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Review

Bacteriophages in food supplements obtained from natural sources

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Abstract. Human gastrointestinal tract is colonized by bacteria that constitute the interstinal microbiota. Changes in the microbiota may lead to several chronic disorders. Bacteriophages are viruses that specifically target bacteria. Several food components contain bacteriophages and probiotics. Bacteriophages have a great specificity for harmful bacteria, helping the growth of good bacteria. Because of their qualities, bacteriophages are considered beneficial component of probiotics that target the pathogenic bacteria and support the natural human microbiota. (www.actabiomedica.it)

Key words: bacteriophage, probiotics, human microbiota, lytic phage, phage supplementation

Introduction

Human gut microbiota and intestinal health are strongly interconnected and they may lead to chronic systemic health diseases. Imbalances of gut microbiota caused by the poor diet, antibiotic use and stress, along with other environmental factors and lifestyle can cause bowel irregularities and intestinal inflammation. Many metabolic and autoimmune diseases as well as the mental health might be associated with gut microbiota. Consequently, the interest in the identification of those dietary supplements that positively modulate the microbial populations of gut is rapidly increasing (1).

Bacteriophages or phage viruses are considered plausible microbial modifier that promotes the intestinal health. Bacteriophages and their bacterial hosts develops a significant relationship by modulating the microbial populations and their evolutional advancement through horizontal gene transfer. These bacteria-infecting viruses show great host specificity that suggests its utility for the selective decrease of pro-inflammatory or pathogenic bacteria. Certainly because of the abundance of bacteriophages in the biosphere and the role they play in various ecosystems like oceans or human gut, bacteriophages are gaining huge interest of scientists and clinicians. As an antibacterial agent, bacteriophages offer more advantages such as safety, specificity, easy genetic engineering and manipulation and effectiveness towards multi-drug resistant bacteria than antibiotics (2).

The use of antibiotics could cause or aggravate the imbalances of microbiota or dysbiosis, whereas phages are able to selectively and subtly modify the gut microbiota. Many bacteriophages are classified as "generally recognized as safe" by the U.S. Food and Drug Administration for the human consumption. Recently, oral bacteriophage ingestion in dietary supplements was established to be tolerable and safe for the healthy adult human population (3).

Therefore, bacteriophages might be used as a new approach for the improvement of human health for both the treatment and the prevention of bacterial in-

fections, reduction of obesity, improvement of certain forms of cancer in which the microbiota of the gastro-intestinal tract play a significant role (1,2).

Microbiome

The microbiome consists of trillions of microorganisms living in the gut of human beings. Microbiome has a critical role in the human health. However, a greater consumption of processed carbohydrates, fats, meats, preservatives can alter the human microbiome. Moreover, overuse of antibiotic destroy both healthy and pathogenic bacteria (4).

With time, an imbalance of the ratio of the good versus the pathogenic bacteria could trigger chronic diseases. Similarly, asthma, allergic disorders, and obesity are associated with unhealthy intestinal bacterial population (5,6).

Incorporating bacteriophages with a probiotic

Almost 70% of the immune system in human beings exist in the gastrointestinal-linked tissues that are called gut-associated lymphoid tissue. This gut-associated immune system depends on the manifestation of healthy intestinal bacteria that protects the body from diseases. On the other hand, poor diet, age, stress and medications can imbalance the ratio good versus pathogenic bacteria. This imbalance among gut microbiome may cause metabolic syndrome, autoimmune disorders, chronic fatigue, diabetes, obesity and non-alcoholic fatty liver disease (7).

A good probiotic would help to maintain microbiome balance and strengthen the immune system. Incorporation of bacteriophages to these probiotics may improve their ability to maintain the intestinal health. Bacteriophages selectively target harmful pathogens, and leave the beneficial microbes to flourish. This technique was commonly used in Europe prior the discovery of antibiotics. Bacteriophages are particularly beneficial when they are used in combination with the probiotics. In fact, probiotics help the beneficial bacteria to grow thousands times higher than their baseline growth rate (6,8).

Difference between bacteria-based probiotics and lytic phage-based probiotics

The use of lytic phages as a major portion of the probiotic diet resembles the use of bacteria-based probiotics that have gradual administration during a certain time period and cause favorable conditioning for the gut microbiome. The main differences among lytic phage-based probiotics and bacteria-based probiotics is that bacteria-based probiotics adds non-pathogenic bacteria to the gastro-intestinal tract, thereby restricting the ability of pathogenic bacteria to colonize the gastro-intestinal tract and move across the intestinal mucosa, whereas the lytic phage-based probiotics target and destroy specific pathogenic bacteria in the gastro-intestinal tract. This approach might lead to the production of a novel class of prophylactic and therapeutic products, effective in the treatment of bacterial infections caused by pathogens that need the gastro-intestinal tract environment to grow and cause the disease. Additionally, phages have the ability to destroy specific bacterial strains without harming other bacteria (9).

Bacteriophages as probiotics

Probiotics consist of live microorganisms that when administered in an adequate amount, induce health benefits to the host. Many researchers classify bacteriophages as probiotics according to the World Health Organization definition (10).

Aleshkin et al analyzed a novel probiotic dietary supplement called polyvalent phage cocktail containing *Escherichia*, *Staphylococci*, *Listeria* and *Salmonella*-targeting bacteriophages that is able to reduce the risk of foodborne infections outbreaks. The bacteriophages that are active against *Escherichia*, *Staphylococci*, *Listeria* and *Salmonella* strains were selected and isolated on the base of their titer, host range activity, sensitivity to hostile factors, ability to maintain their specific characteristics during storage and their safety for the animal models and humans. The resulting data demonstrates the efficiency and safety of the designed polyvalent phage cocktail for humans and animals (11).

Effects of supplemental bacteriophage intake on inflammation and gut microbiota

Bacteriophages give a novel mean of selectively modifying the gut microbiota, affecting the environment of gut and intestine without causing any global distresses that could lead to the microbial dysbiosis. At the opposite, antibiotics may cause the disruption of microbial communities predisposing to dysbiosis or the creation of ecological niches for pathogens, as commonly observed in the *Clostridium difficile* infections (12).

Bacteriophages enhances the fermentative microbial taxa that favor butyrate production, causing a shift toward a healthier gut environment. Both sulfate-reducing bacteria like Desulfovibrio and *E. coli* have been linked to the greater clinical activity indices and the sigmoidoscopy scores during the rectal biopsies of patients suffering of inflammatory bowel disease. Research studies have also showed higher concentration of *Clostridium perfringens*, lower concentration of *Eubacterium spp.* in accordance with the severity of the disease. Another study reported that bacteriophages consumption is able cause a 4-fold decrease of *C. perfringens* and a 4–5-fold rise of two bacteria belonging to the genus *Eubacterium*. Interestinlgy, reduction of *Eubacterium* have been linked with inflammatory conditions of the gastrointestinal tract (13).

Phage consumption is also able to lead to reduction of the interleukin Il-4. Physiologically, the release of Il-4 is associated with Th2 responses that are linked with IgE promotion and the responses of eosinophils to atopy. Although the mechanisms connecting the reduction of Il-4 with the consumption of bacteriophages are unclear, several studies have established that bacterial lipopolysaccharides induce the production of Il-4 through MyD88 and TRAM-dependent pathways that lead to systemic inflammation and increased release of cytokine (14). A possible mechanism the treatment with phage might be the reduction of the circulating lipopolysaccharides that in turn lead to Il-4 reduction. Further experiments are needed to better explore the mechanistic connections between the consumption of phages and Il-4 concentration, and the response human populations with atopic dermatitis and other allergic atopies (1,15).

Administration of bacteriophages

The use of bacteriophages in food products to regulate the microbiota of human gut is currently gaining more and more interest. However, some studies have showed low phage viability under gastrointestinal conditions. In addition, nowadays, processed foods contain only very few phage particles. To improve bacteriophage survival under gastrointestinal conditions, several studies have focused on the encapsulation of these bacteriophages that would lead to the release of bacteriophages in an active form within the intestine. For instance, *Lactococcus*-targeting phages P008 were encapsulated in various matrix materials. The non-encapsulated bacteriophages could not survive the acidic conditions of pH 2.0, whereas, when experimentally encapsulated with milk proteins survived at the pH 2.0. Moreover, these phages were released from their capsules after the incubation for 2 h in the simulated intestinal fluid with pH 6.8. Therefore, phages were released in the gut. Studies have indicated that the encapsulated phages might be included in the food formulation to target the gut and regulate the microbiota (16).

PHAGE Study: Bacteriophages as novel prebiotics

A prebiotic is defined as an indigestible dietary component that selectively enhances specific bacterial species in the intestines to confer a health benefit. The Bacteriophage for Gastrointestinal Health (PHAGE) study done by Tiffany Weir, Colorado State University determined the tolerability and safety of the supplemental consumption of bacteriophage in a healthy adult population with mild to moderate gastrointestinal distress. These bacteriophages are considered safe for the human consumption and they specifically target the pathogenic bacteria of gut and allow the populations of beneficial bacteria to increase. Furthermore, probiotics containing phages have proven their effectiveness in *in vitro* cellular models and animal models, however its efficiency has not fully demonstrated in humans. In a research study involving 40 females and males of age ranging from 18-65 years, the consumption of prebiotic dietary supplement is commercially

available containing a bacteriophage mixture including LL5-Siphoviridae, LH01-Myoviridae, LL12-Myoviridae and T4D-Myoviridae was analyzed for the improvement of the bacterial profiles of the gut as compared to the placebo control. According to this study, therapeutic consumption of a mixture with 4 bacteriophages shows both safety and tolerability in the targeted human population (1,3).

Similar results were reported in another study focusing on the horse gut. It was observed that the concentration and diversity of different strains of *E. coli* were directly associated with the relative concentrations of specific coliphages (17).

Recently, it was observed that bacteriophages could drive the diversification of strains in marine bacteria like flavobacterium. This study presented for the first time the experimental demonstration of the functional diversity caused by bacteriophages within the host bacterial population (18).

Detailed investigations are required to investigate the phages potential role in defining the bacterial population limits in human intestine with the introduction of large amount of bacteria through the dietary intervention. This approach is usually called probiotic treatment and involves viable microbial cells supplemented in an adequate number to alter the bacterial populations in the intestine and produce beneficial effects on the health of humans.

Probiotic microorganisms like bifidobacteria have not been considered affected by phage infections. However, recent genome-based analysis revealed the presence of prophage DNA sequences in nearly all bifidobacterial genomes that have been sequenced until now, indicating that these intestinal commensals are regularly targeted by the phages (19). Probiotic supplementation might alter the relationships between different microbial populations that live in the human gut. Considering the "kill-the winner" hypothesis, the probiotic populations might also be targeted by phages, that would in turn lead to the modification of balance between microbial populations existing before the probiotic treatment. Any bacterial strains more concentrated than other in a specific location will be quickly eliminated by the phage predation (20).

Genetics of phages specific for probiotic bacteria

The development of technological systems like the mixed probiotic cultures supplementation or the selection of probiotic cultures that possess natural immunity against phage infections would be critical to devise appropriate strategies that could limit or avoid the harmful effects caused by the phages on the probiotic cultures. Some interventions such as dairy fermentation procedures might be beneficial to decrease the harmful effects of phage during the probiotic treatment. This method provides a way of counteracting the phage infections after the probiotics administration to animal models (21).

Novel genetic techniques like functional genomics and whole genome sequencing have greatly influenced the probiotic research, helping at understanding the interactive capabilities of these phages with their host and the intestinal microbiota (22).

With the phage metagenomes sequencing along with the sequencing of human gut microbiomes, the specificity and diversity of phages will be better understood. However, further detailed research will be required to understand the real impact of these bacteriophages on the microbial activities and diversity in human gut (20).

Bacteriophages related issues and their solutions

The issue related to the application of bacteriophages in human diet might be considered in quantitative terms (concentration or number of phages in the product), rather than qualitative terms, the way it is tested in chemical antibacterial medications. Furthermore, in principle, the specificity of the phages during their interaction with the bacterial culture restricts the probability of bacteriophage to cause any direct harmful action on the human cells or organism, thus results in the safe decontamination of such bacteriophage-containing products (10). But, theoretically, bacteriophage administration could cause unpleasant effects in humans:

1. Infection of the normal human bacterial flora associated with iatrogenic dysbacteriosis: To overcome this problem, selection of specific

bacteriophage strains was done. This allowed the isolation of phage particles with their lytic activity limited only to specific bacterial species or strains. This precise selection procedure helps to exclude those bacteriophages that might damage *lactobacilli* and *bifidobacteria* while designing the phage cocktails. Similarly the administration of mono- as well as polyvalent bacteriophage preparations for the treatment of acute infection of intestine and deteriorated dysbacteriosis established that these preparations could not lyse the normal microflora in human (23).

2. Human immune reaction stimulation: The results of some clinical research studies showed that bacteriophages may influence the human immune cells contributing to the development of innate and adaptive immunity (cytokines production, antibodies synthesis, T cells proliferation, phagocytosis, phagocytes respiratory bursts) (24). Similarly, it was noted that the systemic and local administrations of the phage therapy did not produce any major anaphylactic reaction to the patients, except the endotoxic reaction. This reaction is caused by the quality of phage composition used and the bacteriolysis reaction (also called Jarisch-Herxheimer reaction) *in situ* (25). However, by minimizing the phage concentration during the prophylactic phage treatment, the reduction of the endotoxin amount produced by bacteriolysis is possible (26).
3. Enhancement of bacterial host virulence caused by the integration of the phage genome the host genome: phage genomes may contain bacterial virulence genes or toxin-encoding genes that could be horizontally transferred to bacteria. To avoid these adverse effects of the phages on human microbiota, directional selection should be applied for the production of strains, therefore only strictly-lytic phages should be incorporated in such composition. With the use of DNA sequencing techniques, these toxin-encoding genes and other virulence factors can be easily controlled (27,28).
4. Introduction of bacterial toxins from the host bacteria used for phage production into the human microbiota: to overcome this issue the quality of phage cocktails is verified for their exo- and endotoxins contents (29). Exotoxins concentration can be strongly reduced by using non-pathogenic bacterial cultures to increase the bacteriophages biomass like *E. coli* phage *K-12* and *L. innocua* phages. The endotoxin content within the composition is analyzed after purification of the sterile filtrate of the phage lysate (30).

Conclusion

The human gastrointestinal tract is inhabited by diverse and abundant populations of bacteria (microbiota) that significantly contribute to the mucosal protection, gastrointestinal immune tolerance regulation, synthesis of vitamin K and fecal matter digestion. Changes in this microbiota could contribute to several chronic degenerative diseases such as ulcerative colitis, Crohn's disease, irritable/inflammatory bowel disease, rheumatoid arthritis or intestinal dysbioses. Phages are usual components of foods and they work better with wide spectrum probiotics. They could be used for the improvement of gastrointestinal health, and for the prophylactic and therapeutic approaches against bacterial infections. Phage-containing probiotics are active in both small and large intestine, and are more effective in small doses administered within hours. Phage targeting naturally affects the dynamics of gut population and have influence on the microbial gene expression. Presently, bacteriophage-containing probiotics are gaining popularity in the United States and other countries.

Finally, after over 90 years of investigation on phages along with their interactions with eukaryotic cells including human and animal cells, scientists have not find any evidences regarding the harmful or negative effect on the human health (26).

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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