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Review

Bacteriophage Control of Foodborne Bacteria†

G. GORDON GREER*

Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, Lacombe, Alberta, Canada T4L 1W1

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ABSTRACT

Bacteriophages are measurable components of the natural microflora in the food production continuum from the farm to the retail outlet. Phages are remarkably stable in these environments and are readily recovered from soil, sewage, water, farm and processing plant effluents, feces, and retail foods. Purified high-titer phage lysates have been used for the species-specific control of bacteria during the pre- and postharvest phases of food production and storage. For example, the inhibition of the phytopathogens *Erwinia amylovara* and *Xanthomonas campestris* has reduced the incidence of diseases such as fire blight in apples and bacterial spot of tomato and peaches. Research on preslaughter treatment of food animals has demonstrated phage control of salmonellosis in chickens, enteropathogenic *Escherichia coli* infections in calves, piglets, and lambs, and *E. coli* O157:H7 shedding by beef cattle. Phages have also been applied to control the growth of pathogens such as *Listeria monocytogenes*, *Salmonella*, and *Campylobacter jejuni* in a variety of refrigerated foods such as fruit, dairy products, poultry, and red meats. Phage control of spoilage bacteria (e.g., *Pseudomonas* spp. and *Brochothrix thermosphacta*) in raw chilled meats can result in a significant extension of storage life. Phage biocontrol strategies for food preservation have the advantages of being self-perpetuating, highly discriminatory, natural, and cost-effective. Some of the drawbacks of biopreservation with phages are a limited host range, the requirement for threshold numbers of the bacterial targets, phage-resistant mutants, and the potential for the transduction of undesirable characteristics from one bacterial strain to another. Most research to date has involved experimentally infected plants and animals or artificially inoculated foods. This technology must be transferred to the field and to commercial environments to assess the possibility of controlling natural contaminants under more realistic production and processing conditions.

Bacteriophages (phages) are obligate parasites, and virulent phages lyse living bacterial hosts. This lytic potential has been exploited in attempts to devise a more natural antimicrobial approach to control bacteria at the various stages of food production. To my knowledge, the only published summary of the nontherapeutic uses of phages is that of Goodridge and Abedon *(28)*, who compiled examples of the use of phages to control bacterial pathogens at the farm and in foods. Those authors proposed the terms *bacteriophage biocontrol* and *bacteriophage bioprocessing* to differentiate the applications of phages on the farm from those during food processing. The prevalence and ecological significance of phages has also been reviewed *(43)*, but that review was focused primarily on the consequences of natural phage contamination and only briefly on the role of exogenously introduced phages in controlling foodborne bacteria.

Here, I have built upon and combined aspects of those previous publications. Thus, the scope is broader and provides a more comprehensive summary and critical evaluation of the results of research where phages have been utilized to control bacteria during the production of foods of plant and animal origin (preharvest) and in perishable foods during refrigerated storage (postharvest). In contrast to the previous review *(28)*, I have extended beyond bacterial pathogens to include detailed information on phage control of foodborne spoilage bacteria, including the effects upon food storage quality and preservation. A more ecological perspective of phage biocontrol is provided, to consider factors influencing phage-bacteria interaction in food production systems and the advantages and drawbacks of such an approach. Although purified phage endolysins (murein hydrolysases) also have the potential to control bacteria *(20)*, there are no known published data on the antibacterial efficacy of phage lysins in foods and these lysins are not considered in this review.

PHAGES AS ANTIMICROBIAL AGENTS

Some researchers have proposed an ecological approach to food preservation *(21, 29)*. This approach stresses the importance of a better understanding of microbial associations in foods when developing control strategies. Some components of the indigenous flora in foods are beneficial from a competitive perspective, and the indiscriminate elimination of the native microflora may be deleterious *(41)*. Thus, broad-spectrum antimicrobials are not necessarily desirable, and a more targeted preservative approach is warranted. One such approach with proven efficacy is preferential manipulation of the microbial ecology of foods using biopreservation with bacteriocinogenic lactic acid

* Author for correspondence. Tel: 403-782-8135; Fax: 403-782-6120; E-mail: greerg@agr.gc.ca.

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bacteria *(72).* Another alternative for selectively modifying the bacterial flora is the use of phages.

Despite the dispute over the discovery of phages, it is generally accepted that they were first described between 1915 and 1917 by both F. W. Twort and F. d'Herelle *(22, 75).* However, d'Herelle was likely the first to consider the therapeutic potential of phages following the discovery in 1917 of lytic phages attacking the dysentery bacillus (*Shigella*) in the feces of convalescing patients *(75).* The recovery from dysentery coincided with the appearance of phages, and researchers speculated that this parasitism was not restricted to dysentery but could be a general phenomenon in other human diseases. This association probably was one of the first published descriptions of biological control.

Subsequently, there have been a multitude of descriptions of phage prophylaxis for the treatment of a variety of diseases of humans and animals, and marketable technologies have been patented. Some researchers have suggested that phages are preferable alternatives to antibiotics *(9, 42, 73).* Although there is considerable literature on the matter, there is a lack of substantive evidence, and the need for clinical trials is critical. The extent of the published literature has been the subject of detailed reviews *(2, 9, 53, 76).*

ECOLOGICAL IMPACT OF PHAGES IN FOODS

Phage infections during the industrial fermentation of dairy products provided some of the first documented evidence for the existence of phages in food production environments *(65).* However, the role of phages in starter culture failure has been intensively studied and reviewed *(65, 85)* and is beyond the scope of the current review. The relevance of phages to the microbial ecology of nonfermented perishable foods is a more recent topic and is more germane to a discussion of the beneficial role of phages.

Perishable refrigerated foods such as red meats, poultry, and produce support complex microbial ecosystems and can harbor relatively large populations of bacteria (2 to 6 log CFU/g). It is reasonable to assume that bacteriophages also exist in association with their bacterial hosts in those food environments. An extensive survey of refrigerated food products was undertaken by Whitman and Marshall *(81),* who recorded 38 phage-host systems in about 50% of retail foods examined, including ground beef, pork sausage, chickens, and raw skim milk. Most of those phages were specific for pseudomonads, and phage numbers were as high as approximately 6 log PFU/g (chicken skin). These researchers concluded that phages were only detectable in foods with $\geq$5 log psychrophilic bacteria per ml or g. Phage-host interactions in foods have been summarized in an authoritative review *(43).*

The ecological significance of phages in a number of environments, including foods, has been the subject of debate and was reviewed in a comprehensive text edited by Goyal et al. *(30).* Native phages may influence the diversity of microbial communities by exerting species-specific control of indigenous bacteria *(11).* In foods *(43)* as in other natural ecosystems *(83),* the ability of phages to manipulate bacterial populations is governed by a complex of interactive factors in those environments. The requirement for a relatively high host cell threshold (4 to 6 log CFU/ml) may limit the impact of phages on bacteria in natural environments *(83).* However, Connerton et al. *(17)* proposed that environmental phage populations in broiler chickens may influence the strains of campylobacters transmitted to chicken meat.

SOURCES OF PHAGES

The successful recovery of phages from foods requires that foods contain relatively large bacterial populations (i.e., 5 log CFU/g). Thus, isolation of the phages specific for the more abundant foodborne spoilage bacteria and indicator organisms is more likely than isolation of phages specific to the less prevalent pathogens. Although there is evidence for the presence of pathogen phages in food products *(3),* those reports are relatively limited compared with the literature concerning phages of coliforms and psychrotrophic spoilage bacteria *(43).* Thus, phages utilized for pathogen control in foods and food production systems usually originate from environmental samples and other nonfood sources such as municipal waste water, feces, sewage, soil, farms, and processing facility effluents *(17, 23, 57, 58).* Those phages employed to control food spoilage bacteria are generally derived from foods and food-processing environments *(31, 32, 43).* Phages specific for spoilage pseudomonads have been recovered from the injection brines used for the production of moisture-enhanced pork and from the effluent stored in the settling tanks at pork-processing facilities *(35).* Lysogenic phages also can be induced by chemical inducers such as mitomycin C *(67).* For example, phages to *Brochothrix thermosphacta (1)* and *Listeria monocytogenes (63)* have been recovered from lysogenic cultures.

PREHARVEST CONTROL OF BACTERIA BY PHAGES

During the preharvest production phases of foods of both plant and animal origin, phage biocontrol strategies have been directed exclusively toward the control of plant and animal pathogens, including those posing a risk to human health. At these initial stages in the food production continuum, phages may be exploited to improve the yield and quality of foods and, in the case of foods of animal origin, to control the dissemination of human pathogens in the environment. Table 1 provides examples of how phages have been utilized to control pathogenic bacteria during the production of foods of fungal, plant, and animal origin. A list of phage biocontrols of plant pathogens has also been compiled *(28).*

Foods of plant origin. Although phages for a number of phytopathogenic pseudomonads, xanthomonads, and erwiniae have been isolated *(16, 25, 78),* there has been only limited research to determine their effects upon the progression of plant disease, fruit yield, and fruit storage. The exceptions include trials in which phages were used to control fire blight on apple trees *(66),* bacterial spot on tomatoes *(8),* and bacterial spot disease of peaches *(62).* Despite

TABLE 1. *Use of bacteriophages to control bacterial pathogens preharvest*

Food production system	Disease/symptom	Bacteriophage host strain	Reference(s)
Cultivated mushrooms	Bacterial blotch	*Pseudomonas tolaasii*	*56*
Tomatoes	Bacterial spot	*Xanthomonas campestris* pv. *vesicatoria*	*8*
Apples	Fire blight	*Erwinia amylovara*	*66*
Stone fruits	*Prunus* bacterial spot	*X. campestris* pv. *pruni*	*62*
Sprouts	Seed contamination	*Salmonella* Enteritidis	*58*
Fish	Red fin disease	*Aeromonas hydrophila*	*84*
	Hemorrhagic ascites	*Pseudomonas plecoglossicida*	*59*
Chickens	Cecal *Salmonella*	*Salmonella* Enteritidis	*68*
	Lethal infection	*Salmonella* Typhimurium	*10*
	Respiratory infection	*Escherichia coli*	*40*
	Growth depression	*Streptococcus faecium*	*39*
Beef cattle	Bacterial shedding	*E. coli* O157:H7	*6*
Calves, piglets, and lambs	Diarrhea, lethal infection	Enteropathogenic *E. coli*	*70, 71*
Sheep	Bacteria in rumen, feces, colon	*E. coli* O157:H7	*13*
Dairy cattle	Mastitis	*Staphylococcus aureus*	*48*
Pigs	Tonsil and cecal *Salmonella*	*Salmonella* Typhimurium	*46*

data indicating phage-specific control of those diseases, there were no data to demonstrate that fruit yield or quality from affected plants was improved by the phages. However, there is evidence that phages can control bacterial blotch (*Pseudomonas tolaasii*) in cultivated mushrooms (*Agaricus bisporus*) with a concomitant 70% reduction in crop loss *(56).*

During the course of studies of phage interaction with *Xanthomonas campestris* pv. *pruni* on peach leaves, phage-resistant bacterial strains were recovered *(62).* Of critical importance, pruniphage resistance was often associated with reduced virulence. Thus, phages of phytopathogenic bacteria may be of benefit in the first instance by the direct lysis of susceptible host bacteria and then by selecting for a surviving population of bacteria with reduced virulence.

Laboratory trials have been conducted to determine the ability of phages to control *Salmonella* in experimentally contaminated broccoli and mustard seeds *(58).* Despite a marginal 1.5-log reduction in bacterial numbers, phage biocontrol may show promise for improving safety during the hydroponic production of sprouts. The authors observed that the limited host specificity of single phages necessitates the use of phage mixtures to ensure broad control of pathogens on infected seeds.

The prospects for the biocontrol of phytopathogenic bacteria have been reviewed *(15, 79).* Vidaver *(79)* provided numerous reasons why phages cannot be recommended as control agents. Some of the more important considerations include phage-resistant bacterial mutants, transduction of undesirable characteristics among bacteria, and environmental conditions. However, Campbell *(15)* was more optimistic in his review of biological control agents in general and stressed the importance of more resources for the in vivo screening, formulation, and production of biocontrol agents. A gap in our current understanding of phage-bacteria interactions is the lack of data demonstrating effects upon yield and quality of horticultural crops in field trials.

Foods of animal origin. Phages have been evaluated under experimental conditions to control infections in a number of diverse species such as fish, chickens, cattle, pigs, and lambs and to control pathogen shedding by asymptomatic carriers (Table 1). Many of these diseases compromise the yield and quality of foods derived from those species, and shedding can have serious environmental consequences. Here, I consider only those publications in which phage therapy may have an impact upon food safety.

In the early 1980s, Smith and Huggins *(69)* used mice as an experimental model to examine phage treatment of enteric *Escherichia coli* infections, and those studies were extended to show that phages could control enteropathogenic *E. coli* infections in calves, piglets, and lambs after oral administration *(70).* The spraying of litter in calf rooms with phage suspensions also could prevent *E. coli* diarrhea *(71).* A critical finding was that phage-resistant *E. coli* recovered from phage-treated experimental animals had reduced virulence *(69, 70).*

Phages of enterohemorrhagic *E. coli* O157:H7 have been characterized, and their antibacterial activities have been determined in vitro in broths *(45, 57, 64).* Some evidence has indicated that phages can control *E. coli* O157:H7 in ruminal fluid *(5)* and that the oral administration of phage mixtures can reduce the duration of *E. coli* O157:H7 shedding by calves *(6).* An important observation during the former study was that phages had no adverse effects on natural rumen fermentation.

Results from a study in an artificial rumen system and in inoculated sheep were not as promising *(7).* Although phages could eliminate *E. coli* O157:H7 in vitro in rumen fluid, they had no effect on fecal shedding of this organism by experimentally infected lambs. These results were recently confirmed by Callaway *(13),* who reported that phages could not significantly reduce the numbers of *E. coli* O157:H7 in the rumen, cecum, and colon of experimentally challenged sheep. As stressed by Bach et al. *(7),* researchers were not successful in using phages to control *E. coli* O157:H7 shedding by lambs possibly because of the time of application, nonspecific adsorption or inactivation of bacteriophages in situ, or the use of a single phage. Phage efficacy

TABLE 2. *Use of bacteriophages to control bacteria in foods*

Food	Bacteriophage host strain	Reference(s)
Melon and apple slices	*Listeria monocytogenes*	*50, 51*
	Salmonella Enteritidis	*49*
Milk	*Staphylococcus aureus*	*26*
	Pseudomonas fragi	*24*
Cheese	*Salmonella* Enteritidis	*54*
Chicken skin	*Campylobacter jejuni*	*4, 27*
	Salmonella Enteritidis	*27*
Retail chicken	*Salmonella* Typhimurium DT104	*44*
Chicken frankfurters	*Salmonella* Typhimurium DT104	*80*
Beef steaks	*Pseudomonas* spp. (spoilage control)	*33*
	Escherichia coli O157:H7	*57*
Vacuum-packed beef	*L. monocytogenes*	*23*
Pork fat	*Brochothrix thermosphacta* (spoilage control)	*37*

is enhanced when mixtures of phages with complementary activities are administered. Thus, successful inhibition of pathogenic *E. coli* in calves required a pool of six or seven phages *(71).* Fecal shedding of *E. coli* O157:H7 by ruminants is intermittent, unpredictable, and of short duration, which makes preslaughter control measures difficult to assess.

Phage control of diseases of chickens has been reported (Table 1), and this research has been reviewed *(42).* Control of *Salmonella* in chickens would have a marked impact on foodborne salmonellosis. Berchieri and coworkers *(10)* reported that some *Salmonella* phages could become established in the ceca of chickens, thus controlling bacterial growth in the gut and reducing mortality. A decade later, Sklar and Joerger *(68)* conducted similar trials in chicks experimentally infected with *Salmonella* Enteritidis. These researchers found that phage mixtures produced inconsistent reductions in the numbers of cecal *Salmonella* Enteritidis and cited numerous environmental, physical, and genetic factors that might limit efficacy. The magnitude of the phage-mediated reduction in salmonellae in live chickens probably would not influence the prevalence of this pathogen on the dressed carcass.

Connerton et al. *(17)* were among the first to examine the ecology of phage-bacteria interactions in broiler chickens naturally contaminated with *Campylobacter jejuni.* These authors speculated that phages associated with broiler chickens could selectively influence the strains of *C. jejuni* entering the food chain. Of critical relevance from a therapeutic perspective is the fact that phage-resistant *C. jejuni* did not dominate and outgrow the phage-sensitive strains.

Some researchers support the view that phages may provide an alternative to conventional antibiotics for disease prevention in animals *(42, 70).* Advantages of a phage antimicrobial strategy would include increased specificity, no toxicity, reduced cost, single dose (self-perpetuating), no concern of phage-resistant pathogens becoming an uncontrollable risk to human health, and reduced virulence of resistant mutants.

Data collected to date on phage prophylaxis for pathogen control in meat animals are inconclusive. Under certain conditions, pathogen proliferation and shedding can be limited by phages, but results are inconsistent across studies and among trials. Although a phage biocontrol strategy for pathogens in animals has potential, the research program is in its infancy and there are a number of challenges to combating intestinal pathogens with phages. Some of the more important issues that need to be resolved include a mechanism of delivery to reduce inactivation in the gastrointestinal tract, time of application, host range of phage mixtures, multiplicity of infection, phage-resistant mutants, and the physiology of the host bacteria in vivo *(7, 42, 68, 70).*

Research to date has largely been conducted with experimentally infected animals, and field trials on naturally infected herds or flocks are needed. Once conditions for an effective phage biocontrol method have been optimized, the remaining critical issue is proof that phages can reduce the persistence of pathogens in the environment and limit the transfer from animals to food during the initial stages of processing.

POSTHARVEST CONTROL OF BACTERIA BY PHAGES

Phages have been successfully used to control spoilage bacteria and human pathogens during the postharvest storage of foods under a variety of environmental conditions. Table 2 provides examples of how phages have been used to extend the storage life and/or improve the safety of fruits, dairy products, chicken, and red meats.

Foods of plant origin. Published data concerning phage biocontrol of bacteria in produce are scarce and essentially limited to the research of Leverentz and colleagues *(49–51),* who examined phage-bacteria interactions on fresh-cut apples and honeydew melons. They also briefly discussed the value of lytic phages in a review of biological control agents in minimally processed fruits and vegetables *(52).* A mixture of four distinct *Salmonella* Enteritidis phages produced a significant reduction in the numbers of salmonellae recovered from artificially inoculated melon slices during storage under refrigeration and abusive temperatures *(49).* None of the surviving bacteria were phage resistant. Using a similar design, those researchers also

found that listeriophage mixtures could control *L. monocytogenes* during the storage of inoculated melon slices at 10°C *(50)* and that phage-nisin combinations were more efficacious than either the nisin or phage treatment alone. A more pronounced reduction of *L. monocytogenes* on melon slices was obtained with a mixture of six phages at a concentration of 8 log PFU/ml that was applied within 1 h of melon processing *(51)*.

Contrary to the results with melons, the numbers of *Salmonella* Enteritidis and *L. monocytogenes* on artificially inoculated apple slices were unaffected by phage treatment. This result was attributed to the increased sensitivity of phages to the more acid environment of the apple slices (pH 4.37) compared with that of the melon slices (pH 5.77). The phages could not persist on apple slices, and phage numbers declined to below detectable levels within 24 h *(49)*.

Leverentz et al. *(50)* conceded that fresh-cut produce would not likely be contaminated with pathogens at the unrealistically high levels used on the experimentally inoculated fruit (i.e., about 3 log CFU per sample) but suggested that the phages could be of value for preventing the growth of foodborne pathogens to levels where they pose a risk (i.e., 3 log CFU or higher). There have been no reports of phages controlling indigenous bacterial populations in produce.

Foods of animal origin. In contrast to the lack of information for foods of plant origin, there are substantially more published data concerning the control of bacteria in foods such as dairy products, poultry, and red meats (Table 2). In addition to safety issues, some of this research has been focused on a role for phages in the control of the bacterial spoilage of foods.

Phages of dairy bacteria have been reviewed in detail *(12, 55)* but the usual focus was their impact on industrial milk fermentation by lactic acid bacteria as opposed their more benevolent qualities as biocontrol agents. Possibly the first evidence of phages for milk spoilage bacteria was published by Whitman and Marshall *(81)*, who recovered *Pseudomonas fragi* phages from raw skim milk. Although phages could reduce *P. fragi* numbers in milk *(24)*, the authors questioned the practical relevance to milk preservation, particularly because of the requirement for unrealistically high numbers of *P. fragi* (5 log CFU/ml). Pseudomonads of milk origin were susceptible to phages isolated from raw beef *(60)*.

Phages specific for pathogens in dairy foods have been described. Gill et al. *(26)* examined factors affecting the interaction of phages with *Staphylococcus aureus* in raw bovine milk. In a more detailed publication, Modi and coworkers *(54)* discussed the results of an investigation of the effects of phages on the survival of *Salmonella* Enteritidis during the manufacture and storage of Cheddar cheese. They concluded that the addition of phages to milk used for cheese production significantly decreased the numbers of *Salmonella* Enteritidis in cheeses made from raw and unpasteurized milk. However, the relatively high initial numbers of *Salmonella* Enteritidis in the artificially inoculated milk (4 log CFU/ml) may not be encountered under usual conditions of commercial cheese production.

In some experimental studies, phages have been used to control salmonellae and *C. jejuni* in fresh and processed chicken (Table 2). Atterbury and coworkers *(3)* isolated and characterized 34 *Campylobacter* phages from retail chicken portions. The ubiquity of phages in commercial poultry supported the conclusion that their use as biocontrol agents would not constitute the introduction of a foreign entity into the product. Under experimental laboratory conditions, homologous phages reduced the numbers of recoverable *C. jejuni* on inoculated chicken skin stored at 4°C *(4)*. Marginal reductions of approximately 1 log CFU were obtained only with high initial numbers of *C. jejuni* (4 to 6 log CFU). Freezing was also bactericidal and could be combined with phages as an additional treatment. Although there was no evidence of phage-resistant bacteria in phage-treated samples, *C. jejuni* was not growing at 4°C, and these authors suggested that this lack of growth may have precluded new mutational events *(4)*.

Goode et al. *(27)* used phages on inoculated chicken skin to produce a 2-log reduction in *C. jejuni* at multiplicities of infection of 100 or 1,000. They also determined that phages could control *Salmonella* Enteritidis on chicken skin inoculated with more commercially relevant numbers of bacteria (i.e., 1 log CFU/cm^2). They speculated that phage resistance would not be an issue with carcass treatments because phages would not likely find their way back to farm environments, where the recycling of phage hosts (fecal shedding) may result in the rapid emergence of phage-resistant pathogens in the intestines of poultry. However, phage treatments in processing facilities could provide conditions for the selection of phage-resistant clones that may occupy niches in processing equipment and continue to be a source of cross-contamination during meat fabrication.

Phages isolated from environmental sources and the Felix 01 phage, which has a broad host range, produced a 2-log reduction in *Salmonella* Typhimurium DT104 inoculated onto chicken legs *(44)* and chicken frankfurters *(80)*.

With notable exceptions *(27)*, most data reporting efficacious phage control of mesophilic pathogens have been derived from laboratory studies using commercially unrealistic numbers of bacteria (i.e., 3 to 6 log CFU). One might also question the practical value of a phage biocontrol strategy for mesophilic pathogens that would not generally proliferate at the usual temperatures at which refrigerated foods are stored (i.e., 4°C). Phage replication requires the metabolic processes associated with host cell growth. During a recent evaluation of the control of *E. coli* O157:H7 in broths, O'Flynn et al. *(57)* reported that phages could eliminate the bacteria at temperatures of 30 or 37°C where the organism was growing but could not lyse the cells in the absence of growth at 12°C. Despite this finding, phages have been found to reduce the numbers of *Salmonella* in cheese *(54)* and on chicken *(27, 44)* in the absence of bacterial growth. The reduction by phages of *Salmonella* Enteritidis on melon slices was independent of storage temperature within the range of 5 to 20°C *(49)*.

An even more extreme example can be found among

studies of *C. jejuni*, which can best be described as thermotrophic (no growth below 31°C and optimum growth at 42°C). In the absence of bacterial growth or phage replication, phages significantly reduced the numbers of *C. jejuni* on experimentally contaminated chicken skin stored at 4°C *(4)*. Although the role of nonspecific lysis is debatable *(4, 27)*, these authors suggested that phages may be able to adsorb to the mesophilic host bacterial cells at nonpermissive growth temperatures and then lyse the cells when the host cell metabolism increases under more permissive growth conditions. Presumably, such conditions may exist when phage-treated enteric pathogens are ingested on chilled foods and then begin to multiply in the intestine.

Although phages in fish and red meats have been recognized for some time, their role as food preservatives has been explored only recently. Research with fish *(18, 19)* led to the discovery of phages in pier water and fish fillets that were active against psychrophilic spoilage pseudomonads and *Shewanella putrefaciens* of marine origin. However, those phages were evaluated only from the perspective of strain differentiation by means of a phage typing scheme, and there has been no further work to examine them as biocontrol agents in fish. In a similar manner, data reported in 1971 *(82)* established the existence of psychrophilic *Pseudomonas* phages in ground beef, but the authors only speculated on the role of these phages in the control of meat spoilage.

After a series of publications appeared between 1982 and 1990, the preservative effects of *Pseudomonas* phages in raw chilled beef were thoroughly examined *(31, 33, 34, 36)*. Research on aqueous extracts of beef muscle and on inoculated refrigerated beef steaks revealed that in the presence of a sensitive host pseudomonad, homologous phages could replicate, limit bacterial growth, and significantly extend the retail shelf life of phage-treated beef by limiting the extent of bacteria-related lean tissue discoloration *(31, 33)*. The interaction of phages with bacteria at meat surfaces was unaffected by temperature (1 to 10°C) but was significantly influenced by the initial numbers of inoculated phages and bacteria. A maximum increase in the retail shelf life of beef required initial bacterial densities in excess of 3 log CFU/cm^2 and phage concentrations exceeding 7 log PFU/cm^2 *(34)*.

In an effort to extend the storage life of naturally spoiling beef, a pool of seven phages with complementary activity was assembled. Despite a modest reduction in the indigenous population of pseudomonads, there was no impact upon the retail shelf life of rib-eye steaks. The authors concluded that the efficacy of the phage pool was limited by a narrow range of specificity and the majority of the natural contaminants resisted phage attack, continued to proliferate, and spoiled the meat *(36)*.

The isolation *(32)*, characterization *(1)*, and activity *(37)* of *B. thermosphacta* phages has been described. Under experimental conditions in the laboratory, these phages limited the growth of *B. thermosphacta* on pork adipose tissue and prevented the development of objectionable off-odors. Phages of *Leuconostoc gelidum* from vacuum-packed pork and phages of a psychrotrophic *Serratia liquefaciens* spoiling processed meats have been identified in preliminary studies, but their influence on the progression of spoilage in vivo has not been determined *(35)*. In these studies, all phages were isolated from spoiled meat and had very limited host range. For both pseudomonads and *B. thermosphacta*, a large proportion of the surviving bacterial population on phage-treated meat were phage-resistant mutants.

TABLE 3. *Considerations for developing a bacteriophage approach to the control of foodborne bacteria*

Advantages
1. Self-perpetuating
2. Selective modification of bacterial flora (specificity)
3. Stable in foods and able to survive processing
4. Natural
5. Ubiquitous and readily isolated
6. Cost-effective
7. Ease of preparation and application
8. Nontoxic to eukaryotic cells
9. No effect on food quality
Disadvantages
1. Limited host range
2. Phage-resistant bacterial mutants
3. Requires large numbers of target bacteria
4. Barriers in food environments
5. Transduction of undesirable characteristics
6. Lysogenic conversion (temperate phages)
7. Antigenicity (immune response, allergenicity)
8. Consumer perception of adding viruses to foods

Most research on phage biocontrol of meatborne bacteria in raw red meats has concerned the control of psychrotrophic spoilage bacteria rather than pathogens. An exception is the study published by Dykes and Moorhead *(23)*, who examined the combined antibacterial effect of nisin and listeriophage. Although phages alone had no effect on the growth of *L. monocytogenes* in broth, there was an enhanced effect when phages and nisin were combined. Unfortunately, no such synergism was demonstrable in vacuum-packed beef. In contrast, a cocktail of three phages reduced *E. coli* O157:H7 from initial numbers of about 3 log CFU to undetectable levels on artificially inoculated beef steaks *(57)*. The researchers speculated that phage cocktails could reduce *E. coli* O157:H7 on beef hides and carcasses. These findings on the variable efficacy in meat and the findings of others continue to support the need for detailed research of factors influencing the interaction of antibacterial agents with target species in complex food systems.

CONSIDERATIONS IN A PHAGE BIOCONTROL STRATEGY

There are many issues that must be considered prior to the development and application of a successful phage biocontrol strategy for foods (Table 3).

Advantages. The advantages of phages compared with more traditional antimicrobial systems for foods have been reviewed as both preharvest therapy *(9, 42)* and postharvest intervention *(49, 52)*. Phage biocontrol has the unique ad-

vantages of being natural, self-perpetuating, and highly specific. Phages are an integral component of the indigenous microflora residing in and on most foods in the retail marketplace, and in the presence of sufficient numbers of a susceptible host they will replicate and continue to reinfect growing bacteria. Because of the specificity of the phage adsorption, infection, and replication processes, they can be used for strain-specific control of a target species without affecting other perhaps desirable constituents of the bacterial flora. Phages are readily isolated from foods and from food production and processing environments, and highly concentrated suspensions can be simply and inexpensively prepared. Another valuable quality is the remarkable stability of phages in foods *(43).*

Campylobacter bacteriophages can survive commercial poultry processing procedures *(3),* a necessary characteristic if phages are to retain their ability to control bacteria during the postprocessing storage of foods. For phages isolated from ground beef, Whitman and Marshall *(82)* examined the responses to a number of environmental stresses, including heat, low pH, osmotic shock, and freezing. They found that some phages were resistant to heating to 60°C, a pH of 4.0, 4 M NaCl, and frozen storage in skim milk and ground beef extract.

The titer of a cocktail of seven *Pseudomonas* phages remained unchanged over 1 year at 4°C in tryptic soy broth (9 log PFU/ml) and for 2 weeks on the surface of rib steaks (6 log PFU/cm^2) stored in air at 8°C *(36).* Phage titers also remained unchanged (7 log PFU/ml) on the surface of vacuum-packaged lean beef tissue stored for 6 months at 2°C *(35).* Thus, phages can remain viable for extended periods of time in refrigerated foods and can then initiate the infectious cycle when susceptible host bacteria grow to sufficient numbers.

The limited data on quality *(33)* would suggest that phages do not impart any undesirable sensory changes in foods, and there is no evidence of toxicity to eukaryotic cells *(9).*

Although the spoilage bacterial hosts contaminating refrigerated foods are psychrotrophic, phages can lyse these hosts at temperatures as low as 1°C *(31, 34).* This is an attribute of considerable importance if a phage biopreservative strategy for refrigerated foods is to be viable.

Disadvantages. Despite these exceptional advantages, some authors have developed plausible arguments questioning the efficacy of phages as antimicrobial agents *(42, 68, 79).* Although these potential drawbacks have been based primarily on observations relating to phage prophylaxis, many of the basic principles are also relevant to food biopreservation. Genetic and ecological factors influencing phage-bacteria interactions have also been discussed.

Transducing phages can transfer undesirable characteristics, such as virulence genes, from one organism to another, and lysogenic conversion can produce bacterial cells that are no longer susceptible to attack. Phages themselves can mutate from lytic virulent phages to temperate phages, which generally form a nonlysing association with host bacteria, resulting in lysis of only a small proportion of the population. Although these transfer and conversion phenomena have been documented with plant and animal pathogens, there is no evidence that they occur in foods. However, there is little doubt that foodborne bacteria can harbor temperate phages *(1).*

Of more critical genetic concern in food systems is the emergence of phage-resistant bacterial mutants, as has been noted both for foodborne pathogens in the preslaughter environment *(68, 71)* and spoilage bacteria during food storage *(37).* However, phages are constantly evolving and may have the potential to counteract this resistance *(47).* The use of mixtures of phages from distinct families also may help resolve issues with bacterial resistance *(9, 52, 68, 77).* Some researchers have not been able to recover phage-resistant bacterial mutants during laboratory trials of phage biocontrol in foods *(4, 49).*

Following a recent investigation of phage control of *E. coli* O157:H7, O'Flynn et al. *(57)* concluded that the frequency of the formation of phage-insensitive bacterial mutants was very low and the mutants appeared to revert to phage sensitivity. This conclusion is supported by data published by Connerton et al. *(17),* who examined the influence of indigenous phages on natural populations of *C. jejuni* in broiler chicks. Under these more realistic conditions with an environmental bacterial population, long-term phage resistance did not appear to be an issue. Despite the disparity in the published literature, phage-resistant bacterial mutants do emerge and are a concern that must be resolved if phage biocontrol is to be practicable. Tanji et al. *(77)* conducted a study of the mechanism of phage resistance in *E. coli* O157:H7 and found that the use of cocktails of phages utilizing distinct cell receptors suppressed the emergence of resistance.

Phage adsorption to receptors on bacterial cell walls requires the chance collision of the phage with a bacterial cell. This initial interaction can be impeded by a number of physical barriers in plants, animals, and foods. Joerger *(42)* carefully considered environmental factors affecting phage interaction with bacteria. For example, the viscous environment of the intestine or rumen could protect bacteria from attack, and the particulate nature of many food environments or nontarget indigenous bacterial contaminants could easily form physical barriers preventing phage-bacteria interactions. Other ecological considerations in designing phage biocontrol strategies include the pH of the food or animal host environments, temperature, immune responsiveness, and physiology of bacterial strains in vivo. A more complete understanding of these environmental influences should provide some explanation for the disparity among phage lytic activities in vitro and in vivo and enable the design of more effective phage biocontrol strategies.

An important impediment to phage biocontrol is the requirement of a threshold density of bacterial host cells. The need for bacterial populations of 3 to 5 log CFU has been reported for phage to have an impact upon these hosts *(34, 43, 83).* Some researchers have found that phages cannot be isolated from naturally contaminated foods where indigenous bacterial numbers are less than 5 log CFU *(81).* The numbers of the target bacterial cells would not be of

concern in spoiling foods, where large populations of spoilage bacteria reside, but would be of critical relevance in controlling pathogens in foods where numbers are usually lower.

Perhaps the most serious obstacle to the phage control of foodborne spoilage bacteria is the diversity of phage types. Research on beef spoilage pseudomonads *(31)* and *B. thermosphacta (32)* had revealed a very restricted host range for phages isolated from spoiled meats, and many of these phages could lyse only their homologous hosts. Even pools of phages with distinct hosts were unable to control the spoilage of naturally contaminated beef *(36)*. Thus, the very factor contributing to the desirable discriminatory power of phages, i.e., a narrow host range, is also a drawback. Barrow and Soothill *(9)* proposed methods for addressing this problem, including adaptation of phages to the resistant strains, identification of phages that attach to cell surface receptors shared by many strains, and use of several phages in combination. Another approach is to breed phages to produce altered host ranges, as described by Hibma et al. *(38)*.

Payne and Jansen *(61)* summarized results from studies of research on phage therapy highlighting the variability in results reported. An important issue was the inability to transfer methods from successful in vitro trials in broth to in vivo trials with living animals. A mathematical model was developed to predict the results of phage therapy by considering the inoculum size and the time of phage application in addition to other factors. The relevance of this predictive model to optimizing phage-bacteria interactions in foods remains to be evaluated.

FUTURE PROSPECTS

A phage biocontrol strategy should be an acceptable, more natural alternative to traditional approaches to food safety and preservation. The ability of exogenous phage suspensions to interact with their bacterial hosts during pre- and postharvest phases of food production has been clearly documented. The consequences of these targeted interactions have been reductions in bacterial numbers, control of infections in plants and animals, and improvement in the quality of stored food.

Unfortunately, most available data have been obtained in studies with experimentally infected horticultural crops and animals or from laboratory trials utilizing artificially inoculated foods. The technology must be transferred to the field to assess the potential of phage biocontrol of natural bacterial contaminants under practical conditions of food production, processing, and storage. Does phage biocontrol in the preharvest and postharvest stages of food production have a measurable impact upon food safety and storage quality? There is considerable commercial interest in phages in both human medicine and agriculture, and patents have been issued. Some examples of the phage companies in North America include Biophage (www.biophage.com) and Intralytix (www.intralytix.com). Intralytix has a food additive petition (FAP) pending with the U.S. Food and Drug Administration to allow the use of *L. monocytogenes*-specific phages on foods, which could reduce the risk of *L. monocytogenes*–associated foodborne illness. FAP approval will also play a critical role in further commercialization of the phage technology in the United States and abroad *(74)*.

Of all the factors with the potential to limit the efficacy of phage control of indigenous bacteria, the most significant concern is the restricted host range in relation to the diversity of susceptibilities in bacteria inhabiting foods. Attempts to isolate phages with a sufficiently broad host range are often futile. A more rewarding and expedient approach would be to breed phages with extended host ranges and then assemble a cocktail of phages with complementary activities. Phages can also be used as a component of a hurdle approach, where they may act synergistically when applied with a compatible antimicrobial agent such as a bacteriocin. As concluded by Campbell *(14)*, ''bacteriophage research is now undergoing a renaissance in which the primary focus is the phages themselves rather than the molecular mechanisms.''

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