruses, especially herpes simplex virus type 1, Epstein-Barr virus type 1, and cytomegalovirus, are common inhabitants of the human oral cavity, and active herpesviruses may induce overgrowth of pathogenic bacteria and oral pathology. Although phages are not aggressive directly toward human cells, their immunomodulatory potential against specific bacteria might alter oral microbiomes. In addition, highly immunogenic phage proteins may act as adjuvants to bacterial and eukaryotic viral antigens. Thus, phages may modulate oral microbiomes and, indirectly, the associated immune responses.

4.5 The interaction between oral phages and human cells

Phages affect cell physiology and cell-cell signaling by inducing anti- (usually) and pro-inflammatory mediators, modulating innate and adaptive immunity, and exerting either human beneficial effects or maladaptive pathogenic immune responses. Phage virions can attach to and be phagocytosed by neutrophils, monocytes, and dendritic cells, and by occupying phagocyte receptors may compromise or act as phagocytosis-facilitating opsonins. Phage DNA attaches to toll-like receptor-9 and activates signaling transduction pathways, which launch immune and inflammatory responses aimed at eliminating invading phages, but can also give rise to maladaptive immune responses. The phage-induced upregulation of antimicrobial defenses might provide an overall beneficial effect, but activation of the toll-like receptor-9 on dendritic cells also stimulates release of interferon-γ and other cytokines having been linked to phage-related colitis.

Internalized phages may modulate phagocytes in a number of ways. In contrast to double-stranded DNA phage activation of toll-like receptor-9, Sweere et al observed that the filamentous single-stranded DNA phage Pf induced toll-like receptor-3-dependent type I interferon in leukocytes, which suppresses tumor necrosis factor and phagocytosis and thus potentially may extend the survivability of the host bacterium and the phage. A Pf phage–infected P. aeruginosa strain was recently shown to aggravate cystic fibrosis. In a murine wound model, vaccination against the Pf phage reduced the morbidity of the Pf phage–infected P. aeruginosa, further affirming a linkage between the Pf phage and pathology. A murine Salmonella typhimurium diarrhea model showed that inflammation promoted free phage production and bacterial spread, suggesting a reciprocal induction between phages and inflammation.

Phage-human cell interactions may also cause pathology in the oral milieu. Preus et al associated morphologically diverse Aggregatibacter phages with severe periodontitis in two siblings with the Papillon-Lefèvre syndrome and in four patients with localized juvenile periodontitis. The authors hypothesized that prophage activation and lysis of A. actinomycetemcomitans exposed the periodontium to disease-producing factors, such as lipopolysaccharide, peptidoglycan, flagellin, and DNA. Also, phage virions may cross the epithelial barrier and interact with immune cells within a tissue, as observed in intestinal epithelial cell lines, and oral phage virions may transverse the periodontal epithelium in a similar manner. To conclude, phages may cause a release of bacterial virulence factors, such as leukotoxin of A. actinomycetemcomitans, and may also induce destructive immune responses.

5 PHAGE THERAPY AND PHAGE-BASED ANTIMICROBIALS

The clinical use of phages to combat bacterial infections is gaining attention as a potentially safe and effective way to resolve life-threatening infections, especially those caused by nosocomial and multidrug-resistant ESKAPE pathogens (Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa, Enterobacter spp) and World Health Organization priority pathogens (E. coli, Proteus spp, Helicobacter pylori, Campylobacter spp, salmonellae, Neisseria gonorrhoeae, S. pneumoniae, H. influenzae,
Shigella spp). Several of these organisms can infect the oral cavity of medically compromised patients and of patients with severe periodontitis.219 Phage therapy has a long history of use in Georgia, Poland, and Russia, and numerous observational studies support the therapeutic utility of phages, but rigorous clinical studies remain to be conducted.220,221 A small controlled clinical trial found a therapeutic *Pseudomonas* phage to be effective and safe against chronic otitis, but recurring infections did occur.222 A randomized controlled trial on the efficacy and safety of *Pseudomonas* phages as topical therapy of burn wounds showed phages to be less effective in reducing bacterial loads than the standard of care therapy using sulfadiazine silver.223 However, the study used low doses of *Pseudomonas* phages due to phage stability issues, lacked preliminary phageograms (sensitivity testing), recruited a small number of patients, and assessed the therapeutic outcome by semiquantitative methods.227 Phage safety was confirmed in a study of 13 patients with severe *S. aureus* infections.224 Oral coliphages were also found to be safe for treatment of diarrhea in children but were unable to improve outcome, perhaps due to insufficient phage coverage and low phage titers.225 Phage therapy targeting intestinal *E. faecalis* in mice was able to attenuate alcoholic liver disease, but a comparable trial has yet to be performed in humans.230 A recent report describes a 15-year-old patient with cystic fibrosis and bilateral lung transplantation who experienced a life-threatening disseminating infection by antibiotic-resistant *Mycobacterium abscessus*, which was successfully treated with a genetically engineered three-phage cocktail.226,227 However, the potential release of genetically engineered phages into the environment raises some concerns.228 *Staphylococcus* phages in intracellular compartments of osteoblasts were inactive but were killing bacteria after being released into extracellular compartments.229

Antimicrobial properties of oral phages and phage enzymes have been studied recently.2 Fusobacterium phage FNU1 was able to disrupt experimental *F. nucleatum* biofilms, as assessed by crystal violet staining and confocal microscopy.19 *Haemophilus* phiKZ-like oral phages may exert therapeutic activity against pathogens of the Pasteurellaceae family.5,29 The multiple antibiotic-resistant *E. faecalis* is an organism of major concern in endodontics. A genetically engineered *Enterococcus* phage ϕEf11-derivative lacked lysogenicity but displayed broadened host range and was able to reduce the level of *E. faecalis* in human dentin specimens by up to 100-fold.230,231 A purified lysin from the ϕEf11 phage was found to be active against 73 of 103 *E. faecalis* strains and able to cause a substantial destruction of *E. faecalis* biofilms.232 ClyR, a chimeric lysin of streptococcal origin, was active against planktonic and sessile *E. faecalis* cells in vitro and in an *ex vivo* dental model.233 A combination of two *Enterococcus* phages (EFDG1 and EFLK1) showed in vitro effectiveness against *E. faecalis*, and several new siphoviruses targeting *E. faecalis* have been identified.234,235 *Enterococcus* phage SHEF2 was able to eradicate *E. faecalis* biofilms on a polystyrene surface and on tooth specimens, and to resolve an *E. faecalis* infection in a zebrafish model system.235 Vancomycin-resistant *E. faecalis* were treated with a synergistic combination of *Enterococcus* phage EFLK1 and vancomycin.236

Numerous *Streptococcus* phages have been identified that eventually may be employed in dental care prevention and treatment.3 Phages engineered to express the *S. mutans*-specific antimicrobial peptide C16G2220 may show anticaries potential.237 The ClyR lysin was active against cariogenic *S. mutans* and *Streptococcus sobrinus* without compromising indigenous *S. sanguinis, S. oralis*, and *S. salivarius*.238,239 High-priority topics in oral phage research also involve identifying phages killing plaque-forming oral Actinobacteria, periodontopathogens, and opportunistic pathogens of nonoral origin. Oral phage research would benefit significantly from having better-characterized propagator host strains and phage isolates.

To conclude, phage safety is well established, but studies are needed to show the best mode of clinical phage application and effectiveness of oral phage therapy. Modern synthetic biology may be the key to create real breakthroughs in the development of commercial phage products.230,231,232,240-242 Commercial phage medicine must also address various clinical, manufacturing, and regulatory challenges and comply with good manufacturing practice.243,246

6 | CONCLUDING COMMENTS

Oral genomic and metagenomic studies have provided repositories with terabyte sequences, but phage sequences usually stay uncharacterized. Oral phages are abundant and highly diverse, but only a few phage isolates have been thoroughly studied to date, and the characterization of oral phages poses a significant research challenge. The identification of phages capable of infecting and possibly altering the pathogenicity of oral bacteria involved in endocarditis, colorectal cancer, and other systemic diseases should help promote oral phage research. Phage interactions with oral bacteria and human cells may modulate the ecology of oral biofilms and immune responses, which, if so, suggests a larger biological role of oral phages than previously thought. The employment of lytic phages and phage enzymes to maintain or restore oral health is particularly exciting, but several hurdles remain. Selection of isolates with the greatest biotechnological potential is contingent upon availability of well-characterized oral phages. Preparation of stable lytic phages is not a trivial issue either, as shown in clinical trials, and phage medicine will require a large-scale production of stable therapeutic phages. To sum up, dentistry is at the very beginning of understanding oral phages, and the coming years will undoubtedly uncover some of their secrets. Insights into the phage-bacterium-human cell molecular interaction seem essential to decipher the role of oral phages in health and disease and for producing “intelligent” phages that would fit specific therapeutic purposes.

REFERENCES

1. Edlund A, Santiago-Rodriguez TM, Boehm TK, Pride DT. Bacteriophage and their potential roles in the human oral cavity. *J Oral Microbiol*. 2015;7:27423.
22. Rohwer F, Edwards R. The phage proteomic tree: a genome-based analysis of mycobacteriophage population revealed through analysis of the human salivary virome. ISME J. 2012;6(5):915-926.

23. Clokie MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage. 2011;1(1):31-45.

24. Shkoporov AN, Clooney AG, Sutton TDS, et al. The human gut virome is highly diverse, stable, and individual specific. Cell Host Microbe. 2019;26(4):527-541.e5.

25. Roux S, Adriaenssens EM, Dutilh BE, et al. Minimum information about an uncultivated virus genome (MIUViG). Nat Biotechnol. 2019;37(1):29-37.

26. Wang X, Wei Z, Yang K, et al. Phage combination therapies for bacterial wilt disease in tomato. Nat Biotechnol. 2019;37(12):1513-1520.

27. Van Belleghem JD, Dabrowska K, Vanechouotte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses. 2019;11(1):10.

28. Davies EV, Winstanley C, Fothergill JL, James CE. The role of temperate bacteriophages in bacterial infection. FEMS Microbiol Lett. 2016;363(5):fnw015.

29. Balasubramanian S, Osburne MS, BrinJones H, Tai AK, Leong JM. Phage induction, but not production of phage particles, is required for lethal disease in a microbiome-replete murine model of enterohemorrhagic E coli infection. PLoS Pathog. 2019;15(1):e1007494.

30. Dalmasso M, de Haas E, Hargreaves KR, Abdon ST, Sullivan MB. Lysogeny in nature: mechanisms, impact and ecology of temperate phages. ISME J. 2017;11(7):1511-1520.

31. Silpe JE, Bassler BL. A host-produced quorum-sensing autoinducer controls a phage lysis-lysogeny decision. Cell. 2019;176(1-2):268-280.e13.

32. Knowles B, Silveira CB, Bailey BA, et al. Lytic to temperate switching of virulent communities. Nature. 2016;531(7595):466-470.

33. Stokar-Avihail A, Tal N, Erez Z, Lopatina A, Sorek R. Widespread utilization of peptide communication in phages infecting soil and pathogenic bacterial. Cell Host Microbe. 2019;25(5):746-755.e5.

34. Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. Nat Rev Microbiol. 2010;8(5):317-327.

35. Samson JE, Magadan AH, Sabri M, Moineau S. Revenge of the phages: defeating bacterial defences. Nat Rev Microbiol. 2013;11(10):675-687.

36. Dy RL, Richter C, Salmond GP, Fineran PC. Remarkable mechanisms in microbes to resist phage infections. Annu Rev Virol. 2014;1(1):307-331.

37. Van Houte S, Buckling A, Westra ER. Evolutionary ecology of prokaryotic immune mechanisms. Microbiol Mol Biol Rev. 2016;80(3):745-763.

38. Croucher NJ, Mostowy R, Wymant C, et al. Horizontal DNA transfer mechanisms of bacteria as weapons of intragenomic conflict. PLoS Biol. 2016;14(3):e1002394.

39. Alseth EO, Pursey E, Luján AM, et al. Bacterial biodiversity drives the evolution of CRISPR-based phage resistance. Nature. 2019;574(7779):549-552.

40. Meeske AJ, Nakandakari-Higa S, Marraffini LA. Cas13-induced cellular dormancy prevents the rise of CRISPR-resistant bacteriophage. Nature. 2019;570(7760):241-245.
47. Bernheim A, Sorek R. The pan-immune system of bacteria: anti-viral defence as a community resource. *Nat Rev Microbiol.* 2020;18(2):113-119.

48. Hampton HG, Watson BNJ, Fineran PC. The arms race between bacteria and their phage foes. *Nature.* 2020;577(7790):327-336.

49. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science.* 2007;315(5819):1709-1712.

50. Bondy-Denomy J, Pawluk A, Maxwell KL, Davidson AR. Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. *Nature.* 2013;493(7429):429-432.

51. Squires SK, Murugan K, Sashital DG. Enzymatic anti-CRISPRs improve the bacteriophage arsenal. *Nat Struct Mol Biol.* 2019;26(4):250-251.

52. Mendoza SD, Niewegowska ES, Govindarajan S, et al. A bacteriophage nucleus-like compartment shields DNA from CRISPR nucleases. *Nature.* 2020;577(7789):244-248.

53. Rollie C, Chevallereau A, Watson BNJ, et al. Targeting of temperate phage genomes drives loss of type I CRISPR-Cas systems. *Nature.* 2020;578(7793):149-153.

54. Hutinet G, Kot W, Cui L, et al. 7-Deazaguanine modifications protect phage DNA from host restriction systems. *Nat Commun.* 2019;10(1):5442.

55. Vidakovic L, Singh PK, Hartmann R, Nadell CD, Drescher K. Dynamic biofilm architecture confers individual and collective mechanisms of viral protection. *Nat Microbiol.* 2018;3(1):26-31.

56. Péres DP, Oliveira H, Melo LD, Sillankorva S, Azeredo J. Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. *Appl Microbiol Biotechnol.* 2016;100(5):2141-2151.

57. Toyofuku M, Nomura N, Eberl L. Types and origins of bacterial viruses. *Cell.* 2001;50(4):470-478.

58. Doron S, Melamed S, Ofir G, et al. Systematic discovery of antiphage defense systems in the microbial pan-genome. *Science.* 2018;359(6379):eaar4120.

59. Simmonds P, Adams MJ, Benko M, et al. Consensus statement: virus taxonomy in the age of metagenomics. *Nat Rev Microbiol.* 2017;15(3):161-168.

60. Barylski J, Enault F, Dutilh BE, et al. Analysis of spounaviruses as a case study for the overdue reclassification of tailed phages. *Syst Biol.* 2020;69(1):110-123. https://doi.org/10.1093/sysbio/syz036

61. Kuhn JH, Wolf YI, Krupovic M, et al. Classify viruses—the gain is worth the pain. *Nat Biotechnol.* 2020;38(1):13-24.

62. DeLong EF, Pace NR. Environmental diversity of bacteria and archaea. *Syst Biol.* 2001;50(4):470-478.

63. Pedulla ML, Ford ME, Houtz JM, et al. Origins of highly mosaic mycobacteriophage genomes. *Cell.* 2003;113(2):171-182.

64. Hatfull GF. Mycobacteriophages. *Microbiology Spectrum.* 2018;6(5):10.1128.

65. Adriaenssens E, Brister JR. How to name and classify your phage: an informal guide. *Viruses.* 2017;9(4):70.

66. Bolduc B, Jang HB, Doucier G, et al. vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria. *PeerJ.* 2017;5:e3243.

67. Adriaenssens EM, Lavigne R, Kropinski AM, Simmonds P. Evaluation of the genomic diversity of viruses infecting bacteria, archaea and eukaryotes using a common bioinformatic platform: steps towards a unified taxonomy. *J Gen Virol.* 2018;99(9):1331-1343.

68. Adriaenssens EM, Sullivan MB, Knezevic P, et al. Taxonomy of prokaryotic viruses: 2018-2019 update from the ICTV Bacterial and Archaeal Viruses Subcommittee. *Arch Virol.* 2020. https://doi.org/10.1007/s00705-020-04577-8

69. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. *Methods Mol Biol.* 2009;501:69-76.

70. Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages using the small drop plaque assay system. *Methods Mol Biol.* 2009;501:81-85.

71. Pelzek AJ, Schuch R, Schmitz JE, Fischetti VA. *Current Protocols: Essential Laboratory Techniques.* Hoboken: John Wiley & Sons Inc; 2008.

72. Castro-Mejía JL, Muhammed MK, Kot W, et al. Optimizing protocols for extraction of bacteriophages prior to metagenomic analyses of phage communities in the human gut. *Microbiome.* 2015;3(1):64.

73. Shkoporov AN, Ryan FJ, Draper LA, et al. Reproducible protocols for metagenomic analysis of human faecal phageomes. *Microbiome.* 2018;6(1):68.

74. Parras-Moltó M, Rodríguez-Galeta A, Suárez-Rodríguez P, López-Bueno A. Evaluation of bias induced by viral enrichment and random amplification protocols in metagenomic surveys of saliva DNA viruses. *Microbiome.* 2018;6(1):119.

75. Cross KL, Campbell JH, Balachandran M, et al. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. *Nat Biotechnol.* 2019;37(11):1314-1321.

76. Goller PC, Haro-Moreno JM, Rodríguez-Valera F, Loeßner MJ, Gomez-Sanz E. Uncovering a hidden diversity: optimized protocols for the extraction of dsDNA bacteriophages from soil. *Microbiome.* 2020;8(1):17.

77. Dunne M, Rupf B, Tala M, et al. Reprogramming bacteriophage host range through structure-guided design of chimeric receptor binding proteins. *Cell Rep.* 2019;29(5):1336-1350.e1334.

78. Kilcher S, Studer P, Muessner C, Klump J, Loeßner MJ. Cross-genus rebooting of custom-made, synthetic bacteriophage genomes in L-form bacteria. *Proc Natl Acad Sci U S A.* 2018;115(5):1253-1260.

79. Raya RR, H’Bert EM. Isolation of phage via induction of lysogens. *Methods Mol Biol.* 2009;501:23-32.

80. Arndt D, Grant JR, Marcu A, et al. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 2016;44(W1):W16-W21.

81. Clark WA. Comparison of several methods for preserving bacteriophages. *Appl Microbiol.* 1962;10:466-471.

82. Kutter E. Phage host range and efficiency of plating. *Methods Mol Biol.* 2009;501:141-149.

83. Hyman P, Abedon ST. Practical methods for determining phage growth parameters. *Methods Mol Biol.* 2009;501:175-202.

84. Kropinski AM. Practical advice on the one-step growth curve. *Methods Mol Biol.* 2018;1681:417-426.

85. Hyman P. Phages for phage therapy: isolation, characterization, and host range breadth. *Pharmaceuticals.* 2019;12(1):35.

86. Ackermann HW. 5500 Phages examined in the electron microscope. *Pharmaceuticals.* 2013;6(8):2306-2318.

87. Barrero-Canosa J, Moraru C, Zeugner L, Fuchs BM, Amann R. 5500 Phages examined in the electron microscope. *Microbiome.* 2019;37:215.

88. Allers E, Moraru C, Duhalme MB, et al. Single-cell and population level viral infection dynamics revealed by phageFISH, a method to visualize intracellular and free viruses. *Environ Microbiol.* 2013;15(8):2306-2318.

89. Barrero-Canosa J, Moraru C, Zeugner L, Fuchs BM, Amann R. Direct-geneFISH: a simplified protocol for the simultaneous detection and quantification of genes and rRNA in microorganisms. *Environ Microbiol.* 2017;19(1):70-82.

90. Ortman AC, Suttle CA. Determination of virus abundance by epifluorescence microscopy. *Methods Mol Biol.* 2009;501:113-126.

91. Allers E, Moraru C, Duhalme MB, et al. Single-cell and population level viral infection dynamics revealed by phageFISH, a method to visualize intracellular and free viruses. *Environ Microbiol.* 2013;15(8):2306-2318.

92. Barrero-Canosa J, Moraru C, Zeugner L, Fuchs BM, Amann R. Direct-geneFISH: a simplified protocol for the simultaneous detection and quantification of genes and rRNA in microorganisms. *Environ Microbiol.* 2017;19(1):70-82.
94 Aruni AW, Mishra A, Dou Y, et al. Filifactor alascis—a new emerging periodontal pathogen. Microbes Infect. 2015;17(7):517-530.

140. Murphy EC, Frick IM. Gram-positive anaerobic cocci—comensals and opportunistic pathogens. FEMS Microbiol Rev. 2013;37(4):520-553.

141. Chou WC, Huang SC, Chiu CH, Chen YM. YMC-2011, a temperate phage of Streptococcus salivarius 57I. Appl Environ Microbiol. 2017;83(6):e03186-16.

142. Siboo IR, Bensing BA, Sullam PM. Genomic organization and molecular characterization of SM1, a temperate bacteriophage of Streptococcus mitis. J Bacteriol. 2003;185(23):6968-6975.

143. Rezaei Javan R, Ramos-Sevillano E, Akter A, Brown J, Brueggemann AB. Prophages and satellite prophages are widespread in Streptococcus and may play a role in pneumococcal pathogenesis. Nat Commun. 2019;10(1):4852.

144. Zheng W, Tan MF, Old LA, et al. Distinct biological potential of Streptococcus gordonii and Streptococcus sanguinis revealed by comparative genome analysis. Sci Rep. 2017;7(1):2949.

145. Bradshaw DJ, Marsh PD, Watson GK, Allison C. Role of Fusobacterium nucleatum and coaggregation in anaerobe survival in planktonic and biofilm oral microbial communities during aeration. Infect Immun. 1998;66(10):4729-4732.

146. Kolenbrander PE, Andersen RN, Moore LV. Coaggregation of Fusobacterium nucleatum, Selenomonas flueggei, Selenomonas infelix, Selenomonas noxia, and Selenomonas sputigena with strains from 11 genera of oral bacteria. Infect Immun. 1989;57(10):3194-3203.

147. Han YW Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol. 2015;23:141-147.

148. Brennan CA, Garrett WS. Fusobacterium nucleatum—symbiont, opportunist and oncobacterium. Nat Rev Microbiol. 2019;17(3):156-166.

149. Eribe ERK, Olsen I. Leptotrichia species in human infections II. J Oral Microbiol. 2017;9(1):1368848.

150. Machuca P, Daille L, Vines E, Berrocal L, Bittner M. Isolation of a novel bacteriophage specific for the periodontal pathogen Fusobacterium nucleatum. Appl Environ Microbiol. 2010;76(21):7243-7250.

151. Kilian M, Schiott CR. Haemophilus and related bacteria in the human oral cavity. Arch Oral Biol. 1975;20(12):791-796.

152. Lloyd-Price J, Mahurkar A, Rahnavard G, et al. Strains, functions and dynamics in the expanded Human Microbiome Project. Nature. 2017;550(7674):61-66.

153. Szafranski SP, Deng ZL, Tomasz J, et al. Quorum sensing of Streptococcus mutans is activated by Aggregatibacter actinomycescentemcomitans and by the periodontal microbiome. BMC Genom. 2017;18(1):238.

154. Norskov-Lauritsen N. Classification, identification, and clinical significance of Haemophilus and Aggregatibacter species with host specificity for humans. Clin Microbiol Rev. 2014;27(2):214-240.

155. Mitchell HL, Dashper SG, Catmull DV, et al. Treponema denticola biofilm-induced expression of a bacteriophage, toxin-antitoxin systems and transposases. Microbiology. 2010;156(Pt 3):774-788.

156. Welch JLM, Dewhirst FE, Borisy GG. Biogeography of the oral microbiome: the site-specialist hypothesis. Annu Rev Microbiol. 2019;73:335-358. https://doi.org/10.1146/annurev-micro-090817-062503

157. Koskella B, Meaden S. Understanding bacteriophage specificity in natural microbial communities. Viruses. 2013;5(3):806-823.

158. Abeles SR, Robles-Sikisaka R, Ly M, et al. Human oral viruses are personal, persistent and gender-consistent. ISME J. 2014;8(9):1753-1767.

159. Ly M, Abeles SR, Boehm TK, et al. Altered oral viral ecology in association with periodontal disease. MBio. 2014;5(3):e01133-14.

160. Zhang Y, Shan T-L, Li F, et al. A novel phage from periodontal pockets associated with chronic periodontitis. Virus Genes. 2019;55(3):381-393.

161. Santiago-Rodriguez TM, Naidu M, Abeles SR, et al. Transcriptome analysis of bacteriophage communities in periodontal health and disease. BMC Genom. 2015;16:549.

162. Wang J, Qi J, Zhao H, et al. Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. Sci Rep. 2013;3:1843.

163. Yost S, Duran-Pinedo AE, Teles R, Krishnan K, Frias-Lopez J. Functional signatures of oral dysbiosis during periodontitis progression revealed by microbial metatranscriptome analysis. Genome Med. 2015;7(1):27.

164. Deng ZL, Szafranski SP, Jarek M, Bhuju S, Wagner-Dobler I. Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci Rep. 2017;7(1):3703.

165. Sandmeier H, van Winkelhoff AJ, Bar K, et al. Temperate bacteriophages are common among Actinobacillus actinomycescentemcomitans isolates from periodontal pockets. J Periodontal Res. 1995;30(6):418-425.

166. Haubeck D, Willi K, Poulsen K, Meyer J, Kilian M. Presence of bacteriophage Aα23 correlates with the population genetic structure of Actinobacillus actinomycescentemcomitans. Eur J Oral Sci. 1997;105(1):2-8.

167. Willi K, Sandmeier H, Asikainen S, Saarela M, Meyer J. Occurrence of temperate bacteriophages in different Actinobacillus actinomycescentemcomitans serotypes isolated from periodontally healthy individuals. Oral Microbiol Immunol. 1997;12(1):40-46.

168. Preus HR, Olsen I, Namork E. Association between bacteriophage-infected Actinobacillus actinomycescentemcomitans and rapid periodontal destruction. J Clin Periodontol. 1987;14(4):245-247.

169. Preus HR, Olsen I, Namork E. The presence of phage-infected Actinobacillus actinomycescentemcomitans in localized juvenile periodontitis patients. J Clin Periodontol. 1987;14(10):605-609.

170. Bensing BA, Siboo IR, Sullam PM. Proteins PblA and PblB of Streptococcus mitis, which promote binding to human platelets, are encoded within a lysogenic bacteriophage. Infect Immun. 2001;69(10):6186-6192.

171. Seo HS, Xiong YQ, Mitchell J, et al. Bacteriophage lysin mediates the binding of Streptococcus mitis to human platelets through interaction with fibrinogen. PLoS Pathog. 2010;6(8):e1001047.

172. Seo HS, Sullam PM. Characterization of the fibrinogen binding domain of bacteriophage lysin from Streptococcus mitis. Infect Immun. 2011;79(9):3518-3526.

173. Willner D, Furlan M, Schmieder R, et al. Metagenomic detection of phage-encoded platelet-binding factors in the human oral cavity. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4547-4553.

174. Schmidt TS, Hayward MR, Coelho LP, et al. Extensive transmission of microbes along the gastrointestinal tract. eLife. 2019;8:e42693.

175. Flynn KJ, Baxter NT, Schloss PD. Metabolic and community synergy of oral bacteria in colorectal cancer. mSphere. 2016;1(3):e00102-16.

176. Kondell PA, Nord CE, Nordenram G. Characterization of Staphylococcus aureus isolates from oral surgical outpatients compared to isolates from hospitalized and non-hospitalized individuals. Int J Oral Surg. 1984;13(5):416-422.

177. Stevens RH, Porras OD, Delisle AL. Bacteriophages induced from lysogenic root canal isolates of Enterococcus faecalis. Oral Microbiol Immunol. 2009;24(4):278-284.

178. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. Int Endod J. 2009;42(4):277-287.

179. Robles-Sikisaka R, Ly M, Boehm T, et al. Association between living environment and human oral viral ecology. ISME J. 2013;7(9):1710-1724.

180. Ly M, Jones MB, Abeles SR, et al. Transmission of viruses via our microbiomes. Microbiome. 2016;4(1):64.
181. Beall CJ, Sulyanto RM, Griffen AL, Leys EJ. Oral bacteriophages are maintained at high levels for months in individuals but infrequently transmitted between mothers and infants. bioRxiv. 2019. https://doi.org/10.1101/633727
182. Kort R, Caspers M, van de Graaf A, et al. Shaping the oral microbiota through intimate kissing. Microbiome. 2014;2(1):41.
183. Saarela M, von Troil-Linden B, Torkko H, et al. Transmission of oral bacterial species between spouses. Oral Microbiol Immunol. 1993;8(6):349-354.
184. Petit MD, van Steenbergen TJ, Scholte LM, van der Velden U, de Graaff J. Epidemiology and transmission of Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans among children and their family members. A report of 4 surveys. J Clin Periodontol. 1993;20(9):641-650.
185. Haubek D, Poulsen K, Asikainen S, Kilian M. Evidence for abscission of Actinobacillus actinomycetemcomitans. J Clin Microbiol. 1995;33(2):395-401.
186. Fazzino L, Anisman J, Chacón JM, Heineman RH, Harcombe WR. Lytic bacteriophages have diverse indirect effects in a synthetic cross-feeding community. ISME J. 2019;14(1):123-134.https://doi.org/10.1038/s41396-019-0511-z
187. Hsu BB, Gibson TE, Yeliseyev V, et al. Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. Cell Host Microbe. 2019;25(6):803-814.e805.
188. Rodriguez-Valera F, Martin-Cuadrado AB, Rodriguez-Brito B, et al. Piggyback-the-winner in host-associated Science. 2009;325(5942):833.
189. Silveira CB, Rohwer FL. Piggyback-the-winner in host-associated microbial communities. NPJ Biofilms Microbiomes. 2016;2:16010.
190. Jończyk-Matysiak E, Weber-Dąbrowska B, Owczarek B, et al. Explaining microbial population genomics through phage predation. Nat Rev Microbiol. 2009;7(11):828-836.
191. Jahn MT, Arkhipova K, Markert SM, et al. A phage protein aids bacterial sibionts in eukaryote immune evasion. Cell Host Microbe. 2019;26(4):542-550.e5.
192. Chen C, Feng P, Slots J. Herpesvirus-bacteria synergistic interaction in periodontitis. Periodontol 2000. 2020;82(1):42-64.
193. Willi K, Sandmeier H, Kulik EM, Meyer J. Transduction of antibiotic resistance markers among Actinobacillus actinomycetemcomitans stains by temperate bacteriophages Aαw23. Cell Mol Life Sci. 1997;53(12):904-910.
194. Chatterjee A, Willott JLE, Nguyen UT, et al. Parallel genomics uncover novel enterococcal-bacteriophage interactions. MBio. 2020;11(2):e03120-19.
195. Williams MA, Santos DC, Collins JJ. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Microbiol Mol Biol Rev. 2019;83(4):e00012-19.
196. Diard M, Bakkeren E, Cornault JD, et al. Inflammation boosts bacteriophage transfer between Salmonella spp. Microbiol. 2019;83(4):349-357.
197. Preus HR, Olsen I, Gjermo P. Bacteriophage infection—a possible mechanism for increased virulence of bacteria associated with rapidly destructive periodontitis. Acta Odontol Scand. 1987;45:49-54.
198. Kutter E, De Vos D, Gvasalia G, et al. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol. 2010;11(1):69-86.
199. Wright A, Hawkins CH, Anggard EE, Harper DR. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clin Otolaryngol. 2009;34(4):349-357.
234. Khalifa L, Gelman D, Shlezinger M, et al. Defeating antibiotic and phage-resistant Enterococcus faecalis using a phage cocktail in vitro and in a clot model. Front Microbiol. 2018;9:326.

235. Al-Zubidi M, Wdziolek M, Court EK, et al. Identification of novel bacteriophages with therapeutic potential that target Enterococcus faecalis. Infect Immun. 2019;87(11):e00512-19.

236. Shlezinger M, Coppenhagen-Glazer S, Gelman D, Beyth N, Hazan R. Eradication of vancomycin-resistant enterococci by combining phage and vancomycin. Viruses. 2019;11(10):954.

237. Guo L, McLean JS, Ysang Y, et al. Precision-guided antimicrobial peptide as a targeted modulator of human microbiota. Proc Natl Acad Sci U S A. 2015;112(24):7569-7574.

238. Xu J, Yang H, Bi Y, et al. Activity of the chimeric lysin ClyR against common gram-positive oral microbes and its anticoagulant efficacy in rat models. Viruses. 2020;12(4):1951-1961.

239. Yang H, Bi Y, Shang X, et al. Antibiofilm activities of a novel chimeric lysin against Streptococcus mutans under physiological and cariogenic conditions. Antimicrob Agents Chemother. 2016;60(12):7436-7443.

240. Ostrov N, Beal J, Ellis T, et al. Technological challenges and milestones for writing genomes. Science. 2019;366(6463):310-312.

241. Yehl K, Lemire S, Yang AC, et al. Engineering phage host-range and suppressing bacterial resistance through phage tail fiber mutagenesis. Cell. 2019;179(2):459-469.e459.

242. Peng H, Borg RE, Dow LP, Pruitt BL, Chen IA. Controlled phage therapy by photothermal ablation of specific bacterial species using gold nanorods targeted by chimeric phages. Proc Natl Acad Sci U S A. 2020;117(4):1951-1961.

243. Henein A. What are the limitations on the wider therapeutic use of phage? Bacteriophage. 2013;3(2):e24872.

244. Young R, Gill JJ. Phage therapy redux–what is to be done? Science. 2015;350(6265):1163-1164.

245. Pirnay JP, Verbeken G, Ceyssens PJ, et al. The magistral phage. Cell. 2019;179(2):459-469.e459.

246. Lehman SM, Mears G, Rankin D, et al. Design and preclinical development of a phage product for the treatment of antibiotic-resistant Staphylococcus aureus infections. Viruses. 2019;11(1):88.

How to cite this article: Szafrański SP, Slots J, Stiesch M. The human oral phageome. Periodontol 2000. 2021;86:79–96. https://doi.org/10.1111/prd.12363