Bacteriocins and Bacteriophages

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Bacteriocins are bactericidal peptides, ribosomally synthesized, with an inhibitory activity against diverse groups of undesirable microorganisms. Bacteriophages are viruses that are able to infect bacterial cells and force them to produce viral components, using a lytic or lysogenic cycle. They constitute a large community in the human gut called the phageome, the most abundant part of the gut virome.

Bacteriophages Bacteriocins Bacterial Gut

1. Relationship of Bacteriophages and Bacteriocins with the Gut Barrier

In the gut mucosa, bacteriophages select specific bacteria by using horizontal gene transfer, influencing their rate of mutation and genetic variability, and thus modulating their abundance and diversity [1][2]. On the capsid they express Ig (Immunoglobulin)-like receptors, which interact with mucin glycoproteins and can regulate innate and acquired immunity [3]. Thus, bacteriophages can influence bacterial composition, modify their function and interaction with epithelial cells, and modulate the glycoproteic mucin layer and control other microorganism populations both directly and indirectly [4]. Moreover, they are dynamic entities that can translocate across the gut barrier and migrate into the peripheral blood and the peripheral tissue, activating the immune system [4]. They also have a complementary action on dendritic cells and can be considered both activators of inflammation and at the same time anti-inflammatory players [5]. The bacteriophages' translocation across the gut barrier has been confirmed by different metagenomics studies that revealed their presence in ascitic, urine and blood samples [5][6]. In this scenario, their actions should not only be considered to be focused on the gastrointestinal tract, but also extended to other sites.

Further, bacteriocins act both on the immune system and on the inhibition of competitive strains by directly influencing the niche competition among commensals [7]. Bacteriocins are commonly used strategically by commensals to colonize and persist in the human gut. Their activities could resemble those of a "probiotic". Indeed, they allow the survival of specific communities in the gastrointestinal tract by selecting strains that are able to resist modification by the host diet, the inhibition of natural defensins, bile salts and other killing factors, and colonization by other species, overall improving gut barrier function and the host immune response [8]. Studies on animal ilea have confirmed the potential effects of bacteriocin against pathogens, which led to positive changes in the gut microbiota composition. This is the case of Bacteriocin Abp118 [9], produced by *Lactobacillus salivarius UCC118* [10] or salivaricin P, produced by another *Lactobacillus salivarius* strain with a probiotic trait [9]. Interestingly, *Lactobacillus salivarius* expresses the srtA gene to tie it to the epithelial cell's surface, before producing protective

bacteriocins [9]. Bactofencin A or bacteriocin 21 produced by *Enterococcus faecalis* are able to kill multidrug resistant-bacteria and contribute to the regulation of the niche competition among intestinal bacteria [7]. Similarly, LAB bacteriocins exert their role against *Staphylococcus Aureus* [11], some vancomycin-resistant enterococci [7], *Salmonella enteritidis* [11], *Clostridium Difficile* [12] and *Listeria monocytogenes* [11]. More studies are needed to test the therapeutic potential of these findings. At the same time, it should be noted that not all changes observed in vitro have also been registered in vivo. This discrepancy is not surprising since several perturbing factors can deeply affect bacteriocin production and their activities.

2. Bacteriophages and Bacteriocins in Bacterial Food Infections

The antibacterial properties of bacteriophages and bacteriocins are exploited in food research. In particular, bacteriocins are used as food preservatives [13] both for dairy products [14] and for meat, fish [15], vegetables and fruits [16], being classified as partially purified bacteriocins, crude-fermented dairy bacteriocins and protective cultures bacteriocins [17].

Bacteriocins are considered natural and safe food additives after being ingested by the gastrointestinal tract [18]. They have the interesting properties of stability, antimicrobial effects, potency, and no flavor alteration [18].

There are different commercially available bacteriocins such as nisin (named Nisaplin) or Pediocin PA-1 against the growth of *Listeria monocytogenes* in meat products [19]. Other bacteriocins, such as those produced by Enterococci, seem to reduce the contamination of cheese due to animal feces [20], while Enterocin AS-48, Enterocin CCM4231 and EJ97 are used to protect both fermented and unfermented vegetables [19]. Bacteriocins can be added to food through the direct inoculation of the producer-strains as a concentrated fermented product [19] or as a gradual-release preparation. They have antimicrobial activity against gram-negative bacteria that infect foods, and this property could be empowered by combining bacteriocins with other compounds (e.g., organic acids, phenolic compounds). Other antimicrobial bacteriocins involved in food protection belong to the class I and II bacteriocins of Bacillus subtilis GAS101 and act against some gram-positive bacterial species such as *Staphylococcus Epidermidis* [21]. Bacteriocins are overall able to reduce the costs of food treatments and at the same time increase the product shelf-life.

Similarly, bacteriophages are used against *Salmonella*, *Campylobacter* and *Enterococcus* on food [22]. The efficacy of bacteriophages is reduced by their spectrum of action, which is oriented against specific serotypes, species or strains of bacteria [22]. However, their narrow spectrum could be more advantageous than antibiotics and may have a lower impact on the other components of the gut microbiota [23].

Bacteriophages can also control the production of some pathogenic toxins such as Cholera, Shiga and Pertussis, and interfere with mechanisms of antibiotic resistance [24]. In the past, they have been used to control the epidemic of bloody diarrhea in Germany caused by *E. coli* strain 0104:H4 through the production of Shiga toxins [25].

Finally, an alteration of gut virome composition could also be found in recurrent *Clostridium Difficile* infection [26]. Interestingly, the presence of a complex of bacteriophages in fecal mass used for therapeutic fecal microbiota transplantation (FMT) has been demonstrated to improve clinical outcomes of this treatment [27].

3. Role of Bacteriophages and Bacteriocins in Extra-Intestinal Diseases

Bacteriophage therapy has strong potential as a treatment for many extra-intestinal diseases, and in particular in those correlated to bacterial infections [28]. Indeed, in chronic bacterial rhino-sinusitis, after twenty days of topical application, a bacteriophage cocktail (P68, K710) is able to control a broad range of *Staphylococcus Aureus* (*S. aureus*) strains, including the methicillin-resistant strain (MRSA) [28]. A common manifestation of invasive *S. aureus* infection is osteomyelitis. *S. aureus* triggers many profound alterations in bone remodeling. Bacteriophage therapy has been applied with promising results, but more data are needed to confirm these findings [29].

In animal models, it has been reported that bacteriophages are able to control *Pseudomonas* lung infections [12]. In fact, two bacteriophages, φMR299-2 and φNH-4, have been proven to induce the formation of a biofilm on lung cells useful in controlling these infections. Hypothetically, they could have beneficial effects in patients with cystic fibrosis who are mostly exposed to this microorganism [30].

Bacteriophage therapy has been also used successfully for acne treatment. Acne has a multifactorial etiology and the inflammatory follicular response caused by the gram-positive skin bacterium *Propionibacterium Acnes* seems to play a primary role. The most common first line treatment is based on topical antimicrobial agents or oral antibiotics. Recent data suggest an alternative treatment, both for acne and for others bacterial skin infections, based on the use of a lytic bacteriophage preparation able to kill specific bacterial cells [31].

Further advantages of bacteriophage treatment have been demonstrated for diabetic foot ulcer healing. A commercial topical preparation of staphylococcal bacteriophage Sb-1 is effective when antibiotic treatment is unsuccessful [32].

On the contrary, bacteriocins such as pyocin were unable to control *Pseudomonas* lung infections in patients with cystic fibrosis. In fact, despite initial positive laboratory experiments, subsequent studies have underlined no evidence of beneficial effects for this bacteriocin [33].

Another frequent infection is provoked by *Streptococcus Pneumoniae*, which is responsible for pneumonia, bacteremia and meningitis, in particular in children. Pneumococcal disease benefit from vaccine and antibiotic treatment, but resistance is increasing. *S. Pneumoniae* is common in the nasopharynx of children, and it produces a circular bacteriocin, known as pneumocyclicin, to attack other bacteria and to protect itself against the immune system. This bacteriocin is similar to the other circular bacteriocins produced by gram-positive bacteria, and it could represent an important target thanks to its correlation to antibiotic resistance mechanisms [34].

Overall, bacteriophage therapy could be very beneficial thanks to its action against all types of pathogens, including those that are multi-drug resistant. A positive aspect of their narrow spectrum is the possibility of preserving the existing microbiome. Bacteriophages also have lower side effects, a wide distribution after their administration, and a possible inhibitory effect on the inflammatory response. They are cost effective and some studies underline their efficacy in comparison with antibiotics [23]. Further, bacteriocins could be very useful in fighting pathogens. They are easier to modify through bioengineering and have targeted activity against specific microorganisms. Numerous studies are trying to prove their use as a natural defense and as an alternative to antibiotics [13] in peculiar cases such as pregnant women and in individuals with contraindications to antibiotic use [35].

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