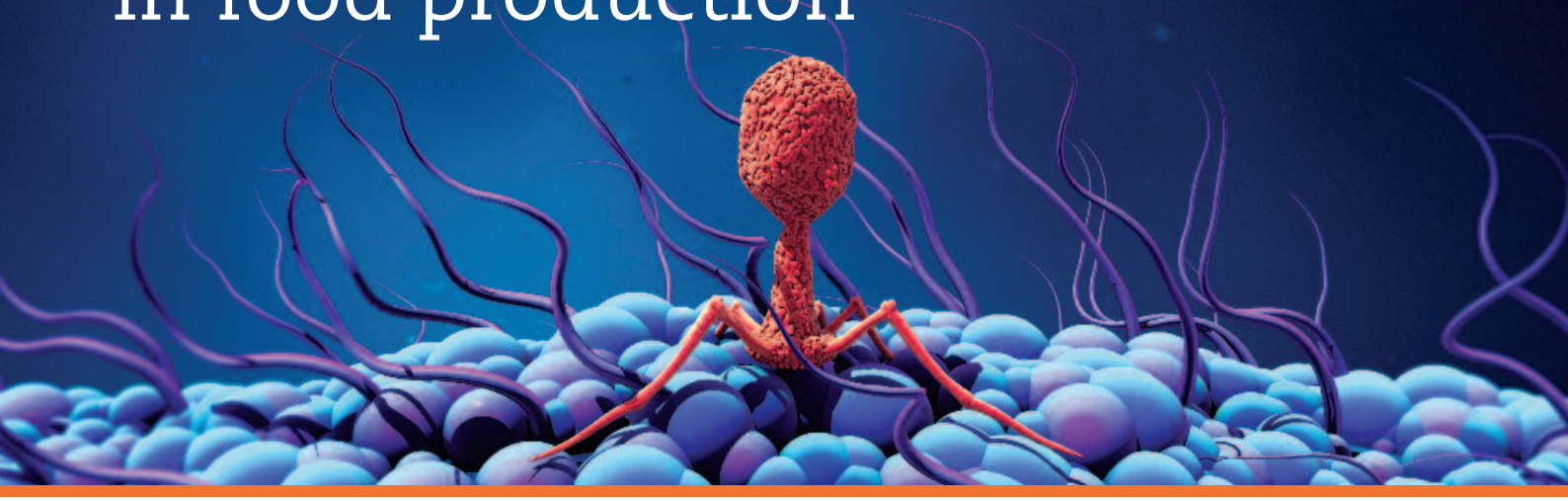
Bacteriophages for environmental decontamination in food production



TEAGASC research is looking at bacteriophages as agents to control *Listeria monocytogenes* in food production/processing.

Bacteriophages are viruses that specifically infect bacterial cells (**Figure 1**). First discovered in 1915 by Frederick Twort, initial research showed their promise in the targeted killing of pathogenic, disease-causing bacteria.

With the discovery of penicillin in 1928 by Alexander Fleming, the research focus turned more to drug therapy. However, in recent years, due to the increasing emergence of antibiotic resistance in pathogenic bacteria, the focus has again turned to the use of bacteriophages to inactivate these bacteria.

In the food industry, controlling the levels of food-borne pathogens is essential to avoid public health issues and for the safety of the products in order to prevent recalls/withdrawals. *Listeria monocytogenes* is a pathogen widely distributed in nature and has the ability of survive many different and hostile environments. It can cause listeriosis, mainly in immunocompromised groups such as infants, the elderly and pregnant women.

The symptoms can vary from gastroenteritis to abortion and encephalitis, with a mortality rate that can be up to 30%. For those reasons, controlling the presence of *L. monocytogenes* in the food industry is important.

Bacteriophages have several characteristics that make them attractive agents for controlling food-borne pathogens. These include their self-perpetuating nature, stability, and specificity in targeting the host bacterium without impacting the other microflora. In food production/processing, bacteriophages have potential application directly on the food, or in controlling the pathogen in the food production/processing environment; for example, in mushroom production.

The use of bacteriophages directly on food has been approved by the United States Food and Drug Administration, and in some cases by the European Union, through the use of products such as the bacteriophage-based ListShield and Listex. However, the use of bacteriophages to control *L. monocytogenes* in the production/processing environment has not been fully assessed.

Endolysin theory

Endolysins (lysins) are phage enzymes that allow new bacteriophage particles to be released from the host cell through degradation of the cell wall. Along with another enzyme, called a holin, the bacteriophages can literally create holes in the inner cell membrane, allowing the endolysin to cleave specific residues of the peptidoglycan structure of the cell wall and destroy it. Endolysins are usually composed of an active domain (amidase) and a cell wall binding domain, specific for the host bacteria. It has been shown previously that, when purified, endolysins, which can be purified from virulent or temperate bacteriophages, have the ability to kill

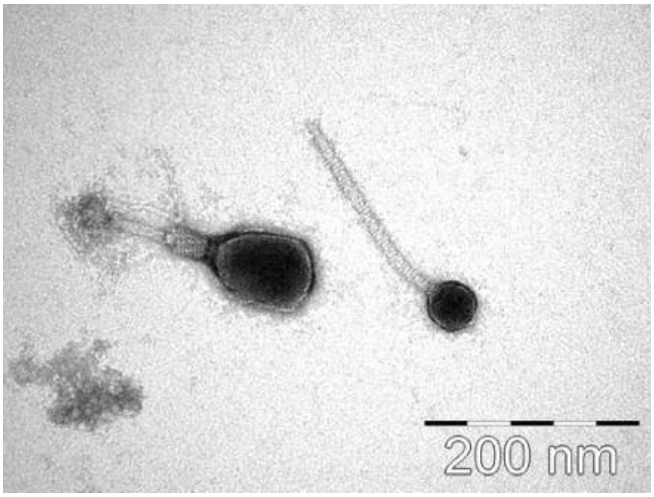


FIGURE 1: Bacteriophages are viruses that specifically infect bacterial cells. Photo provided by Horst Neve.

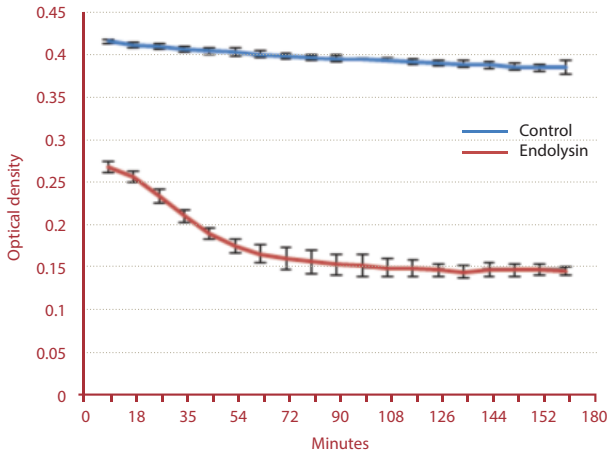


FIGURE 2: In vitro experiment testing purified endolysin against L. monocytogenes.

the target bacteria by 'lysis from without'. Such evidence is the basis for the exploitation of a bacteriophage-based protein targeting *L. monocytogenes* in the food production/processing environment.

Work at Moorepark

At Moorepark, temperate bacteriophages specific for *L. monocytogenes* were isolated from wild mushroom samples. The genome of one of these bacteriophages, phage 293, was sequenced and analysed for the presence of an endolysin gene. The active, or amidase, domain of the endolysin was cloned in *E. coli* in order to produce large amounts of purified protein.

The advantage of this technology, which produces recombinant proteins, is that additional genes that would compromise the safety of the process are not carried. The purified enzyme fragment has been tested in *in vitro* experiments against *L. monocytogenes*, demonstrating antimicrobial activity (**Figure 2**). Tests are still ongoing to characterise the enzyme and its anti-listerial activity against *L. monocytogenes* biofilms. The pilot-scale mushroom production facility at Ashtown will be used as a model food production facility to assess the efficacy of the purified amidase *in vivo*.

Phage biocontrol: some considerations

As with other pathogen control agents in the food industry, bacteriophage-derived products must fulfil certain criteria if they are to be applied:

- effectiveness demonstrated – the efficacy of the phage-derived products depends on the type of matrix they are applied on, and on the concentration of pathogens and bacteriophages or bacteriophage-derived proteins;
- regulatory approval must be obtained;
- production and purification should be economic – large-scale production is possible with endolysins; and,
- safety – there are no known undesirable effects related to bacteriophage applications, although research is still ongoing.

Acknowledgements

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Further reading

Endersen, L., O'Mahony, J., Hill, C., Ross, R.P., McAuliffe, O. and Coffey, A. (2014). 'Phage therapy in the food industry.' *Annual Review of Food Science and Technology*, 5: 327-349. Epub January 9, 2014.

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