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Bacteriophages as Twenty-First Century Antibacterial Tools for Food and Medicine

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Abstract

In recent years it has become widely recognized that bacteriophages have several potential applications in the food industry. They have been proposed as alternatives to antibiotics in animal health, as bio preservatives in food and as tools for detecting pathogenic bacteria throughout the food chain. Bacteriophages are viruses that only infect and lyse bacterial cells. Consequently, they display two unique features relevant in and suitable for food safety. Namely, Their safe use as they are harmless to mammalian cells and their high host specificity that allows proper starter performance in fermented products and keeps the natural microbiota undisturbed. However, the recent approval of bacteriophages as food additives has opened the discussion about 'edible viruses'. In this review, we examine the promising uses of phages for the control of foodborne pathogens and the drawbacks on which more research is needed to further exploit these biological entities.

Keywords: Antibiotics, Bacteriophage, Biocontrol , Food safety, Antibiotic resistance

Introduction

The need for an alternative to antibiotics each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics. At least 23,000 people die annually as a direct result of these infections, while many more die from other conditions that were complicated by an antibiotic-resistant infection. [1] Since the 1980s in the US, newly approved antibiotics have steadily declined and despite the increased awareness and redoubled efforts, the current R&D pipeline remains largely dry. [2] The underlying economic factors make antibiotic development unprofitable, [3] , since it is not commercially viable to develop new drugs if there is a high probability of their becoming ineffective soon after introduction. [4] One of the major drawbacks is the inability to discover completely new antibiotics; those discovered over the last few decades have now been modified to produce new generic forms [5], which is a disincentive to spending money on R&D. Antibiotics were used in poultry industries to reduce Salmonella levels at each step of the production in the farms. Yet Salmonella remains the major cause of foodborne diseases worldwide, with chickens known to be the main reservoir for this zoonotic pathogen. [6][7] It is the second leading cause of bacterial foodborne illness in the US and the great majority of these infections are associated with the consumption of products such as poultry and eggs contaminated with Salmonella. [8] Salmonella have evolved several virulence and antimicrobial resistance mechanisms that allow for continued challenges to our public health infrastructure. [9] The emergence of infectious disease caused by drug resistant bacteria requires alternatives to conventional antibiotics. [10][11][12][13] The search for new drugs is becoming critical because of the growing concern over the failing antibiotic drug discovery pipeline. There is a great deal of interest to investigate alternatives and natural antimicrobial agents, which has also increased due to consumer awareness about the use of chemical preservatives in foodstuff and on food processing surfaces.

Bacteriophages

Bacteriophages (phages) are described as viruses that infect bacteria. Application of phages has been investigated extensively, such as in the indicator of fecal contamination [14] and against antibiotic resistant bacteria. [15]

Lytic phage

When a virulent phage infects a host bacterium, it replicates much faster than the host cell. The whole cycle can be completed in 30–40 min. The phage is a parasite that depends on the host for its propagation, which is influenced by a variety of factors such as temperature, nutrients, light and other environmental forces. [16] It subverts the host's biological function and utilizes the host machinery for reproduction. The host cell undergoes lysis and dies, simultaneously liberating a large number of progeny phages, which are each then ready to start another cycle by infecting new neighbouring bacterial cells. This cycle is known a lytic 'virulent' cycle. The lytic cycle or 'virulent phages' fit in the class of 'natural antimicrobial controlling agents' and are arguably the most abundant biological entities on the planet.

Lysogenic phage

Other particles, called lysogenic phages, are 'temperate' or dormant phages which may take the form of a 'prophage' by integrating with the viral DNA in the host chromosome. They become a part of the host cell and replicate along with the host chromosome for many generations, coexisting as opposed to lysing the host cell. [16]This phenomenon is called 'lysogeny', which also provides immunity against infection by further phage particles of the same type, ensuring that there is only one copy of phage per bacterial cell. The unique characteristics of lysogenic or 'temperate' phages and their potential for exploitation have been demonstrated in a system that restores antibiotic efficiency by reversing pathogen resistance to antibiotics. [17] These phages are genetically engineered to reverse the pathogens' drug resistance, thereby restoring their sensitivity to antibiotics. Unlike conventional phage therapy, the system does not rely on the phage's ability to kill pathogens in the infected host, but instead, on its ability to deliver genetic construct into the bacteria and thus render them sensitive to antibiotics prior to host infection. The transfer of the sensitizing cassette by the constructed phage will significantly enrich antibiotic-treatable pathogens on hospital surfaces. This may hold key advantages to revive the use of old generation antibiotics leading to the use of phage biotechnology synergistically with antibiotics.

Bacteriophages and their role in food safety

The current technologies employed to inactivate bacterial pathogens in foods are not infallible, as proved by the continuous increase in several foodborne diseases caused by pathogens, such as Salmonella, Campylobacter, Escherichia coli, Listeria and others that have an enormous impact on public health. [18] Contaminating bacteria can get access to food during slaughtering, milking, fermentation, processing, storage or packaging. Over the last few years, a number of strategies to minimize the microbial load of raw products have been explored as the use of antibiotics is restricted due to the negative impact on human antimicrobial therapies. Problems of acceptability and deterioration of the organoleptic properties have been described after physical treatments such as steam, dry heat and UV light. Moreover, the extensive use of sanitizers has led to the development of resistant bacteria rendering these procedures less effective. On the other hand, some approaches often used in the food industry to reduce contamination by foodborne pathogens cannot be directly applied to fresh fruits, vegetables and ready-to-eat products. Hence, despite recent advances to avoid transmission of bacterial pathogens throughout the food chain, novel strategies are still required to fulfill consumer demands for minimally processed foods with fewer chemical preservatives.

Bacteriophages for biocontrol of pathogens in food

Bacteriophage-based bio control measurements have a great potential to enhance microbiological safety based, namely, on their long history of safe use, relatively easy handling and their high and specific antimicrobial activity . The concept of combating pathogens in food by means of phages can be addressed at all stages of production in the classic 'farm to fork' approach throughout the entire food chain. Bacteriophages are suitable (i) to prevent or reduce colonization and diseases in livestock (phage therapy), (ii) to decontaminate carcasses and other raw products, such as fresh fruit and vegetables, and to disinfect equipment and contact surfaces (phage bio sanitation and bio control) and (iii) to extend the shelf life of perishable manufactured foods as natural preservatives (bio preservation). Bacteriophages should also be considered in hurdle technology in combination with different preservation methods. [19][20]

Bacteriophages for medical use

The search for alternatives to antibiotics has led many scientists to a treatment practice that's been on the fringes of modern medicine for nearly a century. Bacteriophages—viruses that infect and kill bacteria—were first used in 1919 to treat a wide range of infections. Phage therapy fell out of favor with the advent of antibiotics; the practice has only persisted in some European countries as an experimental treatment. However, earlier this year, phage therapy was highlighted as one of seven approaches to "achieving a coordinated and nimble approach to addressing antibacterial resistance threats" in a 2014 status report from the National Institute of Allergy and Infectious Diseases (NIAID).

First on farms

Renewed interest in phage therapy is due in part to the growing problems posed by antibiotic overuse in the clinic, which has escalated microbial resistance. But even when antibiotics are overprescribed, most people only receive doses in response to illness.

On farms, however, small amounts of antibiotics are routinely used to promote animal growth or prevent disease outbreaks; the practice has been linked to long-term changes to animals' commensal microbiomes, increased transfer of antibiotic-resistant bacteria from animals to farmworkers, and potential risks to human health. Recent governmental initiatives to curb antibiotic use have largely overlooked their use on farms. But a small number of phage-based alternatives are now available from Maryland-based biotech Intralytix, which manufactures sprays that target *Listeria*, *Salmonella*, and *E. coli* O157:H7 in foods and food-processing facilities.

Scientific clarity—understanding why and how to design phage treatments—is a higher priority, according to Gill. In a 2006 Antimicrobial Agents and Chemotherapy study, he and his colleagues attempted to use phages to treat bovine mastitis caused by *Staphylococcus aureus*, a growing concern in the dairy industry. However, only 16 percent of cows treated were cured. High phage concentrations in milk up to 36 hours after treatment also suggested that the virus was being inactivated or destroyed within the gland.

The work highlights one of its many quirks: viruses that look like efficient killers in experiments can fail to cure an infection in the field. Phages may work better at targeting subsets of infections, such as on the skin or deep within organs. Even when bacteriophage therapy is successful, scientists know little about the underlying mechanisms.

Bacteriophage treatments

Ear and lung infections caused by *S. aureus* or *Pseudomonas aeruginosa*, a major concern for cystic fibrosis patients, may be viable targets for clinical phage therapy, according to results from early Phase 1 and 2 clinical trials. In a 2009 Clinical Otolaryngology study, researchers treated 24 patients suffering chronic middle-ear infections caused by antibiotic-resistant *P. aeruginosa* with Biophage-PA, a mixture of six phages, and found significant improvement compared to a placebo.

Another pulmonary pathogen, *Burkholderia cenocepacia*, was successfully targeted by phage in a mouse model of lung disease. The results, published in a 2010 paper in The Journal of Infectious Diseases by Gill and his colleagues, found that in this case, administering phages intraperitoneally treated the disease better than an intranasal spray.

Bone wounds and prosthetic infections, where bacterial biofilms can fester, are another attractive target for potential phage therapy, according to pathologist Catherine Loc-Carillo at the University of Utah. Osteomyelitis caused by drug-resistant bacteria, among other diseases "where we are almost out of antibiotic options are the highest priority [for phage therapy]," she said.

In an alternative approach, other researchers are trying to derive broad-spectrum molecules by understanding how phages kill their hosts. In a 2013 PLOS_ONE study, Rockefeller University researchers identified how phage lysine killed *Bacillus anthracis* by targeting an enzyme essential to cell wall formation in the pathogenic bacteria. They then identified a small-molecule inhibitor that mimicked the interaction of the phage with its host. Found effective against several Gram positive pathogens, the molecule, Otsuka Pharmaceutical's Epimerox, is currently being tested as a potential therapeutic.

Conclusions and future prospects

The use of biological control measurements such as bacteriophage biocontrol seems to be a promising alternative for the management of food contamination as the use of chemical preservatives becomes restricted. Future developments involve safety and technological issues as well as expanding the antimicrobial skills of bacteriophages.

Bacteriophages may act as vectors of undesirable traits (virulence and antibiotic-resistance genes) and temperate phages mediate lysogenic conversion that have raised safety concerns. Recent advance in genomics and phylogenic studies make it possible to understand gene flow among phages and hosts and potentially harmful bacteriophages could be avoided or re-designed without undesirable traits and lacking any gene dissemination systems. As an example, bacteriophages could be genetically engineered to block phage replication once the host has been killed. [21] This would prevent the release of large numbers of phages in a particular environment. Technological challenges to strengthen the future use of bacteriophages as bio preservatives will require the establishment of safe large scale production processes. It is advisable to develop nonvirulent, genetically well-characterized bacteria as hosts in phage propagation. On the other hand, the antimicrobial activity of phages observed in laboratory conditions could be greatly reduced in food systems. Limiting factors are reduced diffusion rates that decrease the chance of host-phage collisions, the microbial load which might also act as a mechanical barrier by providing unspecific phage binding sites and other adverse factors such as temperature, pH and inhibitory compounds. As is the case of any food bio preservatives, bacteriophage efficacy in food should be evaluated on a case-by-case basis. To guarantee proper phage performance, it is wise to isolate phages from the same niche / habitat, as those phages will probably be better adapted to replicate and survive in those conditions. Bacterial fitness and physiological status has a clear influence on the phage infection rate. Phages that selectively bind to the host and replicate in different physiological states should be chosen. There are several strategies to improve and expand the antimicrobial activity of phages. Comparative genomics of phage tail fibre genes involved in the recognition of specific host receptors will lead to approaches to expand the host range. A detailed analysis of the bacterial receptors will also help to understand and predict the development of bacteria insensitive mutants often due to the loss or mutation of these molecules. Both approaches will enhance the use of phages as antimicrobials as well as bacterial detection systems. [22] New phage-derived antibacterial strategies based on phageencode proteins that commit cellular metabolism to phage proliferation, such as endolysins, are currently being evaluated. [23][24] Despite the fact that current research is basically at the experimental stage, the increasing number of publications and emerging companies in this area are paving the way for the future of phage-based bio preservation technologies. Landmarks for the next step towards the establishment of expanded commercial applications will be influenced by the accurate knowledge of bacteriophage biology to allow consumers to feel confident about the safety of 'edible viruses'.

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