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Review

A review of current methods using bacteriophages in live animals, food and animal products intended for human consumption

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ABSTRACT

Bacteriophages are utilised in the food industry as biocontrol agents to reduce the load of bacteria, and thus reduce potential for human infection. This review focuses on current methods using bacteriophages within the food chain. Limitations of research will be discussed, and the potential for future food-based bacteriophage research.

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1. Introduction

The use of bacteriophages to detect bacterial human pathogens has revolutionised modern microbiology. Their use represents a significant divergence from traditional cultivation, and could have the potential to rapidly provide information to diagnose and prescribe a treatment strategy for bacterial infections in humans and animals. However, their use has raised concerns from the public about the inclusion of live viruses in products destined for human consumption. It has also raised concerns about their propagation, safe transport and storage, as well as shelf life in foods that already have to conform to high safety standards.

Bacteriophages are obligate intracellular parasites of bacteria, and are usually specific to one species or even specific to just one strain of that species. Bacterial transmission through the food chain has been recognised as a significant threat to human health for many years. PCR-based technologies are commonly used for the detection of human pathogens in clinical samples (Speers, 2006), as well as for the detection of bacteriophages (del Rio et al., 2008; Martín et al., 2008). It

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might be possible for the existing technology platforms to be adapted to monitor shelf life, effective storage and thus to reassure the public over their inclusion in food products, in terms of biological safety.

This review will focus on current methods utilising bacteriophages as biocontrol agents for food products destined for human consumption. First, live animal research will be discussed. The methods include those where animals are directly inoculated with bacteriophage suspensions in their food and water, or indirectly in to their immediate environment where uptake is facilitated. Next, the review will discuss methods where bacteriophages are applied directly to foods or to their packaging. Finally, methods utilising bacteriophage enzyme preparations will be discussed.

2. Live animal research

The number of naturally occurring bacteria and bacteriophages are likely to fluctuate during passage through animal bodies, and be influenced by diet, contact with other animals, and incidents of disease. This might suggest that a dynamic predator-prey relationship exists within animal populations, and that animals could provide a valuable resource for the discovery of new bacteriophages against a range of pathogenic bacteria. Bacteriophages have been recovered as from live agricultural animals, particularly noted during incidents of disease, such as mastitis (Georgescu et al., 2015). Han et al. (2013) isolated a bacteriophage from the *Myoviridae* family from a sewage outlet effective against *S. aureus* infections in agricultural cattle. The bacteriophage proved to have wide host range against strains isolated from incidents of clinical disease, including activity against some methicillin-resistant strains, as determined by inoculating broth cultures with the bacteriophage at a range MOI of 0, 0.01, 1, 100, with host cell number determined by absorbance at 0 to 6 h. The bacteriophage proved effective at reducing host cell numbers, and demonstrated good potential as a therapeutic agent against infectious disease in cattle. The optimum conditions were determined to be an MOI of 1 with 2 h incubation at 38 °C. This is an important piece of research, as it indicates that bacteriophages with broad host specificity are able to be isolated from the environment and be cultivated on a laboratory scale to demonstrable effect in agricultural animals. Application of this technology could mean a decreased disease load and animal death through infection might allowed for reduced disruption to milk or meat production, and thus to reduce economic burden on farmers through the loss that would be associated with bacterial disease. However, it must be noted that repeat application of bacteriophages could lead to a host immune response. It should also be noted that the co-evolution of bacteria and bacteriophages must be monitored if the effectiveness of the treatment is to be maintained.

The effective delivery of bacteriophages in to animal bodies is an important issue. The bacteriophage must be delivered to the site needed, remain viable during transport and delivery, and to exist in sufficient number to effect the host bacterial population. There are a number of methodological routes by which a bacteriophage culture can be delivered to a live animal (Fig. 1). The most common route is oral delivery, where either the food or water is dosed with the viable bacteriophages, sometimes for individual animals, or sometimes by metaphylaxis.

Ma et al. (2012) reported a method to increase the survival of bacteriophage K through simulated gastric fluid, for use by oral delivery. Bacteriophage K is active against *Staphylococcus aureus*. The bacteriophage was encapsulated in to alginate microspheres with added calcium carbonate to counter acidic conditions, pH 2.5, and added in to the model gastric environment. Non-encapsulated bacteriophage was entirely deactivated, but the presence of the alginate-calcium carbonate resulted in just 0.17 $\log_{10}$ reduction after 2 h. Increased viral survival could be the result of the associated pH increase due to the use of calcium carbonate, as well as the protection afforded by the alginate. This research indicates that the delivery of bacteriophages in to hostile body sites might be possible by adaptation of the delivery vehicle.

In 2014, Wong et al. isolated a *Salmonella*-specific bacteriophage, *st*1, from chicken faeces for potential use a biocontrol agent in live chickens. Analysis by Transmission Electron Microscopy (TEM) indicated that the bacteriophage appeared to resemble members of the *Siphoviridae* family, and demonstrated strong lytic activity against *S.* Typhimurium, and some lytic activity against *S.* Hadar. The live animal model used in this study presented the chickens with a challenge of 10^{10} CFU of *S.* Typhimurium, delivered by intracloacal inoculation. An effective 2.9 $\log_{10}$ reduction of bacterial cells was achieved within 6 h, with no viable

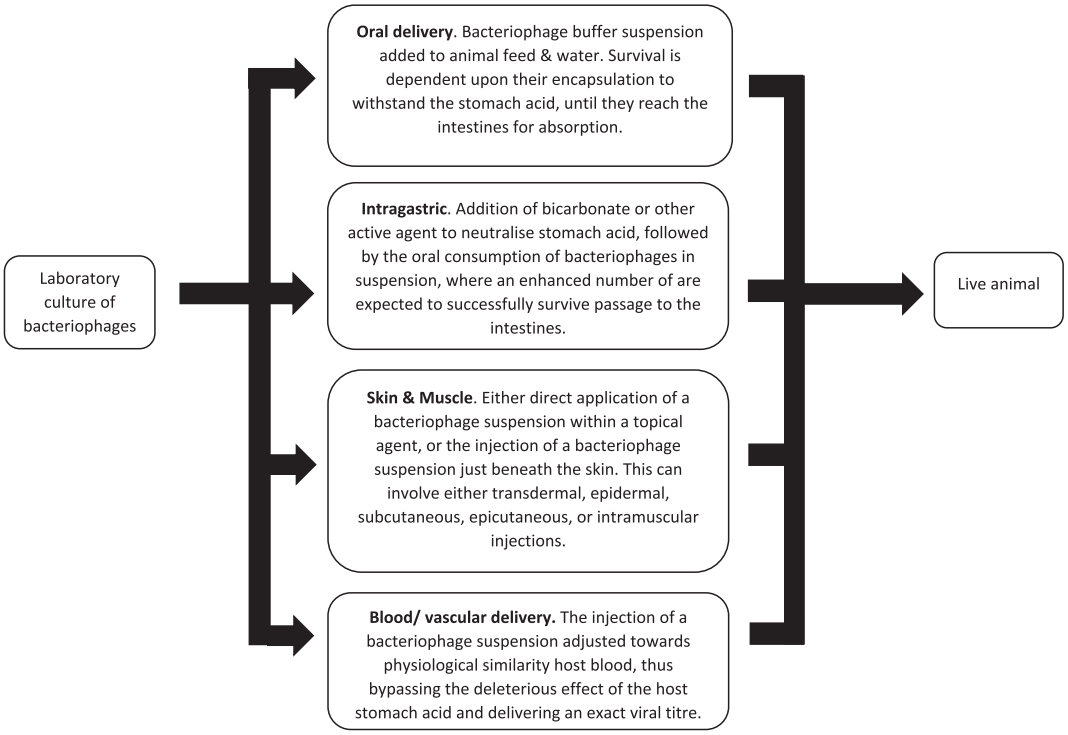

Fig. 1. A brief summation of the methodologies for the delivery for bacteriophages to live animals.