Environmental Protection Authority
Te Mana Rauhī Taiao

PROPOSAL FORM

Proposal form to prescribe certain organisms as not new organisms

for the purposes of the Hazardous Substances and New Organisms (HSNO) Act

Send to the Environmental Protection Authority preferably by email neworganisms@epa.govt.nz or alternatively by post to: Private Bag 63002, Wellington 6140

Name of person or organisation making the proposal

EPA

Postal Address

215 Lambton Quay

Wellington 6011

Date

28 September 2016

Important

If species were not present in New Zealand before 29 July 1998, they are classed as new organisms under the Hazardous Substances and New Organisms (HSNO) Act. As such, they will require HSNO Act approval for propagation or distribution of the organism to occur. Currently, if anyone was to conduct any of these activities without a HSNO Act approval they would be committing an offence under section 109(1) of the Act.

To change its “new organism” status (which means that an organism will not be regulated under the HSNO Act), an organism must be deregulated under section 140(1)(ba) of the HSNO Act, by an Order in Council given by the Governor General prescribing organisms that are not new organisms for the purposes of this Act.

As part of this process, the following form is to be filled in by the person or organisation making a proposal to prescribe certain new organisms as not new organisms.

The information provided in this form will be used in the decision-making process (which is likely to include a public consultation component). Any confidential information must be clearly labelled and included as a separate Appendix.

1. Details of the new organism(s) proposed to be prescribed as not new organism(s)

Please complete this section for each organism proposed to be prescribed as a not new organism.

Name of organism

Listeria phage P100 (P100).

P100 bacteriophage belongs to the order Caudovirales, the family Myoviridae (phages with contractile tails), the subfamily Spounavirinae and the genus Twortlikevirus.

P100 is the active component in the product LISTEX™ P100, which is used as a preventative measure against *Listeria monocytogenes* contamination in food preparation.

Why do you want to prescribe this organism as "not new"?

Including:

- Is there any information on the economic or environmental impacts of the organism?
- What is the benefit of making this organism "not new"?
- Can these benefits be quantified?
- Can these benefits be achieved by alternative means?

The organism *Listeria* phage P100 is a bacteriophage that only infects *Listeria* species and is currently permitted for use in New Zealand under a release with controls approval (APP202089) despite being present in the New Zealand environment before 29 July 1998. The original application was incorrectly made for a release with controls, when in fact it should not have been the subject of New Organisms approval in the first instance (as further described on page 3 of this document under the heading "Has the organism formed a self-sustaining population in New Zealand?"). This has limited the ability to use this organism for public good in the fight against Listeria.

Listeria monocytogenes is a problem in the food industry. The pathogen thrives in food processing conditions and is particularly dangerous since it continues to grow at refrigeration temperatures. As a result it contaminates many convenience foods during their shelf life. *Listeria monocytogenes* has been associated with a number of food poison outbreaks from a broad variety of foods that include milk, cheese and other dairy products, meat and meat products, poultry, fish and seafood, vegetables and fruits.

Infection usually occurs via ingestion of contaminated products. It was estimated that approximately 2,000 hospitalizations and 500 deaths occur annually in the United States alone, as a result of the consumption of foods contaminated with *L. monocytogenes* (Mead, 1999). In New Zealand, around 25 cases occur annually. Of those about 20% are associated with pregnancy or newborn babies. Between 5 and 7% of people affected by the disease die on average each year. The number of cases recorded here is similar to that found in countries with similar public health status.

Food Standards Australia New Zealand (FSANZ) has tested the product and found the use of LISTEX™ P100 to eradicate or decrease *L. monocytogenes* on specific solid ready-to-eat foods to be "technologically justified and demonstrated to be effective in achieving its stated purpose' (referencing the FSANZ approval, and papers that demonstrated the efficacy, e.g. Guenther, 2009).

Many countries have adopted a zero-tolerance policy to *Listeria* bacteria in food, which has led many products being recalled from supermarket shelves with subsequent economic losses. The persistence of *L. monocytogenes* in food products proves that it is difficult to eradicate this pathogen using currently available methods. In addition to all presently available precautionary measures, the application of bacteriophages to eradicate or decrease *L. monocytogenes* will be an attractive approach.
Good Manufacturing Practices (GMP), Hazard Analysis, Critical Control Points (HACCP) and other measures prevent *Listeria* or minimise the optimum conditions for its growth. The application of bacteriophage P100 would be an effective additional measure to control *Listeria* in food.

Describe the biology of the organism

Including:

- What are the biological characteristics of the organism?
- Where is it found overseas?
- Does it cause a disease?
- Does it have potentially beneficial characteristics?
- What adverse effects could making this organism "not new" have on people or the environment, if any? Can these be quantified?

A bacteriophage is a virus that only infects bacteria. P100 is a virulent phage, meaning that it has a purely lytic lifecycle which infects and kills its host cells. Phage P100 has a broad host range and infects >95% of Listeria strains (Carlton et al 2005; Klumpp et al 2008), it is known to infect the following Listeria species:
L. monocytogenes, *L. ivanovii*, *L. innocua*, *L. seeligeri* and *L. welshimeri*.

Bacteriophages are the most abundant biological entities on earth and are present wherever bacteria exist. Bacteriophages rely on their hosts for propagation and can only establish self-sustaining populations where their hosts are continuously abundant (e.g.: wastewater is a rich source of bacteria for P100). In the natural environment, phages and bacteria keep each other in balance.

Some phage isolates, classified as P100, have been sequenced and are well studied with regard to human safety. P100 bacteriophage is unable to infect plant, animal or human cells. It only targets species within the genus *Listeria* (except *L. grayi* strains) with the potential to reduce or eliminate the presence of *Listeria monocytogenes* in food products.

FSANZ concluded that there is minimal scope for hosts to develop resistance to phage infection in food processing environments and that there would be no negative impact on humans caused by ingestion of, or contact with, P100.

Has the organism formed a self-sustaining population in New Zealand?

Including:

- Where has population(s) of the organism been found in New Zealand?
- How does this organism spread?

Listeria phage P100 was isolated for the first time in 1997 from wastewater at a dairy plant in southern Germany (Carlton et al 2005). Almost identical P100 phages have been isolated in other European countries, the US and New Zealand (see Appendix 1).

Micreos obtained DNA of phage strain FWLLm1 from the New Zealand Institute of Environmental Science and Research Ltd (ESR). Phage FWLLm1 was isolated by ESR scientists in New Zealand. The DNA was sequenced at the Swiss Federal Institute of Technology. A 139 kb contig could be assembled from the reads.

Analysis of this sequence using NCBI/BLAST showed that the sequence shared 98% similarity to the type strain. The two sequences are perfectly aligned except for some small insertions in the FWLLm11 genome. Without a doubt, the two phages are variants of the same species of phage.

Furthermore, phage P100 infects all species within the genus Listeria excepti *L. grayi* strains. These host species have a global distribution and are found ubiquitously in the environment. Based on the worldwide distribution of P100 and its hosts found in convenience foods, it is reasonable to assume that self-sustaining populations of this bacteriophage have also established in New Zealand.

Is any person attempting to manage, control or eradicate the organism under any Act or is the organism the subject of an enforcement action or action under a civil penalty regime?

Including:

- If the organism has been part of an official incursion response or other MPI (MAF) response or management activity, describe what happened here including why the response was stood down.

None.

Is there reason to believe that this organism was deliberately imported in contravention of an Act of Parliament? If so, please explain.

None.

Any other information you wish to include?

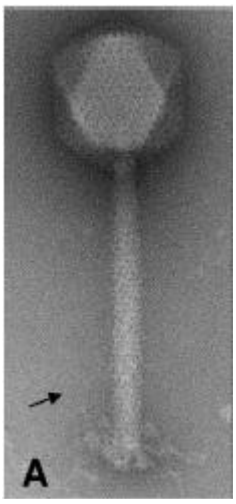
Appendix 1

Bacteriophages are the most abundant self-replicating entities on earth. Estimates for their total numbers range from 10^{30} - 10^{31}. They prey on target bacteria and hence there is significant diversity in different phages.

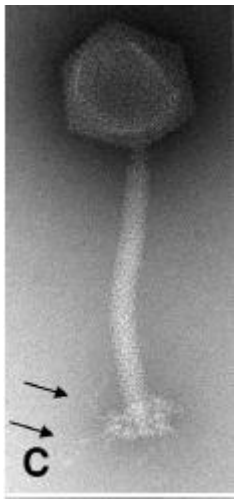
In a natural environment phages and bacteria keep each other in balance. As bacterial numbers rise phage can propagate on the elevated host levels. As a consequence bacterial numbers decline leaving progeny phage without hosts to further increase their numbers. Phage rapidly become inactivated by physical and chemical factors such as UV-light, denaturing and proteolytic compounds or simple adsorption to particles rendering them inactive. These events cycle in a Lotka-Volterra like relationship.

While there are many different types of phages for different bacteria, the number of different phage types that infects a single species is limited. Analysis of 11 isolates of a phage species (φKMV) from different geographical locations (φKMV) revealed only very minor genetic differences with core genome regions being almost completely identical (Ceyssens et al. 2011).

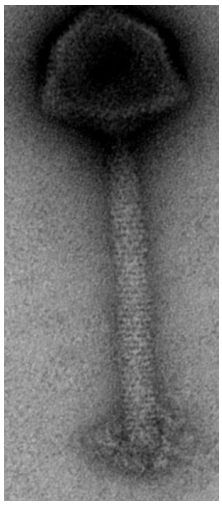
The presence of identical sequences from very different environments has also been investigated leading to the term viriosphere. Certain bacteriophages are found globally (Hambly and Suttle 2005). In one study, identical species were present when they compared phage populations in the Pacific Ocean, Arctic Ocean and Atlantic Ocean (Angly et al. 2006). The authors showed that while diversity of the phage population was large identical species were present. The abundance of a single phage species varied depending on local ecology. This means that the abundance of any one phage species is determined not by its presence in an ecosystem but by the ecosystem itself.



Phage A511



Phage P100



Phage P200
(no sequence data)

Figure heading

Evidence for global distribution of certain phage species such as T4-like E. coli phages and K-like Staphylococcal phages can be found in the literature, as well as numerous other phage species such as phage lambda, T7 and Mu,.

Micreos has 18 different isolates of the phage species. Two of these (A511 and P100) have been sequenced Dorscht et al. 2009). They are 97% identical. This can also be observed in restriction enzyme digests of genomic DNA which are highly similar with small variations. The other 16 isolates all have similar degrees of differences based on similar analysis. They all have similar host ranges (plaque formation on >95% of all strains) although their host ranges are not completely identical. Electron microscopy reveals identical morphology of all these phages (genetic differences don't occur within the structural genes).

There is evidence that this phage species is also found in North America. A publication on q-PCR of a Listeria phage isolated in the US (Lis36) generates the same PCR-product when P100 or A511 DNA is used as a template (For PCR primer sequence info see: Anderson et al . 2011).
While no sequence data is available, phages with identical morphology have been isolated in New Zealand. They are almost certainly members of the SPO1-like Listeria phages.

These Listeria phages were isolated by the Environmental Science and Research (ESR) staff at the Christchurch Science Center (CSC). The complete poster can be viewed at: ?

In an article describing the efficacy of one of these phages to control the growth of Listeria on ready-to-eat poultry products, the authors pointed out the likelihood of this phage belonging to the SPO1-like Listeria phages to be high (?) (Bigot et al. 2011).

There is compelling evidence that phages belonging to the P100 species are found globally and evidence supporting that this is likely to be true for New Zealand as well.

2. References (if applicable)

Carlton, R. M., Noordman, W. H., Biswas, B., De Meester, E. D., & Loessner, M. J. (2005). Bacteriophage P100 for control of Listeria monocytogenes in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. *Regulatory Toxicology and Pharmacology*, *43*(3), 301-312.

Guenther, S., Huwyler, D., Richard, S. and Loessner, M.J. (2009) Virulent Bacteriophage for efficient biocontrol of Listeria monocytogenes in Ready-To-Eat Foods. *Appl Environ Microbiol 75,* 93–100.

Klumpp, J., Dorscht, J., Lurz, R., Bielmann, R., Wieland, M., Zimmer, M., ... & Loessner, M. J. (2008). The terminally redundant, non-permuted genome of Listeria bacteriophage A511: a model for the SPO1-like myoviruses of gram-positive bacteria. *Journal of bacteriology*, *190*(17), 5753-5765.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging infectious diseases*, *5*(5), 607.