THE SCIENCE WORLD
A Monthly e Magazine
ISSN: 2583-2212
July 2022; 2(7), 813-816

Popular Article

Bacteriophage: An Alternative Therapy of Salmonellosis

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Abstract

Bacteriophages (phages) are natural viruses of bacteria and as such can be developed to offer a viable alternative to antibiotics. Salmonella remains to be a large problem for both human and veterinary medicine. WHO classifies salmonellosis as epidemiologically the most severe zoonosis to eradicate, especially considering the fact of intense antibiotic resistance development. In farm animals, phage therapy efficacy perspectives have been widely studied in vitro and in vivo, especially for zoonoses and diseases linked to economic losses (such as mastitis), in pets, studies are still few and rather recent.

Introduction

Bacteriophages are viruses that exclusively infect and kill bacteria. They are highly genetically diverse and the most abundant biological entities on earth, being safe for humans, animals and plants. While infecting bacterial hosts, they promote bacteria destruction, holding therefore a highly efficient antimicrobial activity.

Antibiotic resistance has been an alarming threat in recent decades, subject to much discussion within human medicine, as well as in veterinary medicine. The ongoing debate exposes the fact that bacterial strains continually develop resistance to the existing antibiotics by the means of adaptive mutations. In trying to find a solution for this problem, acceptable alternatives to antibiotics have to be found, e.g. an antibacterial agent that would be able to surpass this constant bacterial resistance development, for example, bacteriophages.

Bacteriophages, also known simply as phages, are bacterial (obligate) parasites or viruses that infect, replicate inside and eventually lyse their host bacterium, letting out hundreds of new phages. Since phages depend on bacteria as their host, they can be used for therapeutic purposes to help the immune system against pathogens by killing the bacteria.

Phage therapy has been known since the end of the 19th century, but with the discovery and rise of antibiotics, the interest in bacteriophages faded away in some countries. However, in recent decades an interest in phage therapy has returned, this time viewed from another perspective.

Bacteriophages, Biological Characteristics and Classification

Bacteriophages are viruses that parasitize bacteria. They are essentially formed of nucleic acids (DNA or RNA) enclosed in a capsid of protein origin. As parasites, they need bacterial cells to survive and reproduce. Bacteriophages are extraordinarily abundant in nature. It is estimated that there are 10^{31} phages present in the biosphere. Surprisingly, the total amount of bacteriophages is 10 times higher than bacteria. They are also very ancient. It is estimated that they have been present on earth for more than three billion years. Their virulence against bacteria is very specific.

Bacteriophages utilize two main types of replications: the lytic cycle and the lysogenic cycle. During the lytic cycle, the virus interrupts the physiological metabolism of the bacterial cell to facilitate the production of the bacteriophage progeny. After the viral replication, the infection results in the lysis of the host bacterial cell. Concerning the lysogenic cycle, after adsorption, the nucleic acid of the bacteriophage integrates with the genome of the host bacterial cell and produces a prophage where it is passed on to subsequent bacterial generations. Chemical or physical factors can activate the prophage, which can exist from the bacterial chromosome, thus starting the lytic cycle.

A temperate bacteriophage has both lytic and lysogenic cycles. Namely, the first cycle plays a key role in the therapeutic use of phage therapy. In fact, in phage therapy, “phage cocktails” are prepared by using lytic phages, which consist of the administration of viruses, which, during in vitro experimentation, show a lytic capacity against target bacterial pathogens.

Today bacteriophages are usually classified into 13 families, over 140 genii, and more than 5300 types of phages. They can be distinguished by shape, structure, nucleic acid and by their interaction with the microbial host. Phages are also distinguished by size: small, medium, large; shape: filiform, spherical; phages with and without head and/or tail, etc.

Salmonellosis

Salmonellosis is caused by *Salmonella* bacteria and it is one of the most common and important infections transmitted between animals and man (zoonosis). Salmonella phages proved very effective in treating mice and chicken infected with Salmonella with a success rate of 90-100% (Lenev, 2013).

Salmonella is a common target for phage therapy because it causes disease in a wide range of endothermic animals as well as humans and causes significant production losses in livestock. Some *Salmonella* serotypes (e.g., *S. enterica* serotype Typhi) are known as 'host-restricted' because they produce a severe, systemic, typhoid-like illness in a single host (or a small number of related hosts). However, phage therapy has mainly focused on 'non-host-restricted' serotypes (principally Enteritidis and Typhimurium) which usually result in a less severe gastrointestinal infection across a much broader range of species and lead to most food borne bacterial infections in developed countries.

Phage therapy has been used for the prevention of *Salmonella* in chickens with high degrees of success. Sklar *et al.* 2001 used phages in a broiler chick model to demonstrate that *Salmonella* colonization of the cecum could be significantly reduced by almost 1 $\log_{10}$ Colony Forming Units (CFU)/g gut contents over 14-days by administering a cocktail of four phages in feed (10^9 Plaque Forming Units (PFU)/g). Additionally, phage treatment appeared to reduce secondary infection signs in the birds as only three out of 10 animals in the phage-treated group showed mild inflammation on the air sacs while 8 out of 10 birds in the untreated control group showed signs of airsacculitis.

Atterbury *et al.*, 2007 selected three lytic phage that was isolated from poultry farms and wastewater in the UK with a broad host range against *S.* Enteritidis, *S.* Hadar and *S.* Typhimurium. All of the phages tested reduced *Salmonella* colonization of the ceca, although only *S.* Enteritidis and *S.* Typhimurium were reduced significantly; by approximately 2.19–2.52 $\log_{10}$ CFU/g compared with the controls. Bacteriophage-insensitive mutants (BIMs) were recovered from phage-treated animals. However, this phage-resistant phenotype was not maintained in vitro following successive sub culturing, nor in vivo when BIMs were introduced into a new group of birds in the absence of phage selective pressure.

In the case of *Salmonella*, pigs can become colonized with the organism during transport and lairage from contaminated trailers and holding pens, resulting in increased pathogen shedding just prior to processing. Increased shedding, in turn, amplifies the likelihood of carcass contamination by magnifying the number of bacteria that enters the processing facility. Treatment

with the anti-*Salmonella* phage cocktail significantly reduced cecal *Salmonella* concentrations (95%; $P < 0.05$) while also reducing (numerically) ileal *Salmonella* concentrations (90%; $P = 0.06$). Additional *in vitro* studies showed that the phage cocktail was also lytic against several non-Typhimurium serovars.

A bacteriophage cocktail as a microencapsulated feed additive and demonstrated it can be an effective and practical way of reducing *Salmonella* colonization and shedding in pigs. The administration of *Salmonella* phages orally exposes them to potentially hostile conditions, such as low pH of the stomach/gizzard and the activities of bile and enzymes in the duodenum, which may impact bacteriophage viability. Various approaches have been used to mitigate against the potential damage that these conditions may cause, including concomitant administration of antacid, microencapsulation with chitosan/alginate microencapsulation with alginate, microencapsulation with antacid/alginate and liposome nanoencapsulation.

In conclusion, the Typhimurium phage and the bivalent salmonella phage are successfully introduced into veterinary praxis and are recommended to veterinary specialists, thereby proving to be promising anti-epizootic medicals.

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