'Not on my watch': eradicating *Listeria* with nature's own biotechnology bacteriophages

Steven Hagens, Chief Scientific Officer, EBI Food Safety, looks at the latest developments of the bacterium *Listeria monocytogenes* and the natural technology that can destroy it.

Listeria, *E. coli*, *Salmonella* – foodborne pathogens, the potential consequences of which we are all too well aware. *Listeria*, in particular, seems to be rearing its ugly head, across the world from Canada, which is facing the devastation of 12 suspected deaths (at the time of writing) from processed foods containing tainted meat, to the UK, which saw a 67 per cent increase in cases from January to June 2007, when compared to the same period in 2006 [1].

Whilst global statistical data on foodborne pathogens are fragmented, data from the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2001 and 2006, show a steady increase in reported listeriosis cases for Denmark, the UK, Germany, Spain, France and Finland, all of which are developed countries with rigorous food safety controls.

So what's happening to prompt these increases in the affluent West with its HACCP controls, meticulous hygiene, extensive technology and constant food-monitoring? Is it just a case of better reporting in these countries when compared to, for example, countries such as Portugal or the former Eastern Block, which have very little historical data? Is it linked to a growing consumption in processed foods most at risk of contamination or is the bacterium itself changing? In 2007, the Panel on Biological Hazards concluded in a report [2] that "after a general decline in the 1990s, the number of cases of listeriosis has increased since 2000 in Europe. The disease is still associated with pregnancy, but it is now predominantly associated with immuno-compromised persons amongst the older section of the population (> 60 years)."

The fact that the increase in illness seems to be occurring far more in a specific risk group (the elderly), rather than in all risk groups, and that these cases are sporadic rather than occurring in groups, may indicate that changes in consumer habits could be partly responsible, for example the growing preference for Ready-to-Eat foods. Even though the primary processors of 'problem' foodstuffs such as hams, cheeses *etc* may have *Listeria* incidence under relative control,

further processing by the next manufacturer in the Ready-to-Eat chain, if care is not taken, provides an opportunity for recontamination, which then reaches the consumer.

Such is the concern of the UK's food watchdog, the Food Standards Agency (FSA), about the rise of listeriosis in compromised people over the age of 60, that in 2007 it asked the Advisory Committee on the Microbiological Safety of Food (ACMSF) to investigate the issue as a priority. A full report by the investigating Ad Hoc Group on Vulnerable Groups will be made to ACMSF sometime in September 2008 and we await their findings with interest. Of particular note is the Group's hypothetical question that the epidemiology of the disease may have changed, together with a possible change in the biology of the bacteria.

Safeguarding your production line

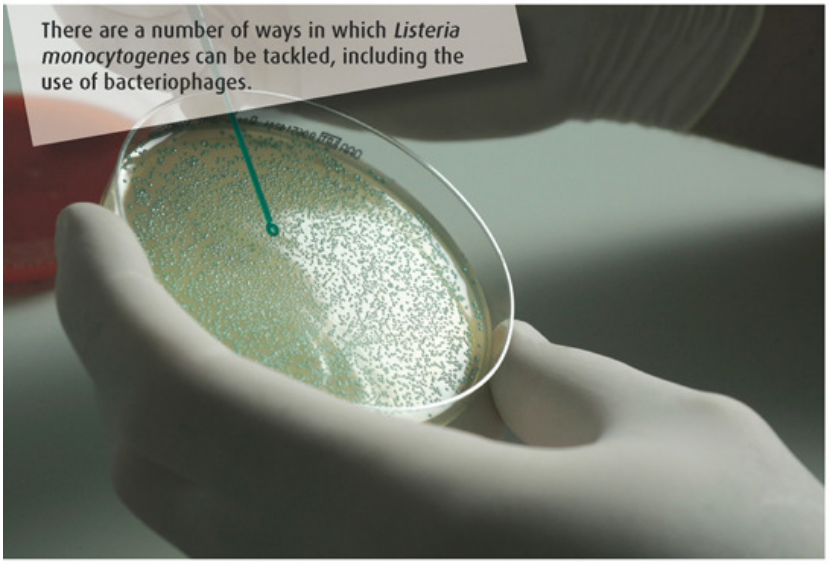
Whilst we may not yet understand the direction that *Listeria monocytogenes* is taking, there are a number of ways in which it can be tackled. The bacteria easily colonise food that has been in contact with a contaminated surface, and can grow in both refrigerated and low-oxygen packaged conditions, such as the aforementioned Ready-To-Eat products. The bacteria are also adept at surviving for long periods of time on processing equipment and household surfaces, therefore increasing the chance of cross or recontamination. Meat, poultry, cheese and fish are the categories most at risk.

Control methods, such as treatment with organic acids, nisin (a bacteriocin) and similar compounds can impair or inhibit the growth of *Listeria* bacteria. However, these substances must be labelled as additives and can affect the organoleptic qualities of the end product. And, crucially, none can be relied upon to completely exclude the presence of *Listeria*. Other methods to eradicate the bacterium from the production line itself include chemical sanitisers, such as chlorine or hydrogen peroxide, which are relatively cheap but do not target the food itself.

Opposites attract

However, it is not all doom and gloom. Nature actually provided the answer billions of years ago, but it is only in the past few years that we have learnt to harness the biological gift for directed and mass-volume use. Bacteriophages (phages) may sound unfamiliar but actually they are omnipresent in the world around us. Indeed, they are the most abundant micro-organisms in our environment, present in anything that contains bacteria, from water to soil to the human gut. On fresh and processed meat and meat products, more than 10^8 viable phages per gram can often be identified, and high numbers of phages are routinely consumed with our food, without any impact on human health or our taste and enjoyment of the product. In nature, phages act as a balance to keep bacteria under control. Each bacteriophage has a specific 'counter' species of bacterium in its sights – you might say nemesis – to which it attaches itself in order to reproduce. It cannot attach itself to any other bacterial species, hence its suitability for use with foods containing desired cultures, such as cheese. In order to survive therefore, each type of phage is constantly seeking its distinct host and, once identified, attaches itself to the cell wall of the bacterium using specific receptors on its surface. Having done so, the phage punctures the cell wall and its DNA is drawn into the bacterium, effectively taking over the cell and destroying the bacterium's ability to function or replicate. This is caused by the phage's own process of reproduction via its DNA, which produces numerous phage proteins. Some of these proteins sequester the host cell and force it exclusively to produce new phages. Once the reproduction is complete, specific proteins weaken the cell wall structure and osmotic pressure causes the cell to disintegrate (lysis) and new phages are released into the environment, destroying the bacterium in the process. In the absence of target bacteria, the phages break down into common biological particles that are naturally absorbed back into the environment.

> ...high numbers of phages are routinely consumed with our food, without any impact on human health.



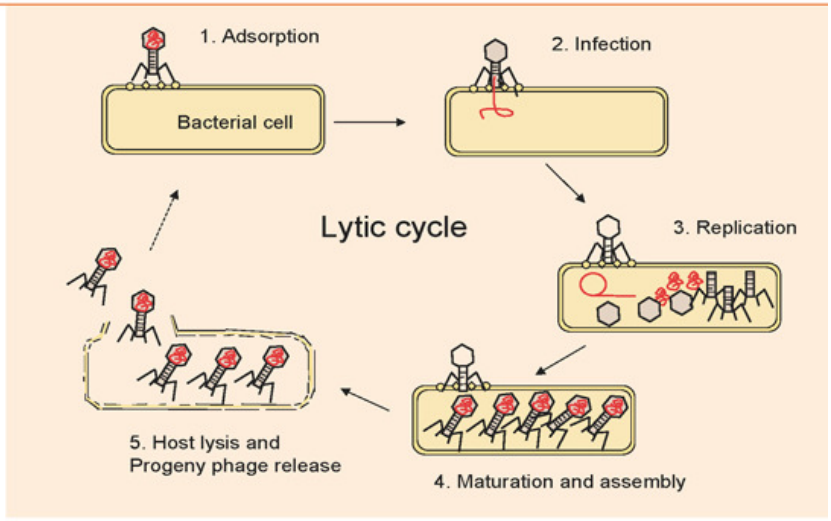
There are a number of ways in which *Listeria monocytogenes* can be tackled, including the use of bacteriophages.

Harnessing bacteriophages for food processing

Whilst there has been extensive study into the use of bacteriophages in both the food industry and the healthcare arena, harnessing their power for convenient and cost-effective use as a topical treatment has not been without its challenges. Indeed, to date, EBI Food Safety is the only company to have brought a product to market that provides consistent safe eradication of *Listeria monocytogenes*. One of the challenges that our microbiological team had to overcome in the food processing environment was the actual meeting between the phages and the bacteria.

In the hygienic conditions enforced by modern food processing, the *Listeria* bacteria may be present in low numbers (when compared with the outside world), residing in nooks and crannies, although, of course, still presenting a strong risk. However, the low numbers and potential surface area mean that a phage has to work harder to find its host. With backing from research institutes such as the Laboratory of Food Microbiology, Institute of Food Science and Nutrition at the Swiss Federal Institute of Technology Zurich, EBI Food Safety launched ListexP100, a solution containing an extremely high concentration of phages. By spraying as a topical liquid application or submerging the food in the solution at the most vulnerable stage of processing, the vast abundance of phages means that susceptible bacterial hosts are found and killed within hours without production having to cease. Kosher and Non GMO accredited, ListexP100 is an innovative processing aid rather than an ingredient, and does not affect the organoleptic properties of the food in any way or provide any other function – indeed the product's integrity is fully protected, and best of all, ListexP100 is completely natural. ListexP100 can be used in meat, poultry and dairy production. Listex For Fish is now available in a marine culture solution for fish processors.

Nobody wants to scaremonger but *Listeria* control does seem to be an increasing problem for food processors. Whilst for many people listeriosis can make them feel slightly unwell, for vulnerable groups, such as the young, the elderly and pregnant women, listeriosis is extremely debilitating and, as we have seen in Canada recently, even deadly. By using bacteriophage technology, products, customers and producers are protected from the potentially devastating impact of a *Listeria* outbreak, or worse.



The lytic cycle demonstrating bacteriophages infecting a bacterium cell, replicating and then releasing phage progeny.

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References

1. Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2001 and 2006.

2. Request for updating the former SCVPH opinion on Listeria monocytogenes risk related to ready-to-eat foods and scientific advice on different levels of Listeria monocytogenes in ready-to-eat foods and the related risk for human illness. Scientific Opinion of the Panel on Biological Hazards (Question No EFSA-Q-2007-064) Adopted on 6 December 2007

3. http://www.food.gov.uk/multimedia/pdfs/committee/acm908vulngroups.pdf

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A quick guide to bacteriophages

- A bacteriophage (from 'bacteria' and the Greek phagein, 'to eat') is a highly specific micro-organism, which infects and kills only bacteria.
- Typically, phages consist of an outer protein hull enclosing genetic material. The genetic material is usually double-stranded DNA between 5 and 500 kilo base pairs long.
- Bacteriophages are usually between 20 and 200 nm in size. A phage is approximately 100 times smaller than a typical bacterium.
- Bacteriophages are highly specific for their target host, and will not affect:

(a) desired bacteria in foods (e.g., starter cultures)
(b) commensals in the gastrointestinal tract
(c) accompanying bacterial flora in the environment

- Phages are generally composed entirely of proteins and nucleic acids, their eventual breakdown products consist exclusively of amino acids and nucleic acids. Thus, they are not xenobiotics and do not leave an ecological footprint.

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