What Makes Bacteriophage Safe?

Phages are viruses that infect bacteria. Phage therapy is the application of phages to humans or to environments to reduce densities of specific bacteria, including, especially, the destruction of pathogenic bacteria. In this report we consider phage-therapy safety issues. We note, first, that species barriers minimize the direct phage interaction with human tissues, particularly by preventing specific interactions such as the phage infection of human cells. Phage impact on human health therefore would occur primarily through indirect effects, such as via the phage infection of bacteria making up human normal flora. However, in most cases the application of phages to bacteria-containing environments (including humans) makes a quantitative difference, not a qualitative one, since phages already exist in many environments, including water, soil, food, and even among human normal flora. Plausible risks associated with any phage exposure should be limited to the killing of otherwise helpful bacteria (already a tradeoff associated with chemical antibiotic usage), modification of specific bacteria to a higher virulence (a real but addressable concern), or due to bacterial toxins associated with phage preparations. These hazards may be mitigated by employing phage therapeutics (i) with narrow host ranges, (ii) that are unable to display lysogeny, (iii) that do not carry toxin genes, (iv) that display minimal tendency towards DNA transduction between bacteria, and (v) which are purified away from bacterial toxins. In other words, exclusion of those traits already observed among many naturally occurring phages with which we make contact on a daily basis. Indeed, following over 80 years of study of phage-animal and phage-human interaction, no evidence has surfaced suggesting that the application of specific phages to the human body will impact negatively on human health.

SPECIES BARRIERS

The human condition is such that biological features of our environment normally are relatively benign. This relative safety occurs in part because most microorganisms are limited in their ability to invade the human body, with contact to the skin less invasive than contact with mucous membranes, contact with mucous membranes less invasive than penetration into body tissues, and penetration into body tissues less invasive than penetration into body cells. Furthermore, in order for microorganisms to cause disease they generally must adhere to host tissues, penetrate those tissues, and then produce some kind of chemical (e.g., a toxin) that either interferes with a normal body process or otherwise directly damages the body. Most of the time microbes, even human pathogens, are not successful in one or more of these steps and, as a consequence, even though microorganisms are constantly probing our body's defenses, for the most part these microbes do not make us sick (89). The primary protections our bodies employ against non-human pathogens are collectively known as *species barriers* (a.k.a., genetic or species immunity). That is, microorganism penetration, replication, or damage will fail to occur due to (i) an absence of specific molecules required for adherence to host tissues, (ii) a lack of specific mechanisms by which penetration into host tissues may be effected, (iii) an absence of toxin production or a lack of human-tissue specificity of any toxins produced, (iv) metabolic (i.e., biochemical) incompatibilities that interfere with microorganism (particularly viral) replication, and (v) metabolic incompatibilities that

interfere with toxin production by potentially pathogenic microbes. As a consequence of these hurdles, the vast majority of microorganisms that humans encounter are incapable of bypassing normal, non-specific defenses against pathogen reproduction and then, should penetration inadvertently occur, nevertheless are incapable of effecting disease.

Viruses are well represented among the microorganisms that commonly surround us, but the vast majority of viruses, so far as science can tell, are incapable of penetrating sufficiently into human cells or tissues to render us harm, or are incapable of damaging human cells or tissues even given penetration. Indeed, of the approximately 90 virus families that are recognized by the International Committee on Taxonomy of Viruses (ICTV), only 19 families cause human disease[1]. In modern biology viruses are typically differentiated in terms of their modes of replication, their type of genomes (e.g., RNA vs. DNA), chemical characteristics of their capsids, their morphology as viewed by an electron microscope, their impact on infected-cell (or tissue) anatomy (or histology), and, most recently, the actual sequence of the nucleotides making up their genes and even entire genomes (e.g., 123). Historically, however, viruses were first distinguished in terms of their host range (the host species they are capable of infecting) and, given infection of a given species, their tissue tropism (tropism is a description of the tissues a pathogen is capable of infecting). Thus, from the classic five-kingdom system of organismal classification we commonly differentiate animal viruses from plant viruses from protozoan viruses from fungal viruses from bacterial viruses. The ICTV—based more on research emphases rather than actual viral diversity— today differentiates virus hosts into eight basic categories: archaebacteria, bacteria, eukaryotic algae, protozoa, fungi, plants, invertebrate animals, and vertebrate animals.

It is rare for pathogens of one kingdom to infect and give rise to disease in another kingdom. Exceptional are a few plant-pathogenic bacteria, such as *Pseudomonas aeruginosa* (175) and *Burkholderia cepacia* (59), which are also pathogenic to humans. Another example is the bacterium *Legionella* which normally exists as a protozoan pathogen, but when spread through contaminated air-conditioning systems can cause atypical pneumonia (84, 151). Additional human or animal pathogens that potentially have protozoan reservoirs include *Listeria*, certain mycobacteria, coliform bacteria, and perhaps even *Escherichia coli* O157 (13). Unlike the above examples of kingdom-jumping bacteria, however, *there is no evidence of a kingdom-jumping virus* (85). The explanation for this rarity is an extension of the idea of species barriers: molecular differences between host species from different kingdoms (e.g., bacteria hosts vs. human hosts) are even greater than those found between different species within the same kingdom (e.g., bovine hosts vs. human hosts). Consequently, though emerging infectious diseases and known zoonoses often have a viral etiology (e.g., AIDS, ebola, influenza, sin nombre virus, SARS…), not only is it typically assumed that the reservoir or original

[1] See www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fr-fst-h.htm for a list of virus families. Human viruses are found among families Adenoviridae, Arenaviridae, Bunyaviridae, Caliciviridae, Coronaviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Herpesviridae, Orthomyzoviridae, Papovaviridae, Paramyxoviridae, Parvoviridae, Picornaviridae, Poxviridae, Reoviridae, Retroviridae, Rhabdoviridae, and Togaviridae. See http://www.kcom.edu/faculty/chamberlain/Website/Lects/VIRAL.HTM for basic descriptions of virion morphologies as well as associated diseases.

host is an animal (essentially by definition for a zoonosis), it is also typically the case that the source animal is either a vertebrate—and an endothermic vertebrates at that (e.g., mice, bats, chimpanzees, etc.)—or an arthropod, perhaps in combination with vertebrate host (e.g., yellow fever and West Nile virus). Even the notoriously broad host-ranged rabies virus is limited to infecting mammals. There are, however, two virus families, Reoviridae and Rhabdoviridae, that possess some members capable of infecting animals and other members capable of infecting plants. Individual viruses, though, are limited in their host range to animal or plant hosts but not some combination of both[2]. See (68) for additional discussion of the relationship, such as it exists, between bacteriophages and viruses of eukaryotic organisms.

BACTERIOPHAGE

The viruses of "Kingdom" Bacteria were first described as invisible entities capable of destroying bacterial cultures and that, like the plant viruses discovered before them (e.g., tobacco mosaic virus), would remain infectious even after suspensions were passed through filters designed to remove bacteria (43, 48, 148). Since the action of bacterial viruses resembled the "eating" of bacterial cultures, the word "phage", which means to eat or devour in Greek, was chosen to describe this phenomenon. It was only decades after their discovery that all researchers accepted bacteriophage as viral. As a consequence, bacterial viruses, even today, are better known as bacteriophages or, simply, as phages.

Though not visible to the naked eye, or even through powerful bright-field light microscopes, nevertheless we are surrounded by these phages. A single drop of seawater can hold literally millions of phages (e.g., 166), and an inadvertent mouthful can contain as many phages as there are people in the U.S. Indeed, total virus estimates worldwide are 10^{30} to 10^{31} (e.g., 68). That is equivalent to 100 million to 1 billion virus particles currently present on Earth for every star in the universe (155) or over 100,000,000,000,000,000,000 (100 quintillion = 100 billion billion) for every human (~10 billion) who has ever lived.

The vast majority of phages, so far as virologists understand, are incapable of harming humans. This is because (i) not all phages are temperate, that is, able to establish "lysogenic" relationships with bacteria, which are symbioses in which the chromosome of a *temperate* phage becomes integrated into the chromosome of a bacterium.; (ii) relatively few temperate phages have been shown to cause bacterial lysogens to display phage-coded bacterial virulence factors (160); (iii) the species these phages are capable of infecting (their host range) does not include humans; (iv) many or most of these phages display limited host ranges even among bacteria and therefore, unless specifically targeted, may be incapable of infecting the bacteria making up the typical human normal

[2] Family Reoviridae includes the plant viruses Fiji disease virus, rice dwarf virus, and rice ragged stunt virus, as well as the vertebrate viruses aquareovirus A, bluetongue virus, Colorado tick fever virus, mammalian orthoreovirus, and rotavirus A. Family Rhabdoviridae includes the plant viruses lettuce necrotic yellows virus and potato yellow dwarf virus as well as the vertebrate viruses bovine ephemeral fever virus, infectious hematopoietic necrosis virus, rabies virus, and vesicular stomatitis Indiana virus.

flora; (v) most of these phages are not even capable of penetrating to the bulk of normal flora—i.e., that found in the alimentary canal—due to the protective action of gastric juices and intestinal proteases, the protein-digesting enzymes; (vi) even given penetration to and infection of normal body bacteria, many phages, such as those incapable of establishing lysogenic relationships with bacteria, do not facilitate changes in bacteria phenotypes that result in changes to bacterial virulence; and (vii) given an absence of bacterial infection there is no evidence that natural bacteriophages otherwise serve as anything other than benign—and transient—components of normal flora.

Analysis of titers of viable phages in foods and water is routinely done, and over the past two decades increasing efforts have been made to ascertain total viral counts, particularly in aquatic systems (e.g., 166), and recently also in soil (9). In the latter study the total number of virus particles (with most assumed to be bacteriophages) were at least 10^7/g and, as the authors speculate based upon estimates of soils to which a known density of phages had been added, perhaps range as high 10^8 viruses/g. In other words, 10 billion naturally occurring viruses may be present in a 100-gram handful of untreated soil. Phages that infect the bacterium *Escherichia coli* and related bacteria (fecal coliforms)—normal constituents of human flora—are even commonly employed as indicators for fecal contamination of water (e.g., 30). That is, fecal contamination may be ascertained by determining counts of phages (coliphages) that are actively capable of infecting these bacteria. That utility is possible because the intestinal contents or feces of certain domestic animals (cows and pigs) and humans contain large amounts of coliphages (141). For cattle, chickens, and other domesticated animals, for example, coliphage counts are frequently found (mean counts among different animals) in the range of 10^4 to 10^7 (10 thousand to 10 million) per gram of feces (1). Fecal contamination, as always, is a concern during food production and coliphages have also been proposed as indicators for the fecal contamination of foods, e.g., of carrots (51), ground beef and poultry meat (72), and animal feed (94). Phages, in short, are a normal part of the human environment, and, in Table 1, we review recent phage isolations directly from humans as well as the immediate human environment. See (2) for a review of phage types and their prevalence in various environments.

PHAGE THERAPY

Phage therapy refers to the process of applying phages to bacteria-containing ecosystems to reduce deleterious bacterial populations. Recently phage therapy has been subject to numerous reviews (4, 8, 17, 35, 39, 49, 70, 81, 82, 92, 99, 131, 143, 146, 147, 149). Historically phage therapy has focused on the application of phages to human bodies to combat bacterial diseases, as well as application to vehicles of pathogen transmission such as water (for reviews of the history of phage therapy see 99, 131, 148, 149). Application to humans (as well as animal models) has ranged from local (e.g., specific areas of skin) to gastrointestinal (usually following oral delivery) to systemic (e.g., intravenous, intraperitoneal, or intramuscular). Systemic application may be employed to combat bacteremias and septicemias, or to deliver phages systemically to more-localized infections.

Phage therapy held great promise during the first half of the 20th century, particularly within a world in which alternative antibacterial therapies were rare, unknown, or otherwise toxic. A lack of understanding of phage biology—combined with the discovery of safe and effective chemical antimicrobials (i.e., antibiotics)—resulted, however, in a decline in interest in phage therapy, particularly by Western medicine. By contrast, extensive use of phages to treat human disease continued to occur within the former Soviet Union and Poland (136-138), as well as France (e.g., 158). The Eliava Institute of Bacteriophage, Microbiology, and Virology in Tbiliisi, Georgia, of the former Soviet Union has been using phage therapy since 1934 (120). Examples of diseases treated with phages include dysentery, food poisoning, typhoid fever, burns, blood poisoning, and infections of the skin, throat, and urinary tract. Phages were administered by drinking, swallowing tablets, aerosols, topical application to lesions, intravenous injections, and in combination with antibiotics. Phages were also used as antiseptics in operating rooms, on surgical instruments, and as prophytics in lesions during surgery. As many as eleven thousand children were given phages as prophytics for many years. No evidence of adverse reactions of any kind to humans has been reported.

Even in the West, and even though we know that specific temperate phages can contribute to specific diseases [e.g., cholera and hemolytic uremic syndrome, as caused by *Vibrio cholera* and *Escherichia coli* O157:H7, respectively (160)], there is no evidence that exposure to phage virion particles, even ones normally associated with disease-causing bacteria, can actually result in the occurrence of human disease (e.g., 49). Indeed, one can identify numerous circumstances, and evidence, consistent with a conclusion that the majority of phage exposure is *not* inherently risky to human health nor, necessarily, even aberrant from the normal human experience. Indeed, in the U.S., tens of millions of individuals have received live virus vaccines that were contaminated with phages, including polio, measles, mumps, and rubella vaccines. Recipients of contaminated vaccines showed no detrimental effects. Because of concern about the safety of phage contaminated vaccines, Milstien et al. (100) isolated phages from a vaccine, produced them in high titer and injected 10^{12} into 6-8 week old monkeys. No adverse reactions to the monkeys were observed. Petricciani *et al*. (115) concluded from additional animal testing that phage contaminated vaccines for humans posed no real threat to public health.

Phage therapy may be defined more broadly than just the application of phages to human bodies to combat bacterial disease. Indeed, at its most inclusive "phage" therapy represents the application of specific pathogens (e.g., such as phages, which are pathogens of bacteria) to selectively reduce or eliminate pathogen-susceptible organisms from specific environments, including natural environments (e.g., forests, lakes, etc., as well as the bodies of humans and other animals), artificial environments (e.g., farms, factories, offices, hospitals, etc.), or even laboratory environments (e.g., to reduce streptomycete numbers on soil dilution plates; 83). In other words, phage therapy is simply another form of biological control—the use of one organism to suppress another; and like other biological controls, the application of phage therapy holds a potential to reduce the usage of anti-pest chemicals, which in the case of phages means a reduction in the application of chemical antibiotics.

Phage therapies can be classified into five categories in terms of the likelihood and nature of human contact. They are:

Category I	Human exposure to the environment to which phages have been applied is unlikely and therefore human exposure to the applied phages is rare;
Category II	Human exposure to the environment is likely, but human exposure to applied phages is greatly reduced;
Category III	Human exposure to the environment is likely *and* human exposure to phages is somewhat likely;
Category IV	Phages are directly applied to humans, but without deliberate introduction of phages deeply into human tissues;
Category V	Phages are deliberately introduced deeply into human tissues.

See Table 2 for a classification of published phage-therapy studies by category.

Category I. Phage (or viral) therapy has been proposed for the control of bacterial disease in non-food plants (53), of various aquatic algal blooms (45, 127) of microbiofouling of marine heat exchanges (78, 128), and in various guises to remove bacteria from non-food organisms growing in tissue culture such as white-clover protoplasts (61, 132). Also consistent with a category I application of phages is phage employment as living tracers, e.g., for following the movement of groundwater or as indicators of fecal contamination from specific sources. For such application it is relevant to this discussion that a stated advantage of phage employment as water tracers is that phages "are neither toxic nor pathogenic for other living organisms as they penetrate only a specific bacterial host" (126). Similarly, sewage treatment plants contain high densities of phages, most of which are readily capable of infecting bacteria normally associated with human flora—and a few of those phages can carry bacterial virulence factors (104, 105, 153, 154) and are potentially aerosolized (19, 29, 34)—yet there is little concern of any association between sewage phages and human disease. This is probably due to little human contact with raw sewage and because well-treated sewage effluent can display significant reductions in the viability of phage contaminants (36, 125, 154). However, total counts of naturally present viruses in even treated sewage effluent can still be enormous [e.g., greater than 10^8/ml total count in a sewage-works lagoon; (22)]. Naturally present viable coliphages found in effluent or sewage-contaminated waters, can range up to 1,000 or more per ml (1).

Category II. In category II, phage densities upon human contact have been significantly reduced due to long time frames between phage application and human contact, or because of various means of processing (e.g., cleaning and disinfection) that have been applied prior to human contact or, more specifically, prior to phage contact with consumers. In Table 2 over 25 studies are listed that are described as category II uses of phages. Note that most of these studies address the application of phages in agricultural settings, typically to eliminate bacteria associated with diseases affecting production, though additionally with phages employed to reduce loads of potential human pathogens.

Reduction in the phage load of foods can occur both pre- and post-harvest, with category II application of phages occurring only pre-harvest. Pre-harvest reduction in phage load occurs due to natural tendencies for phages to decay—due to exposure to sunlight, UV radiation, desiccation, and various chemical and biological antagonists (6, 116, 133, 134, 150, 165, 167)—such that densities decline unless active phage replication occurs. Phage viable counts typically decline in many ecosystems, given an absence of specific host bacteria (and therefore a potential for phage replication)—indeed, phage numbers can decline even given the presence of host bacteria if phage application is done with high ratios of phages to bacteria. Since replication can only occur within phage-susceptible bacteria, and the consequence of phage therapy is the destruction of phage-susceptible bacteria, phage populations tend to decline following both successful and unsuccessful phage therapy. For example, therapeutic phages applied to chicken-feed pellets incubated at 37°C display an approximately 100-fold decline in phage number over a two-week period (135).

Post harvest one can expect that processing, whether post-harvest washing (oftentimes washing includes addition of sodium hypochlorite to the wash water) or subsequent cooking, should result in dramatic declines in both phage viability and total phage numbers. This is particularly so since post-harvest processes are often designed with a reduction in microbial load in mind. Various studies have explored the ability of hypochlorite, for example, to impact on phage viability (23, 27, 67, 76, 77, 95, 119, 129, 145). See (93) for phage removal from strawberries and (112) for a comparison of the impact of hypochlorite on phage suspended in broth vs. milk.

Category III. Category III currently consists mostly of proposed post-harvest applications of phages to foods as a means of reducing the content of potential human bacterial pathogens on food or as a means of interfering with the life cycles of microbes capable of affecting food quality. Since the risk of food spoilage should continue right up to consumer contact, and since the ability to process foods is reduced the closer those foods get to the consumer (i.e., reduced likelihood that phages may be effectively washed or otherwise eliminated via disinfection), the likelihood of human contact with phages should be much greater than with category II application. In cases where phages target potential human pathogens one could envisage that greatest pathogen-killing efficacy could occur where efforts to eliminate phages prior to human contact are *not* employed (since phage removal would be equivalent to anti-pathogen removal). In cases where phages target food-spoilage agents, one similarly might expect a reluctance to remove such agents prior to consumer contact, either because those agents would be employed primarily to prevent spoilage prior to consumer purchase (e.g., during transportation from factory to store, with minimal processing expected in stores) or to prevent spoilage following purchase by the consumer. Thus there exist numerous circumstances for which phages, and even phage infectivity, would be advantageously maintained even given a high likelihood of human contact with high numbers of viable phage.

Though cooking should eliminate phages from some foods (e.g., from meats), in fact not all food to which phages may be applied necessarily are cooked prior to consumption.

Furthermore, handling of foods prior to cooking should result in a spread of phages to hands as well as to common utensils. However, the same could be said for any microorganism found on meats, including pathogenic bacteria that, of course, often are found in association with store-bought meats [and hence the proposal to employ phages to combat potential bacterial pathogens found on meat (50)]. On the other hand, proposals to treat fresh-cut fruit with phages to reduce loads of *Salmonella enteritidis* (88), assuming no subsequent cooking, could result in significant consumer exposure to applied phages. Also potentially included in this category (III) could be the application of phages to workplaces, e.g., as a means of reducing the danger of exposure to agents of bioterrorism (e.g., anthrax; 161), which would also result in significant exposure of humans to phages.

Of significant relevance to considerations on the environmental impact of phage application at the category III level is this pronouncement (75) from the Director, Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA):

> The Food and Drug Administration (FDA) is announcing that Intralytix, Inc., has filed a petition proposing that the food additive regulations be amended to provide for the safe use of a mixture of bacteriophages as an antimicrobial agent on foods, including fresh meat, meat products, fresh poultry, and poultry products… The agency has determined under 21 CFR 25.32(r) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, *neither an environmental assessment nor an environmental impact statement is required*. [emphasis mine]

This pronouncement serves as an indication of the level of concern of the FDA to the casual interaction between humans and phages, which, apparently, is thought neither "individually or cumulative [to] have a significant effect."

Categories IV and V. Though we distinguish these categories into two, it is relevant to point out that phages appear to have some propensity to systemically circulate within animal bodies despite only local application [(122, 139, 162) and possibly (163)], plus may be able to exit systemic circulation at low levels into the gastrointestinal lumen, perhaps via liver uptake followed by elimination in bile (171). Phage DNA (M13) and other DNAs also may be taken up into systemic circulation from the gastrointestinal tract, albeit at relatively low levels, plus, originating in the gastrointestinal tract, ultimately can even cross placental barriers (46, 130). Access of normal flora to systemic circulation—resulting particularly in bacteremias—may not be unusual, especially given acute or chronic injury to normal barriers (91). Thus, categories IV and V basically define opposite ends of a spectrum of deliberate phage exposure to humans in which systemic circulation of phages occurs either with a lower probability or to a lesser extent numerically (category IV) or in which invasive administration of phages occurs presumably with higher phage exposure within body tissues (category V). Included in category IV thus are topical (including to wounds), oral, and intranasal phage application.

Included in category V are intravenous, intraperitoneal, and intramuscular applications of phages.

Consideration of the many potential interactions between phages and human tissue has long been a subject of research, as recently reviewed (58, 99). We can divide up phage-human interactions into a number of areas: (i) Propensity for phages to bypass barriers to microbe invasion of body tissues (e.g., gastric juices, as considered above); (ii) propensity and mechanisms by which phages are actively removed from the body, particularly from systemic circulation; (iii) fate of phages that have been removed from circulation; (iv) potential for phages to infect body cells including exchanging DNA and expressing genes; and (v) interaction of phages with specific immunity, particularly in terms of antibody-mediated immunity as well as the phage potential to serve as allergens. Nevertheless, there is no evidence that even purposeful phage application to human bodies negatively impacts human health (49). Furthermore, there is no evidence that application of phages, even with systemic application, can harm treated animals (140, 141).

SUMMARY

Phage therapies generally are a safe means of combating the proliferation of dangerous and destructive bacteria. This report classifies phage therapies into five categories, distinguished by the likelihood and nature of phage-human contact. Category I is defined in terms of very low likelihoods of human contact with phage-containing environments. Categories III through V are defined in terms of increasing likelihood of invasive phage-human contact. Category II, by contrast, represents phage application for which phage-human contact could occur but, by design, such contact has been rendered unlikely. Though there exist numerous means by which phages can interact with human tissue or human normal flora, in fact there is no evidence that damage to human health can occur via any of these means. The feasibility of the application of procedures to protect the public from pathogens should be a risk/benefit assessment and if the benefit far outweighs the potential hazards then a procedure should be considered for adoption. As an alternative to chemical antibiotics for the removal of pathogenic bacteria, the high degree of safety associated with phage therapy suggests a low risk-to-benefit ratio.

Table 1: Recent Phage Isolations from the Human Environment[3]

Source	Bacteria host[4]	Ref.
bivalves	*Bacteroides fragilis, Escherichia coli*	(7, 21, 38, 40, 47, 87, 101, 103, 111, 117)
carrots	*Escherichia coli*	(51)
cheese	*Propionibacterium freudenreichii*	(56, 57)
kimchee	*Lactobacillus plantarum*	(174)
meat	*Campylobacter, Escherichia coli*	(10, 72)
sauerkraut	*Leuconostoc fallax*	(14, 173)
wine	*Oenococcus oeni* lysogens	(118)
yogurt	*Lactobacillus* spp. lysogens	(80)
dental plaque	*Actinomyces viscosus* (a.k.a., *Actinomyces naeslundii*)	(44, 156, 170)
feces	*Bacteroides fragilis, Escherichia coli*	(42, 55, 60)
saliva	*Enterococcus faecalis*	(11)
vagina	*Lactobacillus* spp. lysogens	(79)

[3] See also Ackermann (2), as adapted from (3), who lists among those environments in which phages have been isolated: "raw and skim milk, butter, butter milk, cheeses (Cheddar, cottage, Swiss), cheese starters (Bel Paese, Emmanthal, Gorgonzola, Mozzarella), cheese wheys, yogurt and yogurt starters… chicken, ground beef, meat starters, salami, steaks; fish fillets, fish sauce… lactic acid beverage, oysters, sake starter, spoiled cabbage, soy sauce mash, [and] wine" .

[4] "lysogens" indicated following host species indicates that these phages were isolated by inducing lysogens obtained from the described environment rather than via the isolation of free phages directly from the environment.

Table 2: Phage Therapy in the Literature as Sorted by Human-Contact Category[5]

Cat.	Year	Bacteria	Description	Ref.
I	1983	*Cyanobacteria*	Phage control of cyanobacteria	(45)
	1984	*Rhizobium trifolii*	Removal of bacteria from protoplast cultures of white clover	(61)
	2001	*Xanthomonas campestris*	Bacterial blight protection of geraniums	(53)
	1964, 1996	Various	Biocontrol agents of marine phytoplankton blooms (note, virus control agents, not necessarily phages)	(127)
	1984, 1989	Various	Use of phages to prevent microbiofouling of marine heat exchangers	(78, 128)
II	1991, 1993, 1995	*Pseudomonas tolaasii*	Control of bacterial blotch of cultivated mushrooms	(106-108)
	2000		Control of pustule disease in abalone	(152)
	1981, 2000	*Aeromonas hydrophila, Edwardsiella tarda*	Bacterial control in eels	(71, 169)
	2001	*Campylobacter jejuni*	Reduction bacteria load in chickens (broilers)	(159)
	1983, 1987	*Escherichia coli*	Control of diarrhea in calves, piglets, and lambs	(90, 140, 141)
	2003	*Escherichia coli*	Protection of poultry from respiratory infection	(73, 74)
	2001	*Escherichia coli*	Review: Phage therapy of cattle	(16)
	1998	*Escherichia coli*	Control of experimental infection of chickens and calves	(15)

[5] Note that we were more selective in the studies presented that were published prior to 1991, limiting ourselves particularly to contact categories I-III. That is, there are a number of pre-1991 phage-therapy studies (as well as a few non-English-language 1991 and newer studies that we could not decipher) that would be included in categories IV or V but which are not included in this table.

	1999	*Lactococcus garvieae*	Protection of disease in yellowtail fish	(110)
	2000	*Pseudomonas plecoglossicida*	Prevention of infection in cultures ayu fish	(113)
	1988	*Pseudomonas tolaasii*	Control of bacterial blotch of cultivated mushrooms	(65)
	1992	rhizobia	Protection of *Bradyrhizobium japonicum* soybean inocula	(18)
	2001	*Salmonella enterica*	Attempts to reduce bacterial load in chickens	(135)
	2001	*Salmonella typhimurium*	Reduction in pre-slaughter Salmonella load in pigs	(86)
	1991	*Salmonella typhimurium*	Reduction of chicken bacterial load and protection from disease	(20)
	2001	*Streptomyces scabies*	Prevention of scab formation via phage-treatment of potato seed tubers	(98)
	1987	*Vibrio anguillarum*	Control in milkfish overwintering ponds	(168)
	2000	*Xanthomonas axonopodis*	Bacterial leaf spot management of mungbean	(28)
	2000, 2002	*Xanthomonas campestris*	Bacterial spot protection of tomato	(12, 54)
	1994	*Xanthomonas campestris*	Protection of peach trees from fire blight	(176)
	1981	*Xanthomonas campestris*	Treatment of cabbage and pepper disease	(52)
	1969	*Xanthomonas pruni*	Protection of peach trees from bacterial spot	(41)
	2002, 2003	General	Review: Phage therapy in aquaculture	(109, 124)
	1976	General	Review: Phage therapy against phytopathogens	(157)
	1958	Various	Treatment of disease of tomato and cabbage	(66)
III	2002	*Brochothrix thermosphacta*	Treatment to prevent pork adipose tissue spoilage	(64)
	2002	*Listeria monocytogenes*	Treatment of raw beef with both phages and nisin	(50)
	1986, 1990	*Pseudomonas* spp.	Prevention (not always successful) of meat spoilage via application of phage	(62, 63)
	2001	*Salmonella enteritidis*	Reduction of bacterial load of fresh-cut fruit	(88)
	2001	*Salmonella enteritidis*	Reduction of bacterial load of cheese and raw milk	(102)
III?	1999	*Listeria monocytogenes*	Sanitization of surfaces (etc.) in food production	(69)

IV	1999	*Clostridium difficile*	Prevention of ileocecits in hamster model	(121)
	1992	*Escherichia coli*	Internal-organ dissemination following oral administration plus prevention of disease	(122)
	2000	*Helicobacter pylori*	Stomach colonization protection in mouse model	(33)
	1999	*Klebsiella pneumoniae*	Case study of oral phage administration to treat purulent meningitis of newborn	(144)
	1994	*Pseudomonas aeruginosa*	Protection of skin graphs in guinea pig model	(142)
	2002	*Pseudomonas* spp., etc.	Review: Phage therapy of burns	(5)
	1999	*Vibiro parahaemolyticus*	Reduction in bacterial load of phage-treated mice	(172)
	2002	Various	"PhagoBioDerm" would treatment	(96)
	2002	Various	Oral application of phages to humans to study neutrophil function and turnover	(163)
	2001	Various	Oral and local phage treatment of cancer-patient bacterial infections	(162)
	1999	Various	Oral and local application to treat imflammatory urologic diseases	(114)
V	2002	*Enterococcus faecium*	Treatment of experimental bacteremia in mouse model	(24)
	2002	*Escherichia coli*	Treatment of experimental infection of mouse model	(32)
	2002	*Escherichia coli*	Phage M13 delivery of DNA encoding bactericidal proteins to bacteria to treat mouse bacteremia	(164)
	1992	*Klebsiella spp.*	Intraperitoneal, internasal, and intravenous administration of phages to various animals as models for disease treatment	(25, 26)
	2002	*Mycobacterium tuberculosis* and *avium*	Trojan horse delivery of virulent phages into macrophages of virulent phage infecting *Mycobacterium smegmatis*	(31)
	2003	*Staphylococcus aureus*	Treatment of Intraperitoneal injections of mouse model	(97)
	2002	*Vibrio vulnificus*	Intravenous phage delivery to mouse model	(37)

Reference List

1. **Abedon, S. T.** 1990. University of Arizona. The Ecology of Bacteriophage T4.

2. **Ackermann, H.-W.** 1997. Bacteriophage ecology, p. 335-339. *In* M. T. Martins, M. I. Z. Sato, J. M. Tiedje, L. C. N. Hagler, J. Döbereiner, and P. S. Sanchez (eds.), Progress in Microbial Ecology (Proceedings of Seventh International Symposium on Microbial Ecology). Brazilian Society for Microbiology.

3. **Ackermann, H.-W. and M. S. DuBow**. 1987. Viruses of Prokaryotes, Volume 1, General Properties of Bacteriophages. CRC Press, Boca Raton, Florida.

4. **Adhya, S.** 2003. Phage therapy, *In* R. Calendar (ed.), The Bacteriophages. Oxford University Press, Oxford.

5. **Ahmad, S. I.** 2002. Treatment of post-burns bacterial infections by bacteriophages, specifically ubiquitous *Pseudomonas* spp. notoriously resistant to antibiotics. Med Hypotheses **58**:327-331.

6. **Ahmed, A. U. and D. L. Sorensen**. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environ.Res. **67**:143-150.

7. **Albert, M., C. Vannesson, and L. Schwartzbrod**. 1994. Recovery of somatic coliphages in shellfish. Water Sci.Technol. **31**:453-456.

8. **Alisky, J., K. Iczkowski, A. Rapoport, and N. Troitsky**. 1998. Bacteriophages show promise as antimicrobial agents. J.Infect. **36**:5-15.

9. **Ashelford, K. E., M. J. Day, and J. C. Fry**. 2003. Elevated abundance of bacteriophage infecting bacteria in soil. Appl.Environ.Microbiol. **69**:285-289.

10. Atterbury, R., Connerton, P., Dodd, C., Rees, C., and Connerton, I. Isolation and characterisation of *Campylobacter*-specific bacteriophage from retail poultry. International Journal of Medical Microbiology 291[Supplement 31], 79-80. 2001.
Ref Type: Abstract

11. **Bachrach, G., M. Leizerovici-Zigmond, A. Zlotkin, R. Naor, and D. Steinberg**. 2003. Bacteriophage isolation from human saliva. Lett.Appl.Microbiol. **36**:50-53.

12. **Balogh, B.** 2002. University of Florida. Strategies for improving the efficacy of bacteriophages for controlling bacterial spot of tomato.

13. **Barker, J., T. J. Humphrey, and M. W. Brown**. 1999. Survival of Escherichia coli O157 in a soil protozoan: implications for disease. FEMS Microbiol.Let. **173**:291-295.

14. **Barrangou, R., S. S. Yoon, F. J. Breidt, P. Fleming, and T. R. Klaenhammer**. 2002. Characterization of six *Leuconostoc fallax* bacteriophages isolated from an industrial sauerkraut fermentation. Appl.Environ.Microbiol. **68**:5452-5458.

15. **Barrow, P., M. Lovell, and A. jr. Berchieri**. 1998. Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. Clin.Diag.Lab.Immunol. **5**:294-298.

16. **Barrow, P. A.** 2001. The use of bacteriophages for treatment and prevention of bacterial disease in animals and animal models of human infection. J.Chem.Technol.Biotechnol. **76**:677-682.

17. **Barrow, P. A. and J. S. Soothill**. 1997. Bacteriophage therapy and prophylaxis: Rediscovery and renewed assessment of potential. Trends in Genetics **5**:268-271.

18. **Basit, H. A., J. S. Angle, S. Salem, and E. M. Gewaily**. 1992. Phage coating of soybean seed reduces nodulation by indigenous soil bradyrhizobia. Can.J.Microbiol. **38**:1264-1269.

19. **Bausum, H. T., S. A. Schaub, K. F. Kenyon, and M. J. Small**. 1982. Comparison of coliphage and bacterial aerosols at a wastewater spray irrigation site. Appl.Environ.Microbiol. **43**:28-38.

20. **Berchieri, A., M. A. Lovell, and P. A. Barrow**. 1991. The activity in the chicken alimentary tract of bacteriophages lytic for *Salmonella typhimurium*. Res.Microbiol. **142**:541-549.

21. **Beril, C., J. M. Crance, F. Leguyader, V. Apaire-Marchais, F. Leveque, M. Albert, M. A. Goraguer, L. Schwartzbrod, and S. Billaudel**. 1996. Study of viral and bacterial indicators in cockles and mussels. Marine Pollution Bulletin **32**:404-409.

22. **Bettarel, Y., T. Sime-Ngando, C. Amblard, and H. Laveran**. 2000. A comparison of methods for counting viruses in aquatic systems. Appl.Environ.Microbiol. **66**:2283-2289.

23. **Binetti, A. G. and J. A. Reinheimer**. 2000. Thermal and chemical inactivation of indigenous *Streptococcus thermophilus* bacteriophages isolated from Argentinian dairy plants. J.Food Prot. **63**:509-515.

24. **Biswas, B., S. Adhya, P. Washart, B. Paul, A. N. Trostel, B. Powell, R. Carlton, and C. R. Merril**. 2002. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect.Immun. **70**:204-210.

25. **Bogovazova, G. G., N. N. Voroshilova, and V. M. Bondarenko**. 1991. Effektivnost' bakteriofaga Klebsiella pneumoniae pri terapii eksperimental'noi klebsielleznoi infektsii.[The efficacy of Klebsiella pneumoniae bacteriophage in

the therapy of experimental Klebsiella infection]. [Russian]. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii **4**:5-8.

26. **Bogovazova, G. G., N. N. Voroshilova, V. M. Bondarenko, G. A. Gorbatkova, Afanas'eva, EV, T. B. Kazakova, V. D. Smirnov, A. G. Mamleeva, I. Glukharev, and E. I. Erastova**. 1992. [Immunobiological properties and therapeutic effectiveness of preparations from Klebsiella bacteriophages]. [Russian]. Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii 30-33.

27. **Bonadonna, L., L. S. Della, E. Veschetti, D. Cutilli, M. Ottaviani, M. Divizia, D. Donia, R. Gabrieli, A. Pana, C. Martini, and P. Anastasi**. 1999. Reduction of microorganisms in sewage effluent using hypochlorite and peracetic acid as disinfectants. Central European Journal of Public Health **7**:130-132.

28. **Borah, P. K., J. K. Jindal, and J. P. Verma**. 2000. Integrated management of bacterial leaf spot of mungbean with bacteriophages of Xav and chemicals. J.Mycol.Plant Pathol. **30**:19-21.

29. **Brenner, K. P., P. V. Scarpino, and C. S. Clark**. 1988. Animal viruses, coliphages, and bacteria in aerosols and wastewater at a spray irrigation site. Appl.Environ.Microbiol. **54**:409-415.

30. **Brion, G. M., J. S. Meschke, and M. D. Sobsey**. 2002. F-specific RNA coliphages: occurrence, types, and survival in natural waters. Water Res. **36**:2419-2425.

31. **Broxmeyer, L., D. Sosnowskai, E. Miltner, O. Chacón, D. Wagner, J. McGarvey, R. G. Barletta, and L. E. Bermudez**. 2002. Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a nonvirulent mycobacterium: A model for phage therapy of intracellular bacterial pathogens. J.Infect.Dis. **186**:1155-1160.

32. **Bull, J. J., B. R. Levin, T. DeRouin, N. Walker, and C. A. Bloch**. 2002. Dynamics of success and failure in phage and antibiotic therapy in experimental infections. BMC Microbiol **2**:35.

33. **Cao, J., Y. Sun, T. Berglindh, B. Mellgard, Z. Li, B. Mardh, and S. Mardh**. 2000. *Helicobacter pylori*-antigen-binding fragments expressed on the filamentous M13 phage prevent bacterial growth. Biochim.Biophys.Acta **1474**:107-113.

34. **Carducci, A., S. Arrighi, and A. Ruschi**. 1995. Detection of coliphages and enteroviruses in sewage and aerosol from an activated sludge wastewater treatment plant. Lett.Appl.Microbiol. **21**:207-209.

35. **Carlton, R. M.** 1999. Phage therapy: past history and future prospects. Archivum Immunologii et Therapiae Experimentalis **47**:267-274.

36. **Castillo, G., M. P. Mena, F. Dibarrart, and G. Honeyman**. 2001. Water quality improvement of treated wastewater by intermittent soil percolation. Water Sci.Technol. **43**:187-190.

37. **Cerveny, K. E., A. Depaola, D. H. Duckworth, and P. A. Gulig**. 2002. Phage therapy of local and systemic disease caused by *Vibrio vulnificus* in iron-dextran-treated mice. Infect.Immun. **70**:6251-6262.

38. **Chai, T.-J., T.-J. Han, and R. R. Cockey**. 1994. Microbiological quality of shellfish-growing waters in Chesapeake Bay. J.Food Prot. **57**:229-234.

39. **Chanishvili, N., T. Chanishvili, M. Tediashvili, and P. A. Barrow**. 2001. Phages and their application against drug-resistant bacteria. J.Chem.Technol.Biotechnol. **76**:689-699.

40. **Chung, H., L. A. Jaykus, G. Lovelace, and M. D. Sobsey**. 1998. Bacteriophages and bacteria as indicators of enteric viruses in oysters and their harvest waters. Water Sci.Technol. **38**:37-44.

41. **Civerolo, E. L. and H. L. Kiel**. 1969. Inhibition of bacterial spot of peach foliage by *Xanthomonas pruni* bacteriophage. Phytopathology **59**:1966-1967.

42. **Cornax, R., M. A. Morinigo, F. Gonzalez-Jaen, A. Carmen, and J. J. Borrego**. 1994. Bacteriophages presence in human faeces of healthy subjects and patients with gastrointestinal disturbances. Zentralbl.Bakteriol. **281**:214-224.

43. **d'Herelle, F.** 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. C.R.Acad.Sci.Ser.D **165**:373.

44. **Delisle, A. L. and J. A. Donkersloot**. 1995. Relationships among *Actinomyces naeslundii* (*A. viscosus*) bacteriophages isolated from sewage and the oral cavity. Microbial Ecology in Health & Disease **8**:121-127.

45. **Desjardins, P. R. and G. B. Olson**. 1983. Viral Control of Nuisance Cyanobacteria (Blue-Green Algae). II. Cyanophage Strains, Stability on Phages and Hosts, and Effects of Environmental Factors on Phage-Host Interactions. California Water Resource Center, University of California, Davis, CA.

46. **Doerfler, W., O. Grtraud, S. Rainer, F. Katja, H. Hilde, W. Petra, and S. Jörg**. 2003. On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. Gene **157**:241-245.

47. **Dore, W. J., M. Mackie, and D. N. Lees**. 2003. Levels of male-specific RNA bacteriophage and *Escherichia coli* in molluscan bivalve shellfish from commercial harvesting areas. Lett.Appl.Microbiol. **36**:92-96.

48. **Duckworth, D. H.** 1976. "Who discovered bacteriophage?". Bacteriol.Rev. **40**:793-802.

49. **Duckworth, D. H. and P. A. Gulig**. 2002. Bacteriophages: potential treatment for bacterial infections. BioDrugs **16**:57-62.

50. **Dykes, G. A. and S. M. Moorhead**. 2002. Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. Int.J.Food Microbiol. **73**:71-81.

51. **Endley, S., L. Lu, E. Vega, M. E. Hume, and S. D. Pillai**. 2003. Male-specific coliphages as an additional fecal contamination indicator for screening fresh carrots. J.Food Prot. **66**:88-93.

52. **Filho, A. B. and H. Kimati**. 1981. Studies on a bacteriophage isolated from *Xanthomonas campestris* 2. Its use in the control of *Xanthomonas campestris* and *Xanthomonas campestris vesicatoria*. Summa Phytopathol. **7**:35-43.

53. **Flaherty, J. E., B. K. Harbaugh, J. B. Jones, G. C. Somodi, and L. E. Jackson**. 2001. H-mutant bacteriophages as a potential biocontrol of bacterial blight of geranium. Hortscience **36**:98-100.

54. **Flaherty, J. E., J. B. Jones, B. K. Harbaugh, G. C. Somodi, and L. E. Jackson**. 2000. Control of bacterial spot on tomato in the greenhouse and field with h-mutant bacteriophages. Hortscience **35**:882-884.

55. **Gantzer, C., J. Henny, and L. Schwartzbrod**. 2002. *Bacteroides fragilis* and *Escherichia coli* bacteriophages in human faeces. International Journal of Hygiene and Environmental Health **205**:325-328.

56. **Gautier, M., A. Rouault, C. Herve, P. Sommer, V. eret, G. Jan, J. M. Fraslin, F. Prevot, and A. Coste**. 1999. Bacteriophages of dairy propionibacteria. Lait **79**:93-104.

57. **Gautier, M., A. Rouault, P. Sommer, and R. Briandet**. 1995. Occurrence of *Propionibacterium freudenreichii* bacteriophages in swiss cheese. Appl.Environ.Microbiol. **61**:2572-2576.

58. **Gorski, A., K. Dabrowska, K. Switala-Jele, M. Nowaczyk, B. Weber-Dabrowska, J. Boratynski, J. Wietrzyk, and A. Opolski**. 2003. New insights into the possible role of bacteriophages in host defense and disease. Medical Immunology **2**:2.

59. **Govan, J. R., J. E. Hughes, and P. Vandamme**. 1996. Burkholderia cepacia: medical, taxonomic and ecological issues. J.Med.Microbiol. **45**:395-407.

60. **Grabow, W. O. K., T. W. Neubrech, C. S. Holtzhausen, and J. Jofre**. 1995. *Bacterioides fragilis* and *Escherichia coli* bacteriophages—Excretion by humans and animals. Water Sci.Technol. **31**:223-230.

61. **Graves, D. A. and R. W. Beck**. 1984. The use of a lytic bacteriophage to remove *Rhizobium trifolii* from protoplast culture of *Trifolium repens*. Plant Sci.Lett. **34**:385-389.

62. **Greer, G. G.** 1986. Homologous bacteriophage control of *Pseudomonas* growth and beef spoilage. J.Food Prot. **49**:104-109.

63. **Greer, G. G. and B. D. Dilts**. 1990. Inability of a bacteriophage pool to control beef spoilage. Int.J.Food Microbiol. **10**:331-342.

64. **Greer, G. G. and B. D. Dilts**. 2002. Control of *Brochothrix thermosphacta* spoilage of pork adipose tissue using bacteriophages. J.Food Prot. **65**:861-863.

65. **Guillaumes, J., G. Houdeau, R. Germain, and J. M. Olivier**. 1988. Improvement of the biocontrol of *Pseudomonas tolaasii* using bacteriophages associated with an antagonistic bacterium. Bull.OEPP **18**:77-82.

66. **Gutermuth, C.** 1958. Kent State University. A study of the action of bacteriophage on some diseases of tomato and cabbage.

67. **Hann, A. C., V. Baubet, and R. Perrin**. 1998. Efficacy and mechanisms of action of sodium hypochlorite on *Pseudomonas aeruginosa* PAO1 phage F116. J.Appl.Microbiol. **85**:925-932.

68. **Hendrix, R. W.** 2002. Bacteriophages: evolution of the majority. Theor.Pop.Biol. **61**:471-480.

69. **Hibma, A. M., S. A. Jassim, and M. W. Griffiths**. 1997. Infection and removal of L-forms of *Listeria monocytogenes* with bred bacteriophage. Int.J.Food Microbiol. **34**:197-207.

70. **Ho, K.** 2001. Bacteriophage therapy for bacterial infections: Rekindling a memory from the pre-antibiotics era. Per.BiologyMed. **44**:1-16.

71. **Hsu, C. H., C. Y. Lo, J. K. Liu, and C. S. Lin**. 2000. Control of the eel (*Anguilla japonica*) pathogens, *Aeromonas hydrophila* and *Edwardsiella tarda*, by bacteriophages. J.Fisheries Soc.Taiwan **27**:21-31.

72. **Hsu, F.-C., Y. S. C. Shieh, and M. D. Sobsey**. 2002. Enteric bacteriophages as potential fecal indicators in ground beef and poultry meat. J.Food Prot. **65**:93-99.

73. **Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue**. 2002. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. Poult Sci **81**:1486-1491.

74. **Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, H. Xie, P. A. J. Moore, and A. M. Donoghue**. 2002. Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). Poult Sci **81**:437-441.

75. **Intralytix Inc.** 2002. Food additive petition for: Control of pathogens on meat by use of a mixture of bacteriophages. Federal Register **67**:47823.

76. **Jette, L. P. and S. Lapierre**. 1992. Evaluation of a mechanical/chemical infectious waste disposal system [see comments]. Infection control and hospital epidemiology **13**:387-393.

77. **Kajiura, T., M. Tanaka, H. Wada, K. Ito, Y. Koyama, and F. Kato**. 2001. Effects of disinfectants on Shiga-like toxin converting phage from enterohemorrhagic *Escherichia coli* O157 : H7. J.Health Sci. **47**:203-207.

78. **Kaminura, K. and M. Araki**. 1984. Control of microbiofouling formed on the heat exchanger by bacteriophage, p. 40-46. *In* Proceedings of the Pacific Congress on Marine Technology (Honolulu, Hawaii).

79. **Kilic, A. O., S. I. Pavlova, S. Alpay, S. S. Kilic, and L. Tao**. 2001. Comparative study of vaginal *Lactobacillus* phages isolated from women in the United States and Turkey: prevalence, morphology, host range, and DNA homology. Clin.Diag.Lab.Immunol. **8**:31-39.

80. **Kilic, A. O., S. I. Pavlova, W. G. Ma, and L. Tao**. 1996. Analysis of *Lactobacillus* phages and bacteriocins in American dairy products and characterization of a phage isolated from yogurt. Appl.Environ.Microbiol. **62**:2111-2116.

81. **Krylov, V.** 2002. Phagotherapy: myths and realities. Russian Academy of Sciences Presidium—Science in Russia **4**:40-46.

82. **Krylov, V. N.** 2001. Phage therapy in terms of bacteriophage genetics: Hopes, prospects, safety, limitations. Russian Journal of Genetics **37**:715-730.

83. **Kurtböke, D. I., C. F. Chen, and S. T. Williams**. 1992. Use of polyvalent phage for reduction of streptomycetes on soil dilution plates. J.Appl.Bacteriol. **72**:103-111.

84. **Kwaik, A., L. Y. Gao, B. J. Stone, C. Venkataraman, and O. S. Harb**. 1998. Invasion of protozoa by Legionella pneumophila and its role in bacterial ecology and pathogenesis. Appl.Environ.Microbiol. **64**:3127-3133.

85. **Lashley, F. R.** 2002. Emerging/reemerging diseases by organisms, p. 389-420. *In* F. R. Lashley and J. D. Durham (eds.), Emerging Infectious Diseases. Trends and Issues. Springer Publishing Co., New York.

86. **Lee, N. and D. L. Harris**. 2001. The effect of bacteriophage treatment to reduce the rapid dissemination of *Salmonella typhimurium* in pigs. Proceedings of the American Association of Swine Veterinarians **32**:555-557.

87. **Legnani, P., E. Leoni, D. Lev, R. Rossi, G. C. Villa, and P. Bisbini**. 1998. Distribution of indicator bacteria and bacteriophages in shellfish and shellfish-growing waters. J.Appl.Microbiol. **85**:790-798.

88. **Leverentz, B., W. S. Conway, Z. Alavidze, W. J. Janisiewicz, Y. Fuchs, M. J. Camp, E. Chighladze, and A. Sulakvelidze**. 2001. Examination of bacteriophage as a biocontrol method for salmonella on fresh-cut fruit: a model study. J.Food Prot. **64**:1116-1121.

89. **Levin, B. R. and R. Antia**. 2001. Why don't we get sick? The within-host population dynamics of bacterial infections. Science **292**:1112-1115.

90. **Levin, B. R. and J. J. Bull**. 1996. Phage therapy revisited: the population biology of a bacterial infection and its treatment with bacteriophage and antibiotics. Am.Nat. **147**:881-898.

91. **Liu, P. V.** 2003. Benign bacteria in blood. ASM News **69**:265.

92. **Lorch, A.** 1999. Bacteriophages: An altertnative to antibiotics? Biotechnology and Development Monitor **39**:14-17.

93. **Lukasik, J., M. L. Bradley, T. M. Scott, M. Dea, A. Koo, W. Y. Hsu, J. A. Bartz, and S. R. Farrah**. 2003. Reduction of poliovirus 1, bacteriophages, *Salmonella montevideo*, and *Escherichia coli* O157:H7 on strawberries by physical and disinfectant washes. J.Food Prot. **66**:188-193.

94. **Maciorowski, K. G., S. D. Pillai, and S. C. Ricke**. 2001. Presence of bacteriophages in animal feed as indicators of fecal contamination. Journal of Environmental Science and Health Part B Pesticides **36**:699-708.

95. **Maillard, J. Y., A. C. Hann, V. Baubet, and R. Perrin**. 1998. Efficacy and mechanisms of action of sodium hypochlorite on *Pseudomonas aeruginosa* PAO1 phage F116. J.Appl.Microbiol. **85**:925-932.

96. **Markoishvili, K., G. Tsitlanadze, R. Katsarava, J. G. Morris, Jr., and A. Sulakvelidze**. 2002. A novel sustained-release matrix based on biodegradable poly(ester amide)s and impregnated with bacteriophages and an antibiotic shows promise in management of infected venous stasis ulcers and other poorly healing wounds. Int J Dermatol **41**:453-458.

97. **Matsuzaki, S., M. Yasuda, H. Nishikawa, M. Kuroda, T. Ujihara, T. Shuin, Y. Shen, Z. Jin, S. Fujimoto, M. D. Nasimuzzaman, H. Wakiguchi, S. Sugihara, T. Sugiura, S. Koda, A. Muraoka, and S. Imai**. 2003. Experimental protection of mice against lethal *Staphylococcus aureus* infection by novel bacteriophage phi MR11. J.Infect.Dis. **187**:613-624.

98. **McKenna, F., K. A. El-Tarabily, G. E. S. T. Hardy, and B. Dell**. 2001. Novel in vivo use of a polyvalent *Streptomyces* phage to disinfest *Streptomyces scabies*-infected seed potatoes. Plant Pathol. **50**:666-675.

99. **Merril, C. R., D. Scholl, and S. L. Adhya**. 2003. The prospect for bacteriophage therapy in Western Medicine. Nature Reviews **2**:489-497.

100. **Milstien, J. B., J. R. Walker, and J. C. Petricciani**. 1977. Bacteriophages in live virus vaccines: lack of evidence for effects on the genome of rhesus monkeys. Science **197**:469-470.

101. **Miossec, L., F. Le Guyader, D. Pelletier, L. Haugarreau, M. P. Caprais, and M. Pommepuy**. 2001. Validity of Escherichia coli, enterovirus, and F-specific RNA bacteriophages as indicators of viral shellfish contamination. J.Shelfish Res. **20**:1223-1227.

102. **Modi, R., Y. Hirvi, A. Hill, and M. W. Griffiths**. 2001. Effect of phage on survival of *Salmonella enteritidis* during manufacture and storage of cheddar cheese made from raw and pasteurized milk. J.Food Prot. **64**:927-933.

103. **Muniain-Mujika, I., R. Girones, and F. Lucena**. 2000. Viral contamination of shellfish: Evaluation of methods and analysis of bacteriophages and human viruses. J.Virol.Meth. **89**:109-118.

104. **Muniesa, M. and J. Jofre**. 1998. Abundance in sewage of bacteriophages that infect *Escherichia coli* 0157:H7 and that carry the Shiga toxin 2 gene. Appl.Environ.Microbiol. **64**:2443-2448.

105. **Muniesa, M. and J. Jofre**. 2000. Occurrence of phages infecting *Escherichia coli* O157:H7 carrying the Stx 2 gene in sewage from different countries. FEMS Microbiol.Let. **183**:197-200.

106. **Munsch, P., J. L. OLIVIER, and G. Houdeau**. 1991. Experimental control of bacterial blotch by bacteriophages, p. 389-396. *In* M. J. Maher (ed.), Mushroom Science. Rotterdam, Netherlands.

107. **Munsch, P. and J. M. Olivier**. 1995. Biocontrol of bacterial blotch of the cultivated mushroom with lytic phages: Some practical considerations, p. 595-602. *In* T. J. Elliott (ed.), Science and Cultivation of Edible Fungi, Vol. II: Proceedings of the 14th International Congress.

108. **Munsch, P., J. M. Olivier, B. Fritig, and M. E. Legrand**. 1993. Biological control of mushroom bacterial blotch with bacteriophages. Developments in Plant Pathology 469.

109. **Nakai, T. and S. C. Park**. 2002. Bacteriophage therapy of infectious disease in aquaculture. Res.Microbiol. **153**:13-18.

110. **Nakai, T., R. Sugimoto, K.-H. Park, S. Matsuoka, K. Mori, T. Nishioka, and K. Maruyama**. 1999. Protective effects of bacteriophage on experimental *Lactococcus garvieae* infection in yellowtail. Dis.Aquat.Org. **37**:33-41.

111. **Nanni, H., L. Bronzetti, G. Fabio, M. Pupillo, and P. Quaglio**. 2000. Microbiological survey of shellfish. Ig.Mod. **114**:113-127.

112. **Parada, J. L. and S. V. De Fabrizio**. 2001. [Stability of *Lactococcus lactis* phages treated with sodium hypochlorite and during storage]. Revista Argentina de Microbiologia **33**:89-95.

113. **Park, S. C., I. Shimamura, M. Fukunaga, K. I. Mori, and T. Nakai**. 2000. Isolation of bacteriophages specific to a fish pathogen, *Pseudomonas plecoglossicida*, as a candidate for disease control. Appl.Environ.Microbiol. **66**:1416-1422.

114. **Perepanova, T. S., O. S. Darbeeva, G. A. Kotliarova, E. M. Kondrat'eva, L. M. Maiskaia, V. F. Malysheva, F. A. Baiguzina, and N. V. Grishkova**. 1995. [The efficacy of bacteriophage preparations in treating inflammatory urologic diseases]. [Russian]. Urologiia i Nefrologiia 14-17.

115. **Petricciani, J. C., T. C. Hsu, A. D. Stock, J. H. Turner, S. L. Wenger, and B. L. Elisberg**. 1978. Bacteriophages, vaccines, and people: an assessment of risk. Proc.Soc.Exp.Biol.Med. **158**:378-382.

116. **Petterson, S. R., P. F. Teunis, and N. J. Ashbolt**. 2001. Modeling virus inactivation on salad crops using microbial count data. Risk Anal **21**:1097-1108.

117. **Pina, S., M. Puig, F. Lucena, J. Jofre, and R. Girones**. 1998. Viral pollution in the environment and in shellfish: Human adenovirus detection by PCR as an index of human viruses. Appl.Environ.Microbiol. **64**:3376-3382.

118. **Poblet-Icart, M., A. Bordons, and A. Lonvaud-Funel**. 1998. Lysogeny of *Oenococcus oeni* (syn. *Leuconostoc oenos*) and study of their induced bacteriophages. Curr.Microbiol. **36**:365-369.

119. **Quiberoni, A.** 1999. Inactivation of *Lactobacillus helveticus* bacteriophages by thermal and chemical treatments. J.Food Prot. **62**:894-898.

120. Radetsky, P. The good virus. The use of bacteriophages to fight antibiotic-resistant bacterial diseases. Discover Magazine 17[11], 50-58. 1996.
Ref Type: Magazine Article

121. **Ramesh, V., J. A. Fralick, and R. D. Rolfe**. 1999. Prevention of Clostridium difficile-induced ileocecitis with bacteriophage. Anaerobe **5**:69-78.

122. **Reynaud, A., L. Cloastre, J. Bernard, H. Laveran, H. W. Ackermann, D. Licois, and B. Joly**. 1992. Characteristics and diffusion in the rabbit of a phage

for Escherichia coli 0103. Attempts to use this phage for therapy. Vet Microbiol **30**:203-212.

123. **Rohwer, F. and R. Edwards**. 2002. The Phage Proteomic Tree: a genome-based taxonomy for phage. J.Bacteriol. **184**:4529-4535.

124. **Ronda, C., M. Vázquez, and R. López**. 2003. Los bacteriófagos como herramienta para combatir infecciones en acuicultura [abstract is in English, manuscript is in Spanish]. Revista AquaTIC **18**:3-10.

125. **Rose, J. B., D. E. Huffman, K. Riley, S. R. Farrah, J. O. Lukasik, and C. L. Hamann**. 2001. Reduction of enteric microorganisms at the Upper Occoquan Sewage Authority Water Reclamation Plant. Water Environ.Res. **73**:711-720.

126. **Rossi, P., N. Doerfliger, K. Kennedy, I. Müller, and M. Aragno**. 1998. Bacteriophages as surface and ground water tracers. Hydr.Earth Syst.Sci. **2**:101-110.

127. **Safferman, R. S. and M. E. Morris**. 1964. Control of algae with viruses. J.Am.Water Works Assoc. **56**:1217-1224.

128. **Sakaguchi, I., K. Shinshima, K. Kawaratani, and O. Sugai**. 1989. Control of microbiofouling using bacteriophage 2. Detection of phages and fundamental study of their lytic effect on fouling bacteria. Denryoku Chuo Kenkyusho Hokoku 1-32.

129. **Schaper, M., A. E. Duran, and J. Jofre**. 2002. Comparative resistance of phage isolates of four genotypes of f-specific RNA bacteriophages to various inactivation processes. Appl.Environ.Microbiol. **68**:3702-3707.

130. **Schubbert, R., U. Hohlweg, D. Renz, and W. Doerfler**. 1998. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Mol.Gen.Genet. **259**:569-576.

131. **Sharp, R. J.** 2001. Bacteriophages: biology and history. J.Chem.Technol.Biotechnol. **76**:667-672.

132. **Shaw, D. R., A. T. Maurelli, J. D. Goguen, S. C. Straley, and R. I. Curtiss**. 1983. Use of UV-irradiated bacteriophage T6 to kill extracellular bacteria in tissue culture infectivity assays. J.Immunol.Meth. **56**:75-83.

133. **Sinton, L. W., R. K. Finlay, and P. A. Lynch**. 1999. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. Appl.Environ.Microbiol. **65**:3605-3613.

134. **Sinton, L. W., C. H. Hall, P. A. Lynch, and R. J. Davies-Colley**. 2002. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste

stabilization pond effluent in fresh and saline waters. Appl.Environ.Microbiol. **68**:1122-1131.

135. **Sklar, I. B. and R. D. Joerger**. 2001. Attempts to utilize bacteriophage to combat *Salmonella enterica* serovar Enteritidis infection in chickens. Journal of Food Safety **21**:15-29.

136. **Slopek, S., A. Kucharewicz-Krukowska, B. Weber-Dabrowska, and M. Dabrowski**. 1985. Results of bacteriophage treatment of suppurative bacterial infections. IV. Evaluation of results obtained in 370 cases. Arch.Immunol.Ther.Exp. **33**:219-240.

137. **Slopek, S., A. Kucharewicz-Krukowska, B. Weber-Dabrowska, and M. Dabrowski**. 1985. Results of bacteriophage treatment of suppurative bacterial infections. V. Evaluation of the results obtained in children. Archivum Immunologii et Therapiae Experimentalis **33**:241-259.

138. **Slopek, S., A. Kucharewicz-Krukowska, B. Weber-Dabrowska, and M. Dabrowski**. 1985. Results of bacteriophage treatment of suppurative bacterial infections. VI. Analysis of treatment of suppurative staphylococcal infections. Archivum Immunologii et Therapiae Experimentalis **33**:261-273.

139. **Slopek, S., B. Weber-Dabrowska, M. Dabrowski, and A. Kucharewicz-Krukowska**. 1987. Results of bacteriophage treatment of suppurative bacterial infections in the years 1981-1986. Arch.Immunol.Ther.Exp. **35**:569-583.

140. **Smith, H. W. and M. B. Huggins**. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J.Gen.Microbiol. **129**:2659-2675.

141. **Smith, H. W., M. B. Huggins, and K. M. Shaw**. 1987. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. J.Gen.Microbiol. **133**:1111-1126.

142. **Soothill, J. S.** 1994. Bacteriophage prevents destruction of skin grafts by *Pseudomonas aeruginosa*. Burns **20**:209-211.

143. **Stokes, G. V.** 2002. Microbial resistance to antibiotics, p. 23-42. *In* F. R. Lashley and J. D. Durham (eds.), Emerging Infectious Diseases. Trends and Issues. Springer Publishing Co., New York.

144. **Stroj, L., B. Weber-Dabrowska, K. Partyka, M. Mulczyk, and M. Wojcik**. 1999. Skuteczne zastosowanie bakteriofagoterapii w ropnym zapaleniu opon mozgowo-rdzeniowych u noworodka. [Successful treatment with bacteriophage in purulent cerebrospinal meningitis in a newborn]. Neurologia I Neurochirurgia Polska **33**:693-698.

145. **Suarez, V. B. and J. A. Reinheimer**. 2002. Effectiveness of thermal treatments and biocides in the inactivation of Argentinian *Lactococcus lactis* phages. J.Food Prot. **65**:1756-1759.

146. **Sulakvelidze, A., Z. Alavidze, and J. G. Morris, Jr.** 2001. Bacteriophage therapy. Antimicrob.Agents Chemother. **45**:649-659.

147. **Sulakvelidze, A. and J. G. Morris, Jr.** 2001. Bacteriophages as therapeutic agents. Ann.Med. **33**:507-509.

148. **Summers, W. C.** 1999. Felix d'Herelle and the Origins of Molecular Biology. Yale University Press, New Haven, Connecticut.

149. **Summers, W. C.** 2001. Bacteriophage therapy. Ann.Rev.Microbiol. **55**:437-451.

150. **Suttle, C. A., F. Chen, and A. M. Chan**. 1992. Marine viruses: Decay rates, diversity and ecological implications, p. 153-163. *In* C. C. Nash, II (ed.), International Marine Biotechnology Conferences "IMBC-91": Short Communications of Invited Lectures. W. Brown Co., Dubuque.

151. **Swanson, M. S. and B. K. Hammer**. 2000. Legionella pneumophila pathogesesis: a fateful journey from amoebae to macrophages. Ann.Rev.Microbiol. **54**:567-613.

152. **Tai-wu, L., J. Xiang, and R. Liu**. 2000. Studies on phage control of pustule disease in abalone *Haliotis discus hannai*. J.Shelfish Res. **19**:535.

153. **Tanji, Y., K. Mizoguchi, T. Akitsu, M. Morita, K. Hori, and H. Unno**. 2002. Fate of coliphage in waste water treatment process and detection of phages carrying the Shiga toxin type 2 gene. Water Sci.Technol. **46**:285-289.

154. **Tanji, Y., K. Mizoguchi, M. Yoichi, M. Morita, N. Kijima, H. Kator, and H. Unno**. 2003. Seasonal change and fate of coliphages infected to *Escherichia coli* O157:H7 in a wastewater treatment plant. Water Res. **37**:1136-1142.

155. **Turner, M. S.** 2000. More than meets the eye. The Sciences **Nov/Dec**:32-37.

156. **Tylenda, C. A., C. Calvert, P. E. Kolenbrander, and A. Tylenda**. 1985. Isolation of Actinomyces bacteriophage from human dental plaque. Infect.Immun. **49**:1-6.

157. **Vidaver, A. K.** 1976. Prospects for control of phytopathogenic bacteria by bacteriophage and bacteriocins. Ann.Rev.Phytopathol. **14**:451-465.

158. **Vieu, J.-F., F. Guillermet, R. Minck, and P. Nicolle**. 1979. Données actuelles sur les applications thérapeutiques des bactériophages. Bull.Acad.Natl.Med. **163**:61.

159. Wagenaar, J. A., Van Bergen, M. A. P., Mueller, M. A., Monie, K., and Carlton, R. M. Phage therapy of *Campylobacter jejuni* colonization in broilers. International Journal of Medical Microbiology 291[Supplement 31], 92-93. 2001.
Ref Type: Abstract

160. **Wagner, P. L. and M. K. Waldor**. 2002. Bacteriophage control of bacterial virulence. Infect.Immun. **70**:3985-3993.

161. Walter, M. H., Baker, D. D., and Timmerman, S. L. Destroying bacterial bio-warfare agents: Growth, stability and genetic characterization of bacteriophage CP-51, lytic on Bacillus anthracis and B. cereus. Abstracts of the General Meeting of the American Society for Microbiology 100, 448. 2000.
Ref Type: Abstract

162. **Weber-Dabrowska, B., M. Mulczyk, and A. Górski**. 2001. Bacteriophage therapy for infections in cancer patients. Clinical and Applied Immunology Reviews **1**:131-134.

163. **Weber-Dabrowska, B., M. Zimecki, M. Mulczyk, and A. Gorski**. 2002. Effect of phage therapy on the turnover and function of peripheral neutrophils. FEMS Immunol.Med.Microbiol. **34**:135-138.

164. **Westwater, C., L. M. Kasman, D. A. Schofield, P. A. Werner, J. W. Dolan, M. G. Schmidt, and J. S. Norris**. 2003. Use of genetically engineered phage to deliver antimicrobial agents to bacteria: an alternative therapy for treatment of bacterial infections. Antimicrob.Agents Chemother. **47**:1301-1307.

165. **Wilhelm, S. W., M. G. Weinbauer, C. A. Suttle, and W. H. Jeffrey**. 1998. The role of sunlight in the removal and repair of viruses in the sea. Limnol.Oceanogr. **43**:586-592.

166. **Wommack, K. E. and R. R. Colwell**. 2000. Virioplankton: viruses in aquatic ecosystems. Microbiol.Mol.Biol.Rev. **64**:69-114.

167. **Wommack, K. E., R. T. Hill, T. A. Muller, and R. R. Colwell**. 1996. Effects of sunlight on bacteriophage viability and structure. Appl.Environ.Microbiol. **62**:1336-1341.

168. **Wu, J. L. and W. J. Chao**. 1987. The epizootic of milkfish vibriosis and its biological control by bacteriophage AS10, *In* K. S. Kou, J. L. Wu, Y. L. Hsu, S. N. Chen, M. C. Tung, I. C. Liao, and H. Y. Chung (eds.), The Memoir of Virology and Pharmacology in Fish Disease. 3. Taipei.

169. **Wu, J. L., H. M. Lin, L. Jan, Y. L. Hsu, and L. H. Chang**. 1981. Biological Control of Fish Bacterial Pathogen, *Aeromonas hydrophila* by Bacteriophage AH 1. Fish Pathol. **15**:271-276.

170. **Yeung, M. K. and C. S. Kozelsky**. 1997. Transfection of *Actinomyces* spp. by genomic DNA of bacteriophages from human dental plaque. Plasmid **37**:141-153.

171. **Yip, Y. L., N. J. Hawkins, G. Smith, and R. L. Ward**. 1999. Biodistribution of filamentous phage-Fab in nude mice. J.Immunol.Meth. **225**:171-178.

172. **Yoon, S. O., S. A. Ju, M. S. Heo, C. R. Jung, and J. W. Ju**. 1999. Characterization of a *Vibrio parahaemolyticus* phage isolated from marine. Journal of the Korean Society for Microbiology **34**:423-433.

173. **Yoon, S. S., R. Barrangou-Poueys, F. Breidt, Jr., T. R. Klaenhammer, and H. P. Fleming**. 2002. Isolation and characterization of bacteriophages from fermenting sauerkraut. Appl.Environ.Microbiol. **68**:973-976.

174. **Yoon, S. S., J. W. Kim, F. Breidt, and H. P. Fleming**. 2001. Characterization of a lytic *Lactobacillus plantarum* bacteriophage and molecular cloning of a lysin gene in *Escherichia coli*. Int.J.Food Microbiol. **65**:63-74.

175. **Yorgey, P., L. G. Rahme, M. W. Tan, and F. M. Ausubel**. 2001. The roles of mucD and alginate in the virulence of Pseudomonas aeruginosa in plants, nematodes and mice. Mol.Microbiol. **41**:1063-1076.

176. **Zaccardelli, M., A. Saccardi, E. Gambin, P. Minardi, U. Mazzucchi, M. Lemattre, S. Freigoun, K. Rudolph, and J. G. Swings**. 1994. *Xanthomonas campestris* pv. pruni bacteriophages on peach trees and their potential use for biological control. Colloques de l'INRA 875-878.