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ORIGINAL PAPER

The impact of bacteriophages on probiotic bacteria and gut microbiota diversity

Marco Ventura · Tommaso Sozzi · Francesca Turroni · Diego Matteuzzi · Douwe van Sinderen

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Abstract The human body is colonized by a vast array of bacteria whose diversity is largely affected by predation of bacteriophages. Here, we discussed the impact of bacteriophages on the composition of human intestinal microbiota as well as on the survival and thus efficacy of probiotic bacteria in the human gut.

Keywords Probiotic bacteria · Bacteriophages · Gut microbiota

Defining the microbial biodiversity of complex environments such as those found in soil or the human intestine has been one of the great conundrums of microbiology. Historically, the approaches that were taken to study the diversity of bacterial populations were completely dependent on classical microbiological tools, being based on the isolation of bacteria using (selective) growth media. Recently, the advent of novel technologies that involve direct sequencing of bacterial DNA contained in environmental samples, i.e., metagenomics approaches, have revolutionized the way of assessing the microbial diversity of the human intestinal microbiota [3, 5]. Importantly, analysis of the enormous amount of bacterial genome sequences that were generated by metagenomics-based investigations has implicated bacteriophages as one of the major forces responsible for shaping the diversity and composition of the gut bacterial community.

In almost all ecosystems that so far have been subjected to in-depth studies, it has been estimated that there are around ten phages for every microbial cell, rendering phages the most abundant biological entities on our planet [12]. By killing bacteria, phages significantly influence global biochemical cycles, while they are also considered to be crucial in driving microbial species diversity due to the fact that phage are species-specific [13]. Bacteriophages are thus considered as guarantors of the microdiversity that is required to efficiently exploit ecological resources. In fact, analysis of the bacterial genome content of variable regions between closely related bacterial strains has lead to the identification of several prophages sequences [2].

In the human intestine, bacterial populations can interact with each other while they must also compete with each other, and phages are expected to have a significant role in driving the biodiversity of this complex ecosystem.

Recently, studies on marine samples have clearly demonstrated that phages influence their bacterial host in a density-dependent manner, essentially infecting a small number of bacterial species at any one time [1, 9]. This is consistent with the "kill-the-winner" dynamics, which suggests that phage predation is preferentially directed against bacteria that are better adapted to a physical environment and that consequently are present at high numbers

M. Ventura (✉) · F. Turroni
Laboratory of Probiogenomics, Department of Genetics, Biology of Microorganisms, Anthropology and Evolution, University of Parma, Parco Area delle Scienze 11a, 43100 Parma, Italy
e-mail: marco.ventura@unipr.it

D. Matteuzzi
Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy

T. Sozzi
Chemin des Abeilles 4, 1010 Lausanne, Switzerland

D. van Sinderen
Alimentary Pharmabiotic Centre and Department of Microbiology, Bioscience Institute, National University of Ireland, Western Road, Cork, Ireland

in that environment [10]. Similar data have been reported for the horse gut, where the diversity and abundance of *Escherichia coli* strains have been shown to be directly linked to the relative abundance of specific coliphages [6]. Recently, it was shown how bacteriophages can drive strain diversification in a marine bacterium, i.e., *Flavobacterium* [8]. This study represents the first direct experimental demonstration of phage-driven generation of functional diversity within a bacterial host population and provides important implications for both phage susceptibility and physiological properties.

Thus, by extension one could argue that in the human gut no specific bacterial strains persist in dominant numbers over time and that there is a dynamic equilibrium of functionally redundant bacterial and viral strains that continuously substitute each other in a "kill-the winner" fashion, thereby maintaining stable metabolic potential and taxonomical diversity.

Bacteria have evolved a range of adaptive strategies that render them differentially successful in various microecological niches, due to their varying abilities to access, sense, respond to and utilize different substrates (e.g., in the case of enteric bacteria, this could apply to the use of different carbon sources that become available through the diet of the host).

An important aspect that deserves a much more detailed investigation is the potential role of phages in defining the limits of bacterial populations in the human intestine when a dietary intervention introduces a considerable amount of bacteria. Such an approach is commonly known as probiotic treatment and consists of the supplementation of viable microbial cells in sufficient numbers so as to modify the intestinal bacterial populations in order to elicit a beneficial effect on human health (Definition according to FAO/WHO—Food and Agriculture Organization of the United Nations/World Health Organization [4]). However, such interventions may not exert any long-lasting effect due to the selection of phages specifically acting on the ingested probiotic strains. In this context, commonly used probiotic microorganisms such as bifidobacteria have for long considered to be free from phage infections [14]. However, genome-based investigations have revealed the existence of prophage sequences in almost all of the bifidobacterial genomes sequenced so far [17], thus indicating that also these intestinal commensals are regularly targeted by phage predation. Probiotic supplementation may cause alterations in the relationships that exist between the different components of microbial population residing in the human intestine. Thus, based on the "kill-the winner" concept, probiotic populations may become the subject of phage predation and may simply lead to a microbial population balance that existed before the probiotic treatment. In fact, any strain that increases its numbers relative to that of other bacteria occupying the same ecological niche will quickly be eliminated by phage predation. It has been shown that daily supplementation of probiotic bacteria to calf rumen as well as to infants lead to the isolation of specific bacteriophage [7, 18]. Moreover, such an assumption would also imply that growth rates will be adjusted to those of the rest of the population unless the bacteria are or will become immune to phage infection [10]. However, since probiotic bacteria are artificially deposited in high numbers as opposed to reaching high numbers due to adaptive advantages, we do not know whether and to what extent the 'kill-the-winner' also applies to probiotic bacteria.

So far, very limited scientific interest has been directed to the identification and the genetics of phages specific for probiotic bacteria [14, 16]. The development of technological systems (e.g., supplementation of mixed probiotic cultures) or selection of novel probiotic cultures possessing natural immune systems against phage infections (e.g., Restriction Modification Systems, CRISPR systems) would be crucial in order to devise suitable strategies that avoid or limit the negative effects of phage infections on probiotic cultures. Interventions such as those commonly used in dairy fermentation processes (rotation of different starter cultures) may be a useful approach in order to reduce the negative effects of phage predation during probiotic therapy. This latter intervention has been proposed as a way to counteract phage predation upon administration of probiotics to animal [11].

Probiotic research has been largely influenced by the advent of novel genetic tools, such as complete genome sequencing (also known as probiogenomics), functional genomics, allowing the understanding of their interactive capabilities with the intestinal microbiota and their host [15]. However, investigations that involve experimental evolution and that are directed to explore the impact of phages on the microbial gut ecosystem have not yet been done.

With the sequencing of viral gut metagenomes [1] together with the human gut microbiomes, our understanding of the diversity and specificity of phages will improve. This knowledge will allow us to better appreciate the impact of phages on the microbiota as well as on specific probiotic bacteria, although further in-depth experimental exploitations will be needed to determine the true impact of these parasites on the microbial diversity and associated activities in the gut.

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