Bacteriophage biocontrol: the technology matures

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Use of bacteriophages (phages) as bacterial biocontrol agents was first envisioned by the phage pioneer Felix d'Herelle [1], and an increasing number of peer-reviewed studies point to the potential of phages to control spoilage and pathogenic bacteria in food. Several such phage-based biocontrol products have recently received regulatory approval and some have been commercialised. Obstacles nevertheless remain before widespread implementation can be achieved. These include consumer acceptance of the addition of 'viruses' to food as well as various commercial-production issues. Reviews of basic principles of phage-based biocontrol can be found elsewhere [2-6]. Here we document recent phage-biocontrol regulatory successes as well as production considerations relevant to phage utilisation as a food additive.

Regulatory approval

Recent regulatory successes include, especially, multiple approvals of phage biocontrol products for use in foods. In 2002 the Environmental Protection Agency (EPA) approved the use of a phage-based pesticide for direct application to plants and surrounding soil. Developed by OmniLytics™ [Salt Lake City, UT, USA], it consists of two phages that lyse the plant pathogens *Xanthomonas campestris* subsp. *vesicatoria* and *Pseudomonas syringae* [7], which are responsible for bacterial spot on tomatoes and peppers and bacterial speck on tomatoes, respectively. In 2006, the Food and Drug Administration (FDA) approved an application by Intralytix [Baltimore, MD, USA] to use a preparation containing six individually purified phages as a *Listeria monocytogenes* biocontrol agent on ready-to-eat (RTE) meat and poultry products [8]. In 2007, the United States Department of Agriculture (USDA) issued two no objection letters for the use of phages targeted toward *E. coli* O157:H7 and *Salmonella,* developed by OmniLytics [9]. These products are approved for use as hide sprays on cattle and could be employed, prior to slaughter, to decrease pathogen transfer to meat.

During the summer of 2007, the FDA and USDA announced the approval of LISTEX™ P100, an anti-*Listeria* natural phage product produced by EBI Food Safety [Wageningen, The Netherlands], as GRAS (generally recognised as safe) for all food products [10]. Since GRAS status exempts the additive in question from formal pre-market safety review [11], it represents a major step forward in the commercialisation of phage-based antimicrobials. In addition, von Jagow & Teufer [12] have recently published an article that clarifies the use of LISTEX as a processing aid under European Union law requiring no mandatory labelling on the final product. These developments are exciting, bode well for the continued future development of phage biocontrol strategies, and indicate the increasing acceptance of phages as natural antimicrobials.

Phage production procedure standardisation

Several issues specific to phage-based antimicrobials, and their intended uses, need to be addressed before widespread regulatory approval can proceed. These issues are related to the types of raw materials used in the production of the phages, the development of standardised good manufacturing practices (GMPs), and the potential for pathogen contamination of the finished phage product. Raw materials include bacteria used for phage propagation, enzymes and growth media as well as materials, used in validation work, that come into direct contact with manufacturing equipment [13]. Bacterial strains used in phage production, for example, should be non-pathogenic. Similarly, confirmation of the identity, purity and quality of raw materials is of utmost importance, including confirmation that raw materials do not contain mycoplasmas, viruses or other microbial or chemical adulterants [13]. A major concern, for example, is the possibility that raw materials derived from animals, especially

ruminants, may enable the spread of transmissible spongiform encephalopathies (TSEs) [14]. Therefore, phages should be produced in a manner that either avoids the use of these materials or only uses materials derived from animals reared and killed in areas where TSEs are not indigenous [13].

Viral contamination is also a concern. Sources include raw materials used in the production process, such as peptone in growth media, but also inadvertent contamination by operators. The Committee for Proprietary Medicinal Products in the UK [15] has outlined a series of guidelines for the production of virus-free biologicals which are applicable to food-grade phage production, regardless of the geographical locale. These guidelines include selecting and testing source materials for the absence of viruses; testing the capacity of the production process to remove or inactivate viruses; and testing the product at various stages of production for viral contamination [15]. The situation is more pronounced in phage production, as opposed to other biological products, because phages share many similarities with human and animal viruses, meaning that it is less likely that phage production processes would remove contaminating viruses [13].

GMPs are a set of documented procedures and controls that are utilised in the manufacture of products [16]. GMPs ensure quality during the entire production process with respect to such things as raw materials, record-keeping of what substances the product comes in contact with during manufacturing process, standards for cleanliness and safety, qualifications of manufacturing personnel, in-house testing, production and process controls, and warehousing and distribution. The main purpose of GMPs is to ensure that the finished product has the required potency, safety, and efficacy. While there are no legal requirements for the development of GMPs for food-grade phage production, without the documentary evidence that is provided by GMPs, there can be no conclusive proof that errors have not been made. The need for GMPs takes on further importance because the issues involved in developing test methods for phage based antimicrobials are complex.

Conclusion

The increasing demand for minimally processed and organic foods necessitates the development of natural antimicrobials to control bacterial contamination. Phages, used either wholly or in part, would seem to have their greatest utility in these foods. As production of phage-based antimicrobials increases, standardised and industry accepted measures of phage efficacy and quality will need to be developed. Consumer acceptance is another issue that must be addressed; the recent recognition of phage-based antimicrobials with two innovation awards [17, 18] is a right step in this direction. Regardless of the obstacles to overcome, it seems that d'Herelle's dream of using phages to control pathogenic bacteria may finally gain widespread acceptance.

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Dr. Goodridge received his PhD from the University of Guelph (Guelph, Ontario, Canada) with a major emphasis in food microbiology and food safety in 2002. Following a Post-Doctoral Fellowship at the University of Georgia, he accepted a faculty position at the University of Wyoming, USA. Currently, Dr Goodridge is an Assistant Professor in the Department of Animal Sciences at Colorado State University, USA. Dr Goodridge is currently focused on the development of novel phage-based diagnostics and methods to control the spread of food-borne pathogens, with a specific focus on development of detection methods and prophylaxis for *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp.