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Phages and the control of bacteria in food

Bacteriophages (phages) were discovered around a century ago through their peculiar property of being filterable entities capable of killing bacteria¹, and were almost immediately put to work to treat bacterial diseases. However, they later became the victim of some ‘snake oil’ salesmanship and eventually the advent of antibiotics saw their use cease in Western countries, although their clinical use continued in the Eastern Bloc. More recently, though, there has been renewed interest in using phages to kill bacteria in numerous applications, including both foodborne pathogen and spoilage organism control².

The biology and characteristics of phages

Phages are viruses that specifically infect and kill bacteria. If you’ve only ever heard of one phage it will be phage T4 which infects *E. coli* (Figure 1, page 21). There are two predominant lifestyles that phages can enter into. In the simplest (the lytic cycle) the virus infects the cell, its nucleic acid is replicated and the genes expressed to make new phage particles. At the end of the replication cycle enzymes are produced to degrade the bacterial cell wall, which becomes so weakened that the cell bursts through its own turgor pressure and the cell is killed.

This process, known as lysis, results in the release of the progeny phages; 100 per cell is not unusual. In the other more complicated life cycle, phages enter a symbiotic relationship, integrating their genetic material into that of the cell. They will then replicate with the cell until environmental conditions change whereupon they enter the lytic cycle, so killing the cell. This second lifestyle is not considered to be immediately useful for food applications.

The initial molecular interaction between the phage and cell is important in determining the ‘host range’ of the phage i.e. the range of

bacterial species or strains that the phage will infect. This can be very narrow (termed specific); I have known of a case where a phage was obtained that would only infect the bacterial strain on which it was isolated and none others of the same species that were tested. It can also be very non-specific as some phages infecting *Listeria monocytogenes* will kill almost any strain of the species that they are exposed to. Getting the host range of any phage product right is important and can allow a very precise grouping of bacteria to be targeted.

It is clear that many of the attributes

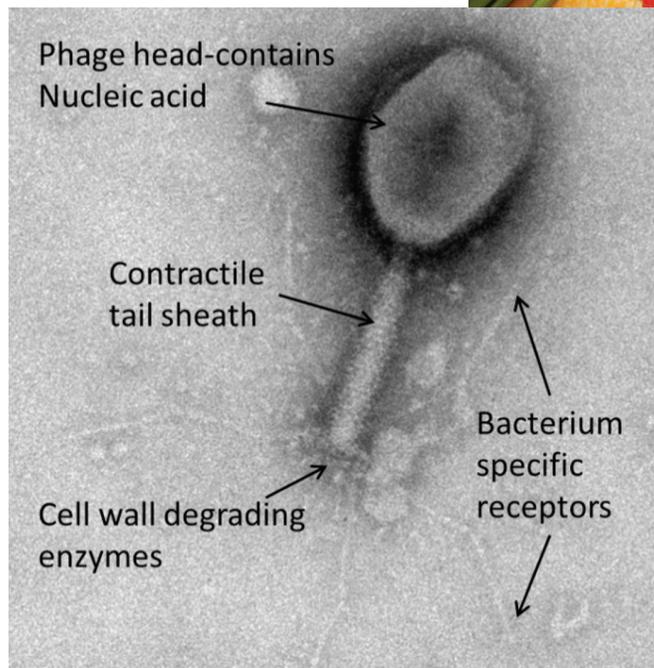
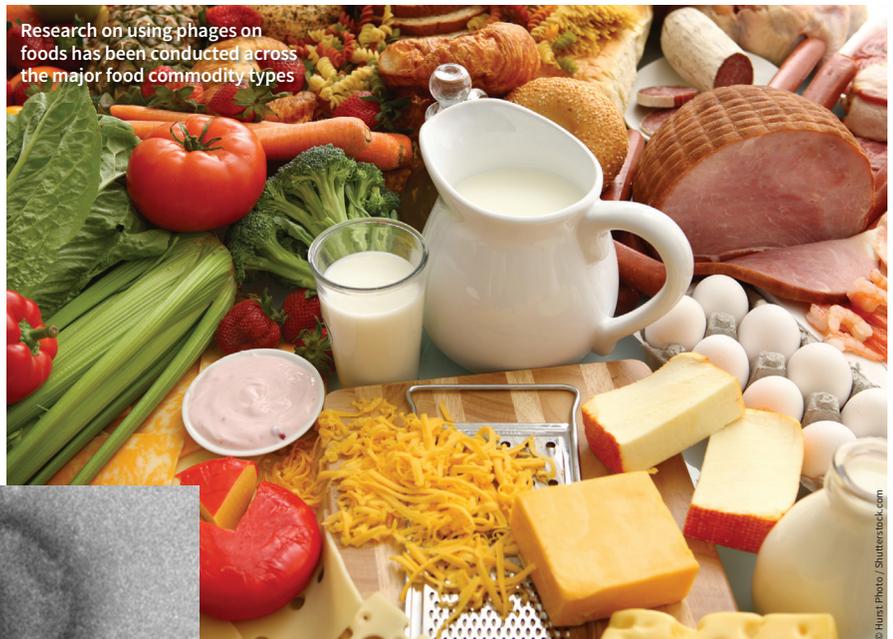


Figure 1: Transmission Electron Micrograph of Phage T4, which infects *Escherichia coli*. Courtesy of Dr. Craig Billington, ESR, Christchurch, New Zealand. The TEM was taken by the Electron Microscopy Unit, University of Otago, Dunedin, New Zealand.

described above make phages ideal for controlling bacteria; they increase in concentration as they replicate, they are entirely natural and they can be very specific in terms of the bacteria they attack.

Phages and food

Phages can be used to control pathogens and spoilage organisms at all points along the farm to fork chain (Figure 2, page 22). During food production they could, for example, be used to control bacteria colonising food animals. Notable in the UK is the work carried out at University of Nottingham on the control of *Campylobacter* in broiler chickens. Similar work has been done with *Salmonella* in pork production and there are a few reports on removal of vibrios from shellfish during depuration.

There is also potential to control contamination immediately prior to, or during, processing. For example, treating the external surfaces of slaughter animals could prevent transfer of pathogens from the hide to the carcass. It may also be possible to attack biofilms present in food

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processing plants with phages³. Phages are useful for this application as they are small enough to diffuse through the polymer layers to reach biofilm bacteria. In addition, some produce enzymes which degrade the extracellular material and lysed bacteria may also release the same sorts of enzymes, thus assisting with the next round of infection.

However, most research at the processing stage has been directed at using phages on foods themselves, and there are now many published studies describing such applications. These experiments have been conducted across the major food commodity types: dairy products⁴, meat products⁵, fruit and vegetables, as well as seafoods⁶. In general these studies show that phages can be successfully used to bring about useful reductions in pathogen concentrations⁷.

There are now a handful of examples of commercially available phages which can be used to control foodborne pathogens; the targets being mainly *Salmonella* and *Listeria monocytogenes*.

In the same way that phages can be used to control pathogens they can also be used to control spoilage bacteria. In the published experiments there is a recurrent observation of rapid inactivation occurring as a result of phage application followed by re-growth of surviving cells. This may seem like a poor result, but if the initial knock down results in shelf life extension then the result has been achieved⁸. A significant problem in the published work seems to be strain coverage; killing only 50% of the strains of a spoilage bacterium is unlikely to be particularly effective⁹. However, with careful selection of phage mixtures ('cocktails') comprehensive strain coverage can be readily obtained.

Potential pitfalls

When applying phages it is unlikely that they will kill all of the bacteria present in/on food, as there will be a small fraction of cells that are inherently resistant; the phages simply do not recognise them. The size of this fraction can be reduced by the use of cocktails, as described above. While resistance may seem to be a problem, distinct public health advances can be made by reducing the presence of pathogens without necessarily killing them all. For example, it is often quoted that if the

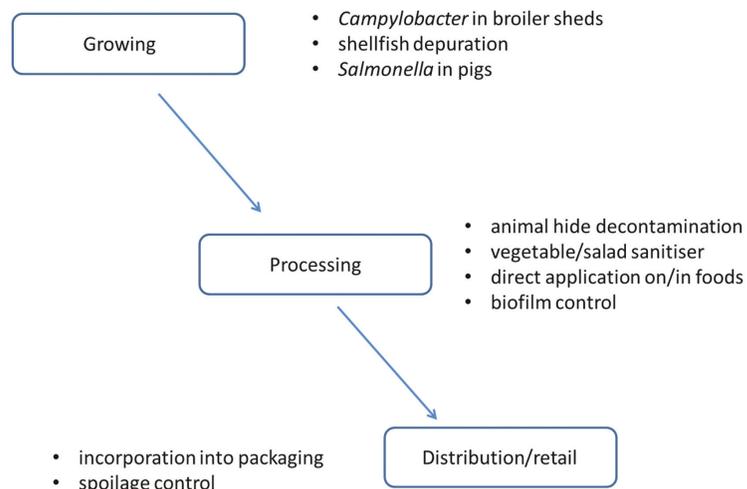


Figure 2: Potential points of phage application along the farm to fork chain

concentration of *Campylobacter* on fresh chicken could be reduced by two logs (99%) then there would be a 30 fold reduction in human campylobacteriosis cases¹⁰.

Another factor is what has become termed the minimum host 'phage replication threshold', i.e. there is a notion that if there are too few host cells present then the application of phages will not 'work'. However, studies have refuted this notion and there are descriptions of how phages and cells interact in suspension¹¹ and on surfaces¹². However, for both liquids¹³ and surfaces¹⁴ around $10^7 - 10^8$ CFU/ml or cm^2 are the sorts of concentrations required for good, rapid, inactivation. That may seem a lot but titres of around 10^{11} phages/ml can be obtained quite easily and subsequently increased through optimisation.

Phages for detecting pathogens

The host specificity of phages and the production of large numbers of progeny phages upon lysis of the cell are properties that have been used to develop sensitive methods for the detection of bacteria¹⁴. These techniques exploit changes to the nucleic acid or head protein (Figure 1, page 21). A common gene used for sensitive measurement is lux which is expressed during replication and detected by bioluminescence a few hours after infection. In another approach phage head proteins have been engineered to display a peptide which could be biotinylated and subsequently detected by streptavidin coated quantum dots¹⁵.

Future prospects

There is ample information available to suggest that carefully selected phages are safe and effective biocontrol agents of unwanted bacteria in all sorts of applications, including foods. Successful use in food animals while they are growing is perhaps a little less developed, as there is comparatively less understanding of the kinetics of phage/pathogen interactions in the gastrointestinal tract. Delivery through the low pH of the stomach to the site of action further down the gastrointestinal tract remains a problem, although solutions such as 'smuggling' them through via chitosan-alginate beads has been tested¹⁶.

An intriguing further development is the possibility of using endolysins¹⁷, which are the enzymes that destroy the bacterial cell wall prior to lysis. These are very good for killing Gram

positive bacteria, but the outer membrane impedes access to the cell walls of Gram negative bacteria. It is reported that resistance to these enzymes has not been detected.

There has been some work looking at attaching phages to packaging materials with the aim of inactivating pathogens or spoilage organisms on the surface of food during storage. However, I am unaware of papers describing useful results for normal plastic food packaging materials and issues such as keeping the phages 'alive' during storage of such packaging materials prior to their use may be difficult to overcome. There have been, however, developments in attaching phages to paper-based packaging materials.

Ultimately, legislation and the attitude of consumers to the presence of phages in food could influence their application. However, phage preparations have been accepted as organic, Halal and as 'Generally Recognised as Safe' in the US. Focus groups have also been reported to consider them as 'green' alternatives to chemical preservatives.

In my opinion the successful use of phages in the food industry will depend on them being used at sufficient concentration to be effective and at points in production and distribution where resistance simply

cannot emerge as a problem. Fortunately, ready-to-eat foods do not, in general, exist in the supply chain under conditions where pathogens can grow (a notable exception would be *L. monocytogenes*) and so the initial kill inflicted by a phage preparation should persist, and resistant cells remain very rare indeed. ☺

About the Author



J. Andrew Hudson has a BSc (Hons) from Bristol University and doctorate from the University of Waikato in New Zealand. His initial foray into food microbiology was at the Meat Industry Research Institute of New Zealand producing predictive growth models for pathogens. Later, he joined New Zealand's Institute of Environmental Science and Research Ltd where he was a science leader in the Food Safety Programme. The main area of interest was the control of foodborne pathogens using bacteriophages. He was also involved in research and consultancy projects in foodborne pathogens with particular focus on *Campylobacter*, pathogenic *Escherichia coli*, *Listeria* and *Yersinia*. In 2015 he moved back to the UK to take up his present role at Fera as Head of Microbiology in Food Quality and Safety. He is a Fellow of both the New Zealand Institute of Food Science and Technology, and the Institute of Food Science and Technology. Andrew has published more than 90 peer-reviewed papers, reviews and book chapters on various topics in food microbiology.

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