THE CONCEPT OF USING BACTERIOPHAGES TO IMPROVE THE MICROBIOLOGICAL QUALITY OF MINIMALLY PROCESSED FOODS

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ABSTRACT
Phages were discovered relatively recently – at the turn of the 19th and 20th centuries. The idea of using bacteriophages for therapeutic purposes was promoted by d’Herelle, who conducted the first successful experiments with prokaryotic viruses. Works of contemporary scientists on phage therapy were, however, halted due to the discovery of penicillin by Alexander Fleming in 1928. Today, when many bacterial strains have developed resistance to common antibiotics offered by the pharmaceutical industry and when new, so far unknown, bacterial strains have appeared, the concept of using bacteriophages to treat bacterial infections has been revived. Considering the food sector, the search for novel solutions that would ensure the appropriate microbiological quality of minimally processed foods may bring an effective method for eradicating bacterial pathogens that induce food-borne infections. The employment of chemical and physical methods of food preservation often lead to the deterioration of its nutritive value and of its physical and organoleptic properties. Minimally processed foods manufactured without any drastic preservation methods can be especially at risk of developing microorganisms, including the pathogenic ones. Low-temperature production processes and cold-storage facilitate the development of psychrophilic microorganisms, while another threat is posed by the high microbiological contamination of raw materials. This work presents a biological method for the eradication of bacteria most commonly found in a food-based environment. The study concept postulated the use of bacteriophages to improve the microbiological quality of food, with special attention paid to minimally processed foods.

Keywords: bacteriophage, food quality, preservation of food, minimally processed foods

INTRODUCTION
Technological operations, and preservation methods in particular, are of key significance in assuring a sufficient quality and safety of food products. Traditional preservation methods are aimed at ensuring that food products have a long shelf-life, but induce changes in their sensory and nutritional properties. Minimal processing is one of the latest concepts proposed in food technology which affords the possibility of manufacturing food products with a fresh appearance and meeting consumer demands (convenience in use, no chemical additives, and increased nutritive value) (Bansal et al., 2014; Ragaert et al., 2007). One of the basic quality attributes of food is its microbiological status. Minimally processed foods have to meet high microbiological quality standards which take into account contamination with their natural microbiota and
with extrinsic microorganisms from the surrounding environment which pose a risk of potential food poisoning (Bansal et al., 2014). Food products of this type include frozen, chilled, chilled and vacuum-packed, as well as chilled and modified atmosphere-packed food products. The problem of ensuring the sufficient microbiological quality of minimally processed foods has not been entirely solved yet (Ragaert et al., 2007; Vermeulen et al., 2018). High microbiological contamination of raw materials, mild preservation methods, and cell sap drip during storage causing water activity to increase, are the main threats posed to minimally preserved foods. For this reason, an important stage of the technological process after cutting and washing raw materials should be the removal of water residues and tissue sap released in centrifuges, which may prevent abrupt development of microflora. Mild preservation methods are not always sufficient to ensure the microbiological safety of foods, and cold conditions of production and storage promote the development of psychrophiles or psychrotrophs. Quality deterioration and curbed usability for the consumption of minimally processed foods are due to the development of microorganisms, natural physiological processes, and the activity of tissue enzymes in raw materials (Bansal et al., 2014).

**PREVENTION OF MINIMALLY PROCESSED FOODS**

A tendency has recently been observed in highly developed countries towards the consumption of natural food products processed to the least possible degree. Producers strive to minimize changes in quality attributes and to reduce the use of chemical additives. Expectations of consumers have contributed to the development of novel types of convenient foods, preserved at lower than normal temperatures. Such products are cold-stored (above 0°C) or stored in a frozen state (up to –18°C and below). Appropriately stored frozen products are microbiologically stable. However, this method of food preservation cannot be deemed effective in bacteria inactivation; it can only induce a bacteriostatic effect due to the inhibition of enzyme-catalyzed metabolic reactions. After food defrosting, cells of pathogenic bacteria recover complete physiological capability and become a potential causative agent of food poisoning (Archer, 2004; Lund, 2000; Manani et al., 2006). Minimally processed food products are additionally preserved using physical, physicochemical or biological methods, like: ohmic heating, radiation, high hydrostatic pressure treatment or alternate (pulsed) electric field; with sous-vide and cook-chill methods, as well as by means of storage in a modified atmosphere and the use of protective edible films or microorganisms (Tirpanalan et al., 2011). The biological method for food preservation exploits bacterial cultures as protective cultures that produce substances with bactericidal or bacteriostatic properties against both bacteria inducing and pathogenic food spoilage. These substances include bacteriocins produced by the lactic fermentation of bacteria, phytoalexins, and chitosans. The food industry also makes use of enzymatic preparations which exhibit antagonistic effects.

Consumer interest in non-processed or minimally processed foods has triggered remarkable advances in the food packaging sector. The most popular packaging and storage techniques include packaging under conditions of increased air purity (CRT), packaging in a modified atmosphere (MPA), and packaging in a controlled atmosphere (CAS) (Tirpanalan et al., 2011).

**USE OF BACTERIOPHAGES TO IMPROVE THE QUALITY OF MINIMALLY PROCESSED FOODS**

In times when consumers are aware of the adverse effects of food environment chemigation, and when they search for as few processed food products as possible or non-processed ones with a natural color and structure, the food industry has to seek out novel solutions to meet their expectations (Lewis et al., 2019). Technologies of minimal processing may evoke changes in the physicochemical properties of food products by modifying their color and texture or diminishing their nutritive value. The use of a biological factor to ensure the desired quality of minimally processed food products may satisfy consumers’ demands through the extension of their stability and lack of negative effects on their physical properties.

Bacteriophages can be used in food biocontrol in order to eradicate pathogenic bacteria from food, minimize food spoilage induced by bacteria development, and reduce bacterial resistance to antibiotics (by
suppressing the expression of antibiotic resistance genes via providing anti-sense DNA of phages; EFSA, 2009).

CHARACTERISTICS OF BACTERIAL VIRUSES

Bacteriophages are obligatory parasites of bacteria that infect viruses replicating in their cells (Anany et al., 2015). Likewise, viruses specific to eukaryotic cells do not fall under the standard definition of life due to their inability to self-reproduce, lack of functional metabolism, and cell morphology. Despite this, they are autonomous structures which undergo processes of evolution and variability. In terms of structure, viruses are made up of the same main components as other cells. Their genomes are composed of nucleic acid, although sometimes in configurations uncommon to cell genomes, like in the example of double-stranded RNA (Brussow et al., 2004). This dual nature of bacteriophages proves them to be organisms from the verge of the animate and inanimate worlds.

Virus particles are composed of genetic material in the form of DNA or RNA nucleic acid surrounded by a shell of structural proteins which form a capsid. Electron microscopy analysis showed most of the phages (96.2%) to be tailed viruses, like, for example, phage T4 (Fig. 1). The tail usually contains a hollow tube coated with a contractile sheath which facilitates the injection of nucleic acid into the host cell. The tail ends with a base plate from which some fibers extend that are composed of two coupled parts – the outer one being responsible for the recognition of appropriate cell receptors, and the inner one fixing the fiber to the base plate. The tail is connected to the capsid by a connecting protein that forms the collar (Anany et al., 2015).

OCCURRENCE OF PHAGES IN THE NATURAL ENVIRONMENT

Bacteriophages are widely abundant in nature. Their special ubiquity may be observed in such natural environments as soil, water, and live organisms (Górski and Weber-Dąbrowska, 2005; Hendrix, 2003). Some literature works (Górski and Weber-Dabrowska, 2005) indicate that there can even be up to 100 phage particles in one cell, depending on environmental factors. The bacteriophage amplification rate is on average 50 times higher than the proliferation rate of cells of their hosts (Marx, 2007).

Phages can be found in all environments that are colonized by viable bacteria: human and animal secretions and excrements, water, sewage, soil (Atterbury et al., 2009; Górski and Weber-Dąbrowska, 2005), as well as in raw materials and food products of plant and animal origin (Weinbauer, 2004). In aquatic environments (fresh and sea waters), the average number of phage particles ranges from $10^7$ to $10^9$ per 1 cm$^3$ (Hendrix, 2003). Similar numbers may be found in soil (Górski and Weber-Dąbrowska, 2005).

Bacteriophages are particles stable in a pH range from 5.0 to 8.0, but at lower temperatures their tolerance extends into pH 4.0–10.0. Due to their rapid inactivation at low pH values, studies are conducted to determine the effects of their microencapsulation or their exposure to the buffering system on their protection against adverse conditions of the anterior sections of the alimentary tract, stomach and duodenum in particular (Carrillo et al., 2005; Kazi and Annapure, 2015; Saez et al., 2011; Wall et al., 2010).

PHAGE INFECTION CYCLE

Bacteriophages interact with susceptible bacterial cells located on tail fibers (families: Myoriridae,
Virulent bacteriophages usually exhibit the lytic cycle, which entails an immediate transition of bacterial into phage metabolism. Processes of cell biosynthesis are arrested and replaced by processes of synthesis of bacteriophage components. The assembly of phage particles proceeds in an infected cell, whereas their release is due to the enzymatic reaction which disrupts the continuity of a host cell wall. The newly synthesized phages released to the environment are capable of infecting other bacterial cells (Anany et al., 2015; Kazi and Annadure, 2015; Kahn et al., 2019).

A state of lysogeny may be induced by moderate phages, with a genome in the form of dsDNA or ss-DNA (filamentous phages). In a lysogenic cycle, the genome of bacteriophages becomes integrated with a bacterial chromosome (episome is being formed) and enters into the latent state (prophage). This state may be interrupted spontaneously or be induced by physical factors (UV light) or chemical ones (antibiotics, e.g. mitomycin C; Lee et al., 2018). The choice between the two developmental cycles is, probably, made by the bacteriophage itself. Once the load of energy in a host cell is sufficient for phage progeny synthesis, bacteriophages enter into the lytic cycle, but when it is too low, the synthesis of a prophage is more likely to ensure the survival of difficult conditions (Anany et al., 2015). Considering the use of bacteriophages in food technology, lytic (virulent) phages should be searched for as their development will lead to rapid damage of bacterial cells (EFSA, 2009; Gutiérrez et al., 2016).

**ATTEMPTS AT BIOCONTROL OF PATHOGENS IN FOOD**

Consumers’ pursuit of ready-to-eat food products and novel technologies aimed at ensuring food safety and quality has intensified along with measures undertaken to reduce the risk of their loss. Gouvea et al. (2016) proved that the absorbent food pad, which contains a cocktail of six bacteriophages: BFSE16, BFSE18, PaDTA1, PaDTA9, PaDTA10, and PaDTA11, used in chilled meat may represent a form of biocontrol to be applicable in food preservation. It offers a perfect system for extending the shelf-life of cold-processed, ready-to-eat foods. In vitro analysis of this system was conducted to check its capability to reduce the initial count of *Salmonella Typhimurium* (10⁶ CFU/cm³ (CFU – colony forming unit)) in the medium. The food pads were covered with phage lysates in three concentrations (10⁶, 10⁷ and 10⁸ CFU/cm³), where the
first one was expected to reduce the bacterial count by one log unit, and the two others to reduce the bacterial counts by ca. 4 log units, at a temperature of 15°C within 48 hours. Incubation of the pads at 10°C facilitated a reduction of the bacterial count in the medium by one log unit on average in the case of all three phage concentrations. It was concluded that, the higher the concentration of bacteriophages, the better their effect on the host.

A cocktail of three Salmonella spp. specific bacteriophages was used by Spricigo et al. (2013) in four different food matrices: pig skin, chicken breast, eggs, and packaged lettuce. The results confirmed the efficacy of the developed bacteriophage mixture as a biocontrol agent for Salmonella spp. under conditions similar to those used in the production processes of the analyzed foods.

A combination of antagonistic bacteria and lytic bacteriophages used to control Salmonella spp. growth during Mung bean seed sprouting was presented by Ye et al. (2010). Antagonistic bacteria were isolated from bean sprouts and tomatoes to evaluate their activity against Salmonella spp. Among the achieved isolates, the Enterobacter asburiae (denoted as “JX1”) exhibited antagonistic activity against a wide array of Salmonella serovars (Agona, Berta, Enteritidis, Hadar, Heidelberg, Javiana, Montevideo, Muenchen, Newport, Saint Paul, and Typhimurium). Bacteriophages lytic to Salmonella spp. were isolated from wastewater and animal excreta. The bacteriophage cocktail was prepared from six isolates of E. asburiae JX1 with Salmonella spp. in a bullion broth. The coupling of E. asburiae JX1 with the phage cocktail reduced the count of Salmonella spp. by over 6 log units compared to the control sample. Their count reached ca. 10⁷ CFU/g in the samples with phages and the protective culture compared to the samples with phages used alone. The injection of phage and of phage + nisin reduced the bacterial count by at least the same order of magnitude as pipetting did. The efficacy of phages depended on both the application method and the initial count of L. monocytogenes.

Protective cultures (e.g., Lactobacillus sakei TH1) may be successfully used as additional protective agents (when coupled with phages) to reduce the growth of Listeria monocytogenes on slices of cooked ham. Research conducted by Holck and Berg (2009) demonstrated that phage addition caused a 10-fold reduction in the count of L. monocytogenes. In turn, a 100-fold reduction was observed after 14 to 28 days of storage in the samples with phages and the protective culture compared to the samples with phages used alone.

The range of bacteriophage application may even be extended once they are immobilized on neutral surfaces. Anany and co-workers (2011) developed a new method for the immobilization of bacterial viruses which is based on differences in the charge between the bacteriophage head (negative charge) and the outer part of the tail fibers (positive charge). In such a configuration, the head was attached to a positively charged carrier (modified cellulose membranes), whereas the tail of the virus remained unbound owing to which it could recognize and capture receptors of bacterial cell walls. The number of immobilized infecting phages on the positively charged cellulose

A promising, chemical-free approach to the inhibition of Salmonella spp. growth in sprouting seeds.

Another area of research (Leverentz et al., 2003) aimed to determine the effect of lytic phages specific to Listeria monocytogenes, using two various methods of virus application, injection and pipetting, on deliberately damaged fresh melon and apple. A phage cocktail reduced the L. monocytogenes population by over 4 log units on the melon samples and below 0.4 log units on the apple samples, compared to the respective control samples. Combined with nisin (belonging to bacteriocins), the phage cocktail reduced the L. monocytogenes population by just under 6 log units when applied on a cut melon and by over 2 log units when administered to apple slices, compared to the control sample. Nisin used alone reduced the bacterial population by over 3 log units in the melon slices and by ca. 2 log units in the apple slices, compared to the control sample. The injection of phage and of phage + nisin reduced the bacterial count by at least the same order of magnitude as pipetting did. The efficacy of phages depended on both the application method and the initial count of L. monocytogenes.

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membranes was significantly higher than on those with non-modified membranes. Phage cocktails acting against *Listeria* sp. or *Escherichia coli* immobilized on these membranes effectively inhibited the growth of *L. monocytogenes* and *E. coli* O157: H7 in ready-to-eat and raw meat at various storage temperatures and packaging conditions. Further work on the industrial application of phages investigated their storage stability. They showed lyophilization to be an effective method for drying phages to preserve their infecting properties when coupled with immobilization on newly developed bioactive carriers.

Commercial poultry products are commonly regarded to be the main sources of campylobacteriosis. Multiple intervention strategies have tended to reduce contamination of broiler carcasses with *Campylobacter* in a slaughterhouse. Atterbury et al. (2003) demonstrated the efficacy of bacteriophages (coupled with freezing) in reducing the count of *Campylobacter jejuni* on artificially contaminated chicken skin.

In addition to the use of bacteriophages as antibacterial agents directly in the food environment, research has been conducted to determine their efficacy in ensuring the microbiological purity of devices intended for direct contact with the product. In the food industry, biofilms are formed by bacteria on the surfaces of the equipment used, especially in places difficult to clean and disinfect. Roy and co-workers (1993) investigated the usability of phages against *Listeria* sp. as disinfectants of contaminated surfaces made of stainless steel and propylene. The surfaces, which were artificially contaminated with *Listeria monocytogenes*, were treated with a suspension of phages from the family *Siphoviridae* (H387, H387-A, and 2671). The phage suspension at a concentration of $3.5 \times 10^8$ PFU/cm$^2$ (PFU – plaque forming unit) was equally effective to a 20 ppm solution of a quaternary ammonium compound (QUATAL). Synergistic activity was observed after the coupled use of two or more phages and after the suspension of phages in a quaternary ammonium base.

In another study, Viazis et al. (2011) determined the effects of the previously described phage cocktail (BEC8) on *Escherichia coli* O157:H7 (EHEC) on food processing surface materials like sterile stainless-steel chips (SSC), ceramic tile chips (CTC), and high-density polyethylene chips (HDPEC). Cultures of EHEC O157:H7 EK27, ATCC 43895, and 472 strains were mixed, inoculated *in situ* onto a surface, and dried. The chips were inoculated at $10^5$, $10^4$, and $10^3$ CFU/chip so as to obtain a multiplicity of infection (MOI) rates of 1, 10, and 100, respectively. Control samples and the phage cocktail (ca. $10^6$ PFU/chip) were administered to the inoculated surfaces and incubated at temperatures of 4, 12, 23, and 37°C. The EHEC survival rate was determined with a standard plate count method on tryptic soy agar. At temperatures of 37°C and 12°C, no bacteria survived onto SSC (detection limit 10 CFU/chip) after BEC8 infection at MOI = 100 after 10 mins, and at a temp. of 23°C after 1 hour. A similar result was obtained for CTC at 37°C after 10 mins and at 23°C after 1 hour. Due to the high specificity of bacteriophages, their use as disinfectants of food contact surfaces may be limited. A good solution would be to develop a phage cocktail containing various viruses with overlapping ranges of bacterial hosts or broad-range host phages (Gutiérrez et al., 2016).

Research is underway on the use of phage reporter systems to detect live cells of pathogenic bacteria in a short time and with a low detection limit. Phages can become a new generation of biocontrol agents and a tool for the rapid detection and identification of pathogens. Today, however, the construction of a reporter phage is still difficult. The development of a new commercial detection kit seems to be the right direction for researchers in the near future (Bai et al., 2016).

**COMMERCIAL BACTERIOPHAGE PREPARATIONS**

There are several companies on the market that have developed and distributed bacteriophage preparations dedicated to the food industry. The Microes Food Safety company has developed a group of products against pathogenic bacteria in food under the PhageGuard brand. Phage preparations against *Listeria* (Listex™ P100), *Salmonella*, and *Escherichia coli* have been approved by the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA). The German company FINK TEC GmbH has developed a preparation against *Escherichia coli* called Secure Shield E1, which has been approved by the FDA. The USDA has also approved the Finalyse® preparation against enterohaemorrhagic bacterium *Escherichia coli* O157: H7 from the Passport Food
Safety Solution company. A similar product, called EcoShield™, from Intralytix Inc., has been approved by the FDA. This company also offers other preparations to be used in food, that have been approved by the FDA: ListShield™ (against Listeria monocytogenes), ShigaShield™ (Shigella spp.), and SalmoFresh™ (Salmonella spp.). In turn, AgriPhage™ preparation from Phagelux can be used against Xanthomonas campestris pv. vesicatoria and Pseudomonas syringae pv. tomato (Moye et al., 2018; Svircev et al., 2018).

Most of the commercial phage preparations exhibit a significant efficacy in reducing the bacteria in the food environment. Ten of them have received temporary GRAS status, granted by the FDA (Kahn et al., 2019). The EFSA report, prepared in 2016, provides results of the assessment of the safety and effectiveness of Listex™ P100 preparation in reducing pathogens in various ready-to-eat products. Studies conducted with three groups of food products (meat and poultry, fish and crustaceans, and dairy products) showed a significant reduction in the population number of Listeria monocytogenes. The report emphasizes the safety of using this preparation when processing these types of food. The P100 bacteriophage poses no health risk, has been found to be non-toxic in studies with rats and is strictly lytic to bacteria and at the same time unable to transduce bacterial DNA. The entire production process of the preparation (phage reproduction and preparation purification) follows the principles of the HACCP system. In addition, the P100 bacteriophage multiplies in the Listeria innocua strain and is non-pathogenic to man. Research conducted by Guenther et al. (2009) confirmed the efficacy of Listex™ P100 preparation. In liquid foodstuffs, the number of bacteria decreased below the detection level, while in solid samples it decreased by up to 5 logarithmic orders compared to the control samples. The efficacy of this preparation has also been confirmed by Lewis et al. (2019), whereas in the study with sliced fruit and fruit juices, Oliveira et al. (2014) recommended the coupled use of Listex™ P100 preparation with other technologies in the case of products with a low acidity. Perhaps an effective solution would be the use of bacteriophages in combination with the spraying of organic acids that are used in food technology. In the research of Oladunjoye et al. (2017), attention was paid to the improved efficacy of Listex™ P100 when used in combination with sucrose monolaurate, i.e. a compound with antimicrobial activity. However, not all studies clearly confirm the efficacy of commercial bacteriophage preparations (Arthur et al., 2016).

The European Food Safety Authority (EFSA) has issued a scientific opinion on the use and functioning of bacteriophages in food production. The EFSA encourages further research aimed at preventing the re-infection of food with bacterial pathogens and at establishing bacteriophage-pathogen and food interactions (EFSA, 2009). Luo et al. (2012) determined the phage-bacterium interaction in the food matrix using non-invasive bioluminescence spectroscopy and imaging. The bacteriophage preparation Coliphage T4r was purchased from Carolina Inc., and its administration on the surface of lettuce leaves effectively reduced bacterial counts both on the surface and inside the leaves.

The EFSA report takes no account of the safety of bacteriophages use in food products (EFSA, 2009).

SAFETY IN THE HOMEOSTASIS OF THE GASTROINTESTINAL TRACT IN HUMANS

Due to the increasingly common multi-drug resistance of bacteria, contemporary medicine exploits bacteriophages in phage therapy. Medical research has provided information on the safety of phage use. Phage therapy is safe and highly effective in children and even in neonates. Owing to the targeted action, it is milder than antibiotic therapy, but its application requires prior in vitro identification of the infection-inducing strain and selection of the appropriate phage (the so-called phage typing) (Biswaas et al., 2002). As in the case of antibiotics, the use of bacteriophages in, i.a., phage therapy, can induce some side effects. However, since these effects are not detrimental to health or life, there is no need to discontinue therapy. Literature works provide examples of temporary, transient gastric ailments, headaches, muscular and joint pains, pains of the face and oral cavity, general debilitation, vomiting, and increased body temperature. Some allergic manifestations and hepatalgia may occur as well. Due to the selective action of phages on individual bacterial strains (EFSA, 2009), phage therapy causes no damage to the physiological bacterial flora of the body, which prevents the development of infections.

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induced by relatively pathogenic bacteria, as it often happens after the eradication of bacterial flora by antibiotics with a wide spectrum of activities (Biswas et al., 2002; Kazi and Annapure, 2015).

THE ROLE OF PHAGES IN THE PATHOGENICITY OF BACTERIA

The presence of active and inactive phages in a host genome imparts special properties to bacteria. This phenomenon has been termed lysogenic conversion. This conversion is especially significant in the case of pathogenic strains, as prophages encode many virulence factors in pathogenicity islands (PAIs). The PAIs are a type of genome island (GEI), namely exogenous DNA segments which were incorporated into a bacterial cell as a result of horizontal transfer of genes and which became integrated with the chromosome (Boyd et al., 2001).

Corynebacterium diphtheriae is an example of a bacterium whose pathogenic effects are due to the presence of prophage in the chromosome. Its pathogenicity is associated with the synthesis of DT toxin by phages. A similar observation was made for Clostridium botulinum which produces a phage-encoded neurotoxin. In this case, genetic elements are encoded by prophages (Boyd et al., 2001). The pathogenicity of Escherichia coli O157:H7, which produces the Shiga toxin (ST), also results from the contribution of prophages. Expression of genes encoding this AB type toxin is regulated by both the phage and the cell protein (Boyd et al., 2001). After cell lysis, a toxin is released as a result of phage induction which acts inside epithelial cells by affecting the 60S subunit of ribosomes in eukaryotic cells, thereby inhibiting protein biosynthesis (cytotoxic effect).

Great concern is aroused by the fact that phages inducing the lysis of Gram-negative bacteria may contribute to the secretion of a high dose of bacterial endotoxins (release of lipopolysaccharides present in the cell wall), which in turn may heavily load the liver and evoke side effects including the cardiogenic shock that was observed in the case of some antibiotics (Dixon, 2004). So far, however, such symptoms have not been observed in phage therapy (Lepper et al., 2002; Soothill et al., 2004). Bacteriophages may mediate in the transfer of antibiotic resistance genes between bacterial strains, though they are not used in practice (Boyd et al., 2001; Gutiérrez et al., 2016). Risk of the development of resistance to bacteriophage infection by bacteria may be effectively minimized by developing a mixture of a few phages which exhibit a wide spectrum of activities (Kahn et al., 2019; Svircev et al., 2018). Another factor which diminishes the efficacy of the therapy is the immunogenicity of bacteriophages. This can, however, be minimized by using a short period and an oral route for administration of the phage cocktail (Górski and Weber-Dąbrowska, 2005).

CONCLUSION

In the case of minimally processed foods at a high microbiological risk (e.g. sprouts and lettuce mixes), the biological fight with both saprophytic and pathogenic bacteria is an alternative to chemical methods, for example, the use of preservatives. Assuming that – being obligatory pathogens of bacteria – the phages accompany the natural microflora of low-processed foods, they may be used as natural abiotic factors against hosts. This concept is based on the isolation of natural bacterial flora directly from the food environment and its proliferation in selective-differentiating media. Next, phages specific to these isolated strains should be typed in the same environment. By this means, a phage cocktail can be obtained that would be composed of phages selective to bacterial microflora typical of a given type of food product. A phage cocktail may be used at a low dose, through direct application to food. Considering phages capability for increasing their dose spontaneously, bacteriophages increase their own number by replicating in host cells when the bacteria that the injection was targeted at appear on the surface of the food. It is also important to develop mixtures of strictly lytic phages, whose developmental cycle will lead to direct eradication of the bacteria. The proper phage cocktail should also contain such a set of bacteriophages that would ensure preparation efficacy in the widest possible range (in different food storage conditions and against many strains of bacteria).

Worthy of note is the lack of adverse effects of bacteriophages on consumers. As specific viruses, they do not destroy either the desirable microflora of food or commensals of the gastrointestinal tract of humans and animals. They are capable of surviving at a wide
range of pH values (from 4.0 to 10.0) and temperatures; and may also survive the environmental stress induced by food processing.

The putative effect of bacteriophages on bacterial pathogenicity is one of the key reasons why they have not been used on a mass scale yet. Today, only such strains are used in phage therapy which are certain to be unable to transfer toxin-encoding genes onto other bacteria, including the so far non-pathogenic ones. Possibilities offered by contemporary genetic engineering could help in the genetic modification of bacterial viruses. Interference into a phage genome would allow the removal of fragments of nucleic acid responsible for the transfer of genes which encode bacterial endotoxins.

In the near future, scientists need to convince consumers of the unconventional preservation method of minimally processed foods with the use of bacterial viruses.

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