USE OF BACTERIOPHAGES AS NOVEL FOOD ADDITIVES

Kathy Walker

FS06 ANR 490/811
Food Regulation in the United States

Michigan State University

October 29, 2006

USE OF BACTERIOPHAGES AS NOVEL FOOD ADDITIVES

I. Introduction

The Federal Food, Drug, and Cosmetic Act defines a food additive as "any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food."[1] Preservatives comprise a major class of food additives. These simple organic and inorganic compounds function as antimicrobials, suppressing the growth of bacteria and fungi. The primary benefits of using preservatives in food are to delay spoilage, thus extending shelflife, and to prevent foodborne illness associated with toxigenic (e.g., *Staphylococcus aureus*) and pathogenic species (e.g., *Listeria monocytogenes*).

With today's emphasis on the availability of fresh foods without chemical preservatives, there is a need for novel preservation strategies. On August 18, 2006, notification for a new food additive appeared in the Federal Register.[2] What made this more than just a "blip on the radar screen" was the fact that this was the first Food and Drug Administration (FDA) approval of a bacteriophage preparation for direct addition to food for human consumption. Unlike the previous generation of antimicrobial preservatives, bacteriophages are viruses - living entities that are the natural predators of bacteria. However, because they are viruses, potential issues exist with their usage that require careful evaluation. In addition, if history is any indication, consumer acceptance of bacteriophage usage may present something of a challenge to the food industry.

[1] 21 U.S.C. § 321 (s).
[2] 71 Fed. Reg. 47729 (2006).

Section II of this paper will provide information concerning the definition and history of bacteriophages. Section III will elaborate on potential and current applications of bacteriophages in the food industry, while Section IV will outline possible issues with their usage as food additives. Conclusions and recommendations will be summarized in Section V.

II. Background Information on Bacteriophages

Bacteriophages were discovered independently in 1915 by Frederick Twort, an English bacteriologist and physician, and in 1917 by Félix d'Herelle, a self-taught French-Canadian bacteriologist. However, it was d'Herelle who first coined the term "bacteriophage" to describe a "microbe" that attacked bacteria and was capable of killing them. d'Herelle was also the first to recognize that this agent of bacterial death was actually a virus.[3]

A. *Definition*

Bacteriophages (or phages) are obligate intracellular parasites that infect bacteria and reproduce by hijacking their host's biosynthetic pathways.[4] Phages are classified as either lytic or lysogenic based upon their replication strategy. A lytic phage infects its bacterial host, replicates its DNA, and produces progeny that are immediately released for further infection, destroying its host in the process. A lysogenic phage, on the other hand, embeds itself into the genome of its bacterial host, establishing a stable relationship with the bacteria that it has infected. This stable relationship is maintained until some

[3] Michael McKinstry and Rotem Edgar, Phages: Their Role in Bacterial Pathogenesis and Biotechnology 430 (**Matthew K. Waldor, David I. Friedman, and Sankar L. Adhya** ed., ASM Press **2005**).
[4] *See* Microbiology and Immunology On-line, [*Bacteriophage*], http://pathmicro.med.sc.edu/mayer/phage.htm (as of October 29, 2006)

stressor, such as DNA damage, disrupts it.[5] Lysogenic phages are capable of transferring genes for toxin production or pathogenicity factors between bacterial populations.[6]

B. *History*

Not long after their discovery, bacteriophages were successfully used to treat certain bacterial diseases, such as dysentery.[7] Large pharmaceutical companies in the United States began to recognize the potential of bacteriophage treatment. In the 1920s and 1930s, Eli Lilly and E.R. Squibb & Sons each marketed a bacteriophage preparation for the treatment of *Staphylococcus* infections.[8] However, a number of factors led to the decline of bacteriophage usage for medical applications in the United States. The most serious challenge came when antibiotics were discovered. Other contributing factors included poorly designed clinical trials, clinical failures (often due to the lack of recognition that phage are specific for a given host bacteria), and overzealous claims for efficacy.[9] Similar issues could arise that negatively impact the use of bacteriophage as antimicrobials in food applications.

III. Proposed Uses of Bacteriophage by the Food Industry

Bacteriophages have traditionally had a negative impact on the food industry. As an example, phage infection of dairy starter cultures disrupts normal fermentation cycles,

[5] John W. Little, Phages: Their Role in Bacterial Pathogenesis and Biotechnology 37 (**Matthew K. Waldor, David I. Friedman, and Sankar L. Adhya** ed., ASM Press **2005**).
[6] Patrick L. Wagner and Matthew K. Waldor, ***Bacteriophage Control of Bacterial Virulence***, 70 Infection and Immunity 3985, **3985** (2002).
[7] Michael McKinstry and Rotem Edgar, Phages: Their Role in Bacterial Pathogenesis and Biotechnology 431 (**Matthew K. Waldor, David I. Friedman, and Sankar L. Adhya** ed., ASM Press **2005**).
[8] *Id*.
[9] *Id*. at 432.

inhibiting lactic acid production. This can result in vats of milk that are slow to set, disrupting manufacturing schedules and resulting in substandard finished product.[10] However, new applications may prove invaluable in controlling foodborne pathogens.

A. *Campylobacter Bacteriophages*

Campylobacter jejuni and *Campylobacter coli* are major causes of acute bacterial enteritis in the developed world.[11] Domestic poultry have been identified as the primary reservoir for these organisms and their presence in undercooked poultry is implicated as the natural source of human infection.[12]

Bacteriophage therapy has been proposed as a novel and effective method of reducing the presence of *Campylobacter* species in the human food chain.[13] Suitable bacteriophages have been identified in the ceca of broiler chickens and their presence negatively correlated with that of their host bacteria.[14] A recent screening of 50 such bacteriophage isolates against strains of *Campylobacter* recovered from human infections yielded two lytic phages that reduced *Campylobacter* in poultry. This suggests that a combination of these two phages could be used to diminish the prevalence of *Campylobacter* in food.

---

[10] Mark E. Johnson and James L. Steele, Food Microbiology: Fundamentals and Frontiers 589 (**Michael P. Doyle, Larry R. Beuchat, and Thomas J. Montville** ed., ASM Press (**1997**).

[11] C.R. Friedman, J. Neimann, H.C. Wegener, and R.V. Taux**,** Epidemiology of *Campylobacter jejuni* Infections in the United States and Other Industrialized Nations 121 (**I. Nachamkin and M.J. Blaser** ed., ASM Press (2000).

[12] D.G. Newell and C. Fearnley, ***Sources of Campylobacter Colonization in Broiler Chickens***, 69 Appl. Environ. Microbiol., 4343, **4343** (2003).

[13] C. Loc Carrillo, R.J. Atterbury, A. El-Shibiny, P.L. Connerton, E. Dillon, A. Scott, and I.F. Connerton, ***Bacteriophage Therapy to Reduce Campylobacter jejuni Colonization of Broiler Chickens***, 71 Appl. Environ. Microbiol., 6554, **6555** (2005).

[14] R.J. Atterbury, E. Dillon, C. Swift, P.L. Connerton, J.A. Frost, C.E.R. Doss, C.E.D. Rees, and I.F. Connerton, ***Correlation of Campylobacter Bacteriophage with Reduced Presence of Hosts in Broiler Chicken Ceca***, 71 Appl. Environ. Microbiol., 4885, **4886** (2005).

B. *Escherichia coli Bacteriophages*

By their nature, ready-to-eat (RTE) foods pose additional safety risks as they receive no further processing prior to consumption (e.g., washing, cooking, etc.). Any bacteria present in the RTE material will remain viable into the gastrointestinal tract (the typical site of infection). This added risk was demonstrated recently by the severe illness and death resulting from the consumption of packaged spinach contaminated by *E. coli* O157:H7.[15] *E. coli* O157:H7 is a highly virulent foodborne pathogen naturally found in the gastrointestinal tract of ruminants and other mammals (e.g., cattle, sheep, pigs, etc.). As a result, these bacteria are difficult to eradicate and frequently enter the human food chain via contact with contaminated fecal material of animals. Bacteriophage therapy may present a likely strategy for reducing the presence of these dangerous microbes. To this end, Raya and colleagues have recently demonstrated that a single oral dose of bacteriophage specific to *E. coli* O157:H7 given to sheep resulted in a two-log reduction (99 percent) of the pathogen.[16]

C. *Listeria monocytogenes Bacteriophages*

Listeria monocytogenes has emerged as a significant foodborne pathogen, one that is especially virulent in individuals with impaired immunity, pregnant women, newborns, and the elderly. The ubiquitous presence of this bacterium in food materials and factory environments, coupled with its ability to grow at low temperatures, render it uniquely capable of infecting refrigerated RTE foods.

---

[15] *See* FDA News, *FDA Statement on Foodborne E. coli O157:H7 Outbreak in Spinach*, http://www.fda.gov/bbs/topics/NEWS/2006/NEW01486.html (as of October 6, 2006).

[16] Raul R. Raya, Peter Varey, Rebecca A. Oot, Michael R. Dyen, Todd R. Callaway, Tom S. Edrington, Elizabeth M. Kutter, and Andrew D. Brabban, **Isolation and Characterization of a New T-Even Bacteriophage, CEV1, and Determination of Its Potential to Reduce *Escherichia coli* O157:H7 Levels in Sheep**, 72 Appl. Environ. Microbiol., 6405, **6405** (2006).

The FDA recently amended the food additive regulations to permit the safe use of a bacteriophage preparation as an anti-listerial agent in RTE meat and poultry products. The preparation as described consists of a combination of six individual lytic phages, selected for activity against different *L. monocytogenes* strains. This cocktail is to be sprayed directly on the surface of the RTE food prior to packaging at a level of approximately 1 milliliter (mL) per 500 square centimeters of surface area. The bacteriophages will remain dormant unless their specific target, *L. monocytogenes*, is encountered, triggering a full infection and destruction cycle. Because they are lytic phages, no viable *Listeria* will remain, and there will be no transfer of problematic genes associated with lysogenic phages. Indeed, the regulation specifies that the phage preparation must test negative for *L. monocytogenes* and that listeriolysin O, a toxin produced by *L. monocytogenes*, be undetectable (at a limit of detection equal to 5 hemolytic units per mL). In evaluating the safety of this new food additive, the FDA considered published reports from animal studies submitted by the petitioner and results from the use of phage therapies against human bacterial infections.[17] Clearly with this amendment, the FDA has demonstrated their belief that bacteriophages are safe for use in the human food chain as antimicrobial food additives.

[17] 71 Fed. Reg. 47729 (2006).

IV. Precautionary Information

Although the FDA has now approved the use of a bacteriophage preparation as a food additive, several factors related to the general use of phage deserve consideration. When viewed together, these factors suggest that great care should be taken in determining which bacteriophages are selected for use, how they are to be manufactured, and the manner in which they are used.

A. *Industrial Preparation*

The specific nature of bacteriophage infection requires the presence of the pathogenic host bacteria if industrial quantities of the phage are to be produced. The initial presence of such a pathogen necessitates the development of adequate separation and/or sterilization technologies to ensure the complete absence of the pathogen in the final bacteriophage preparation.

B. *Selection of Bacteriophage*

Only lytic phages should be used as food additives. Lysogenic bacteriophages have the potential to carry genes to their host bacteria that are associated with toxin production and pathogenicity. One example of this would be the transfer of shiga toxin from enterohemorrhagic *E. coli* to non-pathogenic *E. coli* by bacteriophages.[18]

C. *Immunity*

Bacteria are known to develop resistance to bacteriophage over time, reducing the effectiveness of their antimicrobial properties. This requires constant vigilance and

[18] Jessica S. Tyler, Jonathan Livny, and David I. Friedman, Phages: Their Role in Bacterial Pathogenesis and Biotechnology 131 (**Matthew K. Waldor, David I. Friedman, and Sankar L. Adhya** ed., ASM Press **2005**).

substitution of new phages for “old” phages to which the bacteria have become immune. This cycle is well known in the dairy foods industry.

V. Conclusions

Bacteriophages are bacterial viruses that are ubiquitous in the environment. For almost every bacterial species, there exists at least one bacteriophage that can specifically infect and ultimately destroy that particular bacterial group. They do not harm human or animal cells. Given these characteristics, bacteriophages have proven to be valuable allies in mankind’s fight against disease and show great promise as alternatives to traditional antimicrobials in the control of foodborne pathogens. However, due to the very nature of bacteriophages, care should be exercised if this new technology is to be effectively and safely employed.