

R E V I E W

Bacteriophages presence in nature and their role in the natural selection of bacterial populations

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Abstract. Phages are the obligate parasite of bacteria and have complex interactions with their hosts. Phages can live in, modify, and shape bacterial communities by bringing about changes in their abundance, diversity, physiology, and virulence. In addition, phages mediate lateral gene transfer, modify host metabolism and reallocate bacterially-derived biochemical compounds through cell lysis, thus playing an important role in ecosystem. Phages coexist and coevolve with bacteria and have developed several antidefense mechanisms in response to bacterial defense strategies against them. Phages owe their existence to their bacterial hosts, therefore they bring about alterations in their host genomes by transferring resistance genes and genes encoding toxins in order to improve the fitness of the hosts. Application of phages in biotechnology, environment, agriculture and medicines demands a deep insight into the myriad of phage-bacteria interactions. However, to understand their complex interactions, we need to know how unique phages are to their bacterial hosts and how they exert a selective pressure on the microbial communities in nature. Consequently, the present review focuses on phage biology with respect to natural selection of bacterial populations. (www.actabiomedica.it)

Key words: bacteriophages, spatial and temporal distribution, ecology, microbial communities, natural selection

Introduction

Phages cannot carry out most of the biological processes on their own and require a live host in order to propagate. As a result, phages are obligate parasites to their bacterial hosts and dwell in almost all habitats where bacteria exist (1,2). In addition to bacteria, phages can infect and parasitize archaea and are also found in some eukaryotes (1). Bacteriophages are the most abundant organisms on Earth and have an important role in constant regulation of the diversity, richness, abundance, evolution, and physiology of microbial communities in a particular habitat (1,2). Phages have been extensively studied in the past two

decades for their role in antimicrobial resistance, food safety, food processing, agriculture, environment, and medicine (3-6). However, they are of utmost importance with respect to their effect on bacterial population dynamics in natural environment as well as in laboratory conditions (7).

The coexistence between phages and bacteria seems implausible because phages have a massive proliferative advantage over their bacterial hosts. The reproduction cycles of phage and bacteria occurs in comparable time frames, with both happening within an hour (7). However, after each generation cycle phage produces ~100-200 new phage particles while one bacterial cell divided into two daughter cells only. Conse-

quently, phages would quickly outnumber and destroy the host bacterial population (8,9). Nevertheless, this does not happen. As a result, both have developed ways and means to resist each other's defense mechanism thereby maintaining a balance between their populations (10-12). However, to coevolve, both need to coexist for a certain length of time to allow beneficial mutations to take place (8-10). Hence, understanding phage-bacteria interactions is critical to understand the population dynamics of bacteria in nature. Consequently, the present review aims to gain insight into biology of bacteriophages and their unique role for bacteria and how their interactions exert a selective barrier in the microbial population dynamics in nature.

Discovery of bacteriophage

Phages were independently discovered by Frederick Twort in England (12) and Felix d'Herelle, (13), in Paris at the Pasteur Institute. Twort was trying to produce a vaccine against a virus without a host cell in an artificial medium (14) while D'Herelle was studying an extreme outbreak of dysentery among the soldiers in Paris during the First World War, when he discovered bacteriophage, a bacterium feeding body (15). He applied this to bacterial culture and observed that turbid cultures turned into clear indicating the lytic nature of bacteriophages against bacteria (16). His thesis on the bacteriophage was published in a monograph, "The bacteriophage and its behavior" (17), along with several other books and papers subsequently. This research laid the foundation of "bacteriophagology" (18). A new vocabulary was used to describe post-infection activities and explained the purification and titration of bacteriophage culture in some detail.

Structure of bacteriophages

The bacteriophages biology suggests that phages are well suited to their local host communities (19). Phages possess a structure well-suited to achieve their objectives: identifying a suitable host bacterium, taking advantage of the metabolism of the host and creating several progeny phages that are well built enough

to survive before a new host bacterium is found to infect. The composition of bacteriophage is simple, it consists of genetic material made of either DNA or RNA, double or single stranded, enclosed in a protein capsid. The three basic structural forms of phage (20) include an icosahedral head with a tail, an icosahedral head without a tail and filamentous form (Figure 1).

Phage families and their structure

The family of *Leviviridae* infects bacteria, includes *Enterobacter*, *Acinetobacter* and *Pseudomonas*, consists of small icosahedral bacteriophages with linear, single-stranded RNA genome that functions as messenger RNA encoding four proteins: the coat protein, the replicase, the maturation protein, and the lysis protein (21). This family includes two genera: *Levivirus* (having two species MS2 and BZ13) and *Allolevivirus* (containing Q β and F1).

The *Microviridae* is a family of small icosahedral bacteriophages with a single-stranded, circular plus strand DNA genome, and the minus strand is produced intracellularly to be used as a template for the mRNA generation by transcription. This bacterial virus attacks either the free-living enterobacteria or the obligate intracellular bacterial parasite that lack cell wall like chlamydia or spiroplasma (22).

Another viral family known as *Inoviridae* consists of a rod-shaped filamentous structure with a single-stranded circular plus DNA. Such bacteriophages mostly infects gram-negative bacteria (23).

The *Cystoviridae* family of bacteriophage has nucleocapsid that contains double stranded RNA segments, and the capsid is covered by a lipid membrane layer. These bacteriophages infect *Pseudomonas* bacteria (24). The virion is enveloped with typically two concentric, icosahedral symmetric protein layers.

The *Tectiviridae* is a double-stranded DNA phage family that infects the Gram-negative bacteria. These phages possess an icosahedral capsid, which resembles an adenovirus capsid, that encloses an internal membrane derived from the host, decorated with spikes at fivefold vertices (25) and lack the tail in their structure.

The *Caudovirales* order consists of three bacteriophage families named *Myoviridae* (long contractile tail), *Siphoviridae* (long flexible tail), and *Podoviridae*

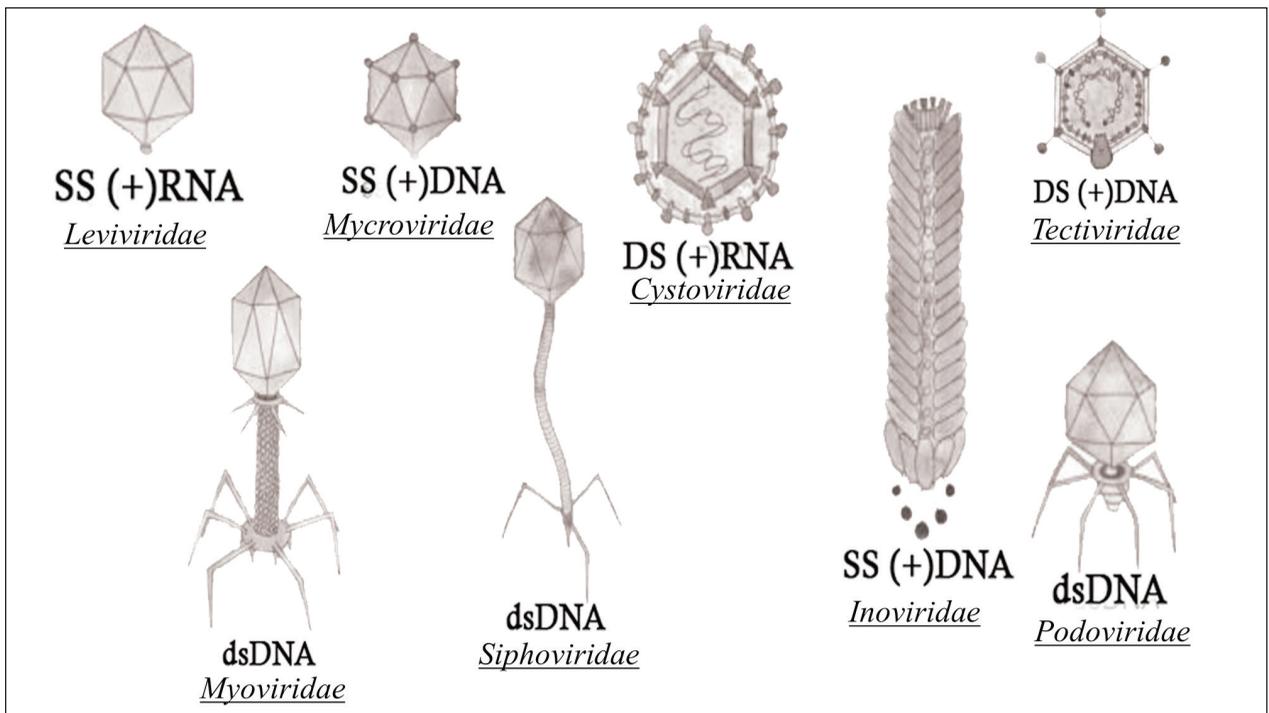


Figure 1. Representation of the bacteriophages families

(short noncontractile tail) having a tail with an icosahedral or prolate capsid containing a linear double-stranded DNA genome (26).

The *Plasmaviridae* is a family of bacteriophages that are membrane enveloped and infect the bacterial host without a cell wall. The genome consists of a circular, supercoiled double-stranded DNA (27).

Life cycle of phages

Phages have various life cycle and can be easily classified on the basis of their life cycle: lytic, lysogenic, pseudo-lysogenic and chronic.

Lytic cycle

In the lytic cycle, the phage injects its DNA into the host bacterium after adsorbing on to the host surface. This induces the shift of the host bacterium's protein machinery to replicate phage DNA and develop 50-200 new infectious phages (28). As a result, the host become weak and eventually ruptures to release the new

phage particles. Lytic life cycle plays an important role in biogeochemical cycling by releasing the organic molecules in nature by degrading the bacterial cells.

Lysogenic cycle

The lysogenic phase of the phage life cycle is characterized by the incorporation of the phage DNA into the host genome, which may survive as a plasmid, and the resulting new phages will have a combination of phage DNA and the host genome (28). Such a transformation of viral DNA might take place without significant metabolic implications for over many generations of bacteria. The phage genes can return to the lytic cycle under certain circumstances that hinder the bacterial state, leading to the release of completely assembled phages.

The uncommon but important phage life cycles

There are two other life cycles of bacteriophages known as pseudo-lysogenic and carrier state. In the pseudo-lysogenic cycle the phage nucleic acid after in-

fecting the host neither form a stable long-term connection (lysogeny), nor induces lytic response, instead the phage nucleic acid stays inside the host cell in an inactive form (29). The phage develops this type of interaction in order to avoid starvation and elimination from the environment when the nutrients are limited, and bacterial hosts are lacking (29).

The carrier state of phage life cycle is chronic because phage causes long-term infection to the bacterial host and the phage progeny is continuously budded off the cell or are passed down to the daughter cells asymmetrically (30).

Bacteriophage distribution in nature

A variety of different forms of bacteriophages are present in different ecological niches. The spatial and temporal distribution of bacteria and phage depends on their range limits and where their ranges overlap. It is widely known that bacteria dwell almost everywhere in every conditions (31), similarly phages exist in all niches wherever their hosts are present, including hypersaline environments (32), polar regions (33), deserts (34), on and within organisms other than bacteria (35,36), fresh and sea water (37) and the soil (38). The isolation of phages from a particular environment and their culturing on bacterial lawns is required to identify phages from a particular environment. Nonetheless, this is not always possible like in unculturable bacterial strains (39).

Epifluorescent microscopy following DNA staining were the first approaches that led to the realization of the phage abundance in marine environment e.g. approximately 10 phages for each bacterial and archaeal cell are present in marine water (40-42). For freshwater ecosystem, similar statistics have been shown, but the situation is less evident for other more complex environments, and the number of phages can be either higher or lower than that of the bacterial or archaeal hosts (43). In this section we will have a look at the phage's distribution in various niches.

Cyanophages of Marine Ecosystem

The studies on marine phages started in the early 1980s, when phages were found in the Black Sea, in-

fecting both unicellular and filamentous cyanobacteria (44). The predominant primary producers of the nutrient-poor ocean areas covering around 70% of the Earth's surface are the two genera of cyanobacteria known as *Prochlorococcus* and *Synechococcus*. The hot oceanic water usually has *Prochlorococcus* between 40° north latitude and 40° south latitude, whereas *Synechococcus* is much harder and is found on both sides of those latitudes (45). PSS2 is the first temperate marine cyanophage to be isolated and its genome sequenced (46). At present the vast majority of the isolated cyanophages are obligately lytic (47). A research in the Red Sea over an annual period found that cyanophages co-vary with *Synechococcus* in abundance and genetic diversity, which is consistent with the hypothesis that cyanophages are an important factor in regulating ecological cyanobacterium succession (48).

Phages in the Animal Environment

Felix D'Herelle reported that bacteriophages are a natural part of healthy animal and human microbiota (49). Electron microscopy findings first revealed the high concentration of phage-like particles in the intestinal microbial systems in the 1960s (50). The concentrations of the virus-like particles in the feces in humans is calculated based on the yield of the total phage DNA (51) and is estimated to be 10^{10} ml⁻¹. Tailed phages tend to be linked to the vast majority of intestinal virus-like particles. In a recent review, in one sample of horse feces, 69 morphologically distinguishable forms of bacteriophages were found in over 200 examined samples (52).

Archaeal Phages

Archaea are as common as the Bacteria in oceans, soils and subterranean environments (53-55). Archae can inhabit in extreme conditions such as hot spring, salt and soda lakes. Archeal viruses are believed to be as abundant as bacterial viruses however so far there are only 50 phages known to infect the Archaea (56). The viruses that can infect Archaea have double stranded DNA genome varying in size from 10kb to 100 kb (57). No archeal RNA virus has been identified so far. These viruses have several unusual morphologies not seen in eukarya or bacterial viruses earlier (58,59).

Phages in Human Gut

Human body is the greatest reservoir of microbiota infested with bacteriophages (60), mostly living inside the gut. Hence, the presence of phages come into consideration as they outnumber the bacterial population present in the body compartments by at least 10 folds (61-63). The human body comprises mucosal surfaces, where microbes inhabit and communicate with their host directly (64). These mucosal membranes serve as an entry point for pathogens (65). Bacteriophages, can bind to mucin glycoproteins through immunoglobulin-like spike present in their capsid *viz* "bacteriophage adherence to mucin" (BAM). BAM plays two significant roles in regulating the bacterial-host interactions: it provides protection for host against the pathogenic bacteria by accommodating bacteriophages with lytic activity that would otherwise demolish the beneficial bacteria leading to local or systemic infection (66); it provides lysogenic bacteria an environment to develop bacterial symbiotic relationship that benefits the human host (67). It has also been demonstrated that the gut epithelium, in response to the bacterial strains, can actively modulate BAM and the bacteriophage composition through hyper-secreting mucins and changing the mucin glycosylation patterns to prevent microbial adhesion and survival (68). The integrated prophages often show genes to enhance bacterial strain's fitness and virulence (69), on the other side the free phages help their bacterial host by killing their competitor bacterial species (70).

Phages in Dairy Environment

Phages are rather unwanted in dairy environment as they can kill the microbial starter cultures, thus contaminating the raw milk (71). The outburst of phages can cause significant economic losses due to delay in the production, wasting of ingredients, low product quality, increase spoilage and even total loss of production (72). The commonly found pathogens in the dairy environment include bacterial pathogens like *Brucella spp.*, *Campylobacter jejuni*, *Bacillus cereus*, Shiga-toxin producing *E.coli*, *Staphylococcus aureus*, *Mycobacterium bovis*, *Salmonella spp.*, and *Yersinia enterocolitica*, viruses, fungi and parasites (73).

However, phages are useful in cheese production. Cheese manufacture process is the most effective process to study the ubiquity of phages in the dairy environment (74). Large volume of raw milk is daily fermented by lactic acid bacteria starters with *Lactococcus lactis* as it is extensively used bacterial species. Consequently, the *Lactococcus lactis* phages are also studied widely followed by *Streptococcus thermophiles* bacteriophage (74,75).

Role of phages in natural selection of bacteria

Although the exact role of natural phages in shaping a microbial community is not fully understood, they might preferentially select some of the bacterial species and eliminate others by various mechanisms such as host selectivity, horizontal gene transfer, driving bacterial evolution and mediating competitions between bacterial communities (76).

The reciprocal selective pressure exerted by phages on bacteria and viceversa suggests that phage-bacteria interactions have a decisive impact on diversity, virulence, and evolution of bacteria (77).

Phage life cycle

Lytic life cycle is of vital importance in removing and eradicating the bacterial species from a particular niche and has been explored extensively with respect to medical applications. However, also the lysogenic, pseudo lysogenic and chronic life cycles deserve a special place in phage-bacteria interactions (78). Lysogenic life cycle contributes to development of bacterial resistance to lytic phages by acting as prophages such as Spi phenotype and via horizontal gene transfer by incorporating a part of bacterial genome and transferring resistance genes to the next host. This results into development of bacterial colonies that are resistant to lytic phages thereby giving them a selective advantage over the susceptible phages (79). Pseudolysogeny is debatable with respect to being real life cycle or just a pause in the phage replication in nutrient deficient conditions, still it has important implications on bacterial population dynamics in each environment. On the other hand, the chronic infection of bacteria by

phages mediated by the carrier life cycle mode is an important method to slow down the growth rate of bacterial species. A classic example of such a life cycle is the M13 bacteriophage infection in *E. coli*. M13 neither lyses the cell nor exists as a prophage and maintains its DNA as an extrachromosomal element, thus utilizing bacterial resources for its continuous replication and packaging into new M13 particles which then are released through pores in bacterial cell membrane without lysing the cells (80). Similar relation is seen between *Pseudomonas syringe* and $\phi 6$ phage which is used to control *P. syringe* in Kiwi plants (81). Similarly, *Campylobacter jejuni* infection with bacteriophages CP8 and CP30 renders it inefficient in colonization in chicken gut thereby effecting its population dynamics (82).

The decrease in growth rate of these bacteria by chronic phages results in a reduction in number of these bacteria as compared to uninfected bacterial species in a particular habitat thus depicting their potential in shaping microbial communities.

Bacterial resistance to phage infections

The survival and existence of bacteria and phages is interdependent. Phages need to infect and replicate inside bacteria while bacteria need to eradicate phages and propagate. In this war between bacteria and phages, bacteria have developed various strategies of offence and defense to get rid of phages that include CRISPR-Cas, prokaryotic argonauts, restriction-modification, surface modification, toxin-antitoxin and abortive infection system in the hosts (83-85). These defense responses vary in many ways, including the number of phages that can be taken care of by these and the cost to bacterial survival (86). Two of these mechanisms surface modification and CRISPR-Cas systems, are often involved in rapid progression of resistance against phages. The simplest method of bacterial resistance to phage is surface modification that involves mutation of the bacterial cell surface receptor used by phage for attachment (87). CRISPR-based immunity on the other hand is a very sophisticated method to degrade phage genomic material (88).

Phage response to bacterial resistance

To counteract the bacterial defense mechanisms, phages also developed a myriad of tools ranging from modification of the attachment proteins to anti CRISPR proteins. Phages can go to any extent to coevolve with the bacteria to maintain their existence (89). For instance, phages have developed an entire bacterial nucleic acid degrading system as discovered in ICP1 vibrio phages that encode a CRISPR-Cas system in their genome (90). Besides that, they manifest point mutations in their genome at sites targeted by the specific host (91-93). Apart from point mutation, phages have developed anti-CRISPR mechanism (Acr gene) that inhibits CRISPR-Cas evolutionary mechanism of bacteria (94). This Acr gene involved in rescuing phages from extinction was identified in temperate *Pseudomonas* phage (95). Studies have suggested that some of the Acr genes are more potent as compared to others, for instance Acr IF1 shows great potency against CRISPR resistant host as compared to the phages that had AcrIF4 (94,95).

Phage host range

It was previously thought that phages were specific for their hosts and this is true for some bacteriophages which are species- and strain-specific, however, their diversity in nature suggests that at least some have a broader host range (96). Phages expand their host range with respect to the evolving bacteria, for example the lytic phage SBW25 ϕ 2 coevolves with its host *Pseudomonas fluorescence* by continually increasing host range to infect the previously resistant strains, this increase in host range however does not increase the infectivity (97,98).

By extending the host range, a single phage can exert selective pressure on various microbial populations leading to the development of evolutionary resistance profiles and mutations in their genomes. Hence, host specificity and range are important factors in natural selection of microbial population in nature and can be used to predict the response of host population and community to phage-based selective pressure (19).

Impact of phages on bacterial population and communities

In phage-bacteria relationship phages are the predators that can modulate bacterial population in many ways such as driving the abundance and diversity of bacterial species; changes in their physiology, competitive ability, and virulence (99). Long periods of repeated interaction between predator and prey brings about their coevolution termed as Red Queen hypothesis (100) in which reciprocal selection cycles change the biotic selective environment of both parties over time (100-103).

Abundance and diversity

Experimental evidence from chemostats and phage-host observations in an open system has shown that populations of phages and hosts oscillate with time for certain bacterial species (104). The most evident effect that a lytic phage has on bacteria is the reduction of its number (105). Several studies have shown that phages have a major role in the bacterial mortality. Phages are 20-30 % more abundant than bacteria and to maintain themselves they cause 10^{24} infections per second (106). These infection account for eliminating 4-50 % of total bacterial population in nature at a given time (106). For example, 20-40% bacteria are eliminated by the phages in marine surfaces indicating the phage capability of out competing bacterial populations in some environmental conditions (95,107). This indicates the enormous predation potential of bacteriophages however evolution of bacterial resistance against phages neutralizes the situation thus maintaining a balance between the two (108). Besides lytic activities phages can reduce the bacterial number by chronic infections whereby a phage decreases the rate at which a bacteria propagates. The decrease in growth rate of one bacterial species with respect to another gives an advantage to the fast-growing species thus changing the microbial community richness (107,108). In addition to this, lysogenic bacteria give an advantage to bacteria conferring them resistance against lytic phages. Large seasonal fluctuations in the bacterial populations of natural aquatic environment is because of lysogenic phages integrated in their genome

indicating the effect of lysogeny on shaping bacterial communities (109).

Phage mediated gene trafficking

Phages can bring about changes in their host genome by several ways. One of the most common method of gene trafficking by phages is incorporation of a part of host genome in their capsid while packaging inside the host. This genetic material is then transferred to other bacterial cells by a process called generalized transduction (110,111). Antibiotic resistance genes are transferred from one bacterium to another through this mechanism. Bacterial populations acquiring these genes act as reservoirs (112). Besides this general transduction there is a specialized transduction in which the phages selectively incorporate targeted sequences from bacterial genome into their own genome. These acquired genetic sequences may provide an edge to phages in changing bacterial physiology in their own favor such as enhanced susceptibility, impaired DNA repair mechanism of bacteria accelerating the mutation rate which might increase phage susceptibility or resistance. In some cases, bacteria may invert one third of their genome in response to selective pressure mediated by phages (113).

Changes in bacterial physiology

Phages may possess genes that are homologous to bacterial metabolic genes and these auxiliary metabolic genes might alter bacterial metabolism in favor of infecting phages (77). For instance, cyanophages may carry genes for photosynthesis that can be used to generate energy after the host stops synthesizing photosynthetic proteins (114). This allows phages to continue replicating in their hosts. In addition, prophages enable bacteria to withstand various stressful environmental conditions. Removal of prophage from *E. coli* revealed that the prophage was integral to the bacteria for withstanding the unfavorable environmental conditions (115).

Bacterial Virulence

Phages can alter bacterial virulence in addition to changing its physiology. Phage mediated addition

of virulence genes in bacteria improves their chances of survival and might increase phage host range thus ultimately benefitting the phages by providing them a chance to continue replication. Occasionally these phage-mediated virulence factors result in an increase in host pathogenicity thereby playing a crucial role in many bacterial infections in human and animals. Phages can enhance bacterial pathogenicity by several ways including transferring genes encoding toxins, duplicating the virulence factors, or changing the regulatory sequences controlling bacterial virulence genes (116). Numerous studies have been conducted in this regard that report phage-mediated toxin encoding gene transfer in bacteria that result in a variety of diseases in humans and animals. In addition, phages may promote biofilm formation, antimicrobial resistance, immune resistance, and increased virulence in their bacterial hosts (89) like in *Vibrio cholerae* (117), *Pseudomonas aeruginosa* (118) and *Shigella dysenteriae* (119).

Phage-bacteria interdependence

It is obvious that a particular phage will survive if its ultimate host will survive so the balance between phage mediated bacterial lysis and bacterial population density is maintained. On the other hand, this can exert selective pressure on both phage and bacterial abundance and distribution (120,121). Eradication of a particular host bacteria from a particular niche means elimination of its parasitic phage too. Phages answer to this situation is increase in host range. Thus, phages infecting multiple hosts have a selective advantage over those which are highly host-specific. Thus, both predator and prey are integral part of each other's existence.

This interaction of phages and bacteria is also influenced by the environment they are in. for instance if the nutrient supply is limited and bacteria cannot grow at an efficient rate, the phages switch from lytic mode to lysogenic mode. Phages can live as phage particles outside bacterial host and have a greater tolerance to the environmental stress (122). Increase tolerance to environmental conditions may increase phages survival during periods of host abundance fluctuations and may give them time to find another suitable host (123).

Phage mediated natural selection of bacterial communities is explained by two hypotheses.

1. **Negative frequency dependent selection (FDS)**
Phages can mediate frequency dependent selection (FDS) of bacterial populations by adapting to the most dominant host strains and having a significant impact on their fitness. Studies in this regard have indicated that phages are well adapted to their local hosts population as compared to those coming from other populations (35,76). Alternatively changes in abundance of a particular phage genotype is correlated to changes in abundance of its respective host (83). Artificial removal of phages from a particular niche is correlated with a resultant increase in number of previously rare bacterial species, while adding phages to particular niche corresponds to an increase in specie richness (124) indicating role of phages in abundance and richness of bacterial populations. Similarly, studies on lysogenic *E. coli* strains have revealed that the relative fitness of the host was highest when the lysogenic phage was rare and vice versa (125).
A recent review on phage-bacteria interaction by Cordero & Polz, 2014 suggest that phage-mediated FDS and other ecological factors such as competition and predation results in stable diversity and rapid turnover of genes in a given population (126).
2. **The Kill the Winner hypothesis**
This model assumes that host growth rate is positively linked with viral fitness, and consequently abundance. Thus, phage infection is proportional to the relative host abundance and this negates inherent fitness advantage of particular bacterial hosts. This model also suggests that viruses can maintain coexistence of bacterial species having varying intrinsic growth rates thus implicating phage involvement in shaping microbial communities (127). A further modification in this model suggested that bacteria face a trade-off between phages resistance and growth rate. This means that the slow growing resistant bacterial species dominate the microbial communities along with the phages attacking fast growing bacterial species (128). This clarifies the fact that there is a cost associated with phage resistance and bacteria pays

that cost by lowering their growth rate. Furthermore, this model explains the inverse abundance distribution observed in marine environments where large proportions of rare microbial communities are highly active (129).

Phage-mediated competition among bacterial species

The specificity of phage-bacteria interaction shapes the relative fitness of bacterial species in a particular habitat. A phage infecting two hosts may have variations in rate of adsorption, time to cell lysis, or burst size across the hosts. As a result, one bacterial species will have an advantage over the other with respect to the presence, but not absence, of the phage (82). If both hosts eventually become resistant to the phages by paying different costs than phage-mediated selection might alter competition between them even in the absence of the phage (76). This competition can become more evident if one host species is less sensitive and the other more susceptible to the same phage. Consequently, the tolerant species would dominate in a particular niche.

Effect of phage-bacteria interactions on non-bacterial species

Phages might have a strong influence on bacterial interactions with their hosts such as animals, plants and humans. An important example of this tricentric interaction is phage-gut microbiota within human gut that plays a central role in shaping human gut microbiome (130). The highest number of bacteriophages in the nature is present in human gut 10^{15} (131) out of the approximate number of phage particles in the biosphere (10^{31}) (77). In fact, Twort in 1915 and d'Herelle in 1917 co-discovered bacteriophages by examining the gut microbiota of humans and animals (13,14). A relatively recent study in mouse model reported that gut phages have measurable effects on diversity and abundance of gut microbiota (34). Another classical example is that of flamingoes which eat cyanobacteria. The decline in flamingo population was found to be a consequence of cyanobacterial degradation by cyanophages (132). Moreover, phages play an important role in phage, bacteria-insect symbiosis (36). Phages

encode virulence genes that are important with respect to protection of wasps from parasitism (133) and they can also reduce the cytoplasmic incompatibility caused by endosymbiont in insects (36).

Role of phage-bacteria interactions in ecosystem

Bacteriophages affect both biotic and abiotic components of ecosystem by changing biogeochemical cycles (134). Release of nucleic acids, proteins and lipids by the phage mediated degradation of bacteria significantly contributing to the biogeochemical cycling of carbon, nitrogen, and phosphorus (135). Marine food web microcosm studies have evidenced strong effect of phages on available phosphorus and the ratios of carbon, nitrogen, and phosphorus (136). In addition to these macro elements, microelements such as iron is also released due to bacterial motility. Moreover, phages in marine environment negatively effects the primary productivity. For instance, increasing virus abundance in seawater, reduces bacterial species and consequently primary productivity of phytoplankton by up to 78% (137). As 50% of global primary productivity can be attributed to phytoplankton, it can be stated that tiny viruses hold an important place in the global food web (138).

Conclusion

It can be concluded here that phages are the main drivers of bacterial diversity, from the genome level to the population level and up to community's level. The mechanism underlying capability of phages might include the frequency-dependent host-specific phage adaptation, correlations between bacterial abundance and associated phage density in a particular habitat, and trade-offs between growth and defense expenditure against phages. This suggests that phage-mediated selection plays a critical role in mediating competition among bacterial hosts thus driving niche diversification. Phage-bacteria interactions could be expressed in terms of predator-prey relationship in which presence of bacteria is necessary to ensure phage existence while presence of phage is necessary to keep bacterial population at bay in natural environment. This check and

balance are the key to shaping microbial population in a particular habitat and is necessary for the coexistence of both parties. This also has large impacts on environment. Phages sitting at the bottom of global food web are the game changers in selecting microbial communities and replenishing macro and micronutrients in the ecosystem by biogeochemical cycling. More studies in this regard are however required to reap the benefits of phages in various biotechnological, environmental, and medical applications.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Received: 3 September 2020

Accepted: 14 October 2020

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