J Cell Signal. 2023;4(3):128-141.

Journal of Cellular Signaling

Review Article

The Human Gut Phageome: Identification and Roles in the Diseases

Mohsen Nabi-Afjadi[1,#,*], Samane Teymouri[2,#], Fatemeh Nafe Monfared[3], Seyed Mostafa Noorbakhsh Varnosfaderani[4], Hossein Halimi[5]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Tarbiat Modares, Tehran, Iran

[2]Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[3]Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[4]Department of Radiology, Afzalipour Hospital, Kerman University of Medical Science, Kerman, Iran

[5]Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[#]These authors contributed equally as the first authors

[*]Correspondence should be addressed to Mohsen Nabi-Afjadi, mohsennabi66@gmail.com

Received date: July 10, 2023, **Accepted date:** August 04, 2023

Citation: Nabi-Afjadi M, Teymouri S, Monfared FN, Varnosfaderani SMN, Halimi H. The Human Gut Phageome: Identification and Roles in the Diseases. J Cell Signal. 2023;4(3):128-141.

Abstract

The human gut is a complex environment that contains a diversity of microorganisms commonly known as the microbiome. Numerous factors influence the composition of human gut bacterial communities, either contributing to homeostasis or the instability associated with a variety of diseases. In this study, we discuss our understanding that proposes among the most influential factors are likely to be bacteriophages, bacteria-infecting viruses that make up a large percentage of the human gut microbiome, demonstrated to have an association with human health and diseases such as inflammatory bowel disease (IBD), cardiovascular disease (CVD), etc. to provide new therapeutic approaches.

Keywords: Phageome, Bacteriophages, Microbiome, Gut health, Cardiovascular disease, Diabetes, Inflammatory bowel disease

Introduction

The human body and the human gut microbiome are referred to as the "superorganism" and the "forgotten organ," respectively [1]. The human body protects a vast and complex ecosystem of microbes, fungi, viruses, bacteria, and other living organisms known as the microbiome [1-3]. The human intestinal microbiome is effective in both health and disease [4]. Most of this microbiota (more than 99%) is located in the lower gastrointestinal tract (GIT) [2,5]. The dynamic balance of the intestinal microbiome is essential for the normal physiology of the host [5]. The intestinal microbiome is involved in nutrient uptake, protection, and a wide range of diseases, from the gastrointestinal tract to the nervous system [1,5]. Most (99%) of microbiome shows focus, particularly on bacterial, viral, fungal, and archaeal populations, are often forgotten [3,6]. Nevertheless, it is necessary for the bacteriophage population that it is not overlooked [7]. Bacteriophages were primarily found in the early 1900s by Twort and d'Herelle [5]. Bacteriophages, viruses that only infect bacteria as a specific strain, are the most common viral components in the human body and are foretold to play an important role in keeping human health through proceedings like horizontal gene transfer [5,8].

Relationships among bacterial populations and bacteriophages play an important role in controlling bacterial biomass, keeping biodiversity, horizontal gene transfer, and directing biogeochemical cycles in the Earth's biosphere [9,10]. Significantly, special and permanent variations in phage composition were identified in various intestinal and systemic conditions like malnutrition, inflammatory bowel

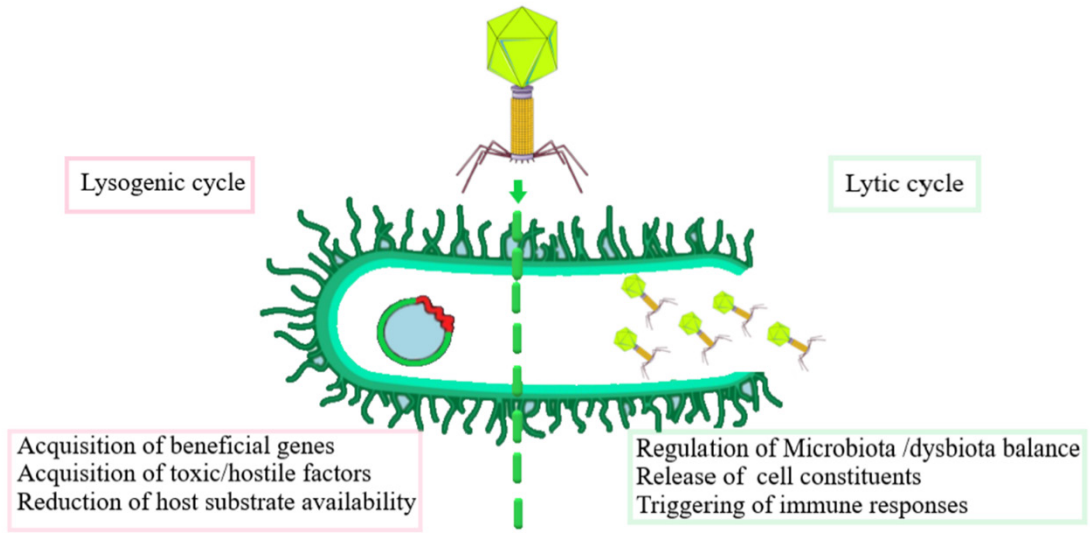

Figure 1: The lysogenic and lytic cycles of the enteric phageome and their local and systemic consequences.

disease (IBD), and acquired immune deficiency syndrome (AIDS). By primary recognition of phages, they were used as an alternative to antibiotic treatment [9-12] (**Figure 1**).

We must perceive the natural interplays of phage in the human microbiome to be able to perform phage therapy safely and efficiently. Lately, the approximation number of bacteriophages in the human intestine has been estimated at nearly 10^{10} per gram of stool [13]. The genetic substance encapsulated in these phages is either RNA or DNA, which has the ability to be single-stranded (ss-) or double-stranded (ds-) [11,13]. The whole population of phages is known as a phageome. In this study, we will concentrate on how to study phageome, classification composition, dynamics, and spatial structure of bacteriophage communities, and the specific characteristic of their interplay with their hosts in a healthy human intestine and its significant roles and functions that act on both human disease and health.

Role of Bacteriophage in Human Disease

It is significant to ponder whether the interaction of phage with commensal bacteria can change the composition of society in a way that affects the operation of the immune system and the diffusion of pathogenic viruses or even bacteria [14]. Whereas phagocytic clearance is the final fate of many circulating phages, phages are internalized by a different set of eukaryotic cells through nonspecific uptake, receptor-associated endocytosis, and uptake of bacterial retaining prophages [15]. Phages can indirectly adjust the immune system. Like effect on a common or pathogenic bacterial host can change the host's infectivity and virulence. For instance, lysogenicity can rise the compatibility of bacteria via a mechanism named "lysogenic conversion" [16]. Phage-encoded proteins take part in human immunity and bacterial virulence. Lysogenic phages permit their bacterial hosts to encode proteins attacking tissue barriers. Maybe the fine investigated is the cholera toxin encoded by a mild phage called *σctx*, which parasitizes *Vibrio cholerae* [17].

The stimulator of the interferon genes (STING) pathway has been indicated to be the great axis for cytosolic dsDNA assay. Whereas there are signs that arranged phages attain the cytosol, there has been no report of STING involvement in phage assays [5]. Bacteriophages present Ig-like receptors (immunoglobulins) on the capsid that react with mucin glycoproteins and can control innate and acquired immunity [18]. There is proof that phageom can affect the innate and adaptation system and play a protective role in the intestinal mucosa [19]. The patient's gut environment can become inflamed and trigger the activation of prophage in *Salmonella*, resulting in bacterial cell death and the development of imbalanced gut bacteria [20]. Intestinal phages may directly rise gut permeability, causing the transfer of bacteria and bacterial products from the intestine into the bloodstream, which provides a chronic inflammatory reaction [21].

Does the Phageome Play a Role in Human Gut Health?

Solving these answers gives an explicit sight of the operation link between human disease and phageome. The constitution of the intestine microbiota is initial at birth, when vast bacterial colonization of the baby happens due to exposure to skin, vaginal, and fecal microbiome [22]. Bacteriophages can affect the combination of bacteria, alter their function and

relationship with epithelial cells, adjust the glycoprotein mucin layer, and adjust other communities of microorganisms. In addition, they are dynamic organisms that can move through the intestinal barrier, transfer into the peripheral blood and tissues, and operate the immune system [23]. The human intestinal phageom represents a prevalent reservoir of several abundant families. These are *Myoviridae*, single-stranded *Microviridae*, *Siphoviridae*, the ubiquitous crAssphage, and many low-frequency families accountable for interpersonal diversity [24]. There is a great deal of interpersonal difference among the phageome of healthy people [25]. Bacteriophages choose certain bacteria in the intestinal mucosa by applying for horizontal gene transfer. They affect their mutation and genetic diversity and therefore modulate their variability and frequency [26].

A study demonstrated that a little set of phages are detected in most healthy individuals [27]. The intestinal microbiome is progressively known as a key factor in human health and different chronic human illness can be associated with gut microbiome disorder [28]. In adulthood, the intestinal phageom is dominated by phages that exhibit a moderate lifestyle [29].

Residual phages on mucosal surfaces enable non-host immunity versus bacterial infection [18]. The role of bacteriophages in gut physiology is more principal than changing the bacterial population by phage infection [28]. Mucosal binding was presented to increase the susceptibility of some bacteria to phage-associated lysis. Significantly, permanent and particular alterations in phageom composition were identified in a number of different intestinal-related and systemic statuses like IBD, AIDS, and malnutrition [30]. In addition, documentation of the transfer proficiency of sterile feces filters in the cure of *C. difficile* infection (CDI) refers to the potential capability of intestinal phages to limit the growth of pathobiont and increase the natural richness of intestinal microbiota [31].

Impact of Medical Interventions on the Phageome

Phages capable of covering bacteria with genes take part in the metabolism of toxins, polysaccharides, and carbohydrates, and in scarce subjects is a source of antibiotic resistance [14]. Bacteriophage therapy has a vigorous potential to cure some extraintestinal illnesses, especially in cases related to bacterial infections [32]. Like the microbiome, everyone has a unique phageom, sensitive to dietary interventions, antibiotics, and illness [11]. Because phages are a significant item of the microbiota, antibiotic disturbance may influence phage communication and increase their amount of proliferation, in that way growing phage-associated gene transfer [33].

Medical methods and cures can influence intestinal phage, containing the well-documented effect of antibiotics. The impress of broad-spectrum antibiotics on the intestinal bacterial population is non-targeted killing, causing a dysbiotic condition [34]. Yang et al. showed that a cocktail of an antiviral medicine exacerbates dextran sulfate sodium (DSS) made colitis in mice while intestinal dormant viruses detected by Toll-like receptors (TLRs) 3 and TLR7 through an interferon-producing (IFN)-β. Although, while phages make up the majority of gut viruses, this investigation did not certain focus on the duty of phages [30].

Fecal microbiota transplantation (FMT) - Transfer of feces material from a healthy donor to a person with a gastrointestinal disorder with the aim of restoring healthy intestinal microbiota. It has been displayed to be a phage component that is very important in FMT and can better performance [35,36].

Cardiovascular Disease (CVD)

Cardiovascular disease (CVD) is a term used to explain different types of disorders and illnesses which influence the blood vessels and eventually the heart. Heart failure, atherosclerosis, and high blood pressure are some of the situations that can lead to CVD [37]. CVD is the main cause of global mortality with 17.3 million deaths worldwide and one in three deaths in the United States each year [38].

Anyhow a study declared that intestinal dysbiosis or abnormal alters in the intestinal microbial flora, can be widely involved in the development of CVD, through the production of metabolic products made by pathogenic bacteria [39]. Diangaran et al. presented the circulating virus in healthy individuals and patients with CVD and found that the viromes of CVD were mainly filled with phages, which accounted for 63% of all viral sequences in comparison with 18% in healthy individuals [40]. The cause of CVD may be bacterial, with a direct connection between the risk of atherosclerotic cardiovascular illness and bacterial metabolites [41]. Some cytokines taking part in CVD are challenging with proof of anti-atherosclerotic effects. Hence, the role of phages in the progress of CVD can be because of modulatory effects on the inflammatory reaction [42].

Impact of Diet on the Phageome

Gut microbiota is well known to be influenced by diet, especially by bacteria. Because phageome composition, population, and variation are affected by microbiomes, it is believed that phageome properties are influenced by diet [24,42-46]. The composition and diversity of phageomes change from infancy to adulthood as a result of dietary changes. To shed light on this issue, it was observed that Microviridae are more abundant in the gut of mice eating high-fat diets and in infants who are breastfed high-fat milk [47,48]. As a consequence, the phage community shifts irreversibly within 24 hours of a high saturated fat diet regardless of the bacterial hosts, Howe and Ringus et al. suggest that diet-induced changes in the phage community are persistent [49].

Furthermore, phageome disruptions in malnourished infants have been observed to play a pathophysiological role by causing a shift in the microbiota into a disease-associated state with age [50,51]. In general, healthy adults with stable lifestyles have stable phageome compositions, populations, and variations [44]. Different dietary regimens alter the proportion and variability of phageomes in the gut, resulting in different phages being incorporated into the genomes of different gut bacterial taxa depending on the diet of the mice [52]. Short-chain fatty acids and fructose, for instance, lead to activating a prophage from the lysogenic gut commensal *Lactobacillus reuteri* and causing it to produce acetic acid, which leads to the release of phages (**Figure 2**) [53]. Moreover, Garmaeva and colleagues investigated the effects of gluten-free diets (GFDs) on virome composition. There were significant changes in the abundance of three distinct viral families, namely crAss-like, Podoviridae, and Virgaviridae, at the nominal level of 0.05 [54]. In a study of obese mice fed a high-fat, high-sugar 'Western' diet, it was demonstrated that their mucosal and luminal viromes were significantly enriched with temperate phages of the Caudovirales order (Bacilli, Negativicutes, and Bacteroidia classes) [55]. Therefore, refined foods may modulate/affect microbiome diversity through phageomes. On the other hand, phageomes population/variation is affected by what we eat daily [46]. In this regard, an impressive number of RNA viruses detected in the gut have been linked to eukaryotic and plant viruses. Moreover, bacteriophages that survive food and water decontamination processes alter/increase gut phageome compositions and populations [56-58]. As a source of bacteriophages, unpasteurized milk can also pose a threat to health by altering the gut microbiome [59]. In other words, our daily food regimen is connected to our phage intake.

Diabetes and Phageome

The gut phageomes are at the root of the development of diabetes since they serve as environmental factors within the gut. Two major phage groups, *Myoviridae* and *Podoviridae*,

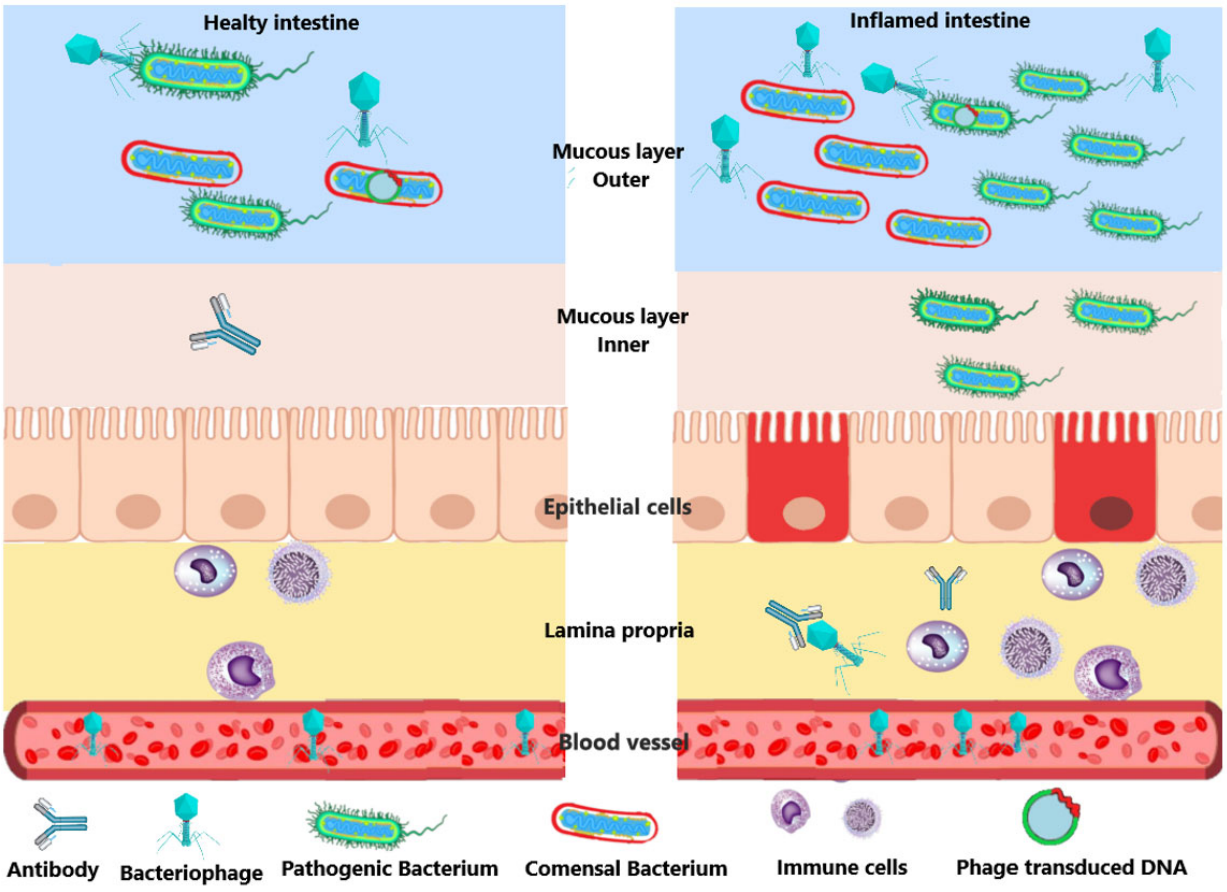

Figure 2: Presence of phages in circulation. Phages may be present in the circulation in a healthy individual **(A)** as well as during the course of intestinal inflammation **(B)**. The higher abundance of phages in the circulation during intestinal inflammation may be due to increased intestinal permeability and the erosion of the mucosal wall.

differed between patients suffering from type 1 diabetes (T1D) and healthy individuals, implicating that diabetes affects gut phage distribution [42,60,61]. In T1D there is a correlation between autoantibodies and amyloid-producing *E. coli* in the gut. The diversity of *E. coli* phages in the intestinal tract of these individuals with T1D is significantly greater compared to healthy controls, and approximately all were lysogenic. It was also found that the ratio of phages to *E. coli* was higher in T1D individuals, which suggests that continual induction of phages or *E. coli* harboring them could be beneficial for individuals with T1D [21]. In addition, it was observed that the abundance of *Bacteroides dorei* populations, containing numerous methylase genes in their genomes, was more prevalent in patients with type 1 diabetes and those at risk of developing islet autoimmunity. As a result, it is possible that *B. Dorei* might alter the autoimmunity of islet cells via gene manipulation [62]. Similarly, the gut phages of T2D patients carried a large number of genes that silently contributed to the pathogenesis of the human disease [42,63,64]. A bioinformatics study based on metagenomic sequencing and intestinal phage types in stool samples from normal and T2DM individuals identified that the number of phages, particularly the T2DM-specific phage *7 pOTU*, in the intestinal tract of diabetic patients was significantly increased [28]. Additionally, *CrAssphage* correlated with *B. Dorei,* but not other *Bacteroides. CrAssphage* and islet autoimmunity, however, did not have a significant relationship [65]. On the other hand, Chen et al. explored the role of extracellular phages in T2D pathogenesis by investigating changes in the extracellular phageome. The results of their study revealed that there was not only an increase in Enterobacteriaceae bacteria but also a significant shift in intestinal phage populations, such that abundances of 7 distinct phages were detected to be elevated specifically associated with Enterobacteriaceae hosts as contributing factors to T2D pathogenesis and development. Furthermore, tissue samples from T2D patients have shown an increase in *podoviridae*, a bacteriophage that infects *E.coli* and *Clostridium* and reproduces alongside its host bacteria as a part of the disease [64]. As the first strategy to prevent the harmful effects of gut microbiota on diabetic patients, fecal virome transplantation (FVT) has been shown to alleviate obesity and type 2 diabetes in mice, by altering the bacterial and viral components of the intestinal microbiota. FVT in mice improves blood glucose tolerance compared to controls by decreasing some effects of a high-fat diet [36,42,65-67]. To sum up, it is unknown how temperate phages contribute to human disease and it remains to be determined if prophages are induced in the gut environment and are responsible for the development of T1D or T2D, or if autoimmunity induces prophage induction.

Inflammatory Bowel Disease (IBD)

Human health and disease have been linked to the intestinal microbiome. As a member of the microbiome, phages have an impact on gut health both directly and indirectly, resulting in systemic health effects. Two major inflammatory bowel diseases are Crohn's disease (CD) and ulcerative colitis (UC) [24,42,68-70]. UC involves inflammation and ulceration only in the colon and rectum of the digestive tract, whereas CD is an inflammation that affects the ileum and colon. Complications from the CD include abscesses, fistulas, and strictures when it affects the entire intestinal wall, whereas complications from UC occur in the inner intestinal lining in the form of crypt abscesses and cryptitis [71]. Active inflammation during a nutrient shortage is a known trigger of bacterial stress, resulting in lysogeny excision. Stressed bacteria typically compete for scarce nutrients in inflammatory diseases such as IBD, resulting in a dysbiotic microbiome, causing an environment conducive to an IBD-associated phage bloom [72]. IBD development and progression are associated with gut phage homeostasis, according to several studies using clinical samples and experimental models [72,73]. When *Salmonella* is exposed to an inflammatory gut environment, prophages are induced, which renders the bacteria lysed, leading to gut dysbiosis [20]. IBD patients have an increased number of virulent phages capable of lysing host bacteria in the gut. Both CD and UC patients exhibit dysbiosis of gut bacteria, which includes an expanded population of potentially pathogenic proteobacteria (e.g., *E. coli* and *Fusobacteria*) and a reduced population of potentially protective Firmicutes (e.g., *Faecalibacterium prausnitzii, Rumininococci*, and *Clostridium clusters IV and XIVa)* [74-76]. *Bacteroides uniformis* and *Bacteroides thetaiotaomicron* have an increased abundance of phages and a decreased abundance of their host bacteria [77]. Furthermore, gut phages can also increase intestinal permeability, which can ultimately lead to bacterial translocation from the gut into the bloodstream, contributing to chronic inflammation [68,78]. In this way, through interaction with the immune system and inducing innate and adaptive immunity, phages can control populations of invasive bacteria in the gut and maintain intestinal barrier function [18]. In addition, phages affect IBD through the activation of TLR3 and TLR7, which induce the release of interferon-β (IFN-β) [79]. The increase in $CD4^+$ and $CD8^+$ T cells in the mesenteric lymph nodes (MLNs) is also associated with their use. Studies conducted *in vitro* also revealed that TLR9 interaction with dendritic cells increases IFN-γ production, thus enhancing intestinal inflammation and the severity of diseases [80].

Anyway, epifluorescent microscopy revealed a ten-fold higher phageome population in CD tissues compared to control tissues when taking biopsies of ulcerated and non-ulcerated tissues [81]. It was found that *Siphoviridae, Myoviridae*, and *Podoviridae morphotypes* were more frequently observed on the mucosa of individuals with non-ulcerated mucosa than on ulcerated tissues. In conclusion, there is a co-occurrence of a reduction in health-associated virulent phages accompanied by a rise in unique temperate phage populations that are predominantly lytic rather than lysogenic [81]. In a study of

metagenomics, a mice model with colitis as well as people with IBD showed a shift from an ordered to a stochastic dysbiosis of intestinal phage populations. The authors noticed that the phage community diversity decreased, subsets of phages expanded, and certain phages' numbers decreased (for example, Clostridiales phages) during colitis [72]. Along with an increase in the number of phages in the gut of CD patients, some phage families, such as the *Caudovirales*, have become more dominant. Caudovirales richness and diversity are inversely correlated with bacterial diversity, suggesting that fluctuations in disease are not associated with host number, but rather are caused by phage [42,82]. It is commonly known that bacteria such as *Bacteroides* are the hosts of the phages. The two most prominent phages are those that infect *Bacteroides fragilis*, which is associated with gut health [83,84]. In CD, *Faecalibacterium prausnitzii* is also a phage that inhibits inflammation. A phage cocktail administered in an experiment targeting pathogenic *Enterobacteriaceae, Streptococcaceae*, and *Staphylococcaceae* caused a reduction in gut *Faecalibacterium* abundance, which is associated with inflammation and altered gut permeability [78,85,86]. As in CD, there is an increased abundance of *Escherichia*, *Streptococcus, Enterobacteria,* and *Caudovirales* phages in the gut mucosa of individuals with UC, and they have a decreased amount of *Microvirides*, indicating that they are directly contributing to inflammatory processes in the gut. Consequently, the changes in bacterial diversity among these subjects were inversely correlated with the changes in phage patterns [60]. Fecal microbiota transplant (FMT) effectiveness is correlated with intestinal *Caudovirales* in humans with UC [31,35,36]. A lower abundance of Caudovirales was detected in UC patients that responded well to FMT than after treatment, suggesting that the intestinal phage community was responsible for worsening the condition. Phage therapy is therefore impacted by phages, so clinicians should be aware of this. It is interesting to observe that the composition of the gut virome of CD and UC patients differed significantly. This suggests that even within IBD, phageomes may be influenced by specific environmental changes [87].

For the treatment of IBD, phage therapy has been proposed as an effective alternative to traditional treatments, such as prebiotics and fecal microbiota transplants [88]. CD patients have an increased rate of phage therapy, which mainly targets the adherent invasive Escherichia coli (AIEC) that maintains intestinal inflammation in IBD [89,90]. In a dextran sulfate sodium (DSS) induced colitis mice model, phage therapy was used against the AIEC strain. Results showed that phage cocktail treatment for a single day drastically decreased the colonization of AIEC strain LF82, which was associated with significantly diminished symptoms over 2 weeks [91]. An experimental trial with a double-blinded placebo-controlled crossover also found that E coli-targeting phages were effective in reducing fecal *E. coli* levels without disturbing the gut microbiota community's composition and increased levels of anti-inflammatory cytokines IL-4 [92,93]. The ability of phages to modulate the balance of intestinal microbiota and immune response is not fully understood, and more research is needed. Overall, changes in population of phageomes in different human diseases are shown in **Table 1**.

Metagenomics and Phageome

The human gut is home to a variety of prokaryotic organisms, including bacteria, bacteriophages, and archaea. Bacteriophages, generally known as phages, can colonize many body organs to perform their desired/undesirable roles; however, their function in the gut, known as phageome, is often overlooked [24,27,94-97]. Virome shows that the most non-pathogenic phages, temperate phages, infect the

Table 1. Changes in population of phageomes in different human diseases.

Disease	Description	Ref.
Cardiovascular disease	The viromes of CVD are mainly filled with phages accounted for 63% of all viral sequences in comparison with 18% in healthy individuals	[42]
Type 1 and 2 diabetes	1) It was found that the ratio of phages to *E. coli* and *B. Dorei* were higher in T1D individuals. 2) In the intestinal tract of diabetic patients, *7 pOTU*, *podoviridae*, and *CrAssphage* were significantly increased	[21,64,65]
Inflammatory bowel disease	1) Both CD and UC patients exhibit dysbiosis of gut bacteria including an expanded population of potentially pathogenic proteobacteria (e.g., *E. coli* and *Fusobacteria*) and a reduced population of potentially protective Firmicutes (e.g., *Faecalibacterium prausnitzii, Rumininococci*, and *Clostridium clusters IV and XIVa.* *2) Bacteroides uniformis* and *Bacteroides thetaiotaomicron* have an increased abundance of phages and a decreased abundance of their host bacteria. 3) *Siphoviridae, Myoviridae*, and *Podoviridae morphotypes* were more frequently observed on the mucosa of individuals with non-ulcerated mucosa than on ulcerated tissues.	[74-76,81]

bacteria by incorporating their genomes into the bacteria genome and subsequently, modify the phenotype of the bacteria by lysogenic conversion into episomes in order to regulate the bacteria biomass [44,98,99]. Phageome diversity, which makes up most of the virome population, is correlated with the bacterial diversity of the gut in different individuals. Therefore, the human gut phageome is highly individual-specific [25,44,82,100]. The use of flow cytometry and microscopic techniques, such as transmission electron microscopy (TEM) and epi-fluorescence microscopy (EFM), is beneficial to reveal the large diversity of viral morphotypes in each individual feces that can be compared against others [81,101]. Overall, various molecular and microscopy based methods available to study the human phageome are listed in **Table 2** and **Figure 3**.

Additionally, the phagosomes were found to be dominated by the Caudovirales order, which is represented by families like *Siphoviridae*, *Podoviridae*, and *Myoviridae* [110]. A metagenomic analysis also indicated that CrAss-like phages constitute the majority of the human gut-associated viruses in 50% of individuals because they account for 90% of metagenomic reads [111-113]. By analyzing fecal viromes, high-throughput metagenomic sequencing can enable us to uncover all phageome phenotypes/families, composition, and function in human gut phageomes. According to preliminary studies of metagenomic sequencing, most sequences of viral genomes (81%-93%) do not align with any known virus genome. This is known as "viral dark matter". Virological dark matter represents a gap in knowledge about the taxonomic composition and population structure of the gut phageome, as well as a bioinformatics problem [114,115]. As well as sequencing techniques, metagenomics analysis of phageomes relies on protocols of storing, separating, and extracting pure nucleic acids from viral particles without contamination as a pre-stage to the metagenomics analysis [116]. First, keep fecal samples from freezing and throwing. To isolate phageomes, re-suspend them in a pH-buffered saline solution or a mixture of sodium chloride and magnesium sulfate [95,117]. To isolate phageome particles, the size of the filter pores must be carefully selected, and the supernatant of the centrifuged fecal sample must be filtered accurately because particle concentration and purity will affect their composition [95,118]. To concentrate and purify the samples before nucleic acid extraction, Polyethylene glycol (PEG) precipitation, Caesium chloride (CsCl) density centrifugation, and Tangential-flow filtration (TFF) will be useful [118-120]. Once the phageoms have been purified and concentrated appropriately, it is time to extract the nucleic acid (RNA or DNA). Both the phenol/chloroform protocol and the formamide/cetyltrimethylammonium bromide (CTAB) method have traditionally been used to extract nucleic acids from bacteria and viruses, respectively [121]. Due to their RNA nature, some plant phageome genomes, such as Pepper mild mottle virus (PMMV), should be converted to cDNA by reverse transcriptase enzyme. When determining the phageome composition, nucleic acid extraction accuracy is extremely crucial, just as it was before [57]. The extracted nucleic acid is then amplified using whole genome amplification (WGA) methods like multiple displacement amplification (MDA), prior to sequencing to assist in an appropriate concentration of the phageome nucleic acid required for library preparation [120,122,123]. To create the phageome genome library, next-generation sequencing (NGS) is used. The NGS process involves cleaving the extracted nucleic acids into shorter fragments, ligating the terminal adapter sequences, amplifying, and sequencing the libraries, and finally assembling the short

Table 2. Various molecular and microscopy based methods available to study the human phageome.

Method	Basis of detection/enumeration	Description	Ref.
Double agar overlay assay (DLA)	Virulent phage particles	Simple, effective, gold standard, shows active virulence	[102]
Transmission electron microscopy (TEM)	Magnification of virus particles	Works well with unknown phages	[103]
Flow cytometry	Viral particles	Can detect different phages in a sample	[104]
NanoSight	Nanoparticle detection by laser-illuminated optical microscopy	Rapid runtime	[105]
qPCR/RT-qPCR	Viral nucleic acid	Precise, reproducible	[105]
Droplet digital PCR (ddPCR)	Viral nucleic acid	No need for internal standards	[106]
Mass spectrometry	Viral protein	Accurate in determining PFU	[107]
Illumina sequencing	Viral nucleic acid library	Not well suited for quantification	[108]
PacBio sequencing	Viral nucleic acid	Prone to sequencing errors	[108]
NanoPore sequencing	Viral nucleic acid (can be amplified if needed)	Compact, rapid, multiple use	[109]

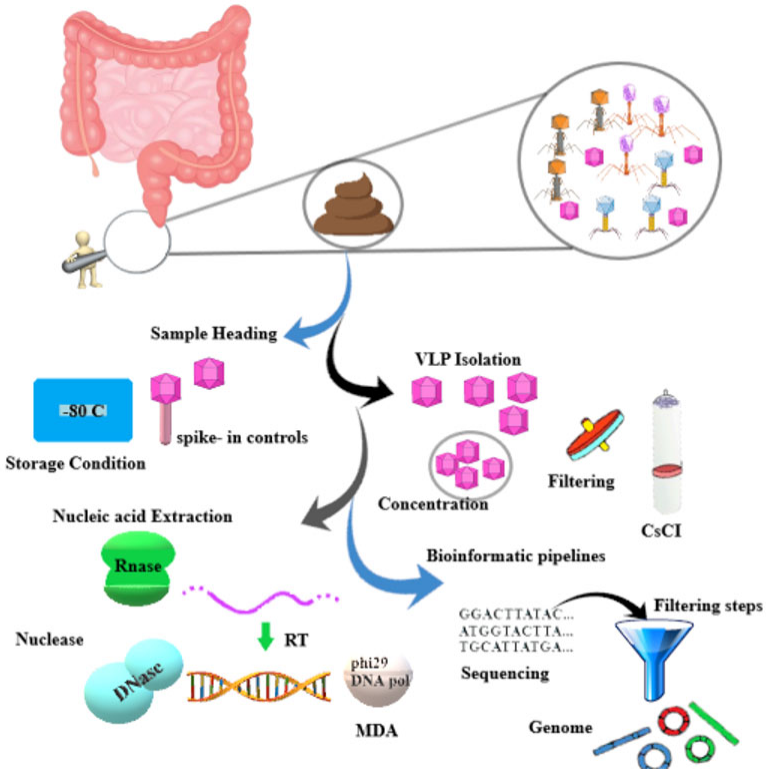

Figure 3. Basis of a viral/phage isolation protocol. Fecal samples are often used as a proxy for human gut virome. Through four main processes, the viral and phage communities of the human gut are analyzed: (i) acquisition and storage of samples, the (ii) concentration of viral particles, (iii) extraction of pure nucleic acids with the elimination of free nucleic acids, and (iv) successful sequencing and bioinformatic analysis of these nucleic.

sequences into larger contigs. NGS platforms such as Illumina HiSeq and Oxford Nanopore, which provide rapid, cost-effective, and advanced long-read sequencing are also available [95]. Pacific Biosciences and Oxford Nanopore have developed long-read sequencing technologies that can also be used to obtain information about methylation patterns by scaffolding large viral genomes. Information about methylation patterns of phageome patterns can facilitate host prediction and study the population structure at a single-virion level [124]. The sequenced fragmented phageome genomes will finally be analyzed with bioinformatics software to identify or characterize the viral contigs by using NGS platforms, such as Illumina HiSeq or MiSeq, Viral MetaGenome Annotation Pipeline (VMGAP), Viral Informatics Resource for Metagenomics Exploration (VIROME), VirSorter, DemoVir, DeepVirFinder, Detection & Analysis of viral and Microbial Infectious Agents by NGS (DAMIAN), Metavir 2 and etc., are of these bioinformatics programs [95,125-128] (**Figure 4**).

Conclusion and Future Directions

Nowadays, more and more evidence suggest that bacteriophages influence the structure and function of the gut microbiome, ultimately affecting health and disease. Gut phage community development and structure are probably crucial to health, implying that the concept of microbiome dysbiosis applies to phages as well. Bacteriophages of the gut are typically induced by prophages found in the local bacterial community. This study has the potential to augment comprehension of the taxonomic data about phageome, while also expediting the investigation of the functional capabilities inherent in phage genomes. Also, it can be conducted from this review that despite the availability of phageome's genome sequence, the identification of these elusive entities from intricate genomic data continues to pose a formidable challenge.

Despite this, more investigations in the future are needed to fully understand the multiple cellular and molecular mechanisms underlying the effects of phageomes in the treatment and/or prevention of diseases such as IBD, CVD, and even multiple cancers, especially colorectal cancer. Furthermore, we suggest that more studies can be conducted in the future to evaluate the prophylactic effects of using bacteriophages in susceptible populations with a familial history of IBD, CVD, etc. to prevent or delay the onset of disease. In addition, bacteriophages could reduce antibiotic resistance and can improve anti-bacterial therapeutic approaches.

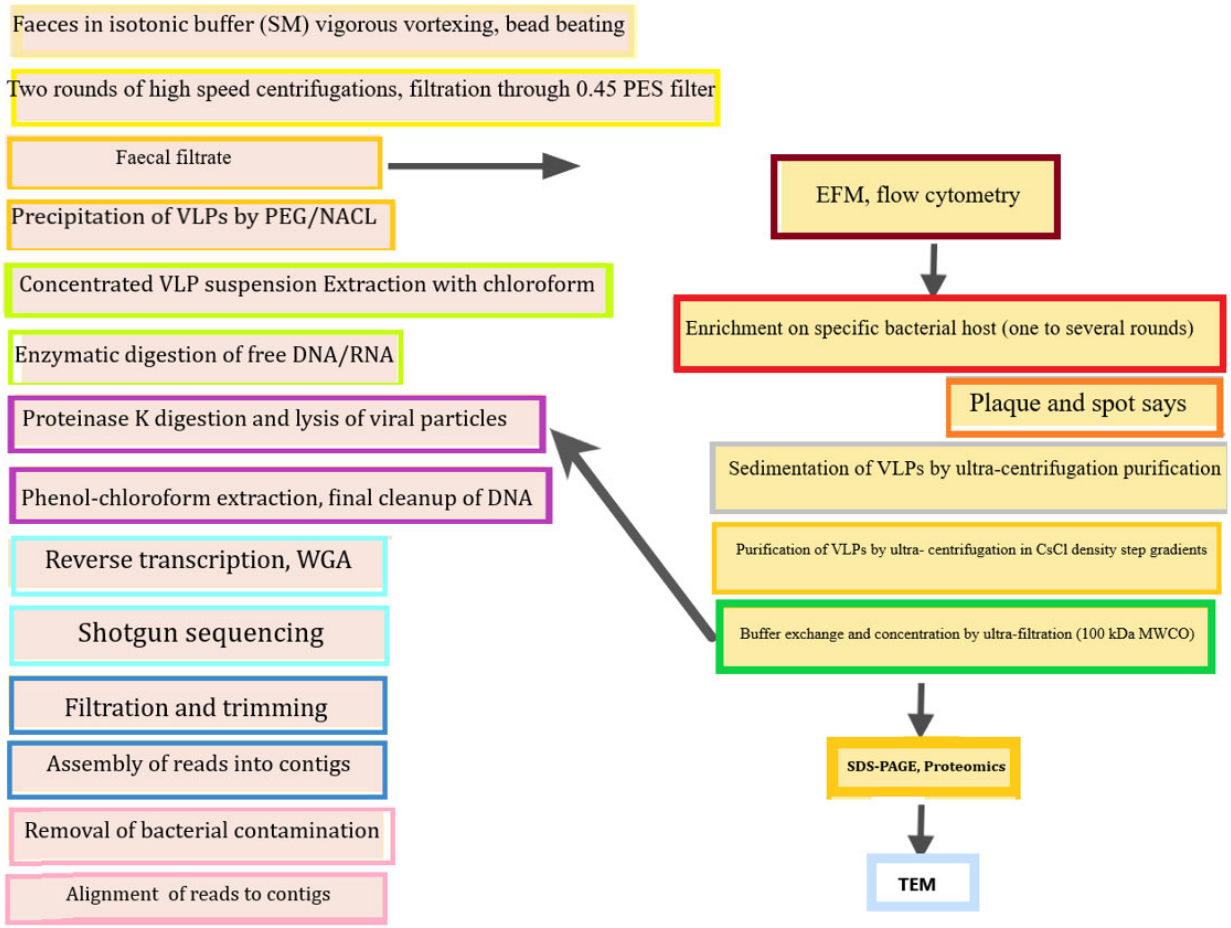

Figure 4. A Generic Gut Phageomics Workflow Combining Metagenomic, Culture-Based, Microscopic, and Proteomic Approaches.

Acknowledgements

Not applicable.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Author Contributions

MN-A conceived and designed the study. MN-A, FNM, ST, SMNV, and HH searched and wrote the manuscript text. FNM created the figures. MN-A supervised the study. All authors read and approved the final manuscript.

Funding

There is no funding for this study.

Data Availability

The data that support the study are all in the article.

Declarations

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Szafrański SP, Slots J, Stiesch M: The human oral phageome. Periodontology. 2000.2021;86(1):79-96.

2. Townsend EM, Kelly L, Muscatt G, Box JD, Hargraves N, Lilley D, et al. The Human Gut Phageome: Origins and Roles in the Human Gut Microbiome. Frontiers in Cellular and Infection Microbiology. 2021;11.

3. Shkoporov AN, Clooney AG, Sutton TDS, Ryan FJ, Daly KM, Nolan JA, et al. The Human Gut Virome Is Highly Diverse, Stable, and Individual Specific. Cell Host and Microbe. 2019;26(4):527-541.e525.

4. Federici S, Nobs SP, Elinav E. Phages and their potential to modulate the microbiome and immunity. Cell Molecular Immunology. 2021;18(4):889-904.

5. Popescu M, Van Belleghem JD, Khosravi A, Bollyky PL. Bacteriophages and the Immune System. Annual Review of Virology. 2021;8:415-435.

6. Maronek M, Link R, Ambro L, Gardlik R. Phages and Their Role in Gastrointestinal Disease: Focus on Inflammatory Bowel Disease. Cells. 2020; 9(4).

7. Fernández L, Duarte AC, Rodríguez A, García P: The relationship between the phageome and human health: Are bacteriophages beneficial or harmful microbes? Beneficial Microbes. 2021;12(2):107-120.

8. Lopetuso LR, Giorgio ME, Saviano A, Scaldaferri F, Gasbarrini A, Cammarota G. Bacteriocins and bacteriophages: Therapeutic weapons for gastrointestinal diseases? International Journal of Molecular Sciences. 2019;20(1).

9. Van Belleghem JD, Dąbrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses. 2019;11(1).

10. Shkoporov AN, Hill C. Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome. Cell Host and Microbe. 2019;25(2):195-209.

11. Górska A, Peter S, Willmann M, Autenrieth I, Schlaberg R, Huson DH. Dynamics of the human gut phageome during antibiotic treatment. Computational Biology and Chemistry. 2018;74:420-427.

12. Bakhshinejad B, Ghiasvand S. Bacteriophages in the human gut: Our fellow travelers throughout life and potential biomarkers of heath or disease. Virus Res. 2017;240:47-55.

13. Ogilvie LA, Jones BV. The human gut virome: Form and function. Emerg Topics Life Sci 2017, 1(4):351-362.

14. Van Belleghem JD, Dąbrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses. 2018;11(1):10.

15. Żaczek M, Górski A, Skaradzińska A, Łusiak-Szelachowska M, Weber-Dąbrowska B. Phage penetration of eukaryotic cells: practical implications. Future Virology. 2019;14(11):745-760.

16. Frobisher Jr M, Brown J. Transmissible toxicogenicity of streptococci. Bulletin of the Johns Hopkins Hospital. 1927;41:167-173.

17. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1996;272(5270):1910-1914.

18. Barr JJ, Auro R, Furlan M, Whiteson KL, Erb ML, Pogliano J, et al. Bacteriophage adhering to mucus provide a non–host-derived immunity. Proceedings of the National Academy of Sciences. 2013;110(26):10771-10776.

19. Barr JJ, Auro R, Sam-Soon N, Kassegne S, Peters G, Bonilla N, et al. Subdiffusive motion of bacteriophage in mucosal surfaces increases the frequency of bacterial encounters. Proceedings of the National Academy of Sciences. 2015;12(44):13675-13680.

20. Diard M, Bakkeren E, Cornuault JK, Moor K, Hausmann A, Sellin ME, et al. Inflammation boosts bacteriophage transfer between Salmonella spp. Science. 2017;355(6330):1211-1215.

21. Tetz G, Brown SM, Hao Y, Tetz V. Type 1 diabetes: an association between autoimmunity, the dynamics of gut amyloid-producing E. coli and their phages. Scientific Reports. 2019;9(1):1-11.

22. Mueller NT, Bakacs E, Combellick J, Grigoryan Z, Dominguez-Bello MG. The infant microbiome development: mom matters. Trends in Molecular Medicine. 2015;21(2):109-117.

23. Łusiak-Szelachowska M, Weber-Dąbrowska B, Jończyk-Matysiak E, Wojciechowska R, Górski A. Bacteriophages in the gastrointestinal tract and their implications. Gut Pathogens. 2017;9(1):1-5.

24. Federici S, Nobs SP, Elinav E. Phages and their potential to modulate the microbiome and immunity. Cellular & Molecular Immunology. 2021;18(4):889-904.

25. Reyes A, Haynes M, Hanson N, Angly FE, Heath AC, Rohwer F, et al. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature. 2010;466(7304):334-338.

26. Lopetuso LR, Giorgio ME, Saviano A, Scaldaferri F, Gasbarrini A, Cammarota G. Bacteriocins and bacteriophages: therapeutic weapons for gastrointestinal diseases? International Journal of Molecular Sciences. 2019;20(1):183.

27. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. Proceedings of the National Academy of Sciences. 2016;113(37):10400-10405.

28. Ma Y, You X, Mai G, Tokuyasu T, Liu C. A human gut phage catalog correlates the gut phageome with type 2 diabetes. Microbiome. 2018;6(1):1-12.

29. Silveira CB, Rohwer FL. Piggyback-the-Winner in host-associated microbial communities. NPJ Biofilms and Microbiomes. 2016;2(1):1-5.

30. Almeida GM, Laanto E, Ashrafi R, Sundberg L-R. Bacteriophage adherence to mucus mediates preventive protection against pathogenic bacteria. MBio. 2019;10(6):e01984-01919.

31. Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, et al. Efficacy of sterile fecal filtrate transfer for treating patients with Clostridium difficile infection. Gastroenterology. 2017;152(4):799-811. e797.

32. Drilling AJ, Ooi ML, Miljkovic D, James C, Speck P, Vreugde S, et al. Long-term safety of topical bacteriophage application to the frontal sinus region. Frontiers in Cellular and Infection Microbiology. 2017;7:49.

33. Fernández-Orth D, Miró E, Brown-Jaque M, Rodríguez-Rubio L, Espinal P, Rodriguez-Navarro J, et al. Faecal phageome of healthy individuals: presence of antibiotic resistance genes and variations caused by ciprofloxacin treatment. Journal of Antimicrobial Chemotherapy. 2019;74(4):854-864.

34. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. The ISME Journal. 2007;1(1):56-66.

35. Bojanova DP, Bordenstein SR. Fecal transplants. what is being transferred? PLoS Biology. 2016;14(7):e1002503.

36. Zuo T, Wong SH, Lam K, Lui R, Cheung K, Tang W, et al. Bacteriophage transfer during faecal microbiota transplantation in Clostridium difficile infection is associated with treatment outcome. Gut. 2018;67(4):634-643.

37. Astudillo AA, Mayrovitz HN. The Gut Microbiome and Cardiovascular Disease. Cureus 2021, 13(4).

38. Akiba Y: Which Comes First. Increased Intestinal Paracellular Permeability or Subepithelial Inflammation? In., vol. 66: Springer; 2021: 3222-3223.

39. Tang WW, Hazen SL. The gut microbiome and its role in cardiovascular diseases. Circulation 2017;135(11):1008-1010.

40. Dinakaran V, Rathinavel A, Pushpanathan M, Sivakumar R, Gunasekaran P, Rajendhran J. Elevated levels of circulating DNA in cardiovascular disease patients: metagenomic profiling of microbiome in the circulation. PloS One. 2014;9(8):e105221.

41. Wang Z, Klipfell E, Bennett B. Intestinal flora Phosphatidylcholine metabolism contributes to cardiovascular disease. Nature. 2011;472:57-63.

42. Townsend EM, Kelly L, Muscatt G, Box JD, Hargraves N, Lilley D, Jameson E. The human gut phageome: origins and roles in the human gut microbiome. Frontiers in Cellular and Infection Microbiology. 2021;2021:498.

43. Samtlebe M, Denis S, Chalancon S, Atamer Z, Wagner N, Neve H, et al. Bacteriophages as modulator for the human gut microbiota: Release from dairy food systems and survival in a dynamic human gastrointestinal model. LWT. 2018;91:235-241.

44. Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, et al. The human gut virome: inter-individual variation and dynamic response to diet. Genome Research. 2011;21(10):1616-1625.

45. Beller L, Matthijnssens J. What is (not) known about the dynamics of the human gut virome in health and disease. Current Opinion in Virology. 2019;37:52-57.

46. Moszak M, Szulińska M, Bogdański P. You are what you eat—The relationship between diet, microbiota, and metabolic disorders—A review. Nutrients. 2020;12(4):1096.

47. Schulfer A, Santiago-Rodriguez TM, Ly M, Borin JM, Chopyk J, Blaser MJ, et al. Fecal viral community responses to high-fat diet in mice. Msphere. 2020;5(1):e00833-00819.

48. Guo M, Liu G, Chen J, Ma J, Lin J, Fu Y, et al. Dynamics of bacteriophages in gut of giant pandas reveal a potential regulation of dietary intake on bacteriophage composition. Science of the Total Environment. 2020;734:139424.

49. Howe A, Ringus DL, Williams RJ, Choo Z-N, Greenwald SM, Owens SM, et al. Divergent responses of viral and bacterial communities in the gut microbiome to dietary disturbances in mice. The ISME Journal. 2016;10(5):1217-1227.

50. Reyes A, Blanton LV, Cao S, Zhao G, Manary M, Trehan I, et al. Gut DNA viromes of Malawian twins discordant for severe acute malnutrition. Proceedings of the National Academy of Sciences. 2015;112(38):11941-11946.

51. Mirzaei MK, Maurice CF. Ménage à trois in the human gut: interactions between host, bacteria and phages. Nature Reviews Microbiology. 2017;15(7):397-408.

52. Kim M-S, Bae J-W. Lysogeny is prevalent and widely distributed in the murine gut microbiota. The ISME Journal. 2018;12(4):1127-1141.

53. Oh J-H, Alexander LM, Pan M, Schueler KL, Keller MP, Attie AD, et al. Dietary fructose and microbiota-derived short-chain fatty acids promote bacteriophage production in the gut symbiont Lactobacillus reuteri. Cell Host & Microbe. 2019;25(2):273-284. e276.

54. Garmaeva S, Gulyaeva A, Sinha T, Shkoporov AN, Clooney AG, Stockdale SR, et al. Stability of the human gut virome and effect of gluten-free diet. Cell Reports. 2021;35(7):109132.

55. Kim MS, Bae JW. Spatial disturbances in altered mucosal and luminal gut viromes of diet-induced obese mice. Environmental Microbiology. 2016;18(5):1498-1510.

56. Liu R, Vaishnav RA, Roberts AM, Friedland RP: Humans have antibodies against a plant virus. evidence from tobacco mosaic virus. PloS One. 2013;8(4):e60621.

57. Zhang T, Breitbart M, Lee WH, Run J-Q, Wei CL, Soh SWL, Hibberd ML, Liu ET, Rohwer F, Ruan Y. RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS Biology. 2006;4(1):e3.

58. Armon R, Araujo R, Kott Y, Lucena F, Jofre J: Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. Journal of Applied Microbiology. 1997;83(5):627-633.

59. Marcó MB, Moineau S, Quiberoni A. Bacteriophages and dairy fermentations. Bacteriophage. 2012;2(3):149-158.

60. Zuo T, Lu X-J, Zhang Y, Cheung CP, Lam S, Zhang F, Tang W, Ching JY, Zhao R, Chan PK. Gut mucosal virome alterations in ulcerative colitis. Gut. 2019;68(7):1169-1179.

61. Sharbatdar Y, Mousavian R, Noorbakhsh Varnosfaderani SM, Aziziyan F, Liaghat M, Baziyar P, Yousefi Rad A, Tavakol C, Moeini AM, Nabi-Afjadi M. Diabetes as one of the long-term COVID-19 complications: from the potential reason of more diabetic patients' susceptibility to COVID-19 to the possible caution of future global diabetes tsunami. Inflammopharmacology. 2023;2023:1-24.

62. Leonard MT, Davis-Richardson AG, Ardissone AN, Kemppainen KM, Drew JC, Ilonen J, Knip M, Simell O, Toppari J, Veijola R. The methylome of the gut microbiome: disparate Dam methylation patterns in intestinal Bacteroides dorei. Frontiers in Microbiology. 2014;5:361.

63. Ma Q, Li Y, Li P, Wang M, Wang J, Tang Z, Wang T, et al. Research progress in the relationship between type 2 diabetes mellitus and intestinal flora. Biomedicine & Pharmacotherapy. 2019;117:109138.

64. Chen Q, Ma X, Li C, Shen Y, Zhu W, Zhang Y, Guo X, Zhou J, Liu C. Enteric phageome alternations in Type 2 diabetes disease. 2019.

65. Cinek O, Kramna L, Lin J, Oikarinen S, Kolarova K, Ilonen J, et al. Imbalance of bacteriome profiles within the Finnish Diabetes Prediction and Prevention study: Parallel use of 16S profiling and virome sequencing in stool samples from children with islet autoimmunity and matched controls. Pediatric Diabetes. 2017;18(7):588-598.

66. Rasmussen TS, Mentzel CMJ, Kot W, Castro-Mejía JL, Zuffa S, Swann JR, et al. Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. Gut. 2020;69(12):2122-2130.

67. Park H, Laffin MR, Jovel J, Millan B, Hyun JE, Hotte N, et al. The success of fecal microbial transplantation in Clostridium difficile infection correlates with bacteriophage relative abundance in the donor: a retrospective cohort study. Gut Microbes. 2019;10(6):676-687.

68. Qv L, Mao S, Li Y, Zhang J, Li L. Roles of Gut Bacteriophages in the Pathogenesis and Treatment of Inflammatory Bowel Disease. Frontiers in Cellular and Infection Microbiology. 2021:1201.

69. Maronek M, Link R, Ambro L, Gardlik R. Phages and their role in gastrointestinal disease: focus on inflammatory bowel disease. Cells. 2020;9(4):1013.

70. Ungaro F, Massimino L, D'Alessio S, Danese S. The gut virome in inflammatory bowel disease pathogenesis: From metagenomics to novel therapeutic approaches. United European Gastroenterology Journal. 2019;7(8):999-1007.

71. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474(7351):307-317.

72. Duerkop BA, Kleiner M, Paez-Espino D, Zhu W, Bushnell B, Hassell B, et al. Murine colitis reveals a disease-associated bacteriophage community. Nature Microbiology. 2018;3(9):1023-1031.

73. Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, et al. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell. 2015;160(3):447-460.

74. Frank DN, Amand ALS, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proceedings of the National Academy of Sciences. 2007;104(34):13780-13785.

75. Hoarau G, Mukherjee P, Gower-Rousseau C, Hager C, Chandra J, Retuerto M, et al. Bacteriome and mycobiome interactions underscore microbial dysbiosis in familial Crohn's disease. MBio. 2016;7(5):e01250-01216.

76. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655-662.

77. Nishiyama H, Endo H, Blanc-Mathieu R, Ogata H. Ecological Structuring of Temperate Bacteriophages in the Inflammatory Bowel Disease-Affected Gut. Microorganisms. 2020;8(11):1663.

78. Tetz GV, Ruggles KV, Zhou H, Heguy A, Tsirigos A, Tetz V. Bacteriophages as potential new mammalian pathogens. Scientific Reports. 2017;7(1):1-9.

79. Sweere JM, Van Belleghem JD, Ishak H, Bach MS, Popescu M, Sunkari V, et al. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. Science. 2019;363(6434):eaat9691.

80. Gogokhia L, Round JL. Immune–bacteriophage interactions in inflammatory bowel diseases. Current Opinion in Virology. 2021;49:30-35.

81. Lepage P, Colombet J, Marteau P, Sime-Ngando T, Doré J, Leclerc M. Dysbiosis in inflammatory bowel disease: a role for bacteriophages? Gut. 2008;57(3):424-425.

82. Lim ES, Zhou Y, Zhao G, Bauer IK, Droit L, Ndao IM, et al. Early life dynamics of the human gut virome and bacterial microbiome in infants. Nature Medicine. 2015;21(10):1228-1234.

83. Kang S, Denman SE, Morrison M, Yu Z, Dore J, Leclerc M, et al. Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. Inflammatory Bowel Diseases. 2010;16(12):2034-2042.

84. Wagner J, Maksimovic J, Farries G, Sim WH, Bishop RF, Cameron DJ, et al. Bacteriophages in gut samples from pediatric Crohn's disease patients: metagenomic analysis using 454 pyrosequencing. Inflammatory Bowel Diseases. 2013;19(8):1598-1608.

85. Martinez-Medina M, Aldeguer X, Gonzalez-Huix F, Acero D, Garcia-Gil JL. Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflammatory Bowel Diseases 2006;12(12):1136-1145.

86. Cornuault JK, Petit M-A, Mariadassou M, Benevides L, Moncaut

E, Langella P, et al. Phages infecting Faecalibacterium prausnitzii belong to novel viral genera that help to decipher intestinal viromes. Microbiome. 2018;6(1):1-14.

87. Gogokhia L, Buhrke K, Bell R, Hoffman B, Brown DG, Hanke-Gogokhia C, Ajami NJ, Wong MC, Ghazaryan A, Valentine JF. Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. Cell Host & Microbe. 2019;25(2):285-299. e288.

88. Oka A, Sartor RB. Microbial-based and microbial-targeted therapies for inflammatory bowel diseases. Digestive Diseases and Sciences. 2020;65(3):757-788.

89. Chervy M, Barnich N, Denizot J. Adherent-Invasive E. coli: Update on the Lifestyle of a Troublemaker in Crohn's Disease. International Journal of Molecular Sciences. 2020;21(10):3734.

90. Lamps LW, Madhusudhan K, Havens JM, Greenson JK, Bronner MP, Chiles MC, Dean PJ, Scott MA. Pathogenic Yersinia DNA is detected in bowel and mesenteric lymph nodes from patients with Crohn's disease. The American Journal of Surgical Pathology. 2003;27(2):220-227.

91. Galtier M, Sordi LD, Sivignon A, De Vallée A, Maura D, Neut C, et al. Bacteriophages targeting adherent invasive Escherichia coli strains as a promising new treatment for Crohn's disease. Journal of Crohn's and Colitis. 2017;11(7):840-847.

92. Febvre HP, Rao S, Gindin M, Goodwin ND, Finer E, Vivanco JS, et al. PHAGE study: effects of supplemental bacteriophage intake on inflammation and gut microbiota in healthy adults. Nutrients. 2019;11(3):666.

93. Sefid F, Bahrami AA, Rajabibazl M, Rahmati M, Kalantar SM, Dastmalchi S, et al. Design of an Epitope Candidate Vaccine Against Iha Protein in Escherichia Coli: an in Silico Approach. Journal of Regeneration, Reconstruction & Restoration (Triple R). 2020;5:e19-e19.

94. Shkoporov AN, Hill C. Bacteriophages of the human gut: the "known unknown" of the microbiome. Cell Host & Microbe. 2019;25(2):195-209.

95. Callanan J, Stockdale SR, Shkoporov A, Draper LA, Ross RP, Hill C. Biases in viral metagenomics-based detection, cataloguing and quantification of bacteriophage genomes in human faeces, a review. Microorganisms 2021;9(3):524.

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

97. Lerner A, Ramesh A, Matthias T. The revival of the battle between David and Goliath in the enteric viruses and microbiota struggle: potential implication for celiac disease. Microorganisms. 2019;7(6):173.

98. Brüssow H, Canchaya C, Hardt W-D. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiology and Molecular Biology Reviews. 2004;68(3):560-602.

99. Ebrahimi K, Shir Ovand S, Mohammedi AaN, Nabi-Afjadi M, Zalpoor H, Bahreini F. Biosynthesis of copper nanoparticles using aqueous thymus daenensis (celak) flora and investigation of its antifungal activity. Journal of Medical Microbiology and Infectious Diseases. 2022;10(3):98-103.

100. Breitbart M, Haynes M, Kelley S, Angly F, Edwards RA, Felts B, et al. Viral diversity and dynamics in an infant gut. Research in Microbiology. 2008;159(5):367-373.

101. Hoyles L, McCartney AL, Neve H, Gibson GR, Sanderson JD, Heller KJ, et al. Characterization of virus-like particles associated with the human faecal and caecal microbiota. Research in Microbiology. 2014;165(10):803-812.

102. Clokie M, Kropinski A, Lavigne R. Bacteriophages: Methods and protocols-Volume III. Methods in Molecular Biology. 2018.

103. Ackermann H-W. Bacteriophage electron microscopy. Advances in Virus Research. 2012;82:1-32.

104. Brussaard CP, Marie D, Bratbak G: Flow cytometric detection of viruses. Journal of Virological Methods. 2000;85(1-2):175-182.

105. Ács N, Gambino M, Brøndsted L. Bacteriophage enumeration and detection methods. Frontiers in Microbiology.2020;2020:2662.

106. Morella NM, Yang SC, Hernandez CA, Koskella B. Rapid quantification of bacteriophages and their bacterial hosts in vitro and in vivo using droplet digital PCR. Journal of Virological Methods. 2018;259:18-24.

107. Wilson IG. Inhibition and facilitation of nucleic acid amplification. Applied and Environmental Microbiology. 1997.63(10):3741-3751.

108. Klumpp J, Fouts DE, Sozhamannan S. Next generation sequencing technologies and the changing landscape of phage genomics. Bacteriophage. 2012;2(3):190-199.

109. Ji P, Aw TG, Van Bonn W, Rose JB. Evaluation of a portable nanopore-based sequencer for detection of viruses in water. Journal of Virological Methods. 2020;278:113805.

110. Dhillon T, Dhillon E, Chau H, Li W, Tsang A. Studies on bacteriophage distribution: virulent and temperate bacteriophage content of mammalian feces. Applied and Environmental Microbiology. 1976;32(1):68-74.

111. Reyes A, Wu M, McNulty NP, Rohwer FL, Gordon JI. Gnotobiotic mouse model of phage–bacterial host dynamics in the human gut. Proceedings of the National Academy of Sciences 2013;110(50):20236-20241.

112. Edwards RA, Vega AA, Norman HM, Ohaeri M, Levi K, Dinsdale EA, et al. Global phylogeography and ancient evolution of the widespread human gut virus crAssphage. Nature Microbiology. 2019;4(10):1727-1736.

113. Guerin E, Shkoporov A, Stockdale SR, Clooney AG, Ryan FJ, Sutton TD, et al. Biology and taxonomy of crAss-like bacteriophages, the most abundant virus in the human gut. Cell Host & Microbe. 2018;24(5):653-664. e656.

114. Aggarwala V, Liang G, Bushman FD. Viral communities of the human gut: metagenomic analysis of composition and dynamics. Mobile DNA. 2017;8(1):1-10.

115. Manrique P, Dills M, Young MJ. The human gut phage community and its implications for health and disease. Viruses. 2017;9(6):141.

116. Thurber RV, Haynes M, Breitbart M, Wegley L, Rohwer F. Laboratory procedures to generate viral metagenomes. Nature Protocols. 2009;4(4):470-483.

117. Adams MH. The stability of bacterial viruses in solutions of salts. The Journal of General Physiology. 1949;32(5):579.

118. Conceição-Neto N, Zeller M, Lefrère H, De Bruyn P, Beller L, Deboutte W, et al. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. Scientific Reports. 2015;5(1):1-14.

119. Feldmann H, Geisbert T, Jahrling P, Klenk H, Netesov S, Peters C, et al. Virus taxonomy: Eighth report of the International Committee on Taxonomy of Viruses. In.: Elsevier/Academic Press London; 2004: 645-653.

120. d'Humières C, Touchon M, Dion S, Cury J, Ghozlane A, Garcia-Garcera M, et al. A simple, reproducible and cost-effective procedure to analyse gut phageome: from phage isolation to bioinformatic approach. Scientific Reports. 2019;9(1):1-13.

121. Shaw KJ, Thain L, Docker PT, Dyer CE, Greenman J, Greenway GM, et al. The use of carrier RNA to enhance DNA extraction from microfluidic-based silica monoliths. Analytica chimica acta 2009;652(1-2):231-233.

122. Džunková M, Garcia-Garcera M, Martínez-Priego L, D'Auria G, Calafell F, Moya A. Direct sequencing from the minimal number of DNA molecules needed to fill a 454 picotiterplate. PloS One 2014;9(6):e97379.

123. Yilmaz S, Allgaier M, Hugenholtz P. Multiple displacement amplification compromises quantitative analysis of metagenomes. Nature Methods 2010;7(12):943-944.

124. Beaulaurier J, Zhu S, Deikus G, Mogno I, Zhang X-S, Davis-Richardson A, et al. Metagenomic binning and association of plasmids with bacterial host genomes using DNA methylation. Nature Biotechnology 2018;36(1):61-69.

125. Roux S, Enault F, Hurwitz BL, Sullivan MB. VirSorter: mining viral signal from microbial genomic data. PeerJ 2015;3:e985.

126. Lorenzi HA, Hoover J, Inman J, Safford T, Murphy S, Kagan L, Williamson SJ. The Viral MetaGenome Annotation Pipeline (VMGAP): an automated tool for the functional annotation of viral Metagenomic shotgun sequencing data. Standards in Genomic Sciences. 2011;4(3):418-429.

127. Wommack KE, Bhavsar J, Polson SW, Chen J, Dumas M, Srinivasiah S, et al. VIROME: a standard operating procedure for analysis of viral metagenome sequences. Standards in Genomic Sciences. 2012;6(3):421-433.

128. Roux S, Tournayre J, Mahul A, Debroas D, Enault F. Metavir 2: new tools for viral metagenome comparison and assembled virome analysis. BMC Bioinformatics. 2014;15(1):1-12.