Applied and Environmental Microbiology, Apr. 2009, p. 2433–2438 Vol. 75, No. 8
0099-2240/09/$08.00+0 doi:10.1128/AEM.02480-08

Temperature-Dependent Phage Resistance of *Listeria monocytogenes* Epidemic Clone II[▽]

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Received 29 October 2008/Accepted 16 February 2009

Listeria monocytogenes **epidemic clone II (ECII) has been responsible for two multistate outbreaks in the United States in 1998-1999 and in 2002, in which contaminated ready-to-eat meat products (hot dogs and turkey deli meats, respectively) were implicated. However, ecological adaptations of ECII strains in the food-processing plant environment remain unidentified. In this study, we found that broad-host-range phages, including phages isolated from the processing plant environment, produced plaques on ECII strains grown at 37°C but not when the bacteria were grown at lower temperatures (30°C or below). ECII strains grown at lower temperatures were resistant to phage regardless of the temperature during infection and subsequent incubation. In contrast, the phage susceptibility of all other tested strains of serotype 4b (including epidemic clone I) and of strains of other serotypes and *Listeria* species was independent of the growth temperature of the bacteria. This temperature-dependent phage susceptibility of ECII bacteria was consistently observed with all surveyed ECII strains from outbreaks or from processing plants, regardless of the presence or absence of cadmium resistance plasmids. Phages adsorbed similarly on ECII bacteria grown at 25°C and at 37°C, suggesting that resistance of ECII strains grown at 25°C was not due to failure of the phage to adsorb. Even though the underlying mechanisms remain to be elucidated, temperature-dependent phage resistance may represent an important ecological adaptation of *L. monocytogenes* ECII in processed, cold-stored foods and in the processing plant environment, where relatively low temperatures prevail.**

Listeria monocytogenes is responsible for an estimated 2,500 cases of serious food-borne illness (listeriosis) and 500 deaths annually in the United States. It affects primarily pregnant women, newborns, the elderly, and adults with weakened immune systems. *L. monocytogenes* is frequently found in the environment and can grow at low temperatures, thus representing a serious hazard for cold-stored, ready-to-eat foods (18, 31).

Two multistate outbreaks of listeriosis in the United States, in 1998-1999 and in 2002, respectively, were caused by contaminated ready-to-eat meats (hot dogs and turkey deli meats, respectively) contaminated by serotype 4b strains that represented a novel clonal group, designated epidemic clone II (ECII) (3, 4). ECII strains have distinct genotypes as determined by pulsed-field gel electrophoresis and various other subtyping tools, and harbor unique genetic markers (6, 8, 11, 19, 34). The genome sequencing of one of the isolates (*L. monocytogenes* H7858) from the 1998–1999 outbreak revealed the presence of a plasmid of ca. 80 kb (pLM80), which harbored genes mediating resistance to the heavy metal cadmium as well as genes conferring resistance to the quaternary ammonium disinfectant benzalkonium chloride (10, 29).

Listeria phages (listeriaphage) have long been used for subtyping purposes (33), and extensive research has focused on the genomic characterization (2, 24, 26, 35), transducing potential (14), and biotechnological applications of selected phages (25). In addition, applications of listeriaphage as biocontrol agents in foods and the processing plant environment have been investigated (12, 15, 22). However, limited information exists on phages from processing plant environments and on the impact of environmental conditions on susceptibility of *L. monocytogenes* strains representing the major epidemic-associated clonal groups to such phages. We have found that strains harboring ECII-specific genetic markers can indeed be recovered from the environment of turkey-processing plants (9). Furthermore, environmental samples from such processing plants yielded phages with broad host range, which were able to infect *L. monocytogenes* strains of various serotypes, and different *Listeria* species (20). In this study, we describe the impact of growth temperature on susceptibility of *L. monocytogenes* ECII strains to phages, including phages isolated from turkey-processing plant environmental samples.

MATERIALS AND METHODS

Bacterial strains, phages, and growth conditions. The strains used in this study are listed in Tables 1 to 3 and were from the *Listeria* strain collection of our laboratory. *L. monocytogenes* F2365 (1985 California outbreak, epidemic clone I [ECI]), H7550 (1998–1999 hot dog multistate outbreak, ECII), and 4b1 (sporadic clinical isolate) were used as serotype 4b reference strains (20), along with strain WS1, implicated in the 2000–2001 outbreak of listeriosis in Winston-Salem, NC, and representing epidemic clone V (ECV) (7). The outbreak-associated strains H7550, J1815, and J1925 have been determined in our laboratory to harbor cadmium resistance plasmids (R. M. Siletzky and S. Kathariou, unpublished observations). Strain H7550-Cd^s was a cadmium-susceptible, plasmid-free derivative of *L. monocytogenes* H7550, derived following repeated passages of the bacteria at 42°C, and was kindly provided by D. Elhanafi. Strains F6854 (serotype 1/2a, 1988 hot dog isolate, representative of ECIII), G3978 (serotype 1/2b), and WSLC 1001 (Weihenstephan *Listeria* Collection, serotype 1/2c) were employed as reference strains for the indicated serotypes (20). Strains from processing plant environmental samples were classified as ECII based on the similarity of

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▽ Published ahead of print on 27 February 2009.

TABLE 1. Susceptibility to phage 20422-1 of *Listeria* strains grown at different temperatures

Strain	Serotype/clonal group	EOP with host cells grown at[a]:			
		20°C	25°C	30°C	37°C
L. monocytogenes					
F2365	4b/ECI	1.0	1.0	1.0	1.0
4b1	4b	1.66	1.56	1.39	1.1
H7550	4b/ECII	$<4.7 \times 10^{-5}$	$<4.3 \times 10^{-5}$	$<3.6 \times 10^{-5}$	3.4×10^{-1}
F6854	1/2a/ECIII	1.19	1.19	1.14	1.1
G3978	1/2b	1.14	1.14	8.2×10^{-1}	6.6×10^{-1}
WSLC 1001	1/2c	7.1×10^{-1}	5.7×10^{-1}	5.4×10^{-1}	6.6×10^{-1}
L. innocua L1307a	ND[b]	8.9×10^{-1}	9.1×10^{-1}	8.9×10^{-1}	7.6×10^{-1}
L. ivanovii SK2797	5	8.6×10^{-1}	8.3×10^{-1}	7.5×10^{-1}	7.3×10^{-1}
L. seeligeri SK2795	ND	1.04	1.09	8.6×10^{-1}	8.8×10^{-1}

[a] EOP values represent the average of duplicate determinations from one representative experiment.
[b] ND, not determined.

their pulsed-field gel electrophoresis patterns (using AscI and ApaI) with the patterns of confirmed ECII outbreak strains and following confirmation that they harbored genetic markers which were unique to ECII, as described previously (9, 19). The phages used in this study were 20422-1, 20125-1, 20131-1, and 805405-1, which were isolated from turkey-processing plant environmental samples in 2004 (20) as well as the broad-host-range phage A511 (kindly provided by Martin J. Loessner). Bacteria were routinely grown in brain heart infusion (BHI) (Difco, Sparks, MD) without shaking at the indicated temperatures (4°C for 3 weeks, 10°C for 12 days, 20°C for 48 h, 25°C for 36 h, 30°C for 24 h, and 37°C for 16 h). Agar cultures were on blood agar plates containing 5% sheep blood (Remel, Lenexa, KS) or BHI agar (BHI with 1.5% agar; Difco).

Phage propagation, infections, and adsorption assays. Phage lysates were prepared using *L. monocytogenes* DP-L862 (serotype 1/2a) as the host, as described previously (20). *L. monocytogenes* DP-L862 (100 μl of overnight culture, ca. 10^8 CFU/ml) was mixed with 100 μl of phage solution (ca. 10^7 PFU/ml) and $CaCl_2$ (final concentration, 10 mM); the mixture was added to 3 ml Luria-Bertani (LB; Difco) soft agar (0.75% agar; Difco) and poured onto regular LB agar (1.5% agar; Difco) plates. After overnight incubation at 37°C, 5 ml of SM buffer (100 mM NaCl, 50 mM Tris, 8 mM $MgSO_4$, 0.1 g/liter gelatin [pH 7.5]) was added to each plate. The plates were incubated at 4°C overnight, and the liquid was filtered with 0.22-μm filters (Millipore, Bedford, MA). Phage infections and plaque enumerations were done as described previously (20) using bacteria grown at the indicated temperatures. Efficiency of plaquing (EOP) was defined as the ratio of the number of plaques formed on a specific host strain over the number of plaques formed on a phage-susceptible reference strain; unless otherwise indicated, the phage-susceptible reference strain used was *L. monocytogenes* F2365. Phage susceptibility of the strains was determined in at least three independent experiments, each done in duplicate.

Phage adsorption assays were done as described previously (20, 32), with the following modifications. Bacteria were grown at the indicated temperature overnight, and the culture was diluted (1:100) in BHI, incubated at 37°C for 2 h with shaking (120 rpm), and mixed with 200 μl phage suspension (prepared as described above). At specific times after infection (0, 0.5, 1.5, 3.5, 6, and 10 h), unadsorbed phage in filtrates of culture supernatants (150 μl, obtained as described above) was enumerated by standard plaque assays using *L. monocytogenes* F2365 as the indicator strain. Phage adsorption was assessed in at least three independent experiments, each done in duplicate.

Duration of phage resistance of *L. monocytogenes* H7550 grown at 25°C. *L. monocytogenes* strain H7550 (ECII) was grown at 25°C for 36 h in BHI. The culture (5 ml) was then centrifuged at 5,000 rpm for 10 min, washed twice with phosphate-buffered saline (0.01 M KH_2PO_4, 0.01 M K_2HPO_4, 0.0027 M KCl, 0.14 M NaCl [pH 7.4]), resuspended in 5 ml phosphate-buffered saline (prewarmed to 37°C), and incubated at 37°C. At the designated times (0, 0.5, 1.0, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 10 h), 200 μl of the cell suspension was mixed with 100 μl of listeriaphage 20422-1 (1×10^7 PFU/ml) in melted LB soft agar supplemented with $CaCl_2$ (10 mM), poured onto BHI agar plates, and incubated for 36 h at 37°C. Plaques were enumerated at the end of this incubation. CFU/ml at the same time points were determined by spreading of dilutions (10^{-4} and 10^{-5}) onto BHI agar plates and incubation overnight at 37°C. *L. monocytogenes* H7550 grown overnight at 37°C was processed identically, as a positive control. EOP determinations were determined as the ratio of plaques formed by 25°C-grown *L. monocytogenes* H7550 at a specific time point following the temperature upshift over the plaques formed by the control strain, 37°C-grown *L. monocytogenes* H7550, at the same time point. Duration of phage resistance of *L. monocytogenes* H7550 grown at 25°C was assessed in two independent experiments, each done in duplicate.

RESULTS

Phage susceptibility of ECII strains is dependent on temperature of growth. Earlier studies characterized the broad-host-range phages 20422-1 and 805405-1, isolated from environmental samples of turkey-processing plants. During those studies, it was noted that the number of plaques formed on lawns of ECII bacteria was 2-fold to 100-fold lower than that obtained with other strains of serotype 4b or of other serotypes or species. Similar results were also obtained with phage A511. In these studies, bacterial growth and phage infections were routinely done at 37°C (20).

To further investigate susceptibility of ECII strains to phage infection, we assessed the plaque-forming potential of phage 20422-1 using a panel of nine strains grown at different temperatures (20, 25, 30, and 37°C). The panel included *L. monocytogenes* strains representing the major clonal groups and serotypes, as well as representatives of other *Listeria* species (Table 1). It was noted that the temperature of growth of the bacteria had pronounced impact on the susceptibility of ECII strain *L. monocytogenes* H7550 to infection by the phage.

TABLE 2. Dependence of *L. monocytogenes* ECII susceptibility to phage infection on the growth temperature of the host cells but not on the temperature of subsequent infection and incubation

Strain	EOP with host cells grown at[a]:			
	37°C		25°C	
	Infection at 37°C	Infection at 25°C	Infection at 37°C	Infection at 25°C
4b1 (serotype 4b)	1.5	9.5×10^{-1}	1.6	9.1×10^{-1}
F2365 (serotype 4b, ECI)	1.0	1.0	1.0	1.0
H7550 (serotype 4b, ECII)	4.2×10^{-1}	4.1×10^{-1}	$<3.4 \times 10^{-5}$	$<3.4 \times 10^{-5}$
F6854 (serotype 1/2a, ECIII)	1.1	9.2×10^{-1}	1.2	1.0

[a] EOP values represent the average of duplicate determinations from one representative experiment.

Plaques were readily obtained when the bacteria were grown at 37°C, even though the number of plaques was lower than that obtained with other strains (Table 1), as also observed earlier (20). However, cells grown at 20, 25, or 30°C appeared to be completely resistant (no visible plaques). All other strains of *L. monocytogenes* in the strain panel, as well as other *Listeria* spp., were susceptible to the phage regardless of the temperature of growth of the bacteria (Table 1). *L. monocytogenes* H7550 grown at 25°C was resistant to the phage regardless of whether the cells were grown in liquid culture or on agar (data not shown). *L. monocytogenes* H7550 grown at 10°C and 4°C was also resistant to phage 20422-1, whereas *L. monocytogenes* F2365 grown at these temperatures formed plaques upon infection (data not shown).

The impact of temperature on susceptibility of *L. monocytogenes* H7550 was specific to the temperature during growth. When grown at 25°C, these bacteria were resistant to infection, regardless of whether infection and subsequent incubation were at 25 or 37°C. Similarly, bacteria grown at 37°C were susceptible, regardless of whether infection and subsequent incubation were at 25 or 37°C (Table 2). Thus, temperature (25 versus 37°C) during infection and subsequent incubation did not have any detectable influence on susceptibility.

Growth temperature-dependent phage resistance is not limited to phage 20422-1 and is a unique characteristic of all tested ECII strains, regardless of source. When grown at 25°C, *L. monocytogenes* H7550 and all other tested ECII strains associated with the 1998–1999 and 2002 outbreaks were resistant not only to phage 20422-1 but to all other tested phages that we had isolated from processing plant environmental samples (20125-1, 20131-1, and 805405-1). Furthermore, following growth at 25°C these strains were

TABLE 3. Phage susceptibility of *L. monocytogenes* ECII and other strains grown at 25°C and 37°C

Strain[a]	Alternative identification	Serotype/clonal group	EOP for listeriaphage with host cells grown at temp shown[b]:				Source or reference
			20422-1[c]		A511		
			37°C	25°C	37°C	25°C	
F2365		4b/ECI	+	+	+	+	29
900		4b/ECI	+	+	+	+	28
4b1		4b	+	+	+	+	20
80	171A	4b	+	+	+	+	9
04-643		4b	+	+	+	+	K. Sperry
05-062		4b	+	+	+	+	K. Sperry
WS1		4b/ECV	$+^r$	$+^r$	+	+	9
F6854		1/2a/ECIII	+	+	+	+	29
G3978		1/2b	+	+	+	+	11
WSLC1001		1/2c	+	+	$+^r$	$+^r$	20
$H7550^{EP*}$		4b/ECII	$+^r$	−	$+^r$	−	9
$H7550\text{-}Cd^s$		4b/ECII	$+^r$	−	$+^r$	−	10
1106^{EN}		4b/ECII	$+^r$	−	$+^r$	−	28
1493^{EN}	L0226	4b/ECII	$+^r$	−	$+^r$	−	9
1495^{EN}	L0315	4b/ECII	$+^r$	−	$+^r$	−	9
1498^{EN}	L0603	4b/ECII	$+^r$	−	$+^r$	−	9
1506^{EN}	L0704	4b/ECII	$+^r$	−	$+^r$	−	9
1513^{EN}	L0719	4b/ECII	$+^r$	−	$+^r$	−	9
$J1735^{EP, CL}$		4b/ECII	$+^r$	−	$+^r$	−	19
$J1815^{EP, FD*}$		4b/ECII	$+^r$	−	$+^r$	−	19
$J1925^{EP, EN*}$		4b/ECII	$+^r$	−	$+^r$	−	19
$H7596^{EP}$		4b/ECII	$+^r$	−	$+^r$	−	19
$H7969^{EP}$		4b/ECII	$+^r$	−	$+^r$	−	19
2688^{EN}		4b/ECII	$+^r$	−	$+^r$	−	28
2483^{EN}		4b/ECII	$+^r$	−	$+^r$	−	K. Boor (17)
2490^{EN}		4b/ECII	$+^r$	−	$+^r$	−	K. Boor (17)
2493^{EN}		4b/ECII	$+^r$	−	$+^r$	−	K. Boor (17)

[a] EP indicates epidemic-associated ECII strains. Strains with the H designation represent the 1998–1999 outbreak, and strains with the J designation represent the 2002 outbreak. Asterisks indicate outbreak strains with cadmium resistance plasmids. CL, EN, and FD indicate ECII strains of clinical, environmental (food-processing plant), and food origins, respectively.

[b] +, susceptible to phage ($1.6 > \text{EOP} > 10^{-1}$); $+^r$, reduced susceptibility to phage ($10^{-1} > \text{EOP} > 10^{-2}$); −, resistant to phage ($\text{EOP} < 10^{-5}$). For each column, the EOP of *L. monocytogenes* F2365 was set at 1.0 at that temperature.

[c] Similar results and EOP values were obtained with listeriaphages 20125-1, 20131-1, and 80505-1.

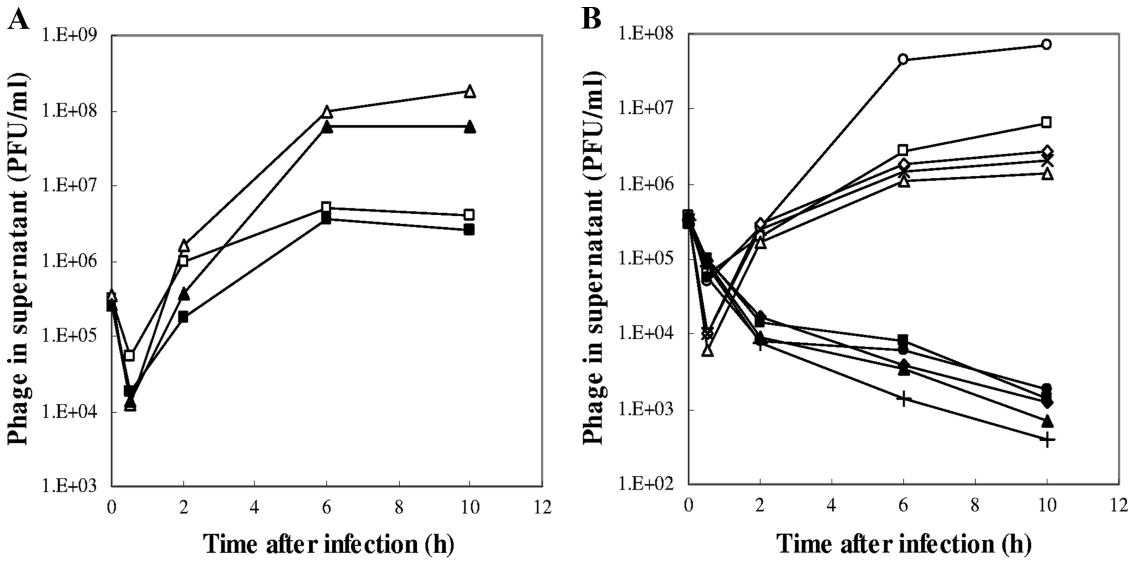

FIG. 1. Results of phage adsorption assays with *L. monocytogenes* ECII strains grown at 25°C and 37°C. (A) Adsorption assays with *L. monocytogenes* strains F2365 (squares) and 4b1 (triangles) done as controls. Bacteria grown at 25°C and 37°C are indicated by closed and open symbols, respectively. (B) Adsorption assays with *L. monocytogenes* ECII strains: H7550 (squares), 1493 (circles), J1735 (triangles), H7596 (diamonds), and 1106 (+ and × for cells grown at 25°C and 37°C, respectively). For strains H7550, 1493, J1735, and H7596, bacterial growth at 25°C and 37°C is indicated by closed and open symbols, respectively. Phage 20422-1 (propagated in *L. monocytogenes* DP-L862) was used in the infections, and the phage titer (PFU/ml) in the supernatant was enumerated at the indicated time points as described in Materials and Methods. Data represent averages of duplicates from one representative experiment.

also resistant to the broad-host-range phage A511 (Table 3). As observed with 20422-1, plaques were readily produced with 20125-1, 20131-1, 805405-1, and A511 following infection of 37°C-grown bacteria (Table 3).

In addition to outbreak-associated ECII strains, isolates that were derived from food-processing plant environmental samples and that harbored ECII-specific genetic markers exhibited the same growth temperature-dependent resistance to the phages (Table 3). Such growth temperature-dependent phage resistance was not observed with any of the other tested serotype 4b strains, either from clinical or from environmental sources (Table 3).

Strains implicated in the 1998-1999 outbreak harbored the cadmium resistance plasmid pLM80 (ca. 80 kb) (29). Examination of a plasmid-free, cadmium-susceptible derivative of H7550 (strain H7550-Cd^s) showed that the presence of this plasmid was not associated with the temperature-dependent susceptibility to phage, as plasmid-harboring and plasmid-free strains had identical phage susceptibility profiles following growth at 25 and 37°C (Table 3). This was in agreement with the finding that all tested strains from the 1998-1999 and the 2002 outbreaks had the same growth temperature-dependent phage susceptibility profile, even though cadmium resistance plasmids were harbored by some, but not others, of the strains from these outbreaks (Table 3).

Phage resistance of ECII strains following growth at low temperature does not reflect absence of phage receptors. Adsorption assays with phage 20422-1 were employed to determine whether the observed phage resistance of 25°C-grown *L. monocytogenes* ECII strains was due to failure of the phage to adsorb onto cells grown at that temperature. *L. monocytogenes* F2365 and 4b1, which were susceptible to the phage regardless of temperature of growth, were used as controls in these adsorption assays. Phage titers (PFU/ml) in the supernatant decreased 30 min after infection of these strains grown at either 25 or 37°C and increased thereafter (Fig. 1A).

Phage adsorption on *L. monocytogenes* ECII was determined with five strains, derived both from outbreaks and from environmental samples. When 37°C-grown *L. monocytogenes* ECII cultures were used, phage concentration in the supernatant decreased 30 min after infection and increased gradually thereafter, with the same pattern as the controls. A similar decrease in phage concentration 30 min after infection was observed with cells grown at 25°C (Fig. 1B), suggesting that adsorption of the phage took place. However, when 25°C-grown cultures were used, the concentration of phage in the supernatant continued to decrease, suggesting progressively increasing adsorption, and no phage amplification was detected, in agreement with the observed resistance of ECII bacteria grown at this temperature (Fig. 1B).

Duration of phage resistance in ECII strains grown at low temperature. When *L. monocytogenes* H7550 was grown at 25°C and subsequently shifted to 37°C and incubated at that temperature for up to 10 h, the bacteria remained resistant to phage 20422-1 for up to 5.5 h; no plaques could be detected (EOP, $<1.0 \times 10^{-5}$). Modest susceptibility was first noted at 6.5 h (EOP, 4.8×10^{-2}). Susceptibility increased thereafter, but at 10 h, it was still ca. 10-fold lower than the control (37°C-grown *L. monocytogenes* H7550 treated identically, and at the same time) (Table 4). A noticeable decrease in CFU/ml of the 25°C-grown cells was noted following 10 h of incubation at 37°C in the presence of the phage (Table 4), in agreement with the observed amplification of the phage at this time point and the expected accompanying host cell lysis.

TABLE 4. Persistence of phage resistance of 25°C-grown *L. monocytogenes* H7550 following temperature upshift to 37°C

Incubation time (h)	Persistence of phage resistance	
	EOP[a]	CFU/ml
0	$<10^{-5}$	4.3×10^8
0.5	$<10^{-5}$	4.1×10^8
1	$<10^{-5}$	4.3×10^8
1.5	$<10^{-5}$	4.2×10^8
2.5	$<10^{-5}$	4.8×10^8
3.5	$<10^{-5}$	4.0×10^8
4.5	$<10^{-5}$	2.2×10^8
5.5	$<10^{-5}$	1.8×10^8
6.5	4.8×10^{-2}	1.3×10^8
10	3.0×10^{-1}	5.6×10^6

[a] Ratio of plaques obtained by the positive control (*L. monocytogenes* H7550 grown at 25°C) over those obtained by 37°C-grown *L. monocytogenes* H7550. EOP values are averages of duplicates from one representative experiment.

DISCUSSION

An unexpected finding of this study was that *L. monocytogenes* ECII strains were resistant to broad-host-range phages when grown at temperatures lower than 37°C. This may be an important attribute in terms of the potential of these organisms to contaminate food and to become implicated in illness, including outbreaks, since relatively low temperatures prevail in the processing plant environment as well as in cold-stored foods. Phage is expected to be present in the food-processing plant environment, and recent surveys in our laboratory indeed indicated that about 10% of environmental samples could yield listeriaphage (20). The resulting advantage to ECII bacteria could enhance their fitness in the processing plant environment, with accompanying increased likelihood for contamination of food. Furthermore, biological control of *L. monocytogenes* ECII strains by phage could be seriously compromised: it is conceivable that application of phages may inadvertently select for these strains, should they be present along with other listeriae in the same low-temperature environment in the processing plant or in food products. In 2006, the FDA approved a mixture of six phages for application on ready-to-eat meat and poultry products (12). Even though we have not tested this phage mixture, the finding that *L. monocytogenes* ECII strains grown at low temperature were resistant to all broad-host-range phages that we tested suggests that other phages, alone or in mixtures, may also be ineffective against these strains, if the bacteria have grown in low-temperature environments.

At this time, the mechanisms mediating phage resistance of *L. monocytogenes* ECII in response to growth at low temperature remain to be characterized. Several mechanisms may prevent successful phage infection, including failure of the phage to adsorb, blocking of phage nucleic acid injection, presence of prophage, restriction-modification systems, and abortive infection of host cells (13). We have found that adsorption blocking was not involved, since phage appeared to be normally adsorbed onto cells grown either at 25°C or at 37°C. Furthermore, PCR with primers based on the nucleotide sequence of selected fragments of the genome of phage 20422-1 (20) failed to detect the phage in the genomic DNA of *L. monocytogenes* H7550 grown either at 25°C or at 37°C (J.-W. Kim, D. Elhanafi, and S. Kathariou, unpublished findings). Such findings suggested that integrated phage was not responsible for the resistance phenotype of 25°C-grown cells. The findings suggest that other mechanisms (e.g., blocking of phage nucleic acid injection, restriction-modification systems, or abortive infection) may be responsible for the observed resistance to phage of *L. monocytogenes* ECII grown at low temperature. Our findings also suggest that the determinants mediating the observed growth temperature-dependent resistance to phage were chromosomally encoded, since the phenomenon was observed in both plasmid-harboring and plasmid-free ECII strains.

Reports of growth temperature-dependent phage susceptibility in other bacteria are relatively rare. Studies with *Lactococcus lactis* revealed a temperature-dependent phage resistance phenotype associated with the restriction-modification system LlaJ1, harbored on the 65-kb plasmid pNP40. Resistance of the bacteria to phages was pronounced at 19°C but decreased as temperature increased to 37°C. The mechanisms underlying the observed impact of temperature on phage resistance appeared to involve transcriptional regulation of *llaJ1* by an unidentified element or elements (30).

Currently unidentified restriction-modification systems of *L. monocytogenes* ECII or other proteins expressed specifically during growth at low temperature may contribute to the observed growth temperature-dependent phage resistance. It is tempting to speculate that the corresponding genes may be unique to *L. monocytogenes* ECII, since the phenomenon appears to be unique to this clonal group. Alternatively, genes may be common to *L. monocytogenes* ECII and other strains, but under differential, temperature-dependent expression in the former. Numerous genes, the transcription of which is under temperature control in *L. monocytogenes* 10403S (serotype 1/2a), have been identified (1, 5, 23, 27). Several key virulence genes, including the gene encoding listeriolysin O, have been known for a long time to be thermoregulated at the transcriptional level (21); transcription of these genes at 37°C, but not below 30°C, was shown to be mediated by temperature-dependent translational control of the key virulence regulator PrfA, based on the secondary structure of *prfA* mRNA (16). It will be of interest to determine whether the observed temperature-dependent phage resistance is part of a larger temperature-controlled regulon in *L. monocytogenes* ECII.

In conclusion, we have shown that susceptibility of *L. monocytogenes* ECII strains to broad-host-range phages was strictly dependent on growth temperature of the bacteria. This is, to our knowledge, the first report of a unique functional attribute of this clonal group that was first recognized in 1998 as important contributor to food-borne listeriosis in the United States. Even though the mechanisms underlying the observed growth temperature-dependent resistance of these strains to phage remain to be elucidated, this attribute may contribute to the ecological fitness of these strains in food products and in the environment, including food-processing plants, and is clearly worthy of further investigations.

ACKNOWLEDGMENTS

Funding for this research was partially provided by USDA grant 2006-35201-17377.

We thank M. J. Loessner for the gift of A511, K. Sperry and K. Boor for bacterial strains, and D. Elhanafi for advice and feedback. We are grateful to Robin Siletzky for technical assistance and all other members of our laboratory for encouragement and support.

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