JOURNAL OF ORAL MICROBIOLOGY
2024, VOL. 16, 2417099
https://doi.org/10.1080/20002297.2024.2417099

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Findings and methodologies in oral phageome research: a systematic review

Xin Chen [a,b], Ting Zou[c,d], Guicong Ding [a,b] and Shan Jiang[c,e]

[a] Shenzhen Children's Hospital, China Medical University (CMU), Shenzhen, China; [b]Department of Stomatology, Shenzhen Children's Hospital, Shenzhen, Guangdong, China; [c]Shenzhen Clinical College of Stomatology, School of Stomatology, Southern Medical University, Shenzhen, Guangdong, China; [d]Central Laboratory, Shenzhen Stomatology Hospital (Pingshan) of Southern Medical University, Shenzhen, China; [e]Department of Periodontology, Shenzhen Stomatology Hospital (Pingshan) of Southern Medical University, Shenzhen, China

ABSTRACT

Background: The oral microbiome serves as both an indicator and a mediator of oral health. Evidence indicates that bacteriophages (phages) are widely present in the oral microbiome and exhibit diverse classifications and interactions with human cells and other microbes. These phages constitute the oral phageome, which potentially exerts significant yet unexplored effects on the interplay between oral and general health.

Methods: Three databases (PubMed/MEDLINE, Embase and Scopus) were searched for metagenomic analyses that investigated the oral phageome. Eligible studies were synthesized based on their methodological approaches and findings.

Results: A total of 14 articles were included in this systematic review. Among the 14 articles included, there were six studies that discussed disease-related alterations, along with a discursive examination of additional variables such as sampling niches, external interventions and methodologies. The phages that infect *Streptococcus Actinomyces Haemophilus*, and *Veillonella* have been discovered to be associated with chronic periodontitis, caries, and pancreatic ductal carcinoma.

Conclusions: This systematic review focuses on findings and methodologies in oral phageome studies, which were conducted using highly heterogeneous methodologies that explored the oral phageome in multiple directions while placing constraints on quantitative statistics. Combining different kinds of sample types, utilizing the characteristics of different methods, involving both DNA and RNA phages, and differentiating lysogenic and lytic phages should be the distinction of further studies.

ARTICLE HISTORY
Received 28 June 2024
Revised 8 October 2024
Accepted 10 October 2024

KEYWORDS
Oral; phageome; methodology; metagenomics; bioinformatics

Introduction

The oral microbiome, which exists throughout the entire oral cavity, from the tooth surface to the oral mucosa, is a complex community that consists of various microorganisms [1]. These microorganisms range from larger organisms, such as fungi or bacteria, to smaller entities, such as viruses and the candidate phyla radiation (CPR), which are measured at the micron scale. Each component of the oral microbiome maintains a balanced microecosystem via close interactions with one another [2]. Within the interplay of microbes, viruses can interact both within the microbiome with bacteria and beyond the microbiome with epithelial tissues [3]. Microbial dysbiosis, a state characterized by a disrupted balance, commonly leads to chronic inflammation, oncogenic metabolism, and, specifically, dental demineralization. This imbalance also gives rise to various oral diseases, such as caries, gingivitis, periodontitis, and even carcinomas or cancers [4]. Furthermore, dysbiosis can serve as an indication or manifestation of underlying issues in distant problems of the body (e.g. atherosclerotic plaques, pneumonia, and rheumatoid arthritis) [5,6]. Therefore, detection with oral samples is highly effective in reflecting current health conditions.

The oral cavity harbors a high load of viruses, which consist predominantly of bacteriophages (phages) [7,8]. Given the advanced findings on the direct or indirect influence of phages on human health, the human virome, which is currently poorly understood, needs to be further explored.

Phages are viruses that infect specific host bacteria, which can be either DNA or RNA-based [9]. The significance of phages and the phageome lies in their relationship with bacteria, which elucidates the causes of various infectious diseases [10]. Phages play a crucial role in manipulating bacterial pathogenicity by influencing the formation and dynamics of the local and general bacteriome [11]. They exhibit

CONTACT Shan Jiang kqshanj@163.com Department of Periodontology, Shenzhen Stomatology Hospital (Pingshan) of Southern Medical University, Shenzhen, Guangdong 518118, China; Guicong Ding dgc_67@sina.com Department of Stomatology, Shenzhen Children's Hospital, Shenzhen, Guangdong 518000, China

Supplemental data for this article can be accessed online at https://doi.org/10.1080/20002297.2024.2417099

characteristic behaviors by switching between two distinct life cycles, namely, the lytic cycle or the lysogenic cycle, to bilaterally influence bacteria. This influence includes integrating their genome into the bacterial genome or producing lysin and holin to cause bacterial lysis [12]. This mechanism positions phages as a potential solution for bacterial infection or dysbiosis, which warrants attention.

Phages that enter the lytic cycle are known as lytic or virulent phages, which colonize and eventually kill host bacteria [13]. The corresponding phages that enter the lysogenic cycle are known as lysogenic nonvirulent or temperate phages. These viruses can integrate their genetic material into the host bacterial genome without immediately causing bacterial death [14]. The different behavior patterns of phages could exert contrary effects on infected bacteria, which can either be killed after bacterial lysis or be equipped with phage genomes [15]. Phages exert their effects mainly through predation on and coevolution with bacteria, thereby indirectly modifying the dynamics of the microbiome [16].

In addition to their effects on bacteria, direct interactions between phages and human cells have also been reported [17]. Phages can be internalized by cells to influence the immune response and deliver phage-encoded genes, which suggests that phages may impact human health [18].

Most fundamental knowledge of phages has been acquired through traditional methods, including phage assays, electron microscopy, genetic analysis, and PCR assays [19]. However, considering the limited understanding of oral phages on a small scale, these methods struggle to adequately fulfill the current demands.

The metagenomic analysis is used in oral phageome research to identify and characterize the components of the oral microbiome, even when the specific organisms or elements are initially unknown [20]. Metagenomic analysis involves a series of procedures, including nucleic acid extraction, pretreatment, library construction, and high-throughput sequencing [21]. After sequencing, the process typically includes the assembly of genetic sequences, which are then complemented by bioinformatic analysis to identify and characterize various aspects of the target ecology [22]. High-throughput sequencing is the key to metagenomic analysis, enabling this technique to acquire large amounts of data to describe the oral phageome. By combining metagenomics and bioinformatics, valuable insights into the oral phageome have been obtained, which confirmed its potential for further exploration [23].

Host prediction is broadly applied in metagenomic studies to differentiate phages from other viruses. Bioinformatic approaches greatly facilitate the identification of virus-host pairings, which are based on the abundance and composition profiles of homologous or identical sequences or clustered regularly interspaced short palindromic repeat (CRISPR) arrays. These features provide identifiable signals for virus-host pairing, enabling the intentional extraction and analysis of phage sequences [24].

However, the metagenomic procedure is rather expensive, and bioinformatic analysis is highly personalized. These barriers hinder the acquisition and application of knowledge regarding the oral phageome. This review aims to provide researchers interested in the field with a comprehensive understanding of oral phageome methodologies and outcomes obtained from metagenomic data by consolidating and evaluating the current body of research.

Materials and methods

Search strategy

This systematic review intends to collect the experience gained from existing oral phageome analyses. The methodology of this systematic review is designed to be comprehensive and replicable, utilizing a structured approach to the search strategy. The articles presented in this review were derived from studies that applied metagenomics to provide a broader picture of the phageome in the oral cavity. To provide an overview of studies that performed metagenomic analyses of phages in the oral cavity, this review adopted the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) [25]. We searched three electronic databases (PubMed/MEDLINE, Embase, and Scopus) for articles published up to 4 July 2024. The search was based on the keywords ‘oral phageome’ and ‘metagenomics’. Studies were included if they met the inclusion criteria for the sampling side, analysis method, and analysis focus. To ensure precision, our search strategy was based on Medical Subject Headings (MeSH) terms from the National Library of Medicine’s thesaurus, and the free text applied across the three databases is shown in Supplementary Table S1. All identified references were imported into and deduplicated by Zotero (version 6.0.23). The title and abstract screening and full-text screening were performed independently by two independent authors (X, C, and T, Z). No limitations were placed on the study date, language, or location.

Inclusion and exclusion criteria

Eligible studies met the following criteria: (1) studies containing primary data on metagenomic phageome analysis from human oral samples; (2) studies

detailing methodologies, including those regarding sampling metagenomic and bioinformatic analyses of phages or phageomes; and (3) studies mentioned in the relevant literature that met the above criteria.

The following studies were excluded: (1) the article was retracted; (2) the article was not published officially; and (3) the article was secondary literature (i.e. review, editorial, or commentary). The articles that remained after full-text screening were assessed for eligibility.

Quality assessment

Two independent reviewers assessed each study based on the items of the STROBE checklist, which consists of items that serve as the primary tool for quality assessment [26]. The checklist covers various aspects of the study, including the title, abstract, introduction, methods, results, discussion, and funding. Each item on the STROBE checklist was scored as 'met', 'partially met', or 'not met' based on the presence and adequacy of the information provided in the study report. The assessment was crucial in evaluating the strength of the inferences and conclusions drawn from the specific published studies, as well as providing recommendations for prospective research. At this stage, a second independent reviewer appraised studies to be included in the review.

Data extraction

The key focus of the data extraction table includes the first author, year of publication, the main findings of the study, information on the subjects (including their nationality, ages, and grouping design), the details of the sampling method (including sample type, sample size, sample volume if applicable, sample preservation, and any other details of the sampling procedure), and the metagenomic analysis (including tools or databases applied for purification, sequencing, and annotation).

Results

Study selection and quality assessment

The literature search strategy returned 220 articles from the database and three articles retrieved from the references. This total was reduced to 83 after 140 duplicates were removed. A further 45 nonrelevant articles were subsequently removed based on title and abstract screening, leaving 38 articles for full-text screening. Subsequently, 14 studies met the inclusion criteria and were subjected to data extraction. The search and screening procedure is illustrated in Figure 1. The result of quality assessment based on STROBE checklist is described in Table 1.

Study characteristics

The included studies focused on adults and were conducted in developed countries. The oral phageome has gained the most interest from American researchers, but researchers from Spain, Japan and China have also made significant contributions to this topic.

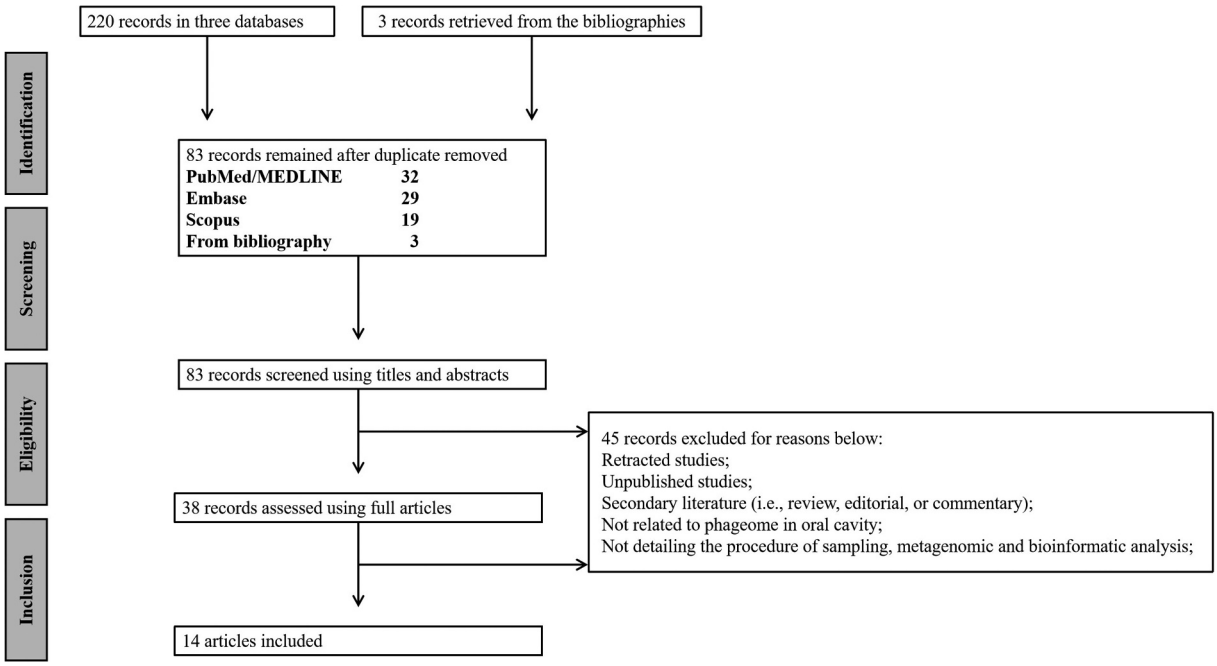
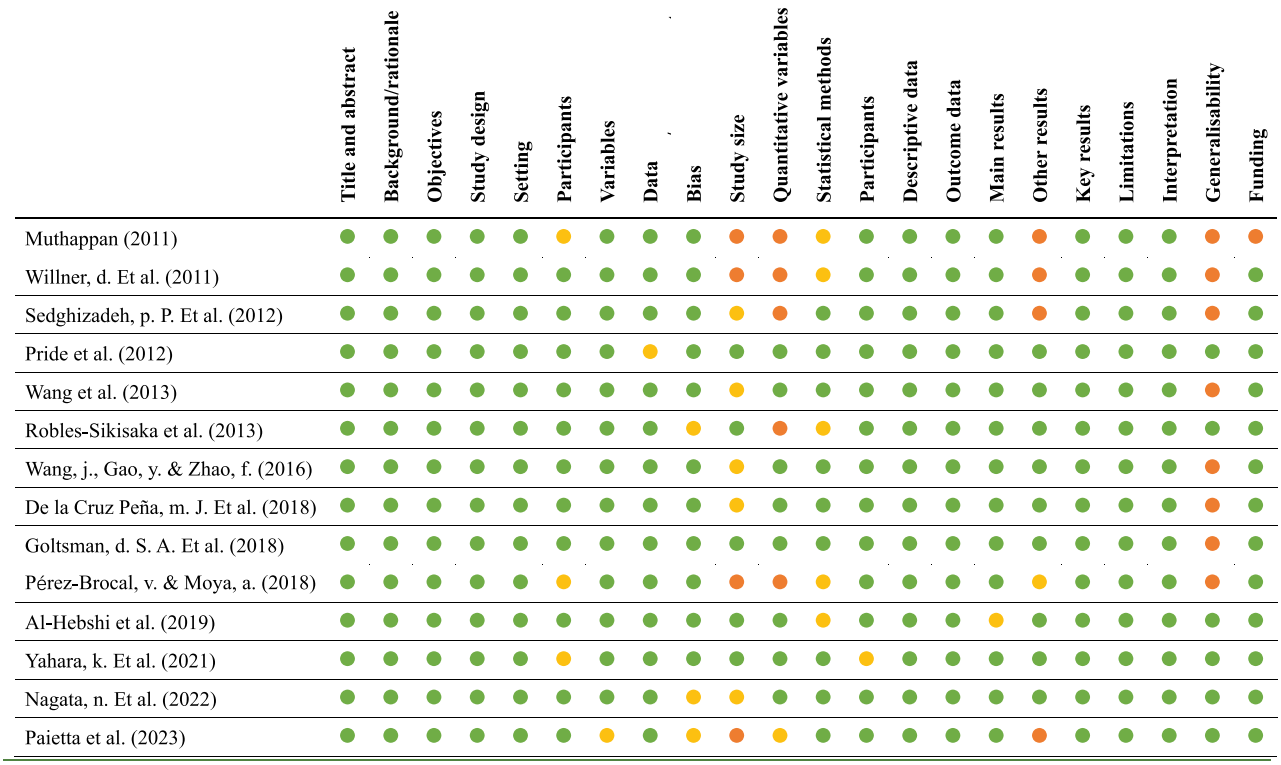


Figure 1.The identification of included articles.

Table 1. Graph showing the results of the quality assessment of included in systematic review done using the STROBE tool.



Sampling, metagenomic, and bioinformatic methodologies

All studies involved healthy individuals, and some studies investigated the oral phageome in sick populations. These sick populations consisted of patients with oral diseases, including bisphosphonate-related osteonecrosis of the jaw (BRONJ) [27], chronic periodontitis [28,29], caries [30], and nonoral conditions, such as preterm pregnancy [31] and pancreatic ductal carcinoma(PDAC) [32]. The main findings and details of the subject recruitment of the included studies are listed in Table 2.

Saliva samples were used in 10 studies [27,29,31–38], whereas mouthwash, plaque, and swab samples were used in one [39], three [28–30], and three studies [28,33,40], respectively. In the reviewed literature, a total of 312 saliva samples were collected, constituting the largest source of metagenomic data. This was followed by mouthwash samples with 72, plaque samples with 61, and the least frequent swab samples with 26. In separate studies, the largest sample sizes for each type of sample were as follows: 101 samples of saliva [31], 72 samples of mouthwash [39], 30 samples of plaque [30], and 19 swabs [33]. Most studies preserved the samples in a buffer solution at −80°C. Sample preparation was relatively consistent across studies and included fasting, abstaining from alcohol, and the prohibition of oral hygiene procedures and antibiotic intake within a specific period. The samples generally underwent different preprocessing procedures, varying from filtration [29,33,35] to enzyme treatment [33–37]. The details of the procedures used for sample management are listed in Table 3.

Current research has primarily concentrated on DNA phages, with the majority of studies utilizing DNA extraction kits for DNA isolation [28–32,34–38,40] However, a subset of these studies also employed alternative methods, such as the cetyltrimethylammonium bromide/formamide technique [33] and the phenol extraction method [27,39]. The sequencing procedure was carried out via Illumina 454 or Ion sequencing, which were applied in 9 (Illumina sequencing) [28,29,31,32,36–40], 3 (454 sequencing) [27,33,34] and 2 (Ion sequencing) [30,35] studies. Bioinformatic methods vary from study to study and focus on different phageome characteristics. However, the procedure involved several widely interesting and relevant, meaningful analyses, including taxonomic assignment, functional annotation, host prediction, composition analysis, and diversity analysis. Several studies combined the above metagenomic-bioinformatic procedures with other analysis methods, including PCR analysis [28,30,33–35,38,40], phage isolation [34,35,37,39,41] or microscopy visualization [34,36,39]. The metagenomic and bioinformatic methodologies used are detailed in Table 4.

Table 2. The main findings and details on subjects' recruitment of the included studies.

Author (year)	Country	Age (years old)	Group	Main Findings
Muthappan (2011)	USA	Not provided	Healthy group: 2	*Streptococcus* pyogenes phage MGAS315 and *Actinomyces* phage Av-1 derived two most abundant sequences encoding hypothetical protein SpyM3_0722 and terminal protein in oral phageome.
Willner, d. et al. (2011)	USA	From 23 to 56	Healthy group: 20	Phage-encoded factors mediating platelet binding were detected in oral cavity, potentially associating oral phages with endocarditis risk.
Sedghizadeh, p. P. et al. (2012)	USA	From 71 to 82	Healthy group: 5 Bisphosphonate-related osteonecrosis of the jaw group: 5	Oral virome were mostly constructed of phages. Moreover, prophages may contributed to antibiotic resistance which have been reported in BRONJ-related microbes.
Pride et al. (2012)	USA	Not provided	Healthy group: 5	Oral virome were mosrly constructed of distinctly distributed phages. Moreover, most phages encoded lysogeny-related genes, which indicated their potential role in shaping oral microbiome.
Wang et al. (2013)	China	From 30 to 65	Healthy group: 9 Chronic periodontitis group: 7	Phages infecting *Actinomyces* were most abundant in healthy plaque phageome, while those infecting *Streptococcus* prevailed in periodontitis swab phageome.
Robles-Sikisaka et al. (2013)	USA	From 23 to 59	Healthy group: 21	*Streptococcus* phage 5093, *Actinomyces* phage AV-1 *Enterobacteriaphage* DE3, and *Haemophilus* phage HP-1 were present uneven distribution within oral phageome.
Wang, j., Gao, y. & Zhao, f. (2016)	USA	From 30 to 65	Healthy group: 20 Chronic periodontitis group: 20	Cross-infective phages were found to influence the structure of the oral microbiome, as revealed by metagenomic analysis.
De la Cruz Peña, m. J. et al. (2018)	Spain	Not provided	Healthy group: 15	SVGs combined with viral metagenomics uncovered the genetic information of uncultured bacteriophages in human saliva.
Goltsman, d. S. A. et al. (2018)	USA	Not provided	Term pregnancy group: 6 Preterm pregnancy group: 4	Strain-level metagenomic analysis uncovered variations in phages during pregnancy, with potential effects on maternal and fetal health.
Pérez-Brocal, v. & Moya, a. (2018)	Spain	From 18 to 25	Healthy group: 72	Widespread bacteriophages from families *Siphoviridae* and *Myoviridae* were found in the oral virome.
Al-Hebshi et al. (2019)	USA	From 6 to 10	Healthy group: 10 Caries group: 20	*Streptococcus* phage M102 was associated with dental caries in children, while *Haemophilus* phage HP1 was associated with the absence of caries in children.
Yahara, k. et al. (2021)	Japan	From 35 to 65	Healthy group: 4	PromethION long-read sequencing exposed a plethora of oral phages, highlighting their complex interactions with bacterial hosts.
Nagata, n. et al. (2022)	Japan Spain Germany	Averaging 70	Healthy group: 45 Pancreatic ductal carcinoma group: 43	Phages infecting *Streptococccus oralis*, *Streptococcus parasanguinis*, *Veillonella atypica*, *Veillonella parvula* were increased in PDAC salivary phageome.
Paietta et al. (2023)	USA	Above 18	Healthy group: 14	Complete genomes of 4 large bacteriophages in the human oral virome were identified, which expanded the understanding of microbe-infecting viral diversity.

Table 3. The details on sampling methodology of the included studies.

Author (year)	Sample size	Volume	Preservation	Sampling details
Muthappan (2011)	Swab: 2	Not provided	Not provided	Not provided
Willner, d. et al. (2011)	Saliva: 20 Swab: 19	3 mL	Phosphate buffer solutions (−80°C)	Oropharyngeal samples were collected and pooled from 19 subjects at baseline. saliva samples were collected at three time points over 3 months.
Sedghizadeh, p. P. et al. (2012)	Saliva: 10	0.5 mL	Todd-Hewitt Buffer (−80°C)	More than 0.5 mL saliva samples were collected at baseline before water- and food-intake
Pride et al. (2012)	Saliva: 45	3 mL	Not provided	Saliva sample was collected in the morning before breakfast prior to any oral hygiene practices, and the saliva was stored at −20°C until further analysis.
Wang et al. (2013)	Swabs: 5 Plaque: 11	Not provided	(−80°C)	Not provided
Robles-Sikisaka et al. (2013)	Saliva:21	Not provided	Not provided	Not provided
Wang, j., Gao, y. & Zhao, f. (2016)	Plaque: 20	Not provided	Phosphate buffer solutions (−80°C)	Plaque samples were collected and pooled from 3-4 sites per subject.
De la Cruz Peña, m. J. et al. (2018)	Saliva: 15	5 mL	Not provided	Saliva sample (5 mL) was collected at baseline before water- and food-intake and oral hygiene
Goltsman, d. S. A. et al. (2018)	Saliva:101	Not provided	Not provided	Not provided
Pérez-Brocal, v. & Moya, a. (2018)	Mouthwash: 72	Not provided	Sterile saline (−20°C)	Oral wash samples were collected at baseline after a thorough oral rinse for 30 seconds
Al-Hebshi et al. (2019)	Plaque: 30	Not provided	Tris-EDTA Buffer (−80°C)	The plaque was pooled by wiping it onto a single sterile gutta – percha point. The point with supragingival plaque sample was placed it into a tube containing sterile TE buffer and stored at – 80°C.
Yahara, k. et al. (2021)	Saliva: 8	1 mL	Not provided	Two samples of 1 mL saliva were successively collected and stored using a kit specialized for microbial and viral DNA/RNA from each of four healthy volunteers.
Nagata, n. et al. (2022)	Saliva: 88	Not provided	RNALater (−80°C)	Saliva samples were collected in the morning of the recruitment day, before breakfast.
Paietta et al. (2023)	Saliva: 14	2 mL	(−80°C)	Saliva samples were collected from healthy adult participants using the passive drool method and Saliva Collection Aid.

Table 4. The details on metagenomic and bioinformatic methodology of included studies.

Author (year)	Sample	Purification	Sequencing	Reference Databases
Muthappan (2011)	Swab	Puregene Kits	Illumina	GenBank
Willner, D. et al. (2011)	Saliva Swab	Cetyltrimethylammonium bromide Formamide method	454 sequencing	GenBank, NCBI
Sedghizadeh, P. P. et al. (2012)	Saliva	Phenol method	454 pyrosequencing	RDP, HGP
Pride et al. (2012)	Saliva	QIAamp Kits	454 sequencing	SEED, MG-Rast, RDPII, VFDB
Wang et al. (2013)	Swab Plaque	QIAamp Kits	Illumina HiSeq	NCBI, SEED, RDP
Robles-Sikisaka et al. (2013)	Saliva	QIAamp Kits	Ion Torrent	Phantom, NCBI
Wang, J., Gao, Y. & Zhao, F. (2016)	Plaque Saliva	QIAamp Kits	Illumina HiSeq	NCBI
De la Cruz Peña, M. J. et al. (2018)	Saliva	QIAamp Kits	Illumina MiSeq	NCBI, PFam
Goltsman, D. S. A. et al. (2018)	Saliva	Powersoil Kits	Illumina HiSeq	NCBI, KEGG, UniRef
Pérez-Brocal, V. & Moya, A. (2018)	Wash	Phenol-chloroform method	Illumina MiSeq	GRCh37
Al-Hebshi et al. (2019)	Plaque	Zymo Kits	Ion Torrent	UniRef90, PFam, MetCyc
Yahara, K. et al. (2021)	Saliva	OMNIgene Kits	Illumina HiSeq	vConTACT, IMG/VR
Nagata, N. et al. (2022)	Saliva	Allprep Kits	Illumina	RDP, NCBI, KEGG
Paietta et al. (2023)	Saliva	High Pure Kits	Illumina NovaSeq	GenBank, NCBI

Factors influencing the observation of the oral phageome

Seven studies discussed factors that influence the observation of the oral phageome, including the subjects, samples, interventions, and methodologies. Six studies reported influential factors related to the characteristics of participants and [27–30,32,35] 2 studies compared the oral phageome in different oral niches [28,33], 1 study reported factors related to external interventions, and 1 study reported factors related to methodologies [33].

Notable components in the oral phageome

Six studies reported the most abundant phages in the oral cavity, which collectively support the predominance of phages that infect *Streptococcus* and *Actinomyces* in the oral phageome [28,29,36,37,39,40]. Additionally, three studies identified characteristic phages associated with certain health conditions. These findings include decreases in *Actinomyces*-infecting phages in the periodontitis plaque phageome, *Streptococcus*-infecting phages in the periodontitis swab phageome, and *Haemophilus*-infecting phages in the caries phageome. Furthermore, increases were detected in the number of phages that infect *Streptococcus* in the caries phageome and the number of phages that infect both *Streptococcus* and *Veillonella* in the PDAC phageome [28,30,32]. A total of 3 studies mutually observed *Streptococcus* phages as temperate phages [33,34,39].

Discussion

Massive heterogeneity of the *methodologies*

The consistency of current methodologies for oral phageome analysis is limited. Participant recruitment, sample selection, metagenomic procedures, and bioinformatic pipelines varied across current studies on the oral phageome. This variability poses a barrier to performing a comprehensive meta-analysis of high-quality evidence, which limits this review to a qualitative level. However, methodological heterogeneity has also helped researchers explore different approaches to understanding the oral phageome, which may pave the way for refining study designs to achieve specific objectives.

Subjects of various backgrounds were selected to reveal correlations between the oral phageome and certain health conditions or environmental exposures. The current recruited participants included not only healthy individuals but also a wide range of individuals suffering from oral diseases, such as BRONJ, caries, and periodontitis, or conditions occurring beyond the oral cavity, including PDAC or preterm pregnancy. These conditions directly affect the oral phageome and cause variations in the oral phageome. Taken together, these findings suggest that the oral phageome is sensitive to local and general health conditions, which extends the understanding of the oral microbiome from a phageome perspective.

The samples used in oral phageome analysis also significantly differed in terms of sample type, sample size collection, preservation procedure, etc. The sample types included saliva, mucosal swabs, plaques, and mouthwash, with saliva being used in the studies that examined the largest samples. Saliva collection is noninvasive and convenient, unlike the collection of plaque and swab samples, which necessitate instruments such as probes or swabs. The collection of mouthwash samples is also simple and rapid, which suggests that mouthwash sampling can be employed to collect large samples, similar to saliva [26]. Mouthwash is a dilution of saliva that also contains minute amounts of components originating from other oral niches, such as plaque or mucosa. Previous analyses that compared the microbiome compositions of saliva and mouthwash samples using 16S rRNA have shown that the bacterial compositions of these samples are similar [27].

Based on current evidence, comparable methods are needed to prove that saliva and mouthwash can both serve as appropriate samples for oral phageome analysis to provide a panoramic view of the oral environment.

Metagenomic procedures were roughly standardized and consisted of DNA extraction and purification, library preparation, and metagenomic sequencing. DNA was most often extracted using DNA extraction kits from different companies, whereas some studies applied traditional chemical methods to extract DNA. Previous experiments compared the efficiency of the DNA extraction kits applied in the included studies. Puregene kits and QIAamp kits were shown to be highly sensitive and consistent [28,29]. However, alternatives are also available. MO BIO Kits outperformed the QIAamp and Zymo Kits, which were applied in the included studies and presented superior decontamination efficiency for solid samples [30]. IHMS protocol Q was also shown to obtain significant amounts of pure viral DNA [31]. As for metagenomic sequencing platforms, a study compared the most commonly applied metagenomic sequencing platforms, including Illumina, 454 and Ion [32]. The three platforms present equally high sensitivity. However, compared with Ion, 454, and Illumina sequencing had better positive predictive values. Therefore, future studies of the oral phageome should select the optimal sequencing platform.

In bioinformatic analysis, tools should be appropriately designed based on specific research objectives. The ever-evolving tools offer a wide range of options for phageome analysis. Given the extremely large variety of bioinformatic tools, a single review that lists current studies is neither applicable nor meaningful. However, a recent review on bioinformatic tools for phage exploration revealed that these tools are most commonly applied to identify either host-encoded determinants of phage-binding, phage-encoded receptor-binding proteins as predictors of host binding, or potential phage-host relationships [33]. This combined work also suggested directions for pharmaceutical research and also highlighted the significance of predicting host-encoded anti-phage systems.

Existing factors affecting the oral phageome

Similar to the oral microbiome, the oral phageome could also be under internal or external influence. Targeted observations that support a specific hypothesis need to thoroughly consider factors related to subject-specific characteristics, sample selection, interventions, and methodologies applied [3].

The specific status of individuals can affect the oral phageome, particularly lifestyle and health conditions. A similar oral phageome was observed among populations that share similar living conditions, which could lead to phageomes with similar bacterial challenges. This similarity may trigger the adaptive behavior of phages and remodel the pattern of the oral phageome [35]. Moreover, the oral phageome structure could also fluctuate with health conditions. The changes in the oral phageome were investigated in patients with BRONJ, chronic periodontitis and PDAC. BRONJ phageomes harbored more lysogenic phages, which influenced bacterial pathogenicity in the oral phageome and decreased viral abundance after antibiotic treatment [27]. Periodontitis reduces the species richness of the oral phageome and increases community structure similarity, which is consistent with bacterial changes in the periodontitis microbiome [29].

The use of different oral niches can also alter the oral phageome [42,43]. Biofilm provides suitable habitats for microbiota colonization prior to adherence and thus may influence the characteristics of phages and the phageome. In bacteria-rich environments, the behavior of phages and the characteristics of the phageome should differ from those observed in environments with lower bacterial density. Previous analysis revealed that *Actinomyces* phages are more abundant in plaque samples, whereas *Streptococcus* phages are more abundant in swab samples, with their abundance being similarly altered to that of their respective host bacteria [29]. Moreover, to address the heterogeneity among samples, samples were pooled from different oral niches to detect sequences of low abundance and ensure sufficient virus biomass [33]. This modification serves as a practical solution to analysis difficulties and provides a preliminary overview of the phageome, but it also introduces greater biases, which should also be considered.

Some interventions that simulate various dietary conditions and survival pressures were found to alter the characteristics of the oral phageome. The ingestion of the mitomycin, nicotine and soy sauce increased the abundance of virulence gene-encoding phages in the oral phageome, whereas the abundance of virulence-encoding phages in the oral phageome did not significantly change in response to the ingestion of red wine, soda or white wine [33]. These findings indicated that the oral phageome could exert disease-inducing effects following exposure to antibiotics, smoking and a heavily seasoned diet. However, changes in phage abundance after different ingestive stimuli could be related either to the stimulation itself or to the dose, which differed from that of the normal diet.

Methodologies, from sampling metagenomic analysis to bioinformatic analysis, presented considerable heterogeneity in the included studies.

Before DNA extraction, filtration and the chloroform method were commonly applied for preprocessing. However, based on their different effects on bacteria and phages, they result in different phageome compositions. Filtration could help omit lysogenic phages integrated into the bacterial genome, whereas the chloroform method could extract lysogenic phages from bacteria by permeabilizing the bacterial membrane. Notably, filtration treatment could both exclude larger phages or include smaller bacteria in the samples, which cannot be corrected by compensatory steps [33]. Moreover, chloroform treatment can lead to the release of bacterial chromosomal DNA, which requires DNase digestion for decontamination.

Streptococcus phages and several other specific phages

Streptococcus phages warrant attention among the investigated DNA phages. Given the broad spectrum of diseases that *Streptococcus* can cause or influence, treatments for these conditions have been developed that involve the application of phage or phage products. These findings on the oral phageome further support the importance of phages that infect *Streptococcus* in the oral environment [44,45]. Previous studies revealed that *Streptococcus* phages, which are mostly prone to be lysogenic phages, dominate oral phages [33,34]. The predominance of *Streptococcus* phages indicates that they may exert stronger effects on oral ecology. *Streptococcus* phages are also linked to the absence of periodontitis and the presence of caries and PDAC, which exhibit parallel trends with those of its host *Streptococcus* [28,29,32]. Previous studies also revealed concordant alterations in host bacteria and specific phages, such as lysogenic phages that infect *Streptococcus* and *Veillonella*, whereas some studies reported discordant relationships, such as phages that infect *Actinomyces* [34]. On the basis of existing evidence, concordant phage–host alterations are correlated with the lysogenicity of phages. Therefore, more evidence of the interaction between *Streptococcus* and its lysogenic phages is needed [33,37].

In addition to phages that infect *Streptococcus*, those that infect *Actinomyces* and *Haemophilus*, which are associated with the absence of periodontitis and caries, respectively, also warrant exploration [28,30]. The correlation between specific phages, including phages that infect *Streptococcus Actinomyces* and *Haemophilus*, and oral diseases indicates potential regulatory effects that oral phages may exert.

The potential of exploring phage–host interactions

Several other notable concepts could be explored further. Horizontal gene transfer (HGT) within the oral phageome introduces additional complexity in understanding coevolution and interactions among phages and bacterial hosts. HGT plays a pivotal role in shaping the genetic diversity of oral phages, allowing them to adapt rapidly to the external environment and transfer antibiotic resistance genes or virulence factors to bacteria [46]. This process may also facilitate the exchange of phage-encoded genes that increase the ability of phages to infect or modulate their bacterial hosts.

Integrated analysis of the bacteriome and clustered regularly interspaced short palindromic repeats (CRISPRs) can also facilitate the understanding of phage–bacteria interactions. CRISPRs in bacteria integrate phage-derived spacers into CRISPRs to defend against phage infection and attack [47]. However, corresponding phage-derived sequences of certain CRISPR spaces in the phageome were observed. These findings indicate that the oral phageome is guarded by CRISPR defense in bacteria [35,37]. CRISPRs may mediate mechanisms that fight bacteria in the oral phageome. Indeed, phages can counter such defenses via anti-CRISPR (ACR) genes [48,49]. However, ACR genes in the oral phageome have not yet been studied. If further studies should focus on this field, the proportion of ACR phages should be considered when their dynamics are suspected. Phages can also obtain CRISPR spaces from different bacterial hosts, suggesting the existence of cross-infection phages (CIPs) within or across the genera *Streptococcus Actinomyces*, *Fusobacterium*, *Aggregatibacter*, *Campylobacter* and *Haemophilus* [17–19]. CIPs can significantly influence the human phageome by promoting genetic diversity and adaptability, thus potentially increasing their resistance to bacterial or environmental pressures [50,51]. CIPs can also modulate the composition of the microbiome by infecting and altering the behavior of commensal bacteria, which can have downstream effects on susceptibility to certain diseases [52]. Given the present knowledge of CIPs, understanding how they function in the oral cavity is crucial for understanding the dynamics of the human phageome and developing phage-based therapies.

Alternative considerations for oral phageome studies

The current findings broaden our knowledge of the oral phageome, yet they are also accompanied by significant barriers that need to be addressed in

future studies. An inclusive understanding of the oral phageome necessitates comprehensive analysis that considers both DNA and RNA phages to capture the full spectrum of phage activity and diversity [53].

Moreover, lysogenic and lytic phages should also be clearly classified because the influence they exert on disease progression can be contrary [54,55]. Phages entering a specific infection cycle can be differentiated by methodology design. For example, extracting DNA via the chloroform method or filtration method tends to include more lysogenic phages and excludes larger phages. The application of bioinformatic analysis tools, such as PhiSpy or Phigaro, can also help differentiate these two types of phages [56–58]. The bioinformatic analysis offers several advantages, including automation, standardization, and effectiveness. However, it is also limited by algorithm deviation and data dependence, which can lead to inaccurate or biased results in cases of low-quality data. Taken together, data from the above variables complicate the prediction of phage host bacteria and the tracing of phage-encoded genes [59]. Therefore, well-designed analytical strategies are needed to decipher the complex interplay between oral phages and human health.

Conclusions

This systematic review synthesized key methodologies and findings from oral phageome studies, revealed heterogeneity in research methodologies and summarized existing knowledge on the oral phageome. Although the variety of approaches in subject recruitment sample selection, metagenomics, and bioinformatic analysis create barriers to quantitative comparisons, they also help explore potential directions for additional research. Specific phages, such as those that infect *Streptococcus Actinomyces* and *Haemophilus*, warrant studies with a more targeted design to reveal their potential to maintain or disrupt oral and general health. Interactions among phages or between phages and bacteria should be considered, including the potential transfer of phage-encoded genes and the effects of CIPs. Moreover, integrating the identification of RNA phages and the differentiation between lytic and lysogenic phages may further facilitate the understanding of the oral phageome.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Medical Science Foundation of Guangdong Province [A2023107]; Natural Sciences Foundation of Shenzhen [JCYJ20230807154030008].

Abbreviations and acronyms

PRISMA	preferred reporting items for systematic reviews and meta-analysis
BRONJ	bisphosphonate-related osteonecrosis of the jaw
PDAC	pancreatic ductal carcinoma
HGT	horizontal gene transfer
CRISPRs	clustered regularly interspaced short palindromic repeats
ACR	anti-CRISPR
CIPs	cross-infection phages

Author contributions statement

Xin Chen: Data curation, Formal Analysis, Investigation, Writing – original draft.

Ting Zou: Data curation, Formal Analysis, Investigation, Review & editing.

Guicong Ding: Conceptualization, Supervision, Writing – review & editing.

Shan Jiang: Conceptualization, Methodology, Supervision, Writing – review & editing

ORCID

Xin Chen http://orcid.org/0009-0006-9040-7024
Guicong Ding http://orcid.org/0000-0003-0918-8620

References

[1] Sedghi L, DiMassa V, Harrington A, et al. The oral microbiome: role of key organisms and complex networks in oral health and disease. Periodontology 2000. 2021;87(1):107–131. doi: 10.1111/prd.12393
[2] Baker JL, Mark Welch JL, Kauffman KM, et al. The oral microbiome: diversity, biogeography and human health. Nature Reviews Microbiol. 2023;22(2):89–104. doi: 10.1038/s41579-023-00963-6
[3] Li X, Liu Y, Yang X, et al. The oral microbiota: community composition, influencing factors, pathogenesis, and interventions. Frontiers in Microbiol. 2022;13. doi: 10.3389/fmicb.2022.895537
[4] Radaic A, Kapila YL. The oralome and its dysbiosis: new insights into oral microbiome-host interactions. Comput Struct Biotechnol J. 2021;19:1335–1360. doi: 10.1016/j.csbj.2021.02.010
[5] Bacali C, Vulturar R, Buduru S, et al. Oral microbiome: getting to know and befriend neighbors, a biological approach. Biomedicines. 2022;10(3):671. doi: 10.3390/biomedicines10030671
[6] Han YW, Wang X. Mobile microbiome: oral bacteria in extra-oral infections and inflammation. J Dent Res. 2013;92(6):485–491. doi: 10.1177/0022034513487559
[7] Martínez A, Kuraji R, Kapila YL. The human oral virome: shedding light on the dark matter. Periodontology 2000. 2021;87(1):282–298. doi: 10.1111/prd.12396

[8] Szafrański SP, Slots J, Stiesch M. The human oral phageome. Periodontology 2000. 2021;86(1):79–96. doi: 10.1111/prd.12363
[9] Dion MB, Oechslin F, Moineau S, et al. Phage diversity, genomics and phylogeny. Nature Reviews MicrobiologyNat Rev Microbiol. 2020;18(3):125–138. doi: 10.1038/s41579-019-0311-5
[10] Torres-Barceló C. The disparate effects of bacteriophages on antibiotic-resistant bacteria. Emerging Microbes & Infections. 2018;7(1):1–12. doi: 10.1038/s41426-018-0169-z
[11] Donlan RM. Preventing biofilms of clinically relevant organisms using bacteriophage. Trends in Microbiol. 2009;17(2):66–72. doi: 10.1016/j.tim.2008.11.002
[12] Catalão MJ, Gil F, Moniz-Pereira J, et al. Diversity in bacterial lysis systems: bacteriophages show the way. FEMS Microbiol Reviews. 2013;37(4):554–571. doi: 10.1111/1574-6976.12006
[13] Young R, Waldor MK, eds. Phage lysis, in *phages*. (WA) (DC), USA: ASM Press; 2014. p. 92–127.
[14] Zhou S, Liu Z, Song J, et al. Disarm the bacteria: what temperate phages can do. Curr Issues Mol Biol. 2023;45(2):1149–1167. doi: 10.3390/cimb45020076
[15] Díaz-Muñoz SL, and Koskella B. Chapter Four - Bacteria–Phage Interactions in Natural Environments. Advances in Applied Microbiology. 2014;89:135–183. doi: 10.1016/B978-0-12-800259-9.00004-4
[16] Flores CO, Meyer JR, Valverde S, et al. Statistical structure of host-phage interactio. Proceedings of the National Academy of Sciences. 2011;108(28):E288–E297. doi:10.1073/pnas.1101595108
[17] Fernández L, Duarte AC, Rodríguez A, et al. The relationship between the phageome and human health: are bacteriophages beneficial or harmful microbes? Beneficial Microbes. 2021;12(2):107–120. doi: 10.3920/BM2020.0132
[18] Żaczek M, Górski A, Skaradzińska A, et al. Phage penetration of eukaryotic cells: practical implications. Future Virology. 2019;(11):745–760. doi: 10.2217/fvl-2019-0110
[19] Peng H, Liu J, Chen IA. eds. Phage engineering and analysis: methods and protocols. New York (NY): Springer US; 2024.
[20] Krishnamurthy SR, Wang D. Origins and challenges of viral dark matter. *Virus Res*. 2017;239:136–142. doi: 10.1016/j.virusres.2017.02.002
[21] Ladoukakis E, Kolisis FN, Chatziioannou AA, et al. Integrative workflows for metagenomic analysis. Front Cell Dev Biol. 2014;2. doi: 10.3389/fcell.2014.00070
[22] Taş N, de Jong AE, Li Y, et al. Metagenomic tools in microbial ecology research. Current Opinion in Biotechno. 2021;67:184–191. doi: 10.1016/j.copbio.2021.01.019
[23] Banar M, Rokaya D, Azizian R, et al. Oral bacteriophages: metagenomic clues to interpret microbiomes. PeerJ. 2024;12:e16947. doi: 10.7717/peerj.16947
[24] Edwards RA, McNair K, Faust K, et al. Computational approaches to predict bacteriophage–host relationships. FEMS Microbiol Rev. 2015;40(2):258–272. doi: 10.1093/femsre/fuv048
[25] Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021:n71. doi: 10.1136/bmj.n71
[26] Cuschieri S. The STROBE guidelines. Saudi J Anaesth. 2019;13(5):31. doi: 10.4103/sja.SJA_543_18
[27] Sedghizadeh PP, Yooseph S, Fadrosh DW, et al. Metagenomic investigation of microbes and viruses in patients with jaw osteonecrosis associated with bisphosphonate therapy. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012;114(6):764–770. doi: 10.1016/j.oooo.2012.08.444
[28] Wang J, Qi J, Zhao H, et al. Metagenomic sequencing reveals microbiota and its functional potential associated with periodontal disease. Scientific Reports. 2013;3(1):1843. doi: 10.1038/srep01843
[29] Wang J, Gao Y, Zhao F. Phage–bacteria interaction network in human oral microbiome. Environ Microbiol 2016;18(7):2143–2158. doi: 10.1111/1462-2920.12923
[30] Al-Hebshi NN, Baraniya D, Chen T, et al. Metagenome sequencing-based strain-level and functional characterization of supragingival microbiome associated with dental caries in children. J Oral Microbiol. 2019;11(1):1557986. doi: 10.1080/20002297.2018.1557986
[31] Goltsman DSA, Sun CL, Proctor DM, et al. Metagenomic analysis with strain-level resolution reveals fine-scale variation in the human pregnancy microbiome. Genome Res. 2018;28(10):1467–1480. doi: 10.1101/gr.236000.118
[32] Nagata N, Nishijima S, Kojima Y, et al. Metagenomic identification of microbial signatures predicting pancreatic cancer from a multinational study. Gastroenterology. 2022;163(1):222–238. doi: 10.1053/j.gastro.2022.03.054
[33] Willner D. Metagenomic detection of phage-encoded platelet-binding factors in the human oral cavity. Proc Natl Acad Sci U S A; 2011;1081:4547–4553. doi: 10.1073/pnas.1000089107
[34] Pride DT, Salzman J, Haynes M, et al. Evidence of a robust resident bacteriophage population revealed through analysis of the human salivary virome. The ISME Journal. 2012;6(5):915–926. doi: 10.1038/ismej.2011.169
[35] Robles-Sikisaka R, Ly M, Boehm T, et al. Association between living environment and human oral viral ecology. The ISME Journal. 2013;7(9):1710–1724. doi: 10.1038/ismej.2013.63
[36] Cruz Peña MJ. Deciphering the human virome with single-virus Genomics and Metagenomics. Viruses. 2018;10(3):113. doi: 10.3390/v10030113
[37] Yahara K, Suzuki M, Hirabayashi A, et al. Long-read metagenomics using PromethION uncovers oral bacteriophages and their interaction with host bacteria. Nature communications. 2021;12(1). doi: 10.1038/s41467-020-20199-9
[38] Paietta EN, Kraberger S, Custer JM, et al. Characterization of diverse anelloviruses, cressdna-viruses, and bacteriophages in the human oral DNA virome from North Carolina (USA). Viruses. 2023;15(9):1821. doi: 10.3390/v15091821
[39] Pérez-Brocal V, Moya A, Zhang C. The analysis of the oral DNA virome reveals which viruses are widespread and rare among healthy young adults in Valencia (Spain. PLOS ONE. 2018;13(2):e0191867. doi: 10.1371/journal.pone.0191867

[40] Muthappan V. Biome representational in silico karyotyping. Genome research; 2011;21(4):626–633.
[41] Wang J, Gao Y, Zhao F. Phage–bacteria interaction network in human oral microbiome. Environmental Microbiol; 2015;18(7):2143–2158. doi: 10.1111/1462-2920.12923
[42] Conceição-Neto N, Zeller M, Lefrère H, et al. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. Scientific Reports; 2015;5(1):16532. doi: 10.1038/srep16532
[43] Lewandowska DW, Zagordi O, Geissberger F-D, et al. Optimization and validation of sample preparation for metagenomic sequencing of viruses in clinical samples. Microbiome. 2017;5(1). doi: 10.1186/s40168-017-0317-z
[44] Rutbeek NR, Rezasoltani H, Patel TR, et al. Molecular mechanism of quorum sensing inhibition in streptococcus by the phage protein paratox. Journal Of Biological Chem. 2021;297(3):100992. doi: 10.1016/j.jbc.2021.100992
[45] Ben-Zaken H, Kraitman R, Coppenhagen-Glazer S, et al. Isolation and characterization of streptococcus mutans phage as a possible treatment agent for caries. Viruses. 2021;13(5):825. doi: 10.3390/v13050825
[46] Nadeem SF, Gohar UF, Tahir SF, et al. Antimicrobial resistance: more than 70 years of war between humans and bacteria. Crit Rev Microbiol. 2020;46(5):578–599. doi: 10.1080/1040841X.2020.1813687
[47] Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science (new York, NY). 2007;315(5819):1709–1712. doi: 10.1126/science.1138140
[48] Landsberger M, Gandon S, Meaden S, et al. Anti-crispr phages cooperate to overcome CRISPR-Cas immunity. Cell. 2018;174(4):908–916.e12. doi: 10.1016/j.cell.2018.05.058
[49] Van Houte S, Ekroth AKE, Broniewski JM, et al. The diversity-generating benefits of a prokaryotic adaptive immune system. Nature. 2016;532(7599):385–388. doi: 10.1038/nature17436
[50] Bouvier T, Del Giorgio PA, Gasol JM. A comparative study of the cytometric characteristics of high and low nucleic-acid bacterioplankton cells from different aquatic ecosystems. Environmental Microbiol. 2007;9(8):2050–2066. doi: 10.1111/j.1462-2920.2007.01321.x
[51] Weitz JS, Poisot T, Meyer JR, et al. Phage–bacteria infection networks. Trends in Microbiol. 2013;21(2):82–91. doi: 10.1016/j.tim.2012.11.003
[52] Piel D, Bruto M, Labreuche Y, et al. Phage–host coevolution in natural populations. Nature Microbiol. 2022;7(7):1075–1086. doi: 10.1038/s41564-022-01157-1
[53] Callanan J, Stockdale SR, Shkoporov A, et al. RNA phage biology in a Metagenomic Era. Viruses. 2018;10(7):386. doi: 10.3390/v10070386
[54] Brady A, Felipe-Ruiz A, Gallego Del Sol F, et al. Molecular basis of lysis–lysogeny decisions in gram-positive phages. Annu Rev Microbiol. 2021;75(1):563–581. doi: 10.1146/annurev-micro-033121-020757
[55] Cheong KH, Wen T, Benler S, et al. Alternating lysis and lysogeny is a winning strategy in bacteriophages due to Parrondo's paradox. Proceedings Of The National Academy Of Sciences. 2022;119(13). doi: 10.1073/pnas.2115145119
[56] Akhter S, Aziz RK, Edwards RA, et al. PhiSpy: a novel algorithm for finding prophages in bacterial genomes that combines similarity- and composition-based strategies. Nucleic Acids Res. 2012;40(16):e126–e126. doi: 10.1093/nar/gks406
[57] Starikova EV, Tikhonova PO, Prianichnikov NA, et al. Phigaro: high-throughput prophage sequence annotation. Bioinformatics (Oxford, England); 2020. doi: 10.1093/bioinformatics/btaa250
[58] Bonilla N, Rojas MI, Netto Flores Cruz G, et al. Phage on tap–a quick and efficient protocol for the preparation of bacteriophage laboratory stocks. PeerJ. 2016;4:e2261. doi: 10.7717/peerj.2261
[59] Mahony J. Biological and bioinformatic tools for the discovery of unknown phage–host combinations. Current Opinion in Microbiol. 2024;77:102426. doi: 10.1016/j.mib.2024.102426