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Isolation and relationship analysis of *Listeria* phages with various serotype hosts and morphological characterization

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Abstract

Listeriosis, caused by *Listeria monocytogenes* (Lm), is a severe foodborne illness with a high fatality rate. *Listeria* phages specifically target and lyse Lm, offer a promising alternative for biocontrol and phage therapy. However, most existing studies focus on the lytic characteristics of *Listeria* phages using limited sample sizes. In this study, a large number of *Listeria* phages were isolated from diverse sources, and their lytic profiles and morphology were characterized. A total of 317 *Listeria* phages were isolated from 90 food-related environmental samples and 196 natural environmental samples collected across seven provinces. The phages were tested for lytic activity against 35 Lm strains representing nine serotypes, and their morphology was characterized using transmission electron microscopy (TEM). Statistical analysis was conducted to evaluate the lytic patterns of phages. The phages were classified into three groups based on their total lysis ratios. Broad Host Range Phages (BHRP) were primarily members of the *Myoviridae*-like phages and demonstrated the ability to lyse a vast majority of nine serotype host strains. Medium Host Range Phages (MHRP) comprised both *Siphoviridae*-like and *Myoviridae*-like phages, and demonstrated lysis of 6–9 serotype strains. Narrow Host Range Phages (NHRP) belonged to the *Siphoviridae*-like phages and exhibited effective lysis of serotype 4 strains. Furthermore, phages isolated from food-related environmental sources demonstrated greater lytic activity against *Listeria* serotypes 1/2b, 4a, and 4c compared to those derived from natural environmental sources. The study first isolated a multitude of *Listeria* phages, elucidated their lytic patterns and ecological distribution, and provided a valuable resource for future research.

Keywords *Listeria monocytogenes*, *Listeria* phage, Lytic profiles, Morphology, Ecological distribution

Introduction

Listeriosis is the third most significant cause of mortality from foodborne pathogens, with a fatality rate of 20 to 30% [1]. *Listeria monocytogenes*, a ubiquitous pathogen responsible for listeriosis, has the capacity to survive and proliferate over a broad range of temperatures (2–45° C) and pH levels (4.6–9.5), as well as in the elevated salt concentrations [2, 3]. *L. monocytogenes*, is a genetically heterogeneous species comprising 14 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e, 4 h and 7) that can be grouped into five distinct serogroups using a multiplex PCR scheme: IIa (1/2a, 3a), IIb (1/2b, 3b, 7),

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IIc (1/2c, 3c), IVb (4b, 4d, 4e) and L (4a, 4c, 4ab) [4–6]. Epidemiological studies have demonstrated that the 1/2a, 1/2b, 1/2c, 4b serotype strains are prevalent in food contamination [7, 8], and the 4b, 1/2a, and 1/2b serotype strains are responsible for 95% of human listeriosis cases [9].

Bacteriophages (phages) are viruses that are capable of specifically infecting and killing bacteria. Lytic phages infect and kill their bacterial host through a process known as lysis. The interest in bacteriophage therapy is growing, which can be attributed to the exceptional lysis specificity of these viruses [10–14].Phages host range refers to the spectrum of bacterial strains a phage can infect. Broad host range phages can lyse diverse strains across multiple serotypes, whereas narrow host range phages are restricted to a few closely related strains. However, no standardised definition distinguishes broad and narrow host range phages [15]. *Listeria*-specific bacteriophages, also referred to as *Listeria* phages, have been isolated from various sources. To date, more than 500 *Listeria* phages have been identified. Nevertheless, only a limited number of virulent bacteriophages with the potential for *Listeria* biocontrol have been fully characterized at the molecular and genomic level [8, 9, 16–18]. Although several studies have been conducted on the lysis characteristics of *Listeria* phages, the sample size was relatively limited, and the analysis was not sufficiently systematic [16, 17, 19].

This study aimed to isolate a diverse range of *Listeria* phages, examine their relationships with various serotype host strains, and investigate their morphological characteristics.

Methods

Sample collection

A total of 90 samples from food-related environments and 196 samples from natural environments were collected from seven provinces. The 90 food-related environmental samples comprise 58 seafood samples (obtained from the seafood markets), 7 swab samples (taken from the surface of pork cutting boards), 3 frozen food samples (frozen meet wontons, frozen hotpot meatballs and frozen dumplings), and 22 market sewage samples. The 196 natural environmental samples include 63 soil samples, 88 sand samples, 2 spring water samples, and 43 seawater samples (Table 1).

Listeria monocytogenes strains

A total of 31 strains (referred to as isolation strains), including 21 laboratory-preserved strains and 10 newly isolated from the samples, were employed to isolate phages (Table 2). A total of 35 strains (referred to as host strains) were employed to assess the lytic activity of the isolated phages. The isolation strains and host strains are represented by nine serotypes (1/2a, 1/2b, 1/2c, 4b, 4d, 4a, 4c, 3a, 3b) of *L. monocytogenes*, respectively (Table 2).

Isolation of phages

In accordance with the methodology outlined in reference [19–21], the samples were subjected to an overnight enrichment process in BHI broth, supplemented with 31 distinct isolation strains and $CaCl_2$. Subsequently, the cultures were collected and filtered. A portion of each filtered culture was combined with an individual isolated strain at a specified optical density (OD). Then the mixtures were incubated for a period of 24 to 48 h. Subsequently, the double-layer agar method applied bacteriophage mixtures to solid agar. The identification of the phages was conducted by observing plaque

Table 1 Samples and isolated phages in this study

Source	Sample types(n)	Samples per region (n)	Isolated phages per Region (n)	Total phages(n)
FE	Seafood (58)	P7 [M1 (12), M2 (8), M3 (7), M4 (13), M5 (11), M6 (7)]	P7 [M1 (3), M2 (7), M3 (7), M4 (4), M5 (5), M6 (4)]	30
	Swab (7)	P1 [7]	P1 [22]	22
	Frozen food (3)	P1 [3]	P1 [3]	3
	Market sewage (22)	P1 [16], P7 [M1 (1), M2 (1), M3 (1), M4 (1), M5 (1), M6 (1)]	P1 [0], P7 [M1 (0), M2 (1), M3 (1), M4 (0), M5 (0), M6 (0)]	2
NE	Soil (63)	P1 [2], P2 [27], P3 [8], P4 [6], P5 [5], P6 [15]	P1 [21], P2 [69], P3 [21], P4 [23], P5 [12], P6 [31]	177
	Sand (88)	P7 [B1 (11), B2 (11), B3 (11), B4 (11), B5 (11), B6 (11), B7 (11), B8 (11)]	P7 [B1 (6), B2 (3), B3 (10), B4 (1), B5 (6), B6 (2), B7 (15), B8 (3)]	46
	Spring water (2)	P3 [2]	P3 [9]	9
	Seawater (43)	P7 [B1 (5), B2 (5), B3 (5), B4 (5), B5 (7), B6 (4), B7 (6), B8 (6)]	P7 [B1 (2), B2 (4), B3 (4), B4 (3), B5 (2), B6 (5), B7 (6), B8 (2)]	28

P1 to P7 represent different provinces, M1 to M6 represent different markets, and B1 to B8 represent different beaches. FE: Food-related environment; NE: Natural environment

Table 2 Isolation strains and host strains used in this study

	Serotypes	Source	Strains
Isolation strain	1/2a	Food	Lm205
	1/2a	Food	Lm043
	1/2a	Patient	Lm261
	1/2a	Food	Lm082
	1/2a	Unknown	*Lm3908*
	1/2b	Food	Lm021
	1/2b	Food	Lm075
	1/2b	Food	Lm097
	1/2b	Unknown	*Lm3910*
	1/2b	Environment	NL01
	1/2b	Environment	NL02
	1/2b	Environment	NL03
	1/2b	Environment	NL04
	1/2b	Environment	NL05
	1/2b	Environment	NL06
	1/2b	Environment	NL07
	1/2c	Environment	Lm959
	1/2c	Environment	Lm1088
	1/2c	Food	*Lm605*
	1/2c	Unknown	*Lm3911*
	1/2c	Environment	NL08
	1/2c	Environment	NL09
	4b	Food	Lm086
	4b	Food	Lm102
	4b	Food	Lm594
	4b	Environment	NL10
	4d	Food	*Lm061*
	4a	Environment	Lm1058
	4c	Food	Lm637
	3a	Food	Lm350
	3b	Food	*Lm083*
	Serotypes	**Source**	**Strains**
Host strain	1/2a	Food	Lm019
	1/2a	Food	Lm0136
	1/2a	Patient	Lm244
	1/2a	Food	Lm329
	1/2a	Patient	Lm841
	1/2b	Environment	Lm716
	1/2b	Food	Lm1290
	1/2b	Environment	Lm1420
	1/2b	Environment	Lm1670
	1/2b	Patient	Lm188
	1/2c	Food	Lm032
	1/2c	Food	Lm058
	1/2c	Food	Lm258
	1/2c	patient	Lm416
	1/2c	Food	Lm038
	4b	Food	Lm0162
	4b	Food	Lm1725
	4b	Food	Lm331
	4b	Food	Lm2869
	4b	Food	Lm2876
	4d	Food	Lm0063
	4d	Food	Lm061
	4d	Food	Lm065
	4a	Environment	Lm1058
	4a	Environment	Lm1331
	4a	Animal	Lm1868
	4c	Food	Lm161
	4c	Food	Lm668
	4c	Food	Lm165
	3a	Food	Lm024
	3a	Food	Lm0093
	3a	Patient	Lm486
	3b	Food	Lm354
	3b	Food	Lm0083
	3b	Food	Lm119

Italicized indicates unsuccessful phage isolation

formation, with the plaques subsequently undergoing purification on three occasions and stored at 4 °C.

Host-range test

The lytic activity of bacteriophages was evaluated by measuring optical density at 600 nm (OD600). In brief, overnight cultures of host bacterial strains were initially diluted 1:100 in BHI medium and incubated at 37 °C with shaking at 200 rpm for 2 h. Subsequently, 100 μL of each bacterial suspension was transferred into individual wells of a 96-well cell culture plate (Corning). Subsequently, 100 μL of either the phage suspension or BHI broth (which served as the negative control) was added to each well and the plate were incubated for 2-3 h at 30°C. The OD600 for each well was determined using a microplate reader (BioTek, Washington, DC, USA). The C value is calculated as follows: C value = (OD600 of negative control wells - OD600 of test wells) / OD600 of negative control wells. A C value exceeding 0.22 was deemed indicative of positive lysis.

Serotype-specific lysis ratio calculation

The serotype-specific lysis ratio was calculated as the proportion of host strains within a given serotype that were lysed by a phage. This was expressed as follows: Serotype-specific lysis ratio = (number of serotype-specific lysis-positive host strains/ total number of tested serotype-specific host strains) $*$ 100%. This ratio was used to assess the efficiency of phage lysis across different serotypes.

Transmission electron microscopy

The purified phages were adsorbed onto formvar and carbon film-coated grids for one minute, and then stained with 1% (w/v) phosphotungstic acid (pH 6.8) for one minute. Following air drying, the grids were observed using a Tecnai 12 transmission electron microscope (FEI, Eindhoven, Netherlands) at 120 kV, with images captured using a charge-coupled device (CCD) camera.

Statistical analysis

The Wilcoxon rank-sum test was utilized to analyze the distribution of binary categorical variables. All statistical analyses were conducted using the R statistical computing software (version 4.4.0).

Results

Phages isolation of samples

A total of 317 *Listeria* phages were isolated, comprising 57 from 90 food-related environmental samples and 260 from 196 natural environmental samples (Table 1). Phages with identical lytic profiles from the same samples were excluded from further analysis, leaving 293 distinct phages for consideration.

Relationship between *Listeria* phages and host strains

In order to elucidate the ecological roles of phages, the phages were categorized into three groups based on total lysis ratios (total lysis ratio = lysis positive host strains / all tested host strains) against host strains: Broad Host Range Phages (BHRP) with a lysis ratio>0.85; Medium Host Range Phages (MHRP), with a lysis ratio between 0.38 and 0.85; and Narrow Host Range Phages (NHRP), with a lysis ratio<0.38. Of the 293 phages, 33 were identified as BHRP, 204 as MHRP, and 56 as NHRP.

Susceptibility of various serotype strains to phage

Broad Host Range phages (BHRP) Among the broad host range phages (BHRP), all nine tested serotype strains (including those frequently associated with food contamination and human listeriosis) were susceptible to the phages. However, a few individual strains, such as Lm331 (4b) and Lm258 (1/2c), exhibited resistance (Fig. 1; Tables 4, 5, 6).

Medium Host Range Phages (MHRP) Among the medium host range phages (MHRP), the susceptibility of the nine serotype strains to phages was found to vary. For serotypes frequently associated with food contamination (1/2a, 1/2b, 1/2c, 4b) and human listeriosis (1/2a, 1/2b, 4b), serotype 4b was found to be the most susceptible to phages, while serotype 1/2b was found to be the least susceptible. Notably, 1/2b strains exhibited low susceptibility to phages derived from natural environmental sources yet demonstrated comparable susceptibility to those derived from food-related environmental sources, similar to that observed in 1/2a and 1/2c strains (Table 3; Fig. 2). A total of 80.39% of the phages tested were found to be effective against strains belonging to 8 or 9 serotypes, as indicated by the number of phages lysing these serotypes divided by the total number of phages in the MHRP (Table 4). The data further indicated that 78.92% of MHRP was effective against strains from all serotypes associated with food contamination (Table 5), and 80.39% were effective against all serotype strains associated with human listeriosis (Table 6). These results were based on the phages lysing at least one strain of each serotype. Moreover, strains from nine serotypes exhibited high susceptibility to MHRP, showing 53.85% susceptibility to phages isolated from food-related environmental sources and 52.12% susceptibility to phages derived from natural environmental sources (Table 4).

Narrow Host Range Phages (NHRP) It was demonstrated that strains of serotype 4a and 4b exhibited high susceptibility to the NHRP, while strains of other serotypes exhibited relatively low susceptibility (Fig. 3; Table 3). Furthermore, strains from five to seven serotypes demonstrated susceptibility to 80.36% of the phages, calculated as the ratio of phages lysing five to seven serotypes to the total number of phages in the NHRP (see Table 4 for details). Additionally, 21.43% of the phages were effective against all serotype strains associated with food contamination (Table 5), and 26.79% were effective against all serotype strains associated with human listeriosis (Table 6). Furthermore, strains from five to six serotypes were susceptible to 75.00%(calculated as the ratio of phages lysing five and six serotypes to total phages sourced from the food-related environment in the NHRP) of phages derived from food-related environmental sources, while strains from 6 to 7 serotypes exhibited susceptibility to 56.25% (calculated as the ratio of phages lysing 6 and 7 serotypes to total phages sourced from the natural environment in the NHRP) of phages derived from natural environmental sources (Table 4).

Phage lysis against different serotype strains

A comparative analysis of phages derived from food-related and natural environmental sources revealed discrepancies in serotype-specific lysis ratios.

Among the 33 broad host range phages (BHRP), the lysis ratios for serotypes were generally high, with all exceeding 90%. However, the lysis ratio for serotype 1/2c strains was relatively low (83.20%) for phages derived from natural environmental sources, and the lysis ratio for serotype 4c strains was also low (79.17%) for phages derived from food-related environmental sources (Fig. 4).

Table 3 Sensitivity of various serotypes strains to different phage groups

Phage group	Source	Serotype 1/2a	Serotype 1/2b	Serotype 1/2c	Serotype 4b	Serotype 4d	Serotype 4a	Serotype 4c	Serotype 3a	Serotype 3b
BHRP	FE	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)	8 (100.00%)
	NE	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)	25 (100.00%)
	Total	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)	33 (100.00%)
MHRP	FE	37 (94.87%)	36 (92.31%)	39 (100.00%)	39 (100.00%)	35 (89.74%)	39 (100.00%)	39 (100.00%)	35 (89.74%)	28 (71.79%)
	NE	152 (92.12%)	135 (81.82%)	162 (98.18%)	165 (100.00%)	157 (95.15%)	164 (99.39%)	163 (98.79%)	133 (80.61%)	137 (83.03%)
	Total	189 (92.65%)	171 (83.82%)	201 (98.53%)	204 (100.00%)	192 (94.12%)	203 (99.51%)	202 (99.02%)	168 (82.35%)	165 (80.88%)
NHRP	FE	3 (37.50%)	4 (50.00%)	2 (25.00%)	8 (100.00%)	7 (87.50%)	8 (100.00%)	7 (87.50%)	5 (62.50%)	0 (0.00%)
	NE	30 (62.50%)	19 (39.58%)	33 (68.75%)	46 (95.83%)	35 (72.92%)	47 (97.92%)	46 (95.83%)	26 (54.17%)	13 (27.08%)
	Total	33 (58.93%)	23 (41.07%)	35 (62.50%)	54 (96.43%)	42 (75.00%)	55 (98.21%)	53 (94.64%)	31 (55.36%)	13 (23.21%)

The numbers in the table represent the positive lysis count (serotype sensitivity rate). The serotype sensitivity rate is calculated as follows: Sensitivity rate = (1- number of serotype strains not lysed by phages / total number of phages tested for this serotype) * 100%. FE, food-related environment; NE, natural environment

Table 4 Number of serotypes lysed by various phage groups

Phage group	Number of sensitive serotypes	Number (proportion) of phages from FE	Number (proportion) of phages from NE	Number (proportion) of total phages
BHRP	9	8 (100.00%)	25 (100.00%)	33 (100.00%)
MHRP	6	0 (0.00%)	4 (2.42%)	4 (1.96%)
	7	6 (15.38%)	30 (18.18%)	36 (17.65%)
	8	12 (30.77%)	45 (27.27%)	57 (27.94%)
	9	21 (53.85%)	86 (52.12%)	107 (52.45%)
NHRP	4	1 (12.50%)	4 (8.33%)	5 (8.93%)
	5	3 (37.50%)	11 (22.92%)	14 (25.00%)
	6	3 (37.50%)	13 (27.08%)	16 (28.57%)
	7	1 (12.50%)	14 (29.17%)	15 (26.79%)
	8	0 (0.00%)	6 (12.50%)	6 (10.71%)

Proportion = (Number of phages lysing the corresponding number of host strains / total number of phages from a specific source in BHRP, MHRP, or NHRP) * 100%. FE, food-related environment; NE, natural environment

Table 5 Number of serotype commonly found in food contamination (1/2a, 1/2b, 1/2c, 4b) lysed by various phage groups

Phage group	Number of sensitive serotypes	Number (proportion) of phages from FE	Number (proportion) of phages from NE	Number(proportion) of total phages
BHRP	4	8 (100.00%)	25 (100.00%)	33 (100.00%)
MHRP	2	1 (2.56%)	7 (4.24%)	8 (3.92%)
	3	3 (7.69%)	32 (19.39%)	35 (17.16%)
	4	35 (89.74%)	126 (76.36%)	161 (78.92%)
NHRP	1	4 (50.00%)	5 (10.42%)	9 (16.07%)
	2	1 (12.50%)	16 (33.33%)	17 (30.36%)
	3	1 (12.50%)	17 (35.42%)	18 (32.14%)
	4	2 (25.00%)	10 (20.83%)	12 (21.43%)

Proportion = (Number of phages lysing the corresponding number of host strains / total number of phages from a specific source in BHRP, MHRP, or NHRP) * 100%. FE, food-related environment; NE, natural environment

Table 6 Number of serotype commonly found in human listeriosis cases (1/2a, 1/2b, 4b) lysedby various phage groups

Phage group	Number of sensitive serotypes	Number (proportion) of phages from FE	Number (proportion) of phages from NE	Number(proportion) of total phages
BHRP	3	8 (100.00%)	25 (100.00%)	33 (100.00%)
MHRP	1	1 (2.56%)	7 (4.24%)	8 (3.92%)
	2	3 (7.69%)	29 (17.58%)	32 (15.69%)
	3	35 (89.74%)	129 (78.18%)	164 (80.39%)
NHRP	1	4 (50.00%)	13 (27.08%)	17 (30.36%)
	2	1 (12.50%)	23 (47.92%)	24 (42.86%)
	3	3 (37.50%)	12 (25.00%)	15 (26.79%)

Proportion = (Number of phages lysing the corresponding number of host strains / total number of phages from a specific source in BHRP, MHRP, or NHRP) * 100%. FE, food-related environment; NE, natural environment.

Among the 204 medium host range phages (MHRP), the lysis ratio for serotype 4a and 4c strains were relatively high (83.76% and 88.03% for food-related environmental sources; 74.55% and 76.36% for natural environmental sources), In contrast, the lysis ratios for serotype 1/2b and 3b strains were notably lower (53.33% and 39.32% for food-related environmental sources; 39.39% and 44.24% for natural environmental sources) (Fig. 4). Notably, the lysis ratios for serotype 1/2b, 4a, and 4c strains were significantly higher for phages derived from food-related

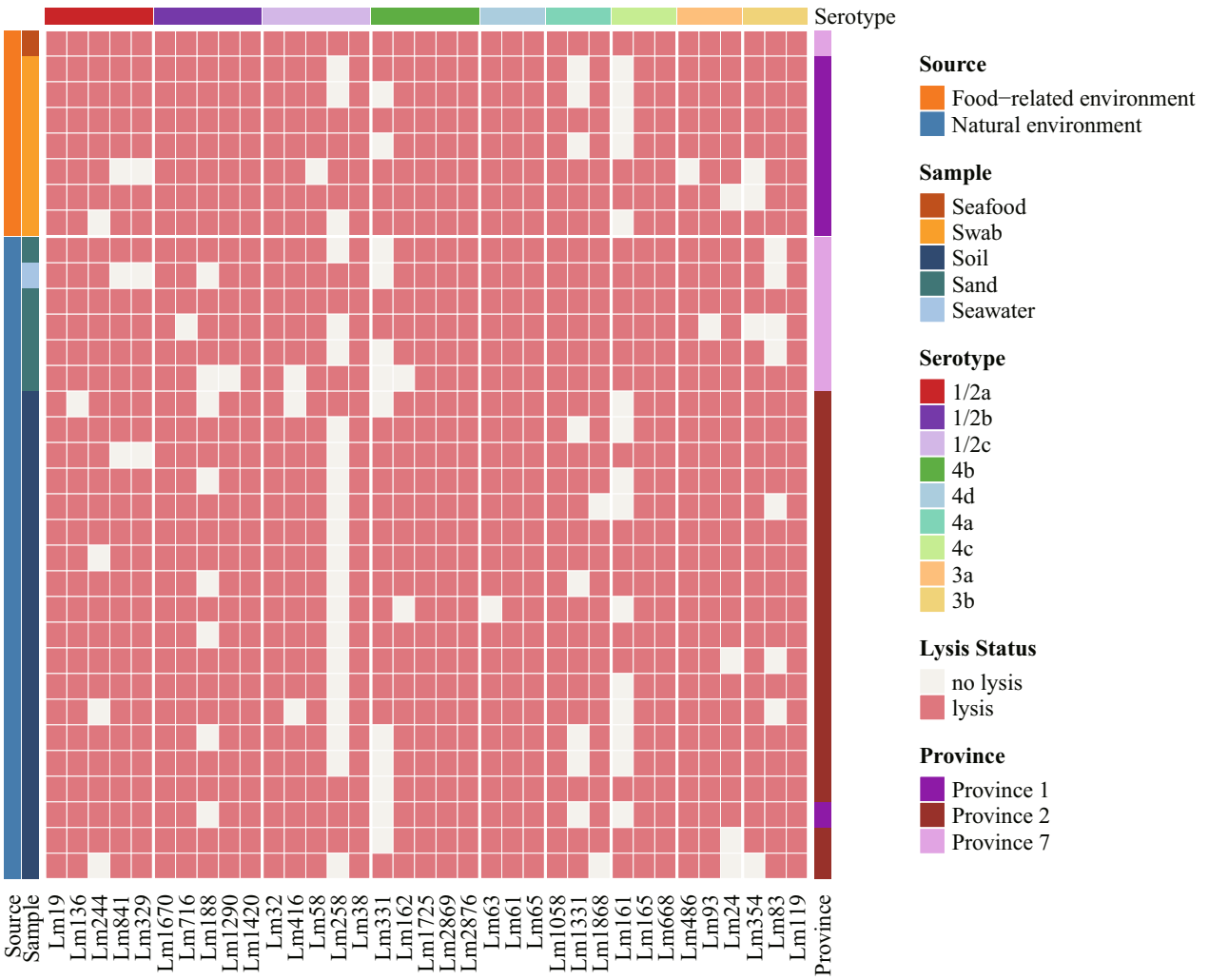

Fig. 1 Lysis Profiles of Broad Host Range Phages (BHRP) (n = 33). Left labels indicate phage sources and sample types, while right labels denote provinces of origin. Columns represent host strains, annotated with their serotypes

environmental sources compared to those from natural environmental sources (53.33% vs. 39.39%, $P = 0.011$; 83.76% vs. 74.55%, P = 0.045; and 88.03% vs. 76.36%, $P < 0.001$; respectively).

Among the 56 narrow host range phages (NHRP), the serotype-specific lysis ratios were generally low, except for serotype 4 strains (4a, 4b, 4c, 4d), which exhibited higher lysis ratios (66.67%, 45.00%, 58.33%, and 41.67% for food-related environmental sources; 72.22%, 47.08%, 79.86%, and 33.33% for natural environmental sources) (Fig. 4). Notably, no phage was found to exclusively target a single serotype.

Morphology of phages by transmission electron microscope

A total of 16 representative phages (8, 4 and 4 for BHRP, MHRP and NHRP) were selected for transmission electron microscopy (TEM) analysis based on their lytic profiles and sources (Fig. 5). The lytic profiles and detailed dimensions of the phages are presented in Fig. 6.

In the BHRP group, three phages were identified as *Siphoviridae*-like phages, with head diameters of 49.93 ± 0.94 nm and contractile tails measuring 6.48 ± 0.27 nm in diameter and 238.65 ± 17.50 nm in length. Furthermore, five phages were classified as *Myoviridae*-like phages, exhibiting head diameters of 78.52 ± 4.99 nm, contractile tails with diameters of 22.80 ± 1.76 nm, and lengths of 168.78 ± 19.92 nm.

In the MHRP group, two phages were identified as *Myoviridae*-like phage, with a head diameter of 77.32 ± 1.43 nm, a contractile tail diameter of 21.46 ± 2.04 nm, and a length of 187.13 ± 4.57 nm. Two phages were classified as *Siphoviridae*-like phages, had head diameters of

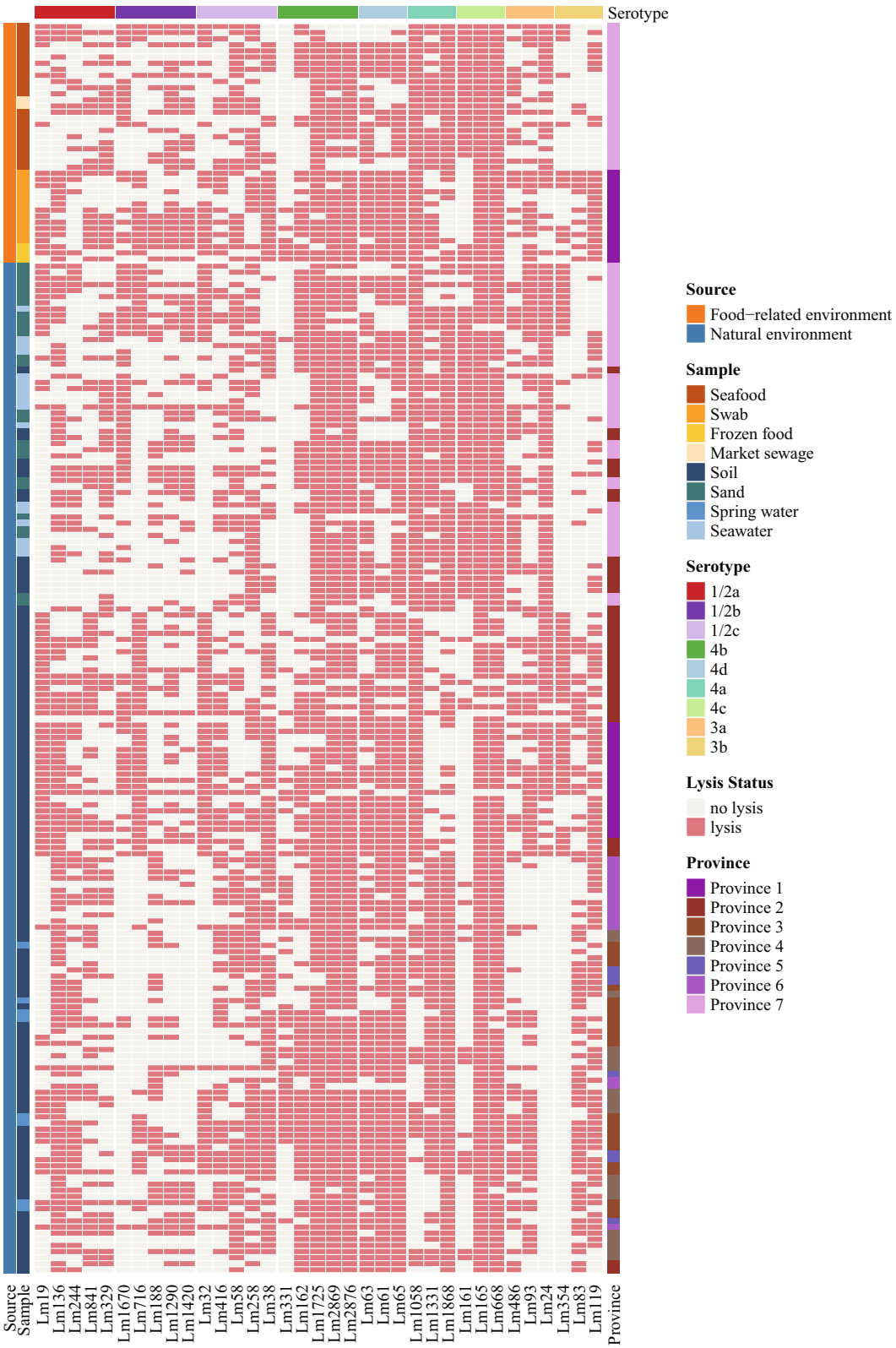

Fig. 2 Lysis Profiles of Medium Host Range Phages (MHRP) (n = 204). Left labels indicate phage sources and sample types, while right labels denote provinces of origin. Columns represent host strains, annotated with their serotypes

Fig. 3 Lysis Profiles of Narrow Host Range Phages (NHRP) (n = 56). Left labels indicate phage sources and sample types, while right labels denote provinces of origin. Columns represent host strains, annotated with their serotypes

56.97 ± 0.26 nm, contractile tail diameters of 9.74 ± 1.29 nm, and tail lengths of 253.56 ± 1.69 nm.

In the NHRP group, four phages were identified as *Siphoviridae*-like phages, with head diameters of 54.39 ± 2.92 nm, contractile tails of 7.97 ± 0.84 nm in diameter, and lengths of 246.22 ± 12.62 nm (Fig. 6).

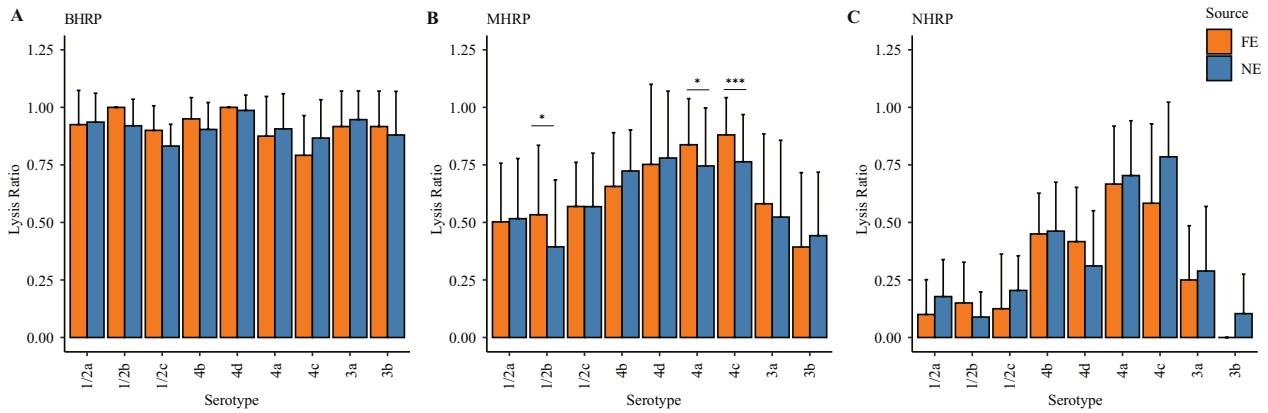

Fig. 4 Comparison of the phage lysis activities by sample environmental sources. **A** Broad Host Range Phages (BHRP) (n = 33). **B** Medium Host Range Phages (MHRP) (n = 204). **C** Narrow Host Range Phages (NHRP) (n = 56). The bars represent the mean + SD. FE, Food-related environment; NE, Natural environment. Statistical analyses were conducted using the Wilcoxon rank-sum test, with the following significance levels: **P*<0.05; ** *P* < 0.01 and ****P* < 0.001

Discussion

Listeriosis is a severe foodborne illness with high fatality rates. It can result in miscarriage, spontaneous preterm labor, preterm birth, stillbirth, and congenital neonatal infections [1, 22]. Phages, as natural antibacterial agents, demonstrate considerable potential for the control of foodborne pathogens and the treatment of infectious diseases [16, 23, 24]. It is important to investigate the interactions between phages and *Listeria* to uncover the molecular mechanisms that could lead to the development of innovative antibacterial strategies. This study first conducted the large-scale endeavor to isolate *Listeria* phages from a multitude of sample environmental sources and diverse provinces, with 317 phages isolated. The host range of these phages was determined using a liquid culture method, which permitted the exploration of their lytic patterns and ecological distribution based on intraspecies serotype classification. Furthermore, their morphological characteristics were observed.

Several studies have provided insights into the morphology and distribution and abundance of phages in marine and soil environments. However, there is a lack of research isolating phages and characterizing their lytic activities [25]. *Listeria*-specific phages have been isolated from a variety of sources, including feces, wastewater, abattoir effluents, soil, farms, food products, and sewage [17, 18, 26–31]. Of the 500 identified *Listeria* phages, only a few have been fully characterized as virulent phages with potential for use in biological control [17, 19, 32–34]. These virulent *Listeria* phages have the capacity to infect a range of major *L. monocytogenes* serotypes (1/2a, 1/2b, 1/2c, 4a, 4ab, 4b, 4c, 4d, 4e) and *Listeria innocua* serotypes 5, 6a, and 6b. To date, no *Listeria* phages have been identified that are capable of lysing *L. monocytogenes* serotypes 3a, 3b, 3c, or *Listeria grayii* [16, 31]. Notably, several phages in this study were observed to lyse *L. monocytogenes* serotypes 3a and 3b strains.

Currently, there is no established reference standard for the lytic activity of phages. Furthermore, the categories such as "broad spectrum" and "narrow spectrum" lack clearly defined cutoff values. Using such terms is inherently subjective and provides limited comparability due to the relatively small sample sizes involved. This study inaugural attempt to categorize phages into three groups: broad host range phages (BHRP), medium host range phages (MHRP), and narrow host range phages (NHRP) based on total lysis ratios. This classification provides a comprehensive characterization of each group and elucidates the ecological roles of phages.

The vast majority of host strains, including those frequently associated with food contamination and human listeriosis, demonstrated susceptibility to BHRP phages in this study, this highlights the potential for their use in biocontrol and phage therapy applications. Further investigation is required to elucidate the resistance mechanisms observed in a few strains. Although *L. monocytogenes* is a well-known foodborne pathogen, 25 of the 33 BHRP phages in this study were isolated from the natural environment. This finding underscores natural environments as a significant reservoir of broad-spectrum phages.

The data indicate that phages in the NHRP group are capable of lysing serotype 4 strains, suggesting that serotype 4 strains exhibit greater ease of identification and lysis by phages. Therefore, serotype 4 strains are the most suitable for isolating *Listeria* phages. An additional potential explanation is the presence of variations in cell wall teichoic acids (WTA) between different serotypes

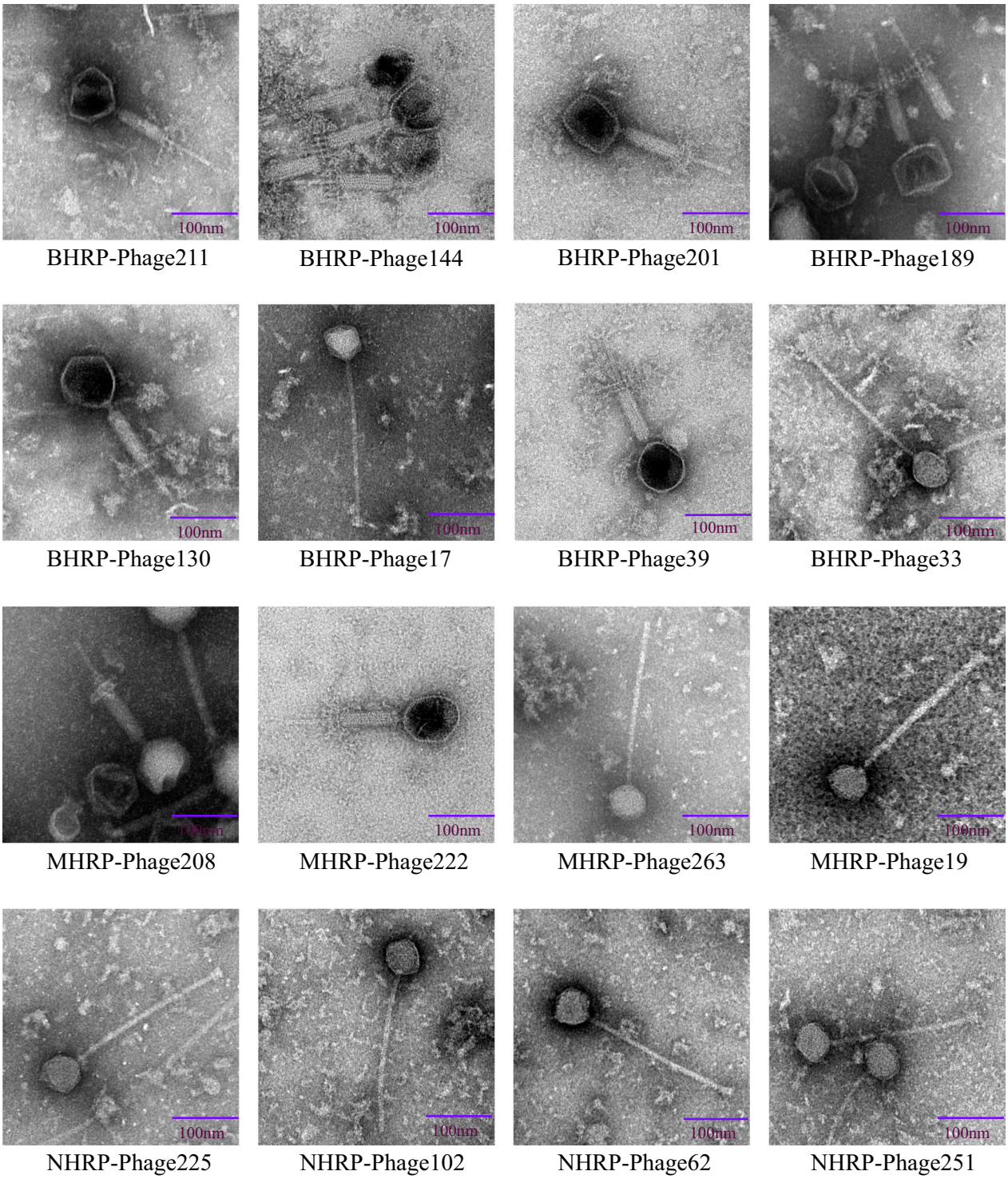

Fig. 5 Transmission electron microscopy images of isolated *Listeria monocytogenes* phages. Phages 17, 33, 263, 19, 225, 102, 62, and 251 belong to the *Siphoviridae*-like phage. Phages 211, 144, 201, 189, 130, 39, 208, and 222 belong to the *Myoviridae*-like phage family

of *L. monocytogenes*. Serotype 4 has WTA with terminal glucose and galactose residues, which are essential for phage adsorption and host lysis [35]. It is noteworthy that the majority of mitomycin C-induced *Listeria* phages (30 out of 39) were capable of lysing hosts of *L. monocytogenes* serotype 4 [36].

Phage	Serotype 1/2a	Serotype 1/2b	Serotype 1/2c	Serotype 4b	Serotype 4d	Serotype 4a	Serotype 4c	Serotype 3a	Serotype 3b	Source	Group	Morphology	Capsid diameter (nm)	Tail width (nm)	Tail length (nm)
211	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.67	1.00	NE	WAP	*Myoviridae*	82.53	19.78	170.51
144	1.00	1.00	0.80	1.00	1.00	1.00	1.00	0.67	0.67	NE	WAP	Myoviridae	76.76	24.17	127.99
201	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.67	FE	WAP	*Myoviridae*	79.29	22.75	181.28
189	1.00	1.00	1.00	1.00	1.00	1.00	0.67	1.00	1.00	FE	WAP	*Myoviridae*	86.64	25.47	186.78
130	1.00	1.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00	NE	WAP	*Myoviridae*	73.91	22.15	163.15
17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	FE	WAP	*Siphoviridae*	50.87	6.21	256.15
39	1.00	1.00	0.80	0.80	1.00	1.00	1.00	1.00	0.67	NE	WAP	*Siphoviridae*	71.98	22.48	182.97
33	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	NE	WAP	*Siphoviridae*	48.99	6.75	221.15
208	0.80	0.80	1.00	0.80	1.00	0.67	1.00	0.33	0.67	FE	MAP	*Myoviridae*	75.89	23.50	191.70
222	0.00	0.20	0.40	0.80	0.67	1.00	1.00	0.00	0.33	NE	MAP	*Siphoviridae*	78.75	19.42	182.56
263	1.00	0.60	1.00	1.00	1.00	0.67	0.67	0.67	0.67	NE	MAP	*Siphoviridae*	56.71	8.45	251.87
19	0.00	0.00	0.60	0.80	1.00	1.00	1.00	0.33	0.33	FE	MAP	*Siphoviridae*	57.23	11.02	255.25
225	0.00	0.00	0.00	0.80	0.67	1.00	1.00	0.00	0.00	NE	NAP	Siphoviridae	54.83	8.76	265.17
102	0.00	0.00	0.20	0.40	0.33	0.67	1.00	0.33	0.00	NE	NAP	*Siphoviridae*	51.70	8.32	245.14
62	0.00	0.00	0.00	0.60	0.67	1.00	1.00	0.00	0.00	FE	NAP	*Siphoviridae*	52.04	6.55	244.92
251	0.00	0.00	0.00	0.60	0.33	0.67	0.67	0.00	0.00	NE	NAP	*Siphoviridae*	59.00	8.26	229.64

Fig. 6 Lytic profiles and morphological characteristics of isolated *Listeria monocytogenes* phages. The numerical values within each cell represent the lysis ratio of each phage against the corresponding serotype strains. FE, Food-related environment; NE, Natural environment

The lysis rates of BHRP phages were observed to be consistently high, while those of NHRP phages were found to be low across all cases in this study. In the MHRP group, food-related environment-sourced phages demonstrated a higher lysis rate against 1/2b strains than natural environment-sourced phages. Furthermore, the results also revealed that 1/2b strains exhibited greater susceptibility to phages derived from food-related environments than those from natural environments. These findings suggest that food-related environments may better support the survival and proliferation of 1/2b strains, aligning with previous studies reporting a high abundance of serotype 1/2b among *Listeria monocytogenes* isolates from food and food-related environments [8, 37–41]. To the best of our knowledge, there is no existing comparative study on isolates from both food-related environmental and natural environmental sources. Current research on *L. monocytogenes* primarily focus on isolates from food-related environmental sources and patients. In contrast, studies on natural environmental isolates are limited and often lack consistency [42–44]. The findings in this study offer insights into the distribution and ecological adaptability of *Listeria* in diverse environments.

It should be noted that this study has few limitations. The experimental design was not sufficiently comprehensive to permit exhaustive measurement of the host range. Accordingly, a diverse range of strains encompassing nine most prevalent serotypes was selected to ensure the attainment of representative results. It is conceivable that the host range may undergo alterations over time due to the co-evolution of phages and bacteria during successive propagation. This study describes the host range and morphology of the isolated *Listeria* phages. Further research is needed to evaluate the potential for more sophisticated applications, elucidate the mechanisms of bacterial resistance to phage, and gain a deeper understanding of the interactions between phages and hosts.

Conclusion

A total of 317 *Listeria* phages were isolated from a diverse array of sources in this study. The lysis patterns for nine serotypes of host strains and the ecological distribution of these phages were analyzed. The phages were classified into three groups based on their total lysis ratios. The majority of phages in the BHRP group are of the *Myoviridae*-like phages and are capable of lysing the majority of host strains, with minimal resistance observed. The phages of the MHRP group include both *Siphoviridae*-like and *Myoviridae*-like phages. Furthermore, phages isolated from food-related sources demonstrated greater lytic activity against *Listeria* serotypes 1/2b, 4a, and 4c compared to those derived from natural environmental sources. The phages in the NHRP group are of the *Siphoviridae*-like phages and are primarily capable of lysing serotype 4 strains. This study offers a valuable resource for the application of *Listeria* phages and provides new insights into the ecological distribution patterns of *Listeria* phages based on their lytic profiles.

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Author contributions
JNC, CYY and YW conceived of the study. LLL (Lingyun Liu), PM, LLL (Lingling Li), XFX, JY, XL, HS, KD, and JDS, performed the experiment; HZ and JP analysed the data; JNC drafted the manuscript. CYY revised the manuscript. All authors reviewed and approved the final manuscript.

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Not applicable.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable

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The authors declare that they have no Conflict of interest.

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