Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages

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ABSTRACT Bacteriocins, antimicrobial peptides, and bacteriophage have attracted attention as potential substitutes for, or as additions to, currently used antimicrobial compounds. This publication will review research on the potential application of these alternative antimicrobial agents to poultry production and processing. Bacteriocins are proteinaceous compounds of bacterial origin that are lethal to bacteria other than the producing strain. It is assumed that some of the bacteria in the intestinal tract produce bacteriocins as a means to achieve a competitive advantage, and bacteriocin-producing bacteria might be a desirable part of competitive exclusion preparations. Purified or partially purified bacteriocins could be used as preservatives or for the reduction or elimination of certain pathogens. Currently only nisin, produced by certain strains of *Lactococcus lactis* subsp. *lactis*, has regulatory approval for use in certain foods, and its use for poultry products has been studied extensively. Exploration of the application of antimicrobial peptides from sources other than bacteria to poultry has not yet commenced to a significant extent. Evidence for the ability of chickens to produce such antimicrobial peptides has been provided, and it is likely that these peptides play an important role in the defense against various pathogens. Bacteriophages have received renewed attention as possible agents against infecting bacteria. Evidence from several trials indicates that phage therapy can be effective under certain circumstances. Numerous obstacles for the use of phage as antimicrobials for poultry or poultry products remain. Chiefly among them are the narrow host range of many phages, the issue of phage resistance, and the possibility of phage-mediated transfer of genetic material to bacterial hosts. Regulatory issues and the high cost of producing such alternative antimicrobial agents are also factors that might prevent application of these agents in the near future.

(*Key words*: antibiotic alternative, bacteriocin, antimicrobial peptide, bacteriophage)

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INTRODUCTION

Antibiotics have been utilized in animal research almost from the time of their discovery and quickly found widespread use in the farm environment as therapeutic agents and as growth promotants. By the early 1950s, a substantial number of publications concerning the growth-promoting effect of antibiotics on poultry had appeared (Branion et al., 1953). The U.S. Food and Drug Administration first approved certain antibiotics for use in animal feed in 1951 and now maintains a list of currently approved products (Center for Veterinary Medicine, 2002). Although exact figures on current use of antibiotics in the farm environment are apparently not available, estimates as high as 10.5 million pounds annually in the United States for the poultry production have been circulated (Mellon et al., 2001).

The efficacy and cost-effectiveness of many of these compounds are at the root of their popularity, but looming or already imposed restrictions or prohibitions on the use antibiotics as growth promotants have drawn attention to possible alternatives (Bedford, 2000; Wierup, 2000; Doyle, 2001). The use of antibiotics for the treatment of animals suffering from parasitic or bacterial infections is rarely questioned as long as the diseases have been diagnosed correctly and the dosage and duration of treatment follow prescriptive measures. But even for these therapeutic applications, it would be desirable to find compounds with fewer ties to human antimicrobial therapies.

Over the years, and especially more recently, a number of strategies for improvements in animal health, productivity, and microbial food safety that did not involve antibiotics have been explored. Probiotics and competitive exclusion preparations have been studied for application in poultry production, and some are already in use (Nurmi and Rantala, 1973; Bailey, 1987; Stavric, 1987; Stavric and D'Aoust, 1993; Corrier et al., 1995; DeLoach, 1998).

The addition of enzymes has been found to be beneficial depending on the feed ingredients used (Choct, 2001;

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Maenz, 2001). Orally administered chicken egg antibodies have shown promise for treatment of intestinal infections in pigs and calves (Yokoyama et al., 1992; Zuniga et al., 1997; Yokoyama et al., 1998), and cytokines have been shown to act as growth promoters and immuno-enhancers for broiler chickens (Lowenthal et al., 1999). Numerous nutritional additives are either already in use or have been proposed as means to reduce or eliminate pathogens or as a means to improve growth and feed conversion. In addition, agents such as bacteriocins, antimicrobial peptides, and bacteriophages have been studied or proposed as potential animal therapeutics. This review will discuss some of the challenges faced by these agents for antimicrobial therapy and their potential for use as growth promotants.

BACTERIOCINS

Bacteriocins are proteinaceous compounds lethal to bacteria other than the producing strain. As a group, bacteriocins are heterogeneous, and they are classified largely based on their molecular weight differences (Klaenhammer, 1993). Some bacteriocins are peptides consisting of only 19 to 37 amino acids, whereas others are large peptides with molecular weights of up to 90,000. Some small bacteriocins contain unusual amino acids originating from modifications of conventional amino acids after translation. The activity spectrum of bacteriocins can be narrow and confined to inhibition of closely related species, or it can be relatively broad and include many different bacterial species.

In contrast to the currently used antibiotics, bacteriocins are often considered more natural because they are thought to have been present in many of the foods eaten since ancient times (Cleveland et al., 2001). The bacteriocin nisin actually has GRAS (generally recognized as safe) status (21 CFR 184.1538). Nisin and other bacteriocins produced by lactic acid bacteria have received a great deal of attention because they are produced by bacteria largely considered beneficial to human health and to food production.

In most cases, bacteriocin production and activity has been demonstrated only in the laboratory. Evidence for a role played by bacteriocins in natural systems such as the intestinal tract is largely circumstantial. The observation that many intestinal bacteria such as *Fusobacterium mortiferum* isolated from chicken ceca (Portrait et al., 2000) are able to synthesize bacteriocins in vitro supports the notion that bacteriocins might be useful for survival in the intestinal tract. Some data from experiments with bacteriocin-producing bacteria also suggest an influence of bacteriocins on the ecology of the intestinal microbiota. For example, an avian *Escherichia coli* strain genetically engineered to produce the bacteriocin microcin 24 lowered intestinal *Salmonella typhimurium* counts in chickens when administered continuously in the water supply (Wooley et al., 1999). Similarly, the bacteriocin-producing *Enterococcus faecium* strain J96 isolated from the crop of a chicken exhibited some protective effect on chicks infected with *S. pullorum* (Audisio et al., 2000).

The administration of bacteriocin-producing bacteria rather than the bacteriocins themselves might be a more cost-effective approach, but significant progress in developing suitable producer strains will have to be made before such an approach will be feasible. Few studies have addressed the fate of bacteriocins in the intestinal tract, but some data suggest that some of the low molecular weight bacteriocins can survive at least some of the intestinal environments and possibly could be administered with feed. For example, Gaenzle et al. (1999) demonstrated that *Lactobacillus curvatus*, a producer of the bacteriocin curvacin, contributed to the inactivation of *E. coli* and strongly inhibited *Listeria innocua* in the gastric compartment of a dynamic model of the stomach and small intestine. During transit through the model system, the bacteriocin was not degraded in the gastric compartment and was degraded in the ileal compartment over approximately 180 min. Jalc and Laukove (2002) introduced nisin into an artificial rumen system and detected some changes in fermentation parameters such as an increase in hemicellulose degradation and acetate and propionate production. It is likely that these changes resulted from changes in microbial activities, but it is not known which bacteria were affected.

Applications of a bacteriocin in poultry processing have been explored by Mahadeo and Tatini (1994) who demonstrated that nisin reduced the number of *Listeria* added to scald water from a poultry processing plant by two orders of magnitude, followed by further reductions upon refrigeration. *Listeria* cells attached to poultry skin were more difficult to inactivate with nisin, and only about one $\log_{10}$ reduction was observed from an initial count of approximately 10^6 cfu. Although gram-negative bacteria such as *Salmonella* are considerably less sensitive to nisin than are many of the gram-positive bacteria, additions of chelating agents such as EDTA and detergents such as Tween 80 have been used to enhance the activity of nisin against gram-negative bacteria. Simultaneous delivery of nisin, EDTA, and Tween 80 under acidic conditions via an alginate or agarose-based gel onto the skin of chicken parts experimentally contaminated with *S. typhimurium* reduced counts of this pathogen by up to 3 $\log_{10}$ after 72 h at 4°C (Natrajan and Sheldon, 2000a). The shelf life of refrigerated broiler drumsticks was extended by 0.6 to 2.2 d by dipping in a nisin-containing solution and storage under nisin-treated PVC overwrap and on nisin-treated tray pads (Natrajan and Sheldon, 2000b).

As with any antimicrobial compound, the issue of resistance also has to be considered for bacteriocins. Although the mechanism of action is not known for all bacteriocins, most of the low molecular weight bacteriocins appear to interact with the bacterial membrane. Resistance is therefore usually the result of changes in the membrane of bacteria targeted by a bacteriocin (Ming and Daeschel, 1993; Mazzotta et al., 1997; Crandall and Montville, 1998), but inactivation by degradation has been observed for nisin (Jarvis, 1967). Until recently, development of resis-

tance to bacteriocins was not considered as affecting resistance to currently used antibiotics; however, Carlson et al. (2001) demonstrated that exposure of *Salmonella* to the bacteriocin microcin-24 can result in microcin-resistant cells exhibiting resistance to multiple common antibiotics. It remains to be seen if this observation is unique or if certain other bacteriocins can produce similar effects. Of concern is also a report by Mantovani and Russell (2001) that nisin-resistant mutants of *Streptococcus bovis* exhibited a 1,000-fold higher resistance to ampicillin than the original nisin-sensitive isolates.

For uses involving purified bacteriocins, cost of the compounds can become a significant barrier. Production of all but the smallest bacteriocins is currently only imaginable by culture of natural or genetically engineered producer organisms. Investments in research and development can be expected to be high, and the size of the market is difficult to predict, but the fact that nisin has found commercial uses indicates that economic aspects are not insurmountable barriers to bacteriocin applications.

ANTIMICROBIAL PEPTIDES

The production of small antimicrobial peptides is not confined to bacteria, but appears to occur in all organisms studied so far. Such natural peptides as well as artificial peptides derived by combinatory chemistry or rational design have attracted great attention in recent years (Hancock, 1997; Hancock and Lehrer, 1998). Numerous publications such as the ones by Gennaro and Zanetti (2000) and van 't Hof et al. (2001) have reviewed general and specific aspects of antimicrobial peptides, their biochemistry, and potential uses, but the scope of this paper does not allow a detailed discussion of all the topics. In general, antimicrobial peptides are small molecules with a molecular mass of 1 to 5 kDa. Their structure usually contains elements that facilitate the interaction with negatively charged membranes, and their mode of action involves the cell membranes of target organisms (Hancock and Rozek, 2002). In this respect, these peptides resemble some of the small bacteriocins such as nisin, and the development of resistance to the eucaryotic peptides might therefore also require changes to the membrane.

Development of strains resistant to antimicrobial peptides from previously sensitive strains has been viewed as difficult if not impossible (Hancock, 1997; van 't Hof et al., 2001), but studies have shown that certain genes can confer increased resistance to antimicrobial peptides. An example is a gene, *rcp*, present in *Legionella pneumophila* (Robey et al., 2001). Some resistance determinants against certain antimicrobial peptides have also been observed in *Staphylococcus* (reviewed by Peschel and Collins, 2001). Whether or not these and other as yet undiscovered determinants can be transferred between bacteria is currently not known.

The ubiquitous nature of antimicrobial peptides suggests that their role in nature has been long standing and must have contributed to an organism's fitness. It is not known if poultry currently used for food production has maintained ancestral levels of antimicrobial peptides or whether intense breeding efforts have led to their decline. Perhaps with increased knowledge of the genome and of gene expression patterns of chickens, this question can be addressed, and, ultimately, selection or perhaps even genetic engineering can restore or increase the activity of poultry antimicrobial peptides. The full range of chicken antimicrobial peptides and their contribution to a chicken's overall health and maintenance of favorable intestinal microbiota are currently not known, but research has already uncovered some of the peptides and some of the gene sequences that potentially code for them. Harwig et al. (1994) purified three antimicrobial peptides (gallinacins) from chicken leukocytes and examined their antimicrobial cativity in vitro. The peptides inhibited *L. monocytogenes* and *E. coli*, and two of the three peptides were also effective against the yeast *Candida albicans*. Three peptides isolated from turkey heterophil granules were tested against *Staphylococcus aureus* and *E. coli*. The three peptides killed *S. aureus*, and two of the three also killed *E. coli* (Evans et al., 1994). In a subsequent study with two chicken and two turkey peptides, antimicrobial activity was demonstrated against *Candida albicans*, *S. enteritidis*, and *Campylobacter jejuni*. *Pasteurella multocida* was not affected (Evans et al., 1995). One of the turkey peptides did not reduce survival of *Bordetella avium*, *E. coli*, and *S. typhimurim*, and none of the peptides neutralized infectious bronchitis virus. mRNA-derived sequences for four of the avian peptides were characterized subsequently (Brockus et al., 1998). A chicken epithelial β-defensin was shown to be inducible by experimental infection with *Haemophilus paragallinarum* (Zhao et al., 2001). This observation suggests a role for these types of peptides in fighting bacterial disease.

Sequences that potentially encode antimicrobial peptides have also been detected in a cDNA library from chicken macrophages (Keeler, unpublished). Among the cDNA was a sequence that could encode a peptide related to cathelicidins. Based on the predicted amino acid sequence, the peptide was synthesized chemically and tested against the three pathogenic bacteria, *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes*. When added to approximately 10^4 cfu/mL of the pathogens in trypticase soy broth, visible growth after 16 h of incubation at 37°C was not detected when the peptide was added in concentrations equal to or higher than 12.5, 25, and 12.5 μg/mL for *E. coli* O157:H7, *S. enteritidis*, and *L. monocytogenes*, respectively (Joerger, unpublished data).

As with bacteriocins, the proteinaceous nature of antimicrobial peptides makes them vulnerable to proteolytic enzymes. This instability is probably of little concern for peptides produced in the immune system or epithelium where bacterial targets are in close range. Some of the peptides such as defensins or bactenecin exhibit toxic effects (Rademacher et al., 1993; Kagan et al., 1994), and proteolysis might limit this effect by reducing the range and concentration of the peptides. In contrast, any intervention that involves injection or ingestion of such pep-

tides will have to deal with the potential for proteolysis and toxicity. Perhaps, the peptides have to be modified chemically to make them more resistant to proteolysis in animals, or their administration might have to include encapsulation methods that protect the peptides from immediate attack. Both strategies would add to the costs of antimicrobial peptide treatment.

Currently, chemical synthesis appears too costly for large-scale production of peptides, and biological production with microorganisms, tissue cultures, or in transgenic animals will probably have to be attempted. Transgenic plants could be used for production of peptides (van t' Hof et al., 2001), and perhaps for some applications, the peptide-containing plant material could be added to animal feed. The prospects for such an approach are currently rather uncertain because important economic and ecological issues need to be resolved; however, in anticipation of the development of plants expressing traits of medicinal interest, the FDA has initiated the process of developing guidelines (Food and Drug Administration, 2002).

Extensive research will be required to identify peptides that influence intestinal microbiota in the same way as currently used antibiotics. Perhaps a more likely use for antimicrobial peptides in the not-too-distant future would be as inhibitors of microbial growth on surfaces and in biological material such as vaccines. Such an application would require smaller amounts of the peptides than feed applications, and enzymatic degradation would also be of less concern.

BACTERIOPHAGES

Bacteriophages are viruses that infect and multiply in bacteria. For many bacteriophages, release into the environment after replication is accompanied by lysis of the host bacterium. This event is easily observed in test tubes and on agar plates (plaque formation), and its exploitation for killing infectious bacteria was suggested almost immediately upon discovery. The enormous suffering caused by bacteria directed the first therapeutic efforts toward treatment of infections in humans, but the initial euphoria about phage as therapeutic agents dissipated with the onset of the antibiotic era. In addition, many earlier trials with phage did not produce desired outcomes. To a limited extent, phage therapy continued to be practiced in some Eastern European countries, but was reexamined in Western Europe and the United States only after problems with antibiotic-resistant bacteria began to surface. Since then, a number of papers reviewing historical aspects of phage therapy and applying mathematical modeling to explain observed phenomena have appeared (Levin and Bull, 1996; Alisky et al., 1998; Pirisi, 2000; Chanishvili et al., 2001; Payne and Jansen, 2001; Sulakvelidze and Morris, 2001; Summers, 2001, Kasman et al., 2002). A consensus appears to have emerged on the feasibility of phage therapy under the right conditions, but it is also clear that significant research efforts will be necessary before phage therapy can be implemented.

Not all phages would be suitable for phage therapy. Detailed studies of potentially useful phages with respect to their interaction with target bacteria and their genetic content will be required. Some phages produce progeny without destroying their bacterial host, others have means to temporarily integrate their genome into that of the bacterium where it is replicated along with the bacterial genome and potentially introduces new traits or modifies the expression of host traits. While part of the bacterial genome, the phage DNA sequences can participate in recombination events, leading to modifications of the phage genomes. There are now numerous accounts of phage encoding virulence genes or of phage integrated into bacterial genomes that influence expression of bacterial genes, among them toxin- or antibiotic-resistance genes (Schicklmaier and Schmieger, 1995; Alisky et al., 1998; Figueroa-Bossi and Bossi, 1999; Schmieger and Schicklmaier, 1999; Mirold et al., 2001). Gene flow among phages and hosts appears to be common, resulting in phage genomes that are mosaics of sequences of different origins. (Hendrix et al., 1999; Juhala et al., 2000) Excision and replication of the integrated phage DNA for production of new phage particles can sometimes lead to inclusion of bacterial sequences that can subsequently be transferred into new host cells. Therefore, phages that infect their hosts, initiate replication and production of progeny immediately, and cause cell lysis are preferred for phage therapy because they lead to relatively fast destruction of many of the bacterial targets, and there are fewer opportunities for interactions with the bacterial genome.

One feature that makes bacteriophage so attractive is their highly discriminatory nature. Most of the known bacteriophages are specialists that interact only with a specific set of bacteria that express specific binding sites; bacteria without these receptors are not affected. This narrow host range is also a significant challenge for phage therapy. For example, there is no known phage that is lytic for all *Salmonella* serovars. More likely, a particular *Salmonella* phage will only lyse a small part of the spectrum of *Salmonella* serovars and even will not be lytic for all members of one particular serovar. This observation was also made in a study on the use of phages to combat infections of chicks with *S. enteritidis* (Sklar and Joerger, 2001). This degree of host specificity necessitates use of phage mixtures for prophylaxis of bacterial infections in most instances.

Chighladze et al. (2001) isolated *Salmonella* phages and combined them into a cocktail that was lytic to 232 of 245 *Salmonella* isolates representing 21 sero and 78 PFGE types. The number of different *Salmonella* phages in the cocktail was not revealed. Application of this cocktail to artificially contaminated surfaces reduced *Salmonella* to undetectable levels after 48 h, and three $\log_{10}$ units of decrease in *Salmonella* counts were reported for phage-treated eggs. Reductions in *Salmonella* counts from artificially contaminated carcasses were also observed.

Use of several different phages for combating even a single bacterial strain has been found necessary in several studies. Kudva et al. (1999) employed a mixture of phage

against a strain of *E. coli* O157:H7, as did Schnabel et al. (1999) in a study with the plant pathogen *Erwinia amylovora*. Sklar and Joerger (2001) also used combinations of *S. enteritidis* phages in their study with young chickens because individual phage did not result in a decrease in cecal counts of *S. enteritidis* at 14 d of age. Almost certainly, phage-based modification of the intestinal microbiota for growth promotion would also require use of several phages in one preparation.

Most of the experimental studies on phage therapy have used model systems that included potentially lethal bacterial infections (Soothill, 1992; Merril et al., 1996; Biswas et al., 2002). In most cases, successful treatment outcomes (survival) were reported. Recently, Huff et al. (2002) described successful application of phage in prevention of *E. coli* infections in broiler chickens. The results from experiments with models not involving bacteremia were not always as encouraging. Smith et al. (1987) reported that phage were able to cure *E. coli*-induced diarrhea in calves. Reynaud et al. (1992) was unable to demonstrate this in a rabbit model. Berchieri et al. (1991) observed reduced mortality in *S. typhimurium*-infected chicks and some reduction of counts for this bacterium in the intestinal tract and the liver. Sklar and Joerger (2001) observed counts of *S. enteritidis* that were lower by 0.3 to 1.3 orders of magnitude in the ceca of phage-treated chickens than in ceca from untreated birds.

Therapy or prophylaxis against intestinal bacteria might be more difficult for several reasons. In contrast to phage therapy for bacteremia, intestinal phage therapy might have little or no support from the immune system. Any target bacteria that escape phage attack or that harbor resistance mechanisms will be able to multiply and perhaps establish population levels comparable to those prior to phage administration. Although earlier concerns based on the studies by Wiggins and Alexander (1985) regarding the minimum number of bacteria required for phage propagation have been rejected recently (Payne and Jansen, 2001), other concerns are still valid.

In a relatively fluid environment such as broth or even blood, mixing and diffusion are relatively unimpeded; the viscosity of intestinal content on the other hand would appear to reduce the chance of bacteria-phage collisions and higher phage concentrations relative to the bacteria might be required for effective treatment. In addition, the large numbers of bacteria found in certain parts of the intestinal tract might not only be mechanical barriers to diffusion of phages, but some of the bacteria might also exhibit nonspecific phage binding.

It has also been proposed that differences between the physiology of bacteria grown under laboratory conditions and of bacteria inhabiting host environments could account for some of the failures of phage therapy attempts. Perhaps a fraction or even most of the bacteria inside the host do no longer express the phage receptors prevalent in laboratory culture. The bacterial physiology could also be such that phage replication is slower or perhaps completely inhibited. Phages that selectively bind to and replicate in bacteria of different physiological states have been recognized. For example, Lui et al. (2002) described phages that replicate in either free-living *Bordetella bronchiseptica* or in the host-associate form of the bacterium.

During phage treatment, phages are exposed to a number of factors that might limit their activity. Phages circulating in the blood stream are potentially subject to attack by antibodies, or they can be removed from circulation by the reticuloendothelial system. Selection of phage variants not as susceptible to removal from circulation in the body is feasible (Merril et al. 1996). Potentially adverse factors encountered in the intestinal tract are pH variations and the presence of enzymes and other digestive compounds such as bile. Phages have been observed in several intestinal systems and have been studied more extensively in the rumen where a dynamic phage population of up to 1.6×10^{10} phage particles per milliliter of rumen fluid was detectable (Klieve and Swain 1993). Most of the phages that target bacteria inhabiting intestinal sites are probably well adapted to the intestinal environment; for example, they remain viable in the fermentative environment of the cecum of chickens (Berchieri et al., 1991; Sklar and Joerger, 2001).

Whether or not phage activity is the same in all compartments of the intestinal system is not known. Sklar and Joerger (2001) observed that chickens experimentally infected with *S. enteritidis* and treated orally with phages were sometimes negative for *Salmonella* in cloacal swab tests even though they harbored significant numbers of *S. enteritidis* in their ceca. Infected chickens not exposed to the phages tested positive for *Salmonella* in cloacal swabs and in cecal samples. These observations might be the result of the testing methodology (swabbing vs. plating of diluted samples), but it is also possible that phage activity was more pronounced in the lower regions of the chickens, thus reducing shedding. Leverentz et al. (2001) suggested that pH can have an effect on phage activity because a phage preparation was able to reduce *S. enteritidis* counts on experimentally contaminated melons but not on apple slices (pH 4.2).

Bacteriophages are generally very stable entities and survive storage relatively well, even when absorbed to solid particles. For example, a titer of 10^9 plaque-forming units (cfu) of a *Salmonella* phage mixed with chicken feed pellets decreased by only approximately two orders of magnitude over 14 d at 37°C (Sklar and Joerger, 2001).

In most respects, the same technical and financial challenges faced by most other large-scale operations involving microorganisms are also present for the production of phages. One significant difference between most current operations and phage production could be safety concerns regarding the bacterial host. If it were necessary to produce therapeutic phage in their pathogenic hosts, relatively costly safety measures to protect plant workers and the surrounding community would have to be implemented. Inherent with propagation of phage on a pathogenic host is also the risk of production of some phage that carry host DNA involved in pathogenicity that could conceivably be transferred to other bacteria. Although the likelihood of such an event might be small, it would be

preferable to produce phage in hosts that are not virulent. Perhaps non-virulent, genetically well-characterized hosts can be found for the propagation of phage, but development of such bacteria will also add to the cost of development of phage preparations.

In vitro assembly of phage particles that contain DNA coding for bactericidal proteins, but not for any function allowing phage replication, could eliminate problems with the release of large numbers of phage capable of infection of new hosts. On the other hand, such phages would also no longer be able to multiply thus potentially reducing the therapeutic effect.

Despite a paucity of experimental data, a certain amount of optimism has been expressed that medical applications will be pursued in the not too distant future (Pirisi, 2000). Phage therapy might become an addition to the collection of antibacterial tools and will be used perhaps primarily when other, cheaper means such as conventional antibiotics fail. Presumably, such occasional application of phage will cause fewer ecological concerns than application of phage to entire flocks or herds of animals. The escape of phage from such facilities is probably inevitable and could be perceived as an environmental risk. The public might consider such a risk as too high, but, as observed with the issue of genetic engineering, might display a more accepting attitude toward medical applications.

For some applications, it might not be necessary to apply active phage, but simply to administer the enzyme(s) used by a particular phage to lyse their bacterial host. Loeffler at al. (2001) demonstrated the feasibility of this approach on mice infected with pneumococci. This approach might be most suitable for the treatment of topic infections or infections of mucous membranes.

Arguably, bacteriophages are the most promising agents that could complement and sometimes replace current antibiotics, but their use on the farm or for food safety applications is uncertain. Small antimicrobial peptides whether of bacterial or eucaryotic origin or created artificially in the test tube will more likely become available for animal applications, but substantial research efforts will still be necessary to realize that possibility. Their use for treatment of diseases will be probably at the forefront; their development as growth promotants will be more difficult, especially since the bacterial targets for the growth promotants are not fully known.

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