13
Bacteriophages of *Listeria*

Steven Hagens and Martin J. Loessner
Institute of Food Science and Nutrition, ETH Zurich, Switzerland
e-mail: martin.loessner@ilw.agrl.ethz.ch

Abstract: Bacteriophages have been shown to influence the evolution of their host and, in several cases, have a major effect on pathogenicity and/or virulence of bacterial pathogens. Several mechanisms allow phages to change the biology and associated phenotypes of their host. This chapter aims at explaining these mechanisms in the context of *Listeria* evolution and pathogenesis, using examples from other pathogens. Our current knowledge on the biology and applications of a few selected *Listeria* phages is reviewed and discussed. The lack of evidence for their influence on the phenotype of lysogenized host bacteria likely reflects our fragmentary knowledge about *Listeria* phages, especially on the molecular level. Clearly, much more research is required to understand the full impact of phages on their hosts, both in an ecological and evolutionary context.

13.1. Bacteriophages

13.1.1. Introduction

Bacterial viruses—known as bacteriophages or phages—are the most abundant self-replicating genetic elements, with estimates of total numbers ranging as high as 10^{31}. These numbers are based on electron microscopy images taken in attempts to enumerate phages in environmental samples. The first such study done on aquatic phages revealed an average of 10^7 virus-like particles (VLPs) per milliliter with typical head–tail morphology (Bergh et al. 1989), and later studies indicated even substantially higher numbers (Wommack and Colwell 2000). With an estimated number of 10^{24} infections per second in order to maintain the total population calculated above, phages are bound to have an enormous impact on bacterial communities both in evolutionary terms and on a global ecological scale (Wilhelm and Suttle 1999).

Phages are highly varied, both in shape and size of their enveloping capsids and in the composition and complexity of their genetic material. They have in common an absolute dependency on their host for replication, featuring no metabolism of their own. In general, phages exhibit very narrow host ranges,

mostly infecting only one particular genus, species, or, in some cases, even specific strains. Recognition of the host by phage receptor-binding proteins is largely but not solely responsible for the observed specificity. *Escherichia coli* phage λ, for example, recognizes the outer-membrane protein LamB of its host, and when this protein was heterologously expressed in various related and unrelated species, successful infection by λ of many, albeit not all, of the modified bacteria could be demonstrated (de Vries et al. 1984).

Phages have been classified based on morphological traits, and the most abundant (96%) and perhaps best-studied of these viruses belong to the order *Caudovirales* (the tailed phages), comprised of large, tailed phages with an isometric capsid containing a dsDNA chromosome. This group is further subdivided into *Myoviridae*, *Siphoviridae*, and *Podoviridae* characterized by long contractile tails, long flexible tails, or short noncontractile tails, respectively. For more information on bacteriophage classification and different types of phages, readers are referred to the website of the International Committee on Taxonomy of Viruses (ICTV): www.ncbi.nlm.nih.gov/ICTVdb/.

In general, bacteriophages have adopted two different life styles. Infection by a strictly virulent phage invariably results in the production of phage proteins, replication of phage DNA, and ends in lysis of the host and release of progeny phage. In contrast, a complex and not fully understood series of events, involving both environmental factors and the physiological state of the host may result in the fate of the temperate phages after infection. The lytic pathway described above for virulent phages is one option, but the infection process may alternatively result in integration of the phage genome into the host bacterial chromosome. This state, known as lysogeny, where the host is called lysogenic and the phage becomes a prophage and is replicated along with the host chromosome, can be maintained over many generations (Little et al. 1999). Subsequent excision of the phage DNA and entry into the lytic cycle again is a very complex and poorly understood process depending on many factors. It has been demonstrated in several cases that phage induction is mediated by the host cell SOS-response to certain stress stimuli. In these cases, it was shown that the more severe the DNA damage is—and thus the less likely host survival becomes—the proportion of prophage excision and lysis increases (Little and Mount 1982). The recent increases in the number of bacterial genome sequences demonstrated that many (if not all) bacterial genomes also contain cryptic phages, which are prophages that have lost essential functions and are no longer able to excise, replicate, and form infectious particles. Nevertheless, the viral DNA is present in the bacterial genomes and may directly on indirectly affect the phenotype of the host cell.

13.1.2. Phages and Pathogenicity

The tremendous impact of phages on the pathogenicity of their host has only recently become the focus of detailed research.

Phages can introduce new bacterial DNA into their host after infection. Although regulated, the normal packaging of phage DNA into capsids can in

certain cases result in incorporation of host DNA into phage particles. This process is called transduction and occurs in two different forms. In *specialized transduction*, improper excision of phage DNA results in packaging of host genes immediately adjacent to the phage genome into infectious phage particles (Matsushiro 1963). This process is unlikely to have a large impact on the pathogenicity of bacteria which are infected by these particles, since only very small portions of host DNA are transduced, and these portions are always those adjacent to the phage integration sites. In *generalized transduction*, however, random host DNA roughly equal in size to a normal phage genome may be packaged into the empty phage heads. The resulting particles are identical to normal phage except for their information content, and they can perform the first two steps of the infectious process, attachment and injection of DNA (Ikeda and Tomizawa, 1965). A schematic overview of generalized transduction is shown in Figure 13.1. It is conceivable that such a more or less randomly selected stretch of host DNA may also contain virulence factors which can, upon recombination with the chromosome of the infected bacteria, influence the phenotype and pathogenicity of the recipient.

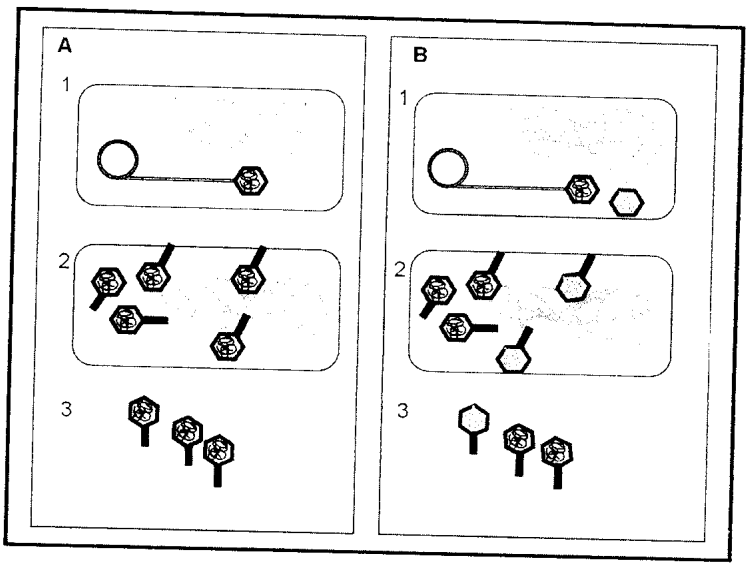

FIGURE 13.1. The principle of generalized transduction is shown here. In *panel A*, normal phage development is depicted. After rolling-circle replication of the phage DNA into multigenome concatemers, the genomes are individually packaged into empty heads (**1**). Phage particles are assembled (**2**), and progeny virions are released to infect new host cell (**3**). In *panel B*, host DNA is packaged into phage head particles (**1**). Particles containing bacterial DNA are normally assembled (**2**), and phage progeny is released. These pseudoinfective particles containing host DNA can inject the mistakenly packaged genetic material into susceptible host bacteria (**3**).

Evidence for this mechanism has been put forward, implicating phages in the distribution of a virulence-associated region in the genome of the animal pathogen *Dichelobacter nodosus* and various other bacteria (Cheetham and Katz 1995).

A phenomenon called lysogenic conversion is often involved in the modulation of host pathogenesis by phage. After incorporation of a temperate phage genome into the host chromosome, most prophage genes are silenced, especially those involved in virus morphogenesis and host cell lysis. In contrast, the genes needed to maintain the lysogenic state are normally expressed during lysogeny. However, bioinformatic analyses have demonstrated that many phage-encoded genes have unknown functions, and it is generally assumed that temperate phages can serve as vectors to introduce novel genetic information into their host that may enhance their fitness in certain environments. These coding sequences may themselves directly specify new properties or act by influencing the expression of existing genes. If this new environment happens to be the human body, the results can be dramatic. Table 13.1. shows several examples of pathogens, their prophages, and the toxins or virulence factors that are encoded by the phages. Such lysogenic conversion has also been reported for *Mycoplasma, Staphylococcus,* and *Streptococcus* (for a comprehensive overview of the state of research concerning these matters, the interested reader is referred to recent overviews on phage-related virulence of pathogens: Waldor et al. 2005).

Interestingly, the transcription promoter for the CTX cholera toxin from *Vibrio cholerae* (Table 13.1.), encoded by genes *ctxA* and *ctxB*, is not phage-regulated but controlled by the master *V. cholerae* virulence regulator, transcription factor ToxR (Skorupski and Taylor 1997). This is in contrast to another well-known example of lysogenic conversion: that of shiga-like toxin (*stx*) converting *E. coli* phages (Table 13.1.), where the transcription of *stx* genes is largely controlled by

TABLE 13.1. Examples of temperate phages encoding pathogenicity and/or virulence factors required for bacterial pathogenesis.

Host species	Phages	Genes	Virulence factor	Reference
Vibrio cholerae	CTXΦ	*ctxA/ctxB*	Cholera toxin CTX	Waldor and Mekalanos (1996)
Escherichia coli (STEC, EHEC)	H19-B 933W	$stx_{1A}/stx_{1B}stx_{2A}/stx_{2B}$	Shiga-like toxins STX1 and STX2	Smith et al. (1983) and O'Brien et al. (1984)
Salmonella enterica	Fels-1 SopEΦ	*nanH* *sopE*	Neuraminidase Type III-translocated G nucleotide exchange factor	Hardt and Galan (1997) and Figueroa-Bossi et al. (2001)
Clostridium botulinum	1D	*botD*	Neurotoxin BoNT	Eklund et al. (1971)
Corynebacterium diphtheriae	β	*tox*	DT	Freeman (1951)

late phage promoters, and the highest levels of STX transcription are observed during lysis of part of the bacterial population (Yee et al. 1993). Virulence modulation after phage induction has recently also been reported for *Staphylococcus aureus* (Goerke et al. 2006). The observations indicate that not only the phage per se, but also the highly specific interaction of both virus and host cell is responsible for full expression of the phenotype.

13.2. *Listeria* Phages

13.2.1. Introduction

The study of phages has provided valuable insights into many genetic principles, such as restriction–modification, the workings of promoters, and the concept of the operon, but at the same time has also contributed to a better understanding of their hosts. Approximately 400 *Listeria* phages have been isolated and described to date. Many of these are specific for *L. monocytogenes*, but phages able to infect *L. ivanovii*, *L. innocua*, *L. seeligeri* and *L. welshimeri* have also been described. However, still very little is known about *Listeria* phage biology, especially on the molecular level. (Sword and Pickett 1961; Jasinska 1964; Hamon and Peron 1966; Audurier et al. 1977; Chiron et al. 1977; Ortel 1981; Rocourt et al. 1982; Ortel and Ackermann 1985; Rocourt et al. 1985; Rocourt 1986; Loessner 1991; Gerner-Smidt et al. 1993; Loessner et al. 1994; Hodgson 2000).

All *Listeria* phages isolated thus far belong to the order *Caudovirales* (tailed phages), and of these most belong to the family of *Siphoviridae* (long, flexible noncontractile tail). The few exceptions belong to the family of *Myoviridae* (long, inflexible contractile tail). To date, no *Podoviridae* (short or missing tail) for *Listeria* have been isolated. Many *Listeria* phages have been characterized and, with three exceptions (see below), all of these are presumably temperate. Most isolates were derived from lysogenic strains either after UV or chemical induction or after spontaneous lysis of their hosts. The few environmental isolates mostly stem from silage or sewage plants.

The temperate *Listeria* phages are extremely host-specific, infecting only particular serovar groups. As mentioned above, this appears to be mainly due to the ability to recognize and attach to specific cell-wall ligands (phage receptors), which in the case of *Listeria* are serovar-specific sugar substituents on the polyribitol phosphate teichoic acids (Wendlinger et al. 1996; Tran et al. 1999).

Currently, only three complete nucleotide sequences of *Listeria* phages are available (A118, PSA, P100) (Loessner et al. 2000; Zimmer et al. 2003; Carlton et al. 2005).

13.2.2. The Temperate Phages

The A118 (Figure 13.2.A) and PSA are temperate phages and belong to the *Siphoviridae* family, characterized by dsDNA-containing isometric capsids and long, flexible tails.

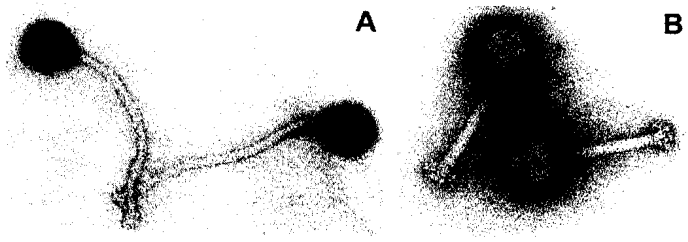

FIGURE 13.2. Electron micrographs of bacteriophages infecting *Listeria*. *Panel A* shows the temperate Siphovirus A118, and *panel B* the broad host range virulent Myovirus A511.

The A118 genome consists of 40,834 bp encoding 72 open reading frames (ORFs). The DNA packaged into phage heads is larger and consists of approximately 43.3 kb, which indicated about 6% redundancy (Loessner et al. 2000). Circular permutation along with terminal redundancy is common among phages and requires the circularization of the phage genome after entry into the host cell. Evidence indicated that after rolling circle replication of A118 phage DNA, sequential packaging starts at a random point of the concatamer, and genomes exceeding the one unit genome size by approximately 2.5 kb of DNA are incorporated into the heads.

Based on sequence similarities, functions could be assigned to 26 of the A118 ORFs. Clustering of genes reflecting different life cycles is common in phages, and especially pronounced in temperate phages. These gene clusters can be divided into those involved in DNA replication, phage morphogenesis, and lysis and those genes necessary for the establishment and maintenance of lysogeny which (as in A118 and PSA) are often oriented in the opposite direction of transcription. Many temperate phages with the same hosts have significant homology over parts of their genomes. Superinfection of lysogenized hosts is common and results in a close encounter of different virus genomes. The theory introducing modular evolution of phage genomes has been postulated more than 25 years ago (Susskind and Botstein 1978) and has since been supported by detailed study of many phages. However, except for a similar arrangement of gene clusters, surprisingly little overall similarity exists between the genomes of A118 (serovar 1/2 host strains) and PSA (Zimmer et al. 2003), the latter of which only infects *L. monocytogenes* serovar 4 strains. This suggests that these phage lineages may have diverged at an earlier stage. Consequently, because phages can only evolve together with their host bacteria, one must assume that the corresponding host strain lineages also diverged relatively early.

While A118 integrates into a region homologous to the *Bacillus subtilis comK* gene which is then disrupted, the integration site of PSA is a t-RNA$_{Arg}$ gene, where the *attB* is functionally complemented by prophage nucleotides. These sites appear to be frequently used by different *Listeria* phages to integrate, and

possible ramifications on host pathogenesis will be discussed at a later stage. t-RNA genes are generally known to harbor prophages, in many different bacteria (Campbell 2003).

The 37,618-bp PSA genome is not circularly permuted nor is it terminally redundant, but features 3'overhanging ends of 10 bp. Such cohesive (cos) ends are the alternative to circular permutation and allow direct circularization of the genome after entry into the host, without the requirement for a recombinase. Of the 57 ORFs found in the PSA genome, functional assignments could be made for 33 putative and confirmed gene products based on sequence homologies to known genes. Gene products from all life cycle-specific regions were identified, and, although there is little homology, they appear to serve similar functions as in A118. Two genes differ radically between the two phages. Because of their different substrates, the phage integrases are completely unrelated. Whereas the A118 integrase is a serine recombinase similar to Tn10 resolvase and *Salmonella* Hin invertase, the PSA integrase is homologous to the *E. coli* XerD protein. XerD is involved in resolving multimeric plasmids containing a *xer* site (Alen et al. 1997; Loessner and Calendar 2006). The endolysins responsible for host cell-wall degradation in lysis encoded by the two phages also differ. Endolysins have a two-domain organization, dividing the enzyme into two functional parts. The C-terminal part is responsible for substrate recognition, and binding to the cell wall is serovar-specific. The cell-wall binding domain (CBD) of phage PSA recognizes ligands on cell walls of serovars 4, 5, and 6, and the A118 CBD binds to serovar 1/2 and 3 cell walls. Both CBDs lack known motifs involved in the recognition of cell-wall anchors. The N-terminal, enzymatically active domains (EAD) also differ significantly from each other. The PSA EAD is an *N*-acetylmuramoyl-L-alanine amidase (Zimmer et al. 2003; Korndoerfer et al. 2006), whereas the A118 EAD is L-alanine-D-glutamate endopeptidase (Loessner et al. 1995). Interestingly, the endolysin of A500, another serovar 4b-specific phage, has a CBD which is almost identical to that of PSA, but its EAD is a peptidase related to Ply118. This supports the theory of modular evolution, with genetic exchange between phages. Phage endolysins have been employed to design attenuated suicide *Listeria monocytogenes* strains for delivery of antigen-encoding eukaryotic expression vectors (Dietrich et al. 1998). Phage endolysins and their applications with a focus on *Listeria* phage endolysins have recently been reviewed (Loessner 2005).

13.2.3. Virulent Phages

Phage A511 (Figure 13.2.B) and P100 are polyvalent, virulent bacteriophages able to infect most strains of different serovars within the genus *Listeria*; they can multiply on about 95% of *L. monocytogenes* strains of serovars 1/2 and 4. They belong to the family of *Myoviridae* with dsDNA-containing isometric capsid and a contractile tail. Sequencing of the complete P100 genome (131,384 bp) was recently completed, and an in-depth analysis of the predicted ORFs was performed (Carlton et al. 2005). A total of 174 putative gene products and

18 tRNAs were predicted. Twenty-five putative assignments could be made comprising phage structural proteins as well as genes responsible for DNA replication, transcription, and lysis. Homology searches did not reveal significant hits for the remaining ORFs. An in-depth analysis did not reveal any homology to genes, proteins, or other factors known or suspected to be involved in pathogenesis or virulence in microorganisms. This result is not surprising since infection invariably leads to host lysis, and the phage would gain nothing from carrying such genes. Phage P100 is highly similar to phage A511, with a large overall homology and some genes being identical on the nucleotide level. The host range, although overlapping for a large part, is not identical. The A511 genome has recently been sequenced completely (Dorscht et al. submitted), and comparison of the two genomes may help to reveal the molecular basis for the differences in specificity.

P35 is a somewhat unusual virulent phage. The virus is a member of the *Siphoviridae* family and can infect approximately 75% of serovar 1/2 strains (Hodgson 2000; Loessner, unpublished information). Its genome sequence was recently completed (Dorscht et al. submitted) and showed P35 life cycle-specific genes to be organized in the clustering typical for temperate *Listeria* phages. However, no lysogeny control region could be identified. It is possible that the module was initially present, but somehow lost as a result of an illegitimate recombination. However, as a consequence, the resulting phage featured a broad host range, because lack of the immunity region also eliminates repression of phage infection upon infection of host cells carrying prophages with homologous repressor proteins. Clearly, being able to lysogenize hosts has both advantages and disadvantages. In order to prevent extinction, at least one single progeny virulent phage has to find a new host and replicate. In environments with low cell density, this is a disadvantage, and the prophage state appears more attractive. Being so intimately linked to your host cells genetic information may also lead to a very narrow host range, however. Perhaps the scarcity of truly virulent *Listeria* phages and the abundance of temperate phages reflect the ecology of the host bacterium. In fact, although listeriae are ubiquitous, they are rarely found in large numbers.

13.2.4. Phage-Based Tools for the Study of Listeria

Within the context of *Listeria* pathogenicity, the most important role phages have played to date is their use as a typing tool in epidemiological studies. Phage typing has been instrumental in establishing food as the primary contamination source in humans (Fleming et al. 1985). Several phage typing sets have been established, and the low cost together with the relative ease of use makes it a useful tool to this day (Audurier et al. 1979, 1984; Rocourt et al. 1985; Loessner and Busse 1990; Loessner 1991; Estela and Sofos 1993; Gerner-Smidt et al. 1993; McLauchlin et al. 1996; van der Mee-Marquet et al. 1997). However, typability of *Listeria* isolates is variable. Strains of serovar 4 exhibit the highest degree of phage sensitivity, followed by strains of serovar 1/2. Strains of serovar

3 are largely resistant to phage infection, only few strains are susceptible to broad-range virulent phages such as A511 and P100. This variability in typing limits the use of phage typing as a universal discriminatory method, and additional phages for typing would be desirable (van der Mee-Marquet et al. 1997; Capita et al. 2002).

Genetically modified *Listeria* reporter bacteriophages can be used for confirming the presence of live *Listeria* cells in a sample, especially contaminated food (Loessner et al. 1996, 1997). Transducing phages will later be considered for their possible role in pathogenesis, but within a research context, they have been employed for studying transposon insertion and resulting mutant phenotypes and in strain construction (Freitag 2000). Another phage-based tool which can readily be used for the study of pathogenesis are two integrative *E. coli/Listeria* shuttle vectors, pPL1 and pPL2. A plasmid vector able to replicate in *E. coli* was equipped with genes encoding two different phage integrases which lead to plasmid integration in the chromosome at the respective phage integration sites after introduction into *Listeria*. The researchers were able to demonstrate full reestablishment of pathogenicity in complementation studies with knock-out mutants of *hly* and *actA*, which had not been possible with nonintegrative plasmids. At the same time, integration of the plasmids had no effect on virulence in wild-type strains (Lauer et al. 2002). These plasmids have also been used to study the contribution to virulence of a second *secA* gene $secA_2$ found in *L. monocytogenes* (Lenz and Portnoy 2002; Lenz et al. 2003), and further plasmid derivatives were used in studying the contribution to virulence of MogR, a transcriptional repressor required for virulence (Grundling et al. 2004).

13.2.5. *Phage Therapy of* Listeria

Because of the unique intracellular life style of *L. monocytogenes* during infection of the mammalian host, it is impossible to employ a classical phage therapy concept in the treatment of the disease. The same barrier that prevents our immune system from attacking and antibiotics from reaching the bacteria would very likely also keep any phages from interacting with and infecting their bacterial hosts.

Within this context, the possibility of using *Listeria* phage as a preventive measure as food additive in high-risk foods should be mentioned. In the first study for biocontrol of *Listeria* with bacteriophage, the researchers were able to demonstrate a significant reduction of bacterial growth in melon and fruit (Leverentz et al. 2003). A recent study has shown that such a phage therapy approach for food using the virulent phage P100 can eradicate or significantly reduce the growth of *Listeria* in soft cheese, depending on the phage-dose, and may thus be able to reduce the risk of contracting the disease (Carlton et al. 2005). Bioinformatic analyses indicated that the P100 genome does not specify any known pathogenicity or virulence factors, and the encoded polypeptides are not likely to act as food allergens. Moreover, an experimental repeated oral-dose

toxicity study in rats showed that P100 had no effects on the health of the treated animals.

Recently, a comprehensive study of the effect of A511 and P100 on *L. monocytogenes* in various foodstuffs treated under different conditions has been completed (Günther et al. submitted). Both phages were highly effective in reducing or eradicating *Listeria* contaminations from almost all food types tested. These promising results appear to be mostly due to the phages used: A511 and P100 are broad host range, strictly virulent phages, and infection invariably leads to host cell lysis.

A phage-based decontamination regimen for surfaces and machines at risk of contamination in food processing may also be considered.

13.2.6. Listeria *Phages and Pathogenesis*

Although most strains, including clinical isolates of *Listeria*, contain prophages—indeed many are polylysogens (Rocourt 1986) containing multiple prophages or cryptic phages—no evident phenotype has yet been associated with the presence of prophages under laboratory conditions nor could a (pro)phage be linked to epidemic strains or outbreaks. However, this situation may reflect our limited understanding of *Listeria* phage genetics and host cell interactions. What seems clear, however, is that the presence of prophages does not attenuate the pathogenic potential or virulence of strains from outbreaks. Phage PSA has been isolated by UV-induction from the genome of the epidemic *L. monocytogenes* strain Scott A (Fleming et al. 1985; Loessner et al. 1994), and the strains isolated from the large Vacherin soft cheese outbreak in Switzerland (Bille 1988) also contain inducible prophages (unpublished information).

Phage A118 integrates itself into a homologue of *B. subtilis comK* gene. In the latter organism, the corresponding gene product represents a global regulator for competence, the complex series of events resulting in the uptake of DNA from the environment. ComK also regulates noncompetence-related genes (van Sinderen et al. 1995). Although both classes of genes are also present in the *Listeria* genomes, they do not seem to be naturally transformable. Whatever the actual function of the gene may be, strains with phage-disrupted *comK* do not show any immediately apparent distinct phenotype under standard laboratory conditions and show no reduced virulence in model systems (Lauer et al. 2002). Again, this might reflect our lack of understanding regarding *Listeria* and biology of its phages. Another possible explanation for the lack of apparent effects of *comK* gene disruption might be the functional replacement by phage-encoded factors.

Within the A118 genome, the putative gene products encoded by ORFs 61 and 66 show a significant similarity to LmaD and LmaC of *L. monocytogenes* (Loessner et al. 2000). Although the function of the four genes in the *lma*DCBA operon of *L. monocytogenes* is not clear, their presence appears to be restricted to the pathogenic strains (Schaferkordt and Chakraborty 1997). Bioinformatic analyses and sequence alignments of A118 gp61 and gp66 also suggested a

possible role of these proteins in the regulation of gene expression, which would correlate well with the localization of the genes within the A118 "early genes" cluster, driving DNA recombination and replication during host cell infection.

The possible role of generalized transduction in the development of pathogenesis has already been mentioned. In one study, the ability of some broad host range phages (including A511 and P35) and more than 50 serovar-specific temperate phages (including A118 and PSA) to transduce bacterial DNA from one *L. monocytogenes* host to another was investigated. Donor cells contained antibiotic resistance markers on transposable elements, and recipients were tested for antibiotic resistance after infection with phages propagated on the donors. As expected, none of the broad host range virulent phages were capable of transduction. However, most of the serovar-specific phages transduced marker DNA in various frequencies. The proportion of transducing particles to normal virions ranged from approximately 5×10^{-2} to 10^{-4}, depending on the strain and phages tested. Both A118 and P35 proved capable of transduction. Frequencies and amount of DNA transduced were higher in A118, but the broader host range of P35 enables transduction into a wider variety of strains. Interestingly, PSA was not capable of transduction. At this time, the unit genome structure was not known, but the later finding that PSA features cohesive ends and therefore packages its DNA dependent on a terminase recognition site (Zimmer et al. 2003) explained the differences reported by Hodgson (2000) and established a link between genome structure and the ability to mistakenly package host bacterial DNA into empty virus particles.

Close inspection of the LIPI-2 pathogenicity island of *L. ivanovii* revealed that it was localized near the same t-RNA_{Arg} gene that temperate phages may use to integrate, and the authors speculate that its initial introduction into *L. ivanovii* may have been mediated by a temperate phage (Dominguez-Bernal et al. 2006). It has previously been postulated that the organization of the virulence gene cluster *prfA-plcA-hly-mpl-actA-plcB* may have been due to phage transduction (Chakraborty et al. 2000). However, there exists no proof or at least preliminary experimental evidence for any of these events.

It may be concluded that the biological basis for the introduction of new genes from related strains is provided by the transducing bacteriophages and that genes acquired through other means may subsequently be distributed in various populations by phages. While transduction is certainly playing a major role in the intraspecies genetic exchange, its role in the evolution of traits is unclear. However, given that the temperate phages isolated from *L. innocua* are generally able to infect *L. ivanovii* and can then reinfect the nonpathogenic host, the interspecies transfer of virulence genes from this animal pathogen to *L. innocua* is at least theoretically possible.

Phages can shape the genetic composition of bacterial strains in any given population. If prophages manage to enhance the fitness or virulence of a particular pathogenic strain, they may eventually contribute to the occurrence of the disease. Therefore, even with our limited understanding of the processes underlying the host evolution driven by phages, it seems obvious that bacterial pathogenesis

can be influenced by bacterial viruses in some way, and further research should be directed to elucidate the precise nature of this influence.

Acknowledgments. We thank Rudi Lurz (Berlin) for providing the electron micrograph of A511, and Julia Dorscht (Munich) and Susanne Guenther (Zurich) for providing unpublished information.

References

Alen C, Sherratt DJ, Colloms SD (1997). Direct interaction of aminopeptidase A with recombination site DNA in Xer site-specific recombination. EMBO J 16:5188–5197.

Audurier A, Rocourt J, Courtieu AL (1977). [Isolation and characterization of "*Listeria monocytogenes*" bacteriophages (author's translation)]. Ann Microbiol (Paris) 128:185–198.

Audurier A, Chatelain R, Chalons F, Piechaud M (1979). [Bacteriophage typing of 823 "*Listeria monocytogenes*" strains isolated in France from 1958 to 1978]. Ann Microbiol (Paris) 130B:179–189.

Audurier A, Taylor AG, Carbonnelle B, McLauchlin J (1984). A phage typing system for *Listeria monocytogenes* and its use in epidemiological studies. Clin Invest Med 7:229–232.

Bergh O, Borsheim KY, Bratbak G, Heldal M (1989). High abundance of viruses found in aquatic environments. Nature 340:467–468.

Bille J (1988). Listeriosis. Schweiz Rundsch Med Prax 77:173–175.

Campbell A (2003). Prophage insertion sites. Res Microbiol 154:277–282.

Capita R, Alonso-Calleja C, Mereghetti L, Moreno B, del Camino Garcia-Fernandez M (2002). Evaluation of the international phage typing set and some experimentál phages for typing of *Listeria monocytogenes* from poultry in Spain. J Appl Microbiol 92:90–96.

Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ (2005). Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul Toxicol Pharmacol 43:301–312.

Chakraborty T, Hain T, Domann E (2000). Genome organization and the evolution of the virulence gene locus in *Listeria* species. Int J Med Microbiol 290:167–174.

Cheetham BF, Katz ME (1995). A role for bacteriophages in the evolution and transfer of bacterial virulence determinants. Mol Microbiol 18:201–208.

Chiron JP, Maupas P, Denis F (1977). Ultrastructure of *Listeria monocytogenes* bacteriophages. C R Seances Soc Biol Fil 171:488–491.

de Vries GE, Raymond CK, Ludwig RA (1984). Extension of bacteriophage lambda host range: selection, cloning, and characterization of a constitutive lambda receptor gene. Proc Natl Acad Sci USA 81:6080–6084.

Dietrich G, Bubert A, Gentschev A, Sokolovic Z, Simm A, Catic A, Kaufmann SH, Hess J, Szalay AA, Goebel W (1998). Delivery of antigen-encoding plasmid DNA into the cytosol of macrophages by attenuated suicide *Listeria monocytogenes*. Nat Biotechnol 16:181–185.

Dominguez-Bernal G, Muller-Altrock S, Gonzalez-Zorn B, Scortti M, Herrmann P, Monzo HJ, Lacharme L, Kreft J, Vazquez-Boland JA (2006). A spontaneous genomic

deletion in *Listeria ivanovii* identifies LIPI-2, a species-specific pathogenicity island encoding sphingomyelinase and numerous internalins. Mol Microbiol 59:415–432.

Eklund MW, Poysky FT, Reed SM, Smith CA (1971). Bacteriophage and the toxigenicity of *Clostridium botulinum* type C. Science 172:480–482.

Estela LA, Sofos JN (1993). Comparison of conventional and reversed phage typing procedures for identification of *Listeria* spp. Appl Environ Microbiol 59:617–619.

Figueroa-Bossi N, Uzzau S, Maloriol D, Bossi L (2001). Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. Mol Microbiol 39:260–271.

Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL (1985). Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N Engl J Med 312:404–407.

Freeman VJ (1951). Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. J Bacteriol 61:675–688.

Freitag N (2000). Genetic Tools for Use with *Listeria monocytogenes*, In: Fischetti VA (ed), Gram-Positive Pathogens, vol. 1. ASM Press, Washington, DC, pp. 488–498.

Gerner-Smidt P, Rosdahl VT, Frederiksen W (1993). A new Danish *Listeria monocytogenes* phage typing system. Apmis 101:160–167.

Goerke C, Koller J, Wolz C (2006). Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in *Staphylococcus aureus*. Antimicrob Agents Chemother 50:171–177.

Grundling A, Burrack LS, Bouwer HG, Higgins DE (2004). *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. Proc Natl Acad Sci USA 101:12318–12323.

Hamon Y, Peron Y (1966). On the nature of bacteriocins produced by *Listeria monocytogenes*. C R Acad Sci Hebd Seances Acad Sci D 263:198–200.

Hardt WD, Galan JE (1997). A secreted Salmonella protein with homology to an avirulence determinant of plant pathogenic bacteria. Proc Natl Acad Sci USA 94:9887–9892.

Hodgson DA (2000). Generalized transduction of serotype 1/2 and serotype 4b strains of *Listeria monocytogenes*. Mol Microbiol 35:312–323.

Ikeda H, Tomizawa JI (1965). Transducing fragments in generalized transduction by phage P1. II. Association of DNA and protein in the fragments. J Mol Biol 14:110–119.

Jasinska S (1964). Bacteriophages of lysogenic strains of *Listeria monocytogenes*. Acta Microbiol Pol 13:29–43.

Korndoerfer, IP, Danzer, J, Schmelcher, M, Zimmer, M, Skerra, A, Loessner, MJ(2006). The crystal structure of the bacteriophage PSA endolysin reveals a unique fold responsible for specific recognition of *Listeria* cell walls. J MOl Biol 364:678–689.

Lauer P, Chow MY, Loessner MJ, Portnoy DA, Calendar R (2002). Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. J Bacteriol 184:4177–4186.

Lenz LL, Portnoy DA (2002). Identification of a second *Listeria* secA gene associated with protein secretion and the rough phenotype. Mol Microbiol 45:1043–1056.

Lenz, LL, Mohammadi S, Geissler A, Portnoy DA (2003). SecA2-dependent secretion of autolytic enzymes promotes *Listeria monocytogenes* pathogenesis. Proc Natl Acad Sci USA 100:12432–12437.

Leverentz B, Conway WS, Camp MJ, Janisiewicz WJ, Abuladze T, Yang M, Saftner R, Sulakvelidze A (2003). Biocontrol of *Listeria monocytogenes* on fresh-cut produce by treatment with lytic bacteriophages and a bacteriocin. Appl Environ Microbiol 69:4519–4526.

Little JW, Mount DW (1982). The SOS regulatory system of *Escherichia coli*. Cell 29:11–22.

Little JW, Shepley DP, Wert DW (1999). Robustness of a gene regulatory circuit. Embo J 18:4299–4307.

Loessner MJ (1991). Improved procedure for bacteriophage typing of *Listeria* strains and evaluation of new phages. Appl Environ Microbiol 57:882–884.

Loessner MJ (2005). Bacteriophage endolysins—current state of research and applications. Curr Opin Microbiol 8:480–487.

Loessner MJ, Busse M (1990). Bacteriophage typing of *Listeria* species. Appl Environ Microbiol 56:1912–1918.

Loessner MJ Calendar R (2006). The *Listeria* Bacteriophages. In: Calendar R (ed). The Bacteriophages, vol. 1. Oxford University Press, New York, pp. 593–601.

Loessner MJ, Estela LA, Zink R, Scherer S (1994). Taxonomical classification of 20 newly isolated *Listeria* bacteriophages by electron microscopy and protein analysis. Intervirology 37:31–35.

Loessner MJ, Wendlinger G, Scherer S (1995). Heterogeneous endolysins in *Listeria monocytogenes* bacteriophages: a new class of enzymes and evidence for conserved holin genes within the siphoviral lysis cassettes. Mol Microbiol 16:1231–1241.

Loessner MJ, Rees CE, Stewart GS, Scherer S (1996). Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable *Listeria* cells. Appl Environ Microbiol 62:1133–1140.

Loessner MJ, Rudolf M, Scherer S (1997). Evaluation of luciferase reporter bacteriophage A511::luxAB for detection of *Listeria monocytogenes* in contaminated foods. Appl Environ Microbiol 63:2961–2965.

Loessner MJ, Inman IB, Lauer P, Calendar R (2000). Complete nucleotide sequence, molecular analysis and genome structure of bacteriophage A118 of *Listeria monocytogenes*: implications for phage evolution. Mol Microbiol 35:324–340.

Matsushiro A (1963). Specialized transduction of tryptophan markers in *Escherichia coli* K12 by bacteriophage phi-80. Virology 19:475–482.

McLauchlin J, Audurier A, Frommelt A, Gerner-Smidt P, Jacquet C, Loessner MJ, van der Mee-Marquet N, Rocourt J, Shah S, Wilhelms D (1996). WHO study on subtyping *Listeria monocytogenes*: results of phage-typing. Int J Food Microbiol 32:289–299.

O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB (1984). Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science 226:694–696.

Ortel S (1981). Lysotyping of *Listeria monocytogenes*. Z Gesamte Hyg 27:837–840.

Ortel S, Ackermann HW (1985). Morphology of new *Listeria* phages. Zentralbl Bakteriol Mikrobiol Hyg [A] 260:423–437.

Rocourt J (1986). Bacteriophages and bacteriocins of the genus *Listeria*. Zentralbl Bakteriol Mikrobiol Hyg [A] 261:12–28.

Rocourt J, Schrettenbrunner A, Seeliger HP (1982). Isolation of bacteriophages from *Listeria monocytogenes* Serovar 5 and *Listeria innocua*. Zentralbl Bakteriol Mikrobiol Hyg [A] 251:505–511.

Rocourt J, Catimel B, Schrettenbrunner A (1985). Isolation of *Listeria seeligeri* and *L. welshimeri* bacteriophages. Lysotyping of *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. seeligeri* and *L. welshimeri*. Zentralbl Bakteriol Mikrobiol Hyg [A] 259: 341–350.

Rocourt J, Audurier A, Courtieu AL, Durst J, Ortel S, Schrettenbrunner A, Taylor AG (1985). A multi-centre study on the phage typing of *Listeria monocytogenes*. Zentralbl Bakteriol Mikrobiol Hyg [A] 259:489–497.

Schaferkordt S, Chakraborty T (1997). Identification, cloning, and characterization of the Ima operon, whose gene products are unique to *Listeria monocytogenes*. J Bacteriol 179:2707–2716.

Skorupski K, Taylor RK (1997). Control of the ToxR virulence regulon in *Vibrio cholerae* by environmental stimuli. Mol Microbiol 25:1003–1009.

Smith HW, Green P, Parsell Z (1983). Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. J Gen Microbiol 129:3121–3137.

Susskind MM, Botstein D (1978). Molecular genetics of bacteriophage P22. Microbiol Rev 42:385–413.

Sword CP, Pickett MJ (1961). The isolation and characterization of bacteriophages from *Listeria monocytogenes*. J Gen Microbiol 25:241–248.

Tran HL, Fiedler F, Hodgson DA, Kathariou S (1999). Transposon-induced mutations in two loci of *Listeria monocytogenes* serotype 1/2a result in phage resistance and lack of N-acetylglucosamine in the teichoic acid of the cell wall. Appl Environ Microbiol 65:4793–4798.

van der Mee-Marquet N, Loessner MJ, Audurier A (1997). Evaluation of seven experimental phages for inclusion in the international phage set for the epidemiological typing of *Listeria monocytogenes*. Appl Environ Microbiol 63:3374–3377.

van Sinderen D, Luttinger A, Kong L, Dubnau D, Venema G, Hamoen L (1995). comK encodes the competence transcription factor, the key regulatory protein for competence development in *Bacillus subtilis*. Mol Microbiol 15:455–462.

Waldor MK, Mekalanos JJ (1996). Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 272:1910–1914.

Waldor MK, Friedmann DI, Adhya SL (2005). Phages: Their Role in Bacterial Pathogenesis and Biotechnology. ASM Press, Washington, DC.

Wendlinger G, Loessner MJ, Scherer S (1996). Bacteriophage receptors on *Listeria monocytogenes* cells are the *N*-acetylglucosamine and rhamnose substituents of teichoic acids or the peptidoglycan itself. Microbiology 142 (Pt 4):985–992.

Wilhelm SW, Suttle CA (1999). Viruses and nutrient cycles in the sea. Bioscience 49:781–788.

Wommack KE, Colwell RR (2000). Virioplankton: viruses in aquatic ecosystems. Microbiol Mol Biol Rev 64:69–114.

Yee AJ, De Grandis S, Gyles CL (1993). Mitomycin-induced synthesis of a Shiga-like toxin from enteropathogenic *Escherichia coli* H.I.8. Infect Immun 61:4510–4513.

Zimmer M, Sattelberger E, Inman RB, Calendar R, Loessner MJ (2003). Genome and proteome of *Listeria monocytogenes* phage PSA: an unusual case for programmed +1 translational frameshifting in structural protein synthesis. Mol Microbiol 50:303–317.

Zink R, Loessner MJ, Scherer S (1995). Characterization of cryptic prophages (monocins) in *Listeria* and sequence analysis of a holin/endolysin gene. Microbiology 141 (Pt 10):2577–2584.