Review

CellPress

Exploiting gut bacteriophages for human health

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The human gut contains approximately 10^{15} bacteriophages (the 'phageome'), probably the richest concentration of biological entities on earth. Mining and exploiting these potential 'agents of change' is an attractive prospect. For many years, phages have been used to treat bacterial infections in humans and more recently have been approved to reduce pathogens in the food chain. Phages have also been studied as drug or vaccine delivery vectors to help treat and prevent diseases such as cancer and chronic neurodegenerative conditions. Individual phageomes vary depending on age and health, thus providing a useful biomarker of human health as well as suggesting potential interventions targeted at the gut microbiota.

Bacteriophages and the human host

Bacteriophages (phages) are bacterial viruses that can attack and kill a target bacterium within minutes of infection. Phages bind to specific targets on the bacterial cell surface and so each individual phage generally targets a very narrow range of strains of the same bacterial species. Phages replicate principally by means of one of two main lifecycles, the lytic cycle or the lysogenic (or temperate) cycle [1]. In the lytic cycle, the phage infects the bacterial cell and replicates by redirecting the cell metabolism to produce new phage particles, which are in turn released during programmed cell lysis. In the lysogenic cycle, the phage DNA integrates into the bacterial genome without inducing cell lysis. The phage genome (termed a prophage) can then replicate in concert with the host chromosome until such time that a lysis event is induced. However in some cases, pseudolysogeny can occur where the phage exists as a plasmid-like prophage, without inducing a lytic cycle or integrating into the bacterial chromosome [2].

Since the early 20th century [3], it has been recognised that phages can play a role in human health by taking advantage of their ability to destroy pathogens. To date, phages have been mainly deployed in much the same way as antibiotics and/or preservatives. They have been recently used to help in controlling pathogenic bacteria on foods [4] and in some countries have been used as phage therapy to combat pathogenic bacteria infection in humans [5] (Figure 1). A unique feature of phages as bactericidal agents is that they will only replicate in the presence of their target bacteria, and are unlikely to affect non-target species, creating a 'narrow spectrum, self-replicating' antimicrobial. On the negative side, target bacteria may become resistant to any particular phage or the contaminating bacterium may be outside of the 'target range' of the deployed phage. Nonetheless, there is no obvious reason why phages should not be used to a far greater extent in controlling bacteria of concern in both food and medical applications.

In general, to minimise the development of bacterial resistance observed with the use of a single phage, cocktails of phages are targeted against a particular pathogen. However, phages are often sourced from complex communities of bacteria, where they presumably play a role in contributing to population structure and stability. One such reservoir of phages is the human gut, home to a diverse and abundant phage community that varies depending on age and health condition. It has been estimated that the human gut contains as many as 10^{15} individual phage particles [6,7], making it perhaps the most densely populated ecological niche in nature. The first metagenomic study of the combined phage metagenome (phageome) in a healthy adult gut provided insights into the complexity of this vast community [8]. However, little is known about the role of the phageome in maintaining community structure, or of any possible impact on human health. It seems self-evident that if phages have the potential to modulate the gut microbiota, then in turn they can have an indirect but important impact on host–microbe interactions and thus on host health [7]. Furthermore, phages can translocate through the gut mucosa to local lymph nodes and internal organs, leading to intimate interactions with the host immune system [9–11]. An unanswered question is to what extent the composition and flux in the phageome could be used as biomarkers of the microbiota, and thus as indirect biomarkers of health or disease in the host (Figure 1). It is tempting to speculate that since the phageome must be a reflection of the microbiota at any given point, it could be used as a surrogate reporter for this complex community.

The aim of this review is to explore the recent uses of phages to preserve human health. This includes using phages in food safety applications and curing bacterial infections in humans, as well as the concept of using

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Keywords: bacteriophage; phageome; human health; phage therapy.

0966-842X/$ – see front matter
 http://dx.doi.org/10.1016/j.tim.2014.02.010

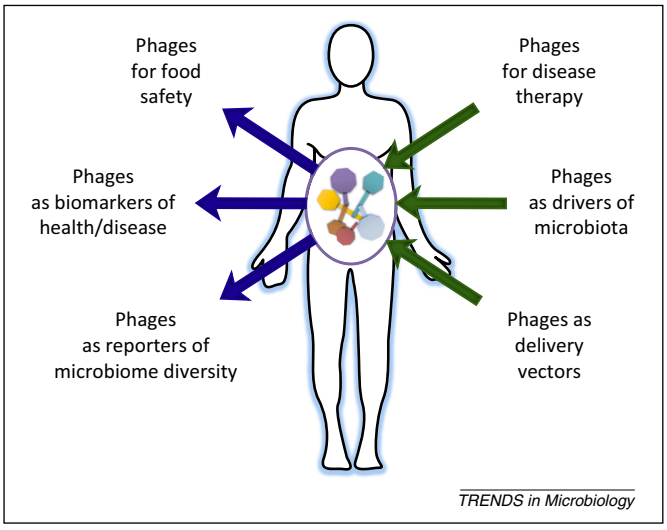

Figure 1. Potential applications for bacteriophages (phages) in human health.

phages as delivery vectors to cure several diseases. In addition, recent findings that highlight the composition and evolution of the human gut phageome in healthy individuals and patient groups are also addressed.

Phages as tools for food safety

Strategies to prevent the contamination of food with pathogenic bacteria have been extensively reviewed [4,12–14]. They include the direct application of phages at the primary production level in chickens, calves, pigs, and cattle [15], on food contact surfaces in food processing facilities [16], and on ready-to-eat foods [17].

One concern about using phages for the production of safe foods is that it is necessary to confirm their safety for human health after consumption of 'phage-treated' foods. It is reassuring to consider that to date no negative effects of phages on human health have been reported [14]. An oral toxicity of phage study on rats demonstrated that the ingested phages have no effect on rat behaviour or physical appearance [18]. However, data is not readily available regarding the impact of phages contained in 'phage-treated' foods on the composition of the human gut microbiota and any potential consequences for the host in the long-term. We know that changes in the diet composition influence the phageome in healthy individuals, presumably by influencing the microbiota [19]. Despite the narrow host range displayed by phages, the impact of phages used as bioprotectants in the food chain on the gut microbiota and consequently their influence on human health in the long-term warrants further investigation.

Phage as agents for health

The use of phages against pathogenic bacteria infections in humans is a longstanding field of research. Phages have also emerged as promising tools for curing cancer, neurodegenerative diseases, and other health disorders.

Phages against pathogenic bacteria

Phage therapy against pathogenic bacteria in humans has a long history, with reports of successful phage therapy as long ago as 1921 [3]. Phages represent a promising alternative to antibiotics, particularly in the context of the emergence of antibiotic-resistant bacteria. Recent examples illustrating the potential of phage therapy concern the successful use of phages against *Pseudomonas aeruginosa* in murine and cell line models of lung infection [20], and against *Staphylococcus aureus* and *P. aeruginosa* infections in murine models of diabetic wounds, the world's leading cause of non-traumatic lower limb amputation [21–23].

Not all phages work equally well in all settings. For example, the impact of phages against O104:H4 enteroaggregative *Escherichia coli* was not uniform throughout the gut of mice [24]. The viability of phages seems to be dependent on the tissues and the incubation conditions [25]. Bacteria also rapidly evolve resistance in the presence of single phages whereas the use of multiple-phage cocktails can delay the acquisition of resistance and prolong phage efficacy against the target bacteria [26]. Repeated serial passage on an ancestral bacterium increases the infection capacity of phage, implying that evolutionary insights may well be useful to improve phage therapy [27]. The lysogenic capacity of some phages could also prove a barrier to the efficacy of phage therapy. One example is the strong predilection for lysogenisation of phages targeting *Clostridium difficile*, preventing effective *C. difficile* elimination, with a potential risk of virulence gene acquisition [28,29]. This demonstrates the need of a comprehensive characterisation of phage cocktails before they are used in phage therapy. In addition, EU regulations for the clinical use of phages are unclear. It has been suggested that phages should be regarded as human biological medicinal products defined by the European directive 2001/83/EG and that phage therapy should be tailored to each patient bacterial infection, even if this possibility is not fully compatible with the existing directive [30,31].

Phages in the treatment of health disorders

Phage specific activity against bacteria is not the only aspect of their biology that has been examined, and other impacts of phages on the host can be utilised to help in the treatment of various diseases. For example, phages can mediate inhibition of the production of reactive oxygen species (ROS) by immune cells exposed to endotoxins and this indicates a potential protective role for phages against oxidative stress [32,33].

The filamentous M13 phage has also been used in treatment models for Alzheimer's and Parkinson's diseases because of its ability to bind to the typical β-amyloid and α-synuclein plaques in the brain, resulting in plaque disaggregation [34]. In murine cancer treatment models, phages could significantly inhibit cancer tumour formation, promote the regression of tumours by recruiting inflammatory cells and inducing cytokines, and contribute to long-term survival of mice [35–37]. Engineered phages can also be used as delivery vectors of anticancer agents such as anticancer proteins, efficiently inducing selective death by apoptosis of cancer cells and consequently the regression of breast tumours [38], and genes in cancer gene therapy [39].

The potential of phages to be actors in human health is demonstrated by their diverse applications to prevent or