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Characterization of Two Virulent Phages of *Lactobacillus plantarum*

Mariángeles Briggiler Marcó,[a] Josiane E. Garneau,[b] Denise Tremblay,[b] Andrea Quiberoni,[a] and Sylvain Moineau[b]

Instituto de Lactología Industrial (UNL-CONICET), Facultad de Ingeniería Química, Santa Fe, Argentina,[a] and Département de Biochimie, de Microbiologie et de Bio-informatique, Faculté des Sciences et de Génie, Groupe de Recherche en Écologie Buccale, Faculté de Médecine Dentaire, Université Laval, Félix d'Hérelle Reference Center for Bacterial Viruses, Québec City, Québec, Canada[b]

We characterized two *Lactobacillus plantarum* virulent siphophages, ATCC 8014-B1 (B1) and ATCC 8014-B2 (B2), previously isolated from corn silage and anaerobic sewage sludge, respectively. Phage B2 infected two of the eight *L. plantarum* strains tested, while phage B1 infected three. Phage adsorption was highly variable depending on the strain used. Phage defense systems were found in at least two *L. plantarum* strains, LMG9211 and WCSF1. The linear double-stranded DNA genome of the *pac*-type phage B1 had 38,002 bp, a G+C content of 47.6%, and 60 open reading frames (ORFs). Surprisingly, the phage B1 genome has 97% identity with that of *Pediococcus damnosus* phage clP1 and 77% identity with that of *L. plantarum* phage JL-1; these phages were isolated from sewage and cucumber fermentation, respectively. The double-stranded DNA (dsDNA) genome of the *cos*-type phage B2 had 80,618 bp, a G+C content of 36.9%, and 127 ORFs with similarities to those of *Bacillus* and *Lactobacillus* strains as well as phages. Some phage B2 genes were similar to ORFs from *L. plantarum* phage LP65 of the *Myoviridae* family. Additionally, 6 tRNAs were found in the phage B2 genome. Protein analysis revealed 13 (phage B1) and 9 (phage B2) structural proteins. To our knowledge, this is the first report describing such high identity between phage genomes infecting different genera of lactic acid bacteria.

Lactobacilli are widely used in a variety of food fermentation processes, where they contribute to the flavor and texture of final products. They also produce organic acids, and the resulting low pH protects fermented products from degradation by spoilage microorganisms (15). In recent years, the industrial relevance of lactobacilli has been significantly enhanced by their increasing use as probiotics (12) or as a biotechnological tool (32).

Lactobacillus plantarum is commonly found as part of the natural microflora of fermented foods (dairy, vegetables, and meats) (12, 53, 68). This lactic acid bacterium may also be added as a starter or adjunct culture, in both cases improving the organoleptic characteristics of the final products (2, 12, 14, 15, 48, 49). Additionally, many *L. plantarum* strains possess documented probiotic properties, and marketed functional foods contain these strains (12, 53). *L. plantarum* can be used as a probiotic starter culture in the production of functional foods, taking advantage of, among others, its ability to grow in milk. However, the increasing use of *L. plantarum* as a starter or adjunct culture can lead to phage infections in industrial environments, with adverse effects on the final product (25, 51).

Phage infection is still one of the persistent causes of substandard dairy fermentation processes (60). Virulent phages can lyse starter cultures, yielding low-quality products that lead to economic losses. Consequently, efficient control measures to minimize problems caused by phage attacks become essential. In order to carry out successful antiphage strategies, knowledge about phage population and biology is needed (27, 39).

To date, over 30 *L. plantarum* phages, isolated from several sources, have been reported (16, 70, 72). All belong to the *Caudovirales* order (tailed phages, double-stranded DNA genome) (1, 70), and members belonging to each of the three *Caudovirales* families have been isolated: *Siphoviridae* (19 phages), *Myoviridae* (5 phages), and *Podoviridae* (1 phage). Other *L. plantarum* phages have been reported but not classified (70). Therefore, *L. plantarum* phages are relatively diverse and found in a wide variety of niches.

To our knowledge, only four *L. plantarum* phage genomes have been sequenced. Phage g1e (*Siphoviridae*, temperate) was isolated from plant materials and has a 42,259-bp genome with a G+C content of 43.1% and 62 open reading frames (ORFs) (37). Phage Sha1 (*Siphoviridae*, temperate) was isolated from kimchi and has a 41,726-bp genome with a G+C content of 40.6% and 58 putative ORFs (72). Phage JL-1 (*Siphoviridae*, virulent) was isolated from fermented cucumbers (43) and possesses a 36,700-bp genome with a G+C content of 39.4% and 52 ORFs. Finally, phage LP65 (*Myovidiae*, virulent) was isolated from fermented meat and has a very large genome of 131,573 bp with a G+C content of 37.3% and 165 ORFs (10).

Other *L. plantarum* phages have been analyzed in some detail; studies mainly included thermal and chemical sensitivities, and there were some preliminary genetics studies (9, 16, 44, 54, 65, 74). Overall, research on *Lactobacillus* phages has progressed over the past decade, but our knowledge of their biology and genetic composition is still limited and lags somewhat behind that of other industrially relevant phages (70).

The aim of this work was to carry out the characterization of two available *L. plantarum* phages. Phages ATCC 8014-B1 and ATCC 8014-B2 (herein referred to as B1 and B2, respectively) were previously isolated from corn silage and anaerobic sewage sludge (21).

MATERIALS AND METHODS

Bacterial strains, phages, and culture conditions. *L. plantarum* strains were grown at 37°C in MRS broth (Difco). *L. plantarum* ATCC 8014 was

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Address correspondence to Sylvain Moineau, Sylvain.Moineau@bcm.ulaval.ca.

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used as the host strain for phages B1 and B2. For phage amplification, MRS was supplemented with 10 mM $CaCl_2$ (MRS-Ca). Phage stocks were prepared as described previously (56) and stored as lysates at 4°C. Phage counts, expressed as PFU per milliliter, were obtained using the double-layer plaque titration method (64). Bacterial strains are maintained at the INLAIN Collection (Argentina) and the Félix d'Hérelle Reference Center for Bacterial Viruses of the Université Laval (Canada; www.phage.ulaval.ca) as frozen stocks in MRS broth containing 15% (vol/vol) glycerol. Phages B1 and B2 as well as the host *L. plantarum* ATCC 8014 were purchased from the American Type Culture Collection (Manassas, VA; www.atcc.org).

Electron microscopy. Ten microliters of 2% phosphotungstic acid (pH 7.0) was put in a clean sterile petri dish. A 200-mesh Formvar-carbon-coated copper grid (Pelco International) was deposited face down on the staining solution for 30 s. Then, 10 μl of a purified phage suspension (10^{10} PFU ml^{-1}) was mixed with the stain by pipetting up and down. After 90 s, the grid was deposited face up on blotting paper. The grid was dried for 5 min and observed at 80 kV using a JEOL 1230 transmission electron microscope (62).

Microbiological assays. The host range of *L. plantarum* phages B1 and B2 was assessed by spotting 5 microliters of 10^{-2} and 10^{-4} dilutions of a high-titer lysate (10^9 PFU ml^{-1}) on top of agar containing one of the eight *L. plantarum* strains tested (see Table 1). To study the phage adsorption process, *L. plantarum* cultures were grown in MRS to an optical density at 600 nm of 0.6 to 0.8, after which they were in contact with phage B1 or B2 at a final concentration of 10^3 PFU ml^{-1}. The phage-containing cultures were incubated at 37°C for 15 min, and then we proceeded as described elsewhere (22). To determine the presence of active natural defense mechanisms against phages B1 and B2, the efficiency of plaquing (EOP) was calculated by dividing the phage titer on the test *L. plantarum* strain by the titer of the phage on the phage-sensitive host strain *L. plantarum* ATCC 8014. For phage-host systems showing reduced EOP values, two phage plaques obtained on the restrictive strain were purified and propagated on the same strain. The lysate obtained (modified phage) was titrated on both strains (original sensitive host and the restrictive strain) to determine a second EOP value. Modified phages were then propagated again on *L. plantarum* ATCC 8014, and the resulting lysate (unmodified phage) was titrated on both strains (4).

Phage DNA preparation and sequencing. Genomic DNA of phages B1 and B2 was isolated using a Maxi lambda DNA purification kit (Qiagen) with modifications (19). The restriction profiles of phage B1 and B2 DNA were compared to confirm differences. Restriction endonucleases (Roche Diagnostics) were used as recommended by the manufacturer. The DNA fragments were separated in a 0.8% agarose gel, stained with ethidium bromide, and photographed under UV illumination. Genome sequencing was performed at the Plateforme d'ADN génomique de l'Université Laval (Université Laval, Québec, Canada) using a GS-FLX Titanium apparatus (Roche) and the 454 pyrosequencing technique. For phage B1, 39,144 reads were generated and assembled into a single contig with a coverage of 430-fold. For phage B2, 4,670 reads were generated and assembled into a single contig with a coverage of 18-fold. The extremities of the genomes were determined by sequencing ligated phage DNA preparations using converging PCR primers at the genomic platform of the Centre Hospitalier de l'Université Laval with an ABI Prism 3100 apparatus.

Bioinformatics analysis. Sequence analyses were performed using BioEdit (30). Open reading frames (ORFs) were first identified using the GenMark program (46) and were further confirmed with ORFinder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). An ORF was considered valid if it had AUG, UUG, or GUG as the starting codon, encoded at least 29 amino acids (aa), and was preceded by an *L. plantarum* Shine-Dalgarno sequence (AGAAAGGAGGTGATC) (5). Function was attributed to an ORF using Blast2go (http://blast2go.bioinfo.cipf.es/start_blast2go) and BLASTp (NCBI [http://blast.ncbi.nlm.nih.gov/Blast.cgi]). The annotations were supported by searching for protein functional domains using the NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and EMBL InterProScan (http://www.ebi.ac.uk/Tools/InterProScan/). The tRNAs were identified using the tRNAscan-SE server (http://lowelab.ucsc.edu/tRNAscan-SE) and the ARAGORN program (41). Codon usage was determined through the DNA 2.0 Web server (Menlo Park, CA) and the Count-codon program available on the Kazusa DNA Research Institute Web page (http://www.kazusa.or.jp/codon/). The bacterial codon usage for the *L. plantarum* host strains was obtained from the Kazusa DNA Research Institute database.

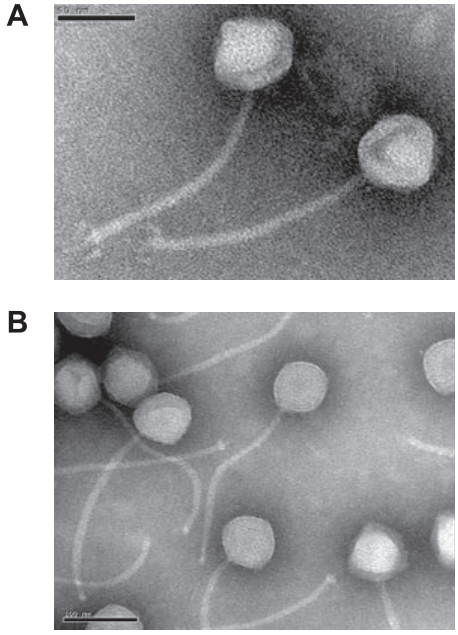

FIG 1 Electron micrographs of the phages B1 (A) and B2 (B). Bars, 50 nm (A) and 100 nm (B).

Analyses of phage B1 and B2 structural proteins. Phage lysates were concentrated with polyethylene glycol (PEG) and purified using two CsCl gradients (61). Purified phages were recovered by ultracentrifugation using a Beckman SW41 Ti rotor at 35,000 rpm (210,053 × *g*) for 3 h, followed by a second ultracentrifugation using a Beckman NVT65 rotor at 60,000 rpm (342,317 × *g*) for 18 h. The phage preparations were then dialyzed against phage buffer (0.05 M Tris-HCl [pH 7.5], 0.1 M NaCl, 8 mM $MgSO_4$). Purified phages (4×10^{11} PFU ml^{-1}) were treated as described elsewhere (62). Briefly, phages were mixed with 4× loading buffer and boiled for 5 min. The samples were sonicated for 5 s with an ultrasonic Sonifier W-350 cell disrupter. Proteins were then separated by migration on a 12% SDS-polyacrylamide gel (1.5 mm thick). The Coomassie-stained protein bands of interest were excised from the gel and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Centre Protéomique de l'Est du Québec (Université Laval, Quebec, Canada). These results were analyzed using the Scaffold Proteome software (13, 33, 55). Purified phage lysates were also directly analyzed by LC-MS/MS.

Nucleotide sequence accession numbers. The complete genome sequences of phages B1 and B2 have been deposited in GenBank under accession numbers JX486087 and JX486088, respectively.

RESULTS AND DISCUSSION

Electron microscopy. Both B1 and B2 phages have long noncontractile tails (Fig. 1) and belong to the *Siphoviridae* family, as do most characterized *L. plantarum* phages (70). Phage B1 has an icosahedral capsid with an estimated diameter of 54 ± 3 nm and a

TABLE 1 Host range and adsorption rates of phages B1 and B2 on *L. plantarum* strains

Strain	Source[a]	Phage B1		Phage B2	
		EOP	Adsorption (%)	EOP	Adsorption (%)
ATCC 8014	ATCC	1.0	99.6 ± 4.8	1.0	90.8 ± 3.0
WCSF1	Human saliva (NCBIM collection)	1.5×10^{-2}	19.0 ± 1.4	—[b]	0
LMG9211	Human saliva (BCCM collection)	4.0×10^{-3}	2.3 ± 0.4	—	92.4 ± 1.8
PLN	NSLAB (INLAIN collection)	—	0	1.0	98.5 ± 1.5
SMQ-1113	Industrial strain	—	1.1 ± 0.7	—	14.4 ± 3.9
SMQ-1114	Industrial strain	—	8.2 ± 2.3	—	3.2 ± 1.0
SMQ-1115	Industrial strain	—	9.5 ± 2.5	—	12.3 ± 1.6
SMQ-1116	Industrial strain	—	13.7 ± 3.6	—	10.4 ± 6.4

[a] ATCC, American Type Culture Collection; BCCM, Belgian Coordinated Collections of Microorganisms; NCBIM, National Collection of Industrial and Marine Bacteria; NSLAB, nonstarter lactic acid bacteria; INLAIN, Instituto de Lactología Industrial.
[b] —, not determined, as the phage does not infect the strain.

tail of 157 ± 10 nm in length and 8 ± 1 nm in width. The baseplate appears somewhat complex, with spikes or fibers (Fig. 1A). Phage B2 has an icosahedral capsid with a diameter of 74 ± 2 nm and a tail of 240 ± 3 nm in length and 10 ± 1 nm in width (Fig. 1B). Other investigators previously reported a larger capsid diameter (110 nm) and a much longer tail (500 nm) for *L. plantarum* phage B2 (54). Although dimensions may vary due to the use of different electron microscopes and methodologies (59), this cannot explain such large differences. At this time, it is unclear why such a discrepancy exists.

Microbiological assays. The results of the host range and adsorption tests are presented in Table 1. Each phage exhibited a distinctive host range but shared a common host (*L. plantarum* ATCC 8014). Phage B1 also replicated on *L. plantarum* strains WCSF1 and LMG9211, but the EOP was reduced. Surprisingly, under the conditions tested, the adsorption of phage B1 on strains LMG9211 and WCSF1 was very low, although clear plaques were formed. This low adsorption could be due to a limited number of phage receptors (in comparison with *L. plantarum* ATCC 8014) or their availability on the cell surface. Similar results were reported for *Lactobacillus paracasei* phages (8). Conversely, phage B2 was amplified on *L. plantarum* strain PLN and on its host ATCC 8014 (Table 1). Interestingly, phage B2 adsorbed well to strain LMG9211 without forming plaques (Table 1), suggesting the presence of phage resistance mechanisms in this strain (39). In general, phages were not able to adsorb on the other *L. plantarum* strains tested, suggesting the absence of receptors or perhaps adsorption blocking mechanisms (39).

Restriction/modification systems. As indicated above, *L. plantarum* LMG9211 and WCSF1 seemed to carry a natural defense system, as the EOP of phage B1 was reduced (Table 1). Phage plaques were recovered from these two hosts (LMG9211 and WCSF1), purified, and amplified on each strain. These amplified phages had an EOP of 1.0 on *L. plantarum* ATCC 8014. When these phages were propagated again in their original host, *L. plantarum* ATCC 8014, the EOP values were reduced and similar to those shown in Table 1. This temporary host-specific immunity suggests the presence of a classical restriction/modification (R/M) system in both strains (52). Besides, the same specificity might be involved in both systems, since an EOP value of 1 was obtained when LMG9211-amplified phage was tested on *L. plantarum* WCSF1 and when WCSF1-amplified phage was tested on *L. plantarum* LMG9211. A type I restriction/modification system was previously identified in the genome of *L. plantarum* WCSF1, though its functionality was not demonstrated (36, 63).

Genome analysis. Phages B1 and B2 have linear double-stranded DNA genomes comprising 38,002 bp and 80,618 bp, respectively. Nes et al. (54) reported a relatively similar genome size for phage B2 (73 kbp), which was calculated from the addition of the molecular sizes of DNA restriction fragments. Phage B1 has the highest GC content (47.6%) reported to date for an *L. plantarum* phage. The GC content of phage B2 was much lower, at 37.0%, but is similar to the GC content of the *L. plantarum* myophage LP65 (10). The GC content of the host strain *L. plantarum* ATCC 8014 was previously estimated at 45.1% (50), whereas genome sequencing of strain WCSF1 revealed a GC content of 44.5% (36, 63). The genomes of two other *L. plantarum* strains also have GC contents of 44.5 to 44.7% (71, 75). The GC contents were similar throughout the genomic sequences of both phages, although some noncoding regions in phage B2 were AT rich. The lower GC content of phage B2 may suggest that some genetic elements were derived from phages infecting other hosts (23, 31).

The phage genomic DNA was also digested with various restriction enzymes (EcoRV, HindIII, MluI, and SalI), and the profiles obtained were similar to the theoretical profiles obtained from the genomic data (NEBcutter), suggesting the absence of modified nucleotides (data not shown). The profile obtained for phage B2 was similar to that reported elsewhere (54). Analysis of the genome extremities indicated that phage B1 is a *pac*-type phage, like *L. plantarum* phages fri, JL-1, and LP65 (10, 43, 65), whereas phage B2 was classified as a *cos*-type phage, similar to SC921 phage (74). The *cos* site is 11 nucleotides long (5′-TGAGC GCCCTA-3′) (data not shown).

Sixty ORFs were identified for phage B1 and 127 ORFs for phage B2 (Tables 2 and 3; Fig. 2 and 3). They covered 93% (B1) and 87% (B2) of the genome length. A total of 56 ORFs (93%) for phage B1 and 65 ORFs (51%) for phage B2 had homology to previously characterized genes in public databases. However, a protein function could be attributed to products of only 25 ORFs (42%) for phage B1 and 37 ORFs (29%) for phage B2. The predominant starting codon was ATG for both phages (90% for B1, 86% for B2). Interestingly, four B1 ORFs share some identity with B2 ORFs, namely, B1 ORF15 and B2 ORF33, B1 ORF18 and B2 ORF36, B1 ORF22 and B2 ORF40, and B1 ORF35 and B2 ORF99. Of interest, ORF18 of phage B1 is likely involved in host recogni-

TABLE 2 Open reading frames deduced from the genome of *L. plantarum* phage B1 and their predicted functions

ORF[a]	Start (bp)	Stop (bp)	Predicted protein			Putative RBS and start codon[b]	Predicted function	Best match(es) (extent[c]; % amino acid identity)	E value	Aligned protein	
			Size (aa)	Molecular mass (kDa)	pI					Size (aa)	GenBank accession no.
1	242	553	103	11.4	6.3	ATAAAGGAGATAACGga**ATG**	Terminase small subunit	clP1_010 (*P. damnosus* phage clP1) (99/103; 96)	3.0E−64	139	YP_004934175
2	550	1845	431	48.9	8.2	AACATCGGGTTTCCCga**ATG**	Terminase large subunit	clP1_009 (*P. damnosus* phage clP1) (430/431; 99)	0	431	YP_004934174
								ORF440 (*L. plantarum* phage phiJL-1) (293/431; 68)	0	440	YP_223885
3	1861	3402	513	58.4	4.8	ATAACGGAGGAGTTAaaacA**TG**	Portal protein	clP1_008 (*P. damnosus* phage clP1) (508/513; 99)	0	513	YP_004934173
								ORF506 (*L. plantarum* phage phiJL-1) (284/506; 56)	0	506	YP_223886
4	3320	4132	270	30.6	8.8	CGAAAGGCGGATTGAttatca**ATG**	Capsid protein	clP1_007 (*P. damnosus* phage clP1) (266/270; 99)	0	270	YP_004934172
								ORF273 (*L. plantarum* phage phiJL-1) (121/270; 45)	2.0E−72	273	YP_223887
5	4232	4762	176	19.1	4.7	ATTGAGGAGGAGAAAccatc**ATG**	Scaffold protein	clP1_006 (*P. damnosus* phage clP1) (174/176; 99)	1.0E−118	176	YP_004934171
								ORF184 (*L. plantarum* phage phiJL-1) (70/161; 43)	1.0E−27	184	YP_223888
6	4780	5640	286	30.9	4.8	CGGAGGAACTTAAACa**ATG**	Major capsid protein	clP1_005 (*P. damnosus* phage clP1 (284/286; 99)	0	286	YP_004934170
								ORF286 (*L. plantarum* phage phiJL-1) (198/286; 69)	7.0E−138	286	YP_223889
7	5688	5915	75	7.2	4.4	CAAAAGACCGCTAGC**ATG**	Minor capsid protein	clP1_004 (*P. damnosus* phage clP1 (74/75; 99)	9.0E−37	75	YP_004934169
								ORF64b (*L. plantarum* phage phiJL-1) (33/49; 67)	6.0E−11	64	YP_223890
8	5943	6314	123	14.1	5.0	ATAATTAACGTACCCgtatgg**GTG**	DNA packaging	clP1_003 (*P. damnosus* phage clP1) (113/115; 98)	6.0E−74	115	YP_004934168
								ORF113 (*L. plantarum* phage phiJL-1) (69/113; 61)	2.0E−42	113	YP_223891
9	6311	6589	92	10.2	9.5	GGGGTTCAGGTTCTT**ATG**		clP1_002 (*P. damnosus* phage clP1) (90/92; 98)	5.0E−57	92	YP_004934167
								ORF94 (*L. plantarum* phage phiJL-1) (49/93; 53)	3.0E−27	94	YP_223892
10	6546	6959	137	15.1	9.3	GAACGTGCCGTTATCa**ATG**	Head to tail joining	clP1_001 (*P. damnosus* phage clP1) (123/125; 98)	5.0E−85	125	YP_004934166
								ORF125 (*L. plantarum* phage phiJL-1) (77/121; 64)	3.0E−46	125	YP_223893
11	7035	7388	117	13.2	8.0	CGCTAGGGGGTGTCAcaag**ATG**		ORF117 (*L. plantarum* phage phiJL-1) (64/117; 55)	2.0E−40	117	YP_223894
12	7407	8033	208	22.7	4.3	AATGAGGAGTGTAaaaaaat**ATG**	Major tail protein	clP1_057 (*P. damnosus* phage clP1) (208/208; 100)	5.0E−150	208	YP_004934222
								ORF199 (*L. plantarum* phage phiJL-1) (131/195; 67)	4.0E−92	199	ZP_03964227

13	8064	8468	134	15.2	5.0	AAAAAGGACGGTACCaacaaa**ATG**		clP1_056 (*P. damnosus* phage clP1) (134/134; 100)	1.0E−93	134	YP_004934221
								ORF139 (*L. plantarum* phage phiJL-1) (71/138; 51)	7.0E−37	139	YP_223896
14	8546	8785	79	8.7	9.9	GAAGCCGAGGCCGTC**ATG**		clP1_055 (*P. damnosus* phage clP1) (79/79; 100)	9.0E−48	79	YP_004934220
								ORF140 (*L. plantarum* phage phiJL-1) (23/79; 29)	5.1E−2	140	YP_223897
15	8789	12043	1084	110.9	9.6	TCGGAGGAGGTTAACga**ATG**	Tape measure protein	clP1_054 (*P. damnosus* phage clP1) (1067/1084; 98)	0	1084	YP_004934219
								ORF1133 (*L. plantarum* phage phiJL-1) (97/172; 56)	5.0E−51	1133	YP_223898
16	12347	12877	176	19.1	9.0	ATTACCGAGCTGGCCg**ATG**	Minor tail protein	clP1_053 (*P. damnosus* phage clP1) (169/176; 96)	1.0E−118	273	YP_004934218
								ORF441 (*L. plantarum* phage phiJL-1) (47/101; 47)	1.0E−23	441	YP_223899
17	12892	15390	832	90.7	5.0	ATATAGATAGGAGTG**ATG**	Prophage tail superfamily	clP1_052 (*P. damnosus* phage clP1) (653/839; 78)	0	829	YP_004934217
								ORF738 (*L. plantarum* phage phiJL-1) (270/455; 59)	0	738	YP_223900
18	15371	17479	702	75.7	5.4	CGACAGGAGGAGTTAaaca**ATG**	Tail/host recognition	clP1_051 (*P. damnosus* phage clP1) (648/702, 92)	0	702	YP_004934216
								ORF749 (*L. plantarum* phage phiJL-1) (66/189; 35)	3.0E−26	749	YP_223901
19	17491	17862	123	14.0	5.4	AGTTAGGAGGCCGAAcc**ATG**		clP1_050 (*P. damnosus* phage clP1) (118/119, 99)	2.0E−79	119	YP_004934215
21	18031	18363	110	12.3	6.7	AAAAAGAATTAAAGGagt**ATG**	Holin	clP1_048 (*P. damnosus* phage clP1) (99/106, 93)	2.0E−64	106	YP_004934213
22	18363	19604	413	45.5	9.6	GATAACGAGGTACAAta**ATG**	Endolysin	clP1_047 (*P. damnosus* phage clP1) (399/413, 97)	0	413	YP_004934212
								ORF398 (*L. plantarum* phage phiJL-1) (316/393; 80)	0	398	YP_223905
23	20279	20395	38	3.9	6.7	ATAACGGCGTTAGTTat**GTG**		clP1_046 (*P. damnosus* phage clP1) (36/38, 95)	8.0E−18	71	YP_004934211
24	20346	20810	154	17.6	5.2	CCACATGTGGCTCGCtactg**GTG**	Endonuclease	clP1_045 (*P. damnosus* phage clP1) (138/140 (99)	3.0E−99	140	YP_004934210
								ORF134 (*L. plantarum* phage phiJL-1) (46/129; 36)	2.0E−13	134	YP_223908
25	20813	21559	248	28.2	4.6	AGTGAGGAGGACTAAac**ATG**		clP1_044 (*P. damnosus* phage clP1) (248/248, 100)	0	248	YP_004934209
								ORF246 (*L. plantarum* phage phiJL-1) (118/233; 51)	4.0E−71	246	YP_223909
26	21950	22609	219	23.6	5.7	CGAGGAGAGATAAGC**ATG**	Helicase (NTP[d] binding)	clP1_043 (*P. damnosus* phage clP1) (219/219, 100)	5.0E−157	219	YP_004934208
								ORF224 (*L. plantarum* phage phiJL-1) (169/218; 78)	3.0E−121	224	YP_223910

(Continued on following page)

TABLE 2 (Continued)

ORF[a]	Start (bp)	Stop (bp)	Predicted protein: Size (aa)	Predicted protein: Molecular mass (kDa)	Predicted protein: pI	Putative RBS and start codon[b]	Predicted function	Best match(es) (extent[c]; % amino acid identity)	E value	Aligned protein: Size (aa)	Aligned protein: GenBank accession no.
27	22616	24556	646	72.7	5.1	AAAGGGGAAATAAAGcact**ATG**	DNA primase	clP1_042 (*P. damnosus* phage clP1) (641/646, 99)	0	646	YP_004934207
								ORF637 (*L. plantarum* phage phiJL-1) (285/634; 45)	7.0E−180	637	YP_223911
29	24921	25337	138	15.7	5.1	ATTGAGGAGGAAATGta**ATG**		clP1_040 (*P. damnosus* phage clP1) (105/138, 76)	2.0E−68	138	YP_004934205
30	25340	25639	99	11.3	7.8	AAACGGGAGGATTATTaaat**ATG**		clP1_039 (*P. damnosus* phage clP1) (64/99, 65)	4.0E−39	99	YP_004934204
31	25600	26091	163	18.6	10.3	ACTAAGGGGGTGAAAac**ATG**	Replication protein	clP1_038 (*P. damnosus* phage clP1) (143/150, 95)	4.0E−102	150	YP_004934203
32	25959	26450	163	19.2	5.2	CGCGGGCACGTCATCt**ATT**		clP1_037 (*P. damnosus* phage clP1) (115/123, 93)	8.0E−80	123	YP_004934202
33	26488	26685	65	9.5	6.2	TCATAGGAGGTAATTat**ATG**		clP1_036 (*P. damnosus* phage clP1) (62/65, 95)	2.0E−37	66	YP_004934201
34	26687	27151	154	17.8	5.1	AAAAAGGGGAATTATtaac**ATG**	Replicase	clP1_035 (*P. damnosus* phage clP1) (153/154, 99)	4.0E−106	154	YP_004934200
								ORF153 (*L. plantarum* phage phiJL-1) (74/156; 47)	3.0E−35	153	YP_223913
35	27167	27487	106	11.8	4.6	AGTAAAGGGGTAAAAcg**ATG**	DNA binding	clP1_034 (*P. damnosus* phage clP1) (100/106, 94)	2.0E−67	106	YP_004934199
								ORF97 (*L. plantarum* phage phiJL-1) (45/94; 48)	1.0E−18	97	YP_223877
37	27725	29155	476	53.3	9.1	TGTACGGAGGGATTGca**ATG**	Helicase	clP1_033 (*P. damnosus* phage clP1) (470/476, 99)	0	476	YP_004934198
								ORF467 (*L. plantarum* phage phiJL-1) (291/446; 65)	0	467	YP_223915
38	29199	29471	90	10.2	4.8	ATCAAGCAAGGGAGGta**ATT**		clP1_032 (*P. damnosus* phage clP1) (64/88, 73)	2.0E−39	102	YP_004934197
39	29458	29832	124	13.8	4.4	AGAAAAGGGGTATTTtg**ATG**		clP1_031 (*P. damnosus* phage clP1) (119/124, 96)	3.0E−71	125	YP_004934196
40	29834	30253	139	16.0	5.4	TGAAAGGATTGATTAac**ATG**		clP1_030 (*P. damnosus* phage clP1) (125/143, 87)	3.0E−85	149	YP_004934195
41	30225	30659	144	16.4	5.3	AAACTAAAAGTCACGa**ATG**		clP1_029 (*P. damnosus* phage clP1) (98/139, 71)	4.0E−68	139	YP_004934194
42	30659	31102	147	16.2	9.0	AAAAAGGGGTAATTGaata**ATG**		clP1_028 (*P. damnosus* phage clP1) (129/147, 88)	5.0E−89	147	YP_004934193
43	31095	32072	325	36.9	5.3	ATTATGGAGGTTGTGaaag**ATG**	Structural protein	clP1_027 (*P. damnosus* phage clP1) (306/325, 94)	0	324	YP_004934192
44	32073	32468	131	14.3	4.5	AAATCGGAGGTTATTtaa**ATG**		clP1_026 (*P. damnosus* phage clP1) (129/131, 98)	1.0E−89	131	YP_004934191
45	32601	32840	79	9.1	6.0	CGAAAGGACGAGGGAtaa**ATG**		clP1_025 (*P. damnosus* phage clP1) (78/79, 99)	4.0E−50	79	YP_004934190

46	32827	33183	118	13.7	9.3	ATCATGGAGGACGACa**ATG**		clP1_024 (*P. damnosus* phage clP1) (117/118, 99)	2.0E−81	118	YP_004934189
								ORF114 (*L. plantarum* phage phiJL-1) (54/104, 52)	8.0E−31	114	YP_223917
48	33518	33706	62	7.6	5.2	AGGAAAGTGGTAATAaaa**ATG**		clP1_023 (*P. damnosus* phage clP1) (61/62, 98)	3.0E−35	76	YP_004934188
49	33703	33831	42	4.7	8.5	GGATATGAGGTGATCga**ATG**		clP1_022 (*P. damnosus* phage clP1) (39/42, 93)	3.0E−6	42	YP_004934187
50	33857	34030	57	6.2	12.1	AACAAAGGGGTCTTAtatt**ATG**		clP1_021 (*P. damnosus* phage clP1) (42/44, 95)	6.0E−18	57	YP_004934186
51	34030	34275	81	9.3	5.6	AAAAAGGGGCCAAGta**ATG**		clP1_020 (*P. damnosus* phage clP1) (78/81, 96)	6.0E−51	81	YP_004934185
								ORF77 (*L. plantarum* phage phiJL-1) (40/75, 53)	2.0E−3	77	YP_223874
52	34341	34634	97	11.5	9.5	AGAACGTCATGGGTCg**ATG**		clP1_019 (*P. damnosus* phage clP1) (94/97, 97)	1.0E−64	119	YP_004934184
53	34621	34920	99	11.1	9.8	TAAAAGGCGGCGAGAtt**ATG**		clP1_018 (*P. damnosus* phage clP1) (99/99, 100)	1.0E−65	99	YP_004934183
54	34917	35108	63	7.2	4.4	ATAAAGGGGATAAAAgt**ATG**		clP1_017 (*P. damnosus* phage clP1) (61/63, 97)	4.0E−37	63	YP_004934182
55	35077	35238	53	6.1	6.0	CGAAAAGGGGTTTTTaa**ATG**		clP1_016 (*P. damnosus* phage clP1) (52/53, 98)	1.0E−27	53	YP_004934181
56	35251	35685	144	16.8	5.0	AATTAGGAGGGTTTTTacc**ATG**		clP1_015 (*P. damnosus* phage clP1) (136/144, 94)	4.0E−95	144	YP_004934180
57	35678	36124	148	17.1	6.1	ACGGAGGTTGAAATCa**ATG**		clP1_014 (*P. damnosus* phage clP1) (132/148, 89)	3.0E−94	148	YP_004934179
58	36117	36512	131	14.9	9.8	ATCGAGGTGAAGCTAc**ATG**		clP1_013 (*P. damnosus* phage clP1) (1126/131, 96)	3.0E−87	131	YP_004934178
59	36514	37032	172	19.1	10.0	TACTGGGAGGTGTTAtgac**ATG**	Endonuclease	clP1_012 (*P. damnosus* phage clP1) (167/172, 97)	1.0E−119	172	YP_004934177
60	37025	37441	138	15.7	9.1	TGAAAGGTGATAATA**ATG**		clP1_011 (*P. damnosus* phage clP1) (136/138, 99)	4.0E−93	141	YP_004934176

[a] Only the ORFs with significant hits to those of other proteins in the database are included.
[b] RBS, ribosome binding site. Underlined codons correspond to bases identical to the *L. plantarum* RBS consensus sequence; uppercase letters represent the RBS sequence; boldface indicates the starting codon; lowercase letters indicate spacer nucleotides between the RBS and start codon.
[c] Number of identical amino acids/total number of amino acids.
[d] NTP, nucleoside triphosphate.

TABLE 3 Open reading frames deduced from the genome of *L. plantarum* phage B2 and their predicted proteins

ORF[a] or tRNA	Start (bp)	Stop (bp)	Predicted protein			Putative RBS and start codon[b]	Predicted function	Best matches (extent[c]; % amino acid identity)	E value	Aligned protein	
			Size (aa)	Molecular mass (kDa)	pI					Size (aa)	GenBank accession no.
12	3401	2769	210	25.1	5.2	TAAAAGGAGGAATAAaatt**ATG**	Fatty acid/phospholipid synthesis protein	PlsX gene product (*Clostridium ljungdahlii* DSM 13528) (29/154; 19)	9.0E−2	337	YP_003779451
15	4262	4393	43	4.8	3.9	AAAAGGGAGGACTTAgc**ATG**	C.D.[d]: transcription factor	EUBREC_3432 (*Eubacterium rectale* ATCC 33656) (15/43; 35)	2.0E−3	209	YP_002939292
16	4320	4802	160	18.6	9.3	ACAAGAAAGATGATT**ATG**	C.D.: HNH endonuclease	pls32_p096 (*Bacillus subtilis* subsp. *natto*) (54/122; 44)	8.0E−26	135	YP_004243694
17	4815	5321	168	18.7	5.1	TCTAAGGGGGTGAACac**ATG**	Terminase small subunit	pls32_p095 (*Bacillus subtilis* subsp. *natto*) (61/166; 37)	3.0E−22	163	YP_004243693
18	5476	5988	170	19.7	4.9	AAAAGGTAGGTAAAGtcaa**ATG**		gp089 (*Lc. lactis* phage 949) (33/114; 29)	8.0E−11	171	YP_004306249
tRNA	6246	6317					tRNA-Asn				
tRNA	6925	6997					tRNA-Leu				
tRNA	6999	7072					tRNA-Met				
20	7143	7403	86	10.2	5.3	GAAAGAGAGGAAAACt**ATG**		ORF78 (*L. plantarum* phage LP65) (81/86; 95)	1.0E−40	109	YP_164713
tRNA	7742	7814					tRNA-Gly				
21	7937	9646	569	65.0	5.5	TATAACGAGGTGATAtg**TTG**	Terminase large subunit	ORF5 (*L. delbrueckii* phage c5) (211/522; 40)	9.0E−131	559	ACA63297
22	9830	11137	435	48.0	4.8	ACAGAAGATACGGTT**ATG**	Portal protein	ORF5 (*L. delbrueckii* phage LL-Ku) (106/384; 28)	3.0E−37	404	AAV30165
23	11133	12236	367	40.6	4.3	TGTTAGGAGGTAATGaca**ATT**	Major capsid protein/protease	pls32_p090 (*Bacillus subtilis* subsp. *natto*) (93/201; 46)	1.0E−38	313	YP_004243688
24	12240	13526	428	45.9	5.3	GATTTGGAGGTCTAAtta**ATG**	Major capsid protein	ORF7 (*L. delbrueckii* phage LL-Ku) (106/381; 28)	1.0E−21	395	AAV30167
25	13657	14115	152	15.9	4.5	TGATAGGAGGGAATActa**TTG**	Tail protein	SPC35_0138 (enterobacterial phage SPC35) (37/90; 41)	5.0E−9	162	YP_004306621
27	14453	14881	142	15.9	5.4	TTTAGTGAGGTGAGAaa**ATG**	Head-tail adaptor	HMPREF9104_01875 (*Lactobacillus kisonensis* F0435) (23/87; 26)	1.0E−3	120	ZP_09556164
28	14832	15323	163	18.4	6.2	AAACGGCGGTTCATCtg**GTG**	Head-tail joining protein	LSL_0288 (*Lactobacillus salivarius* phage Sal2) (31/126; 25)	7.6E−2	130	YP_535185
29	15447	15704	85	9.7	4.8	GTTCAGAATAAAATA**GTG**	Tail protein	ORF10 (*L. casei* phage phiAT3) (23/83; 28)	5.0E−2	123	YP_025035
30	15723	16334	203	21.5	4.7	AAGAAAGAGGTAATTacta**ATG**	Major tail protein	OF23 (*L. plantarum* phage Sha1) (61/209; 29)	8.0E−06	212	ADW01304
31	16442	16861	139	15.6	4.4	AAAAATAAAATATTTag**ATG**		LAR_1055 (*Lactobacillus reuteri* JCM 1112) (31/95; 33)	1.0E−5	132	YP_001842051
33	17115	22778	1,887	199.5	10.1	TGCAAGGAGGGTTTTaa**ATG**	Tail tape measure protein	LSL_0794 (*L. salivarius* phage SalI) (243/686; 35)	4.0E−93	1,274	YP_535687
34	22802	24613	603	66.1	6.0	ATACGAGGGGTAATCccctc**GTG**	Minor structural protein	ORF27 (*L. plantarum* phage Sha1) (321/596; 54)	1.0E−177	590	ADW01308
35	24669	27053	794	88.0	4.7	GATTAGGAGGTAATGga**ATG**	Minor structural protein	ORF28 (*L. plantarum* phage Sha1) (339/768; 44)	0	789	ADW01309
36	27065	30406	1,113	118.8	4.9	GAGTAGGAGGTTATCaa**ATG**	Tail fiber/Host specificity protein	ORF29 (*L. plantarum* phage Sha1) (373/588; 63)	0	929	ADW01310
37	30403	30624	73	8.0	4.7	CTGGAGGATAAAATCaa**ATG**		ORF30 (*L. plantarum* phage Sha1) (61/71; 86)	3.0E−34	84	ADW01311

38	30639	31061	140	16.1	4.7	AAATAGGAGGAAATTaaact**ATG**		ORF96 (*L. plantarum* phage LP65) (46/123; 37)	5.0E−20	125	YP_164731
39	31078	32277	399	41.8	4.6	AGAAAGGAATGATTTggt**TTG**	P53-like protein	ORF32 (*L. plantarum* phage Sha1) (115/179; 64)	3.0E−65	294	ADW01313
40	32351	33742	463	50.7	9.4	TAAAAGGAGACAAAAAag**ATG**	Lysin	ORF88 (*L. plantarum* phage LP65) (388/45; 86)	0	464	YP_164723
41	33760	34098	112	12.9	5.3	ATAAGGGAGGTTCACcac**ATG**		phig1ep16 (*L. plantarum* phage phig1e) (26/92; 28)	4.0E−5	118	YP_003084354
42	34085	34693	202	21.1	4.4	AGGGGAGAAATAAACA**TG**		ORF35 (*L. plantarum* phage Sha1) (53/95; 56)	5.0E−29	176	ADW01316
43	34751	35725	324	38.3	7.7	GGGAATGGTGAGATAca**ATG**	Recombinase/integrase	gp131 (*Lc. lactis* phage 949) (80/324; 25)	8.0E−12	330	YP_004306291
45	38528	36027	833	95.1	7.9	ATTAGTCAGATGAAGat**ATA**	DNA polymerase III alpha subunit	yorL (*Bacillus* phage SPBc2) (279/833; 34)	7.0E−128	1,305	NP_046685
47	40897	39467	476	54.9	5.7	ATAGAGGAGGAAAAAta**ATG**	DNA polymerase III alpha subunit	yorL (*Bacillus* phage SPBc2) (171/472; 37)	3.0E−72	1,305	NP_046685
tRNA	42308	42237					tRNA-Arg				
tRNA	42595	42522					tRNA-Arg				
53	43264	42965	99	11.7	7.8	TTATTGGAGGACATAtt**ATG**		ORF148 (*L. plantarum* phage LP65) (39/95; 41)	2.0E−15	93	YP_164783
54	43897	43340	185	21.4	9.2	ATTAAGAACATTACC**ATG**		ORF145 (*L. plantarum* phage LP65) (76/121; 63)	6.0E−46	157	YP_164780
55	44582	44277	101	11.7	5.0	TGATTGGAGCAGTGAata**ATG**		ORF21 (*L. plantarum* phage LP65) (32/86; 38)	2.0E−10	95	YP_164656
57	45726	45028	232	24.3	5.2	AGAAAGAGGTTTATTttaa**ATG**	Endolysin	ORF121 (*L. plantarum* phage LP65) (92/204; 46)	1.0E−33	193	YP_164756
58	46337	45804	177	20.7	8.8	AGGGAGAAATTAAATc**ATG**		LRATCC53608_1805 (*L. reuteri* ATCC 53608) (30/97; 31)	5.0E−3	174	CCC04558
60	46931	46671	86	9.7	5.8	AGCGAGGAAAACGGCcg**GTG**	Growth inhibitor	ORF6 (*L. reuteri*) (33/87; 38)	1.0E−10	94	CAC03499
63	47855	47460	131	15.9	7.8	GAAAGAGAGGTAAATaatg**ATG**		ORF32 (*L. plantarum* phage LP65) (81/124; 66)	1.0E−52	130	YP_164667
65	48770	48306	154	17.1	4.4	AAGACGGAGGTAAAAta**ATG**	Nucleoside deoxyribosyltransferase	lb338_phage_72 (*L. paracasei* phage Lb338-1) (56/156; 36)	5.0E−27	164	YP_002790751
66	49087	48770	105	12.0	7.7	AGATGGAGAGTGCTAaag**ATG**	Glutaredoxin	nrdH (*L. plantarum* JDM1) (31/86; 36)	5.0E−10	76	YP_003062153
68	49575	49234	113	13.2	4.1	GGAAAAGTGATTGTA**ATG**		ORF127 (*L. plantarum* phage LP65) (41/64; 65)	3.0E−16	74	YP_164762
69	50369	49665	234	26.5	9.4	CAGAGTCAGTTAGTCgg**GTG**	Nicotinamide mononucleotide transporter	ORF125 (*L. plantarum* phage LP65) (78/229; 34)	3.0E−34	259	YP_164760
70	51724	50492	410	46.2	5.7	TACTAGAGGGAGAACtta**ATG**	DNA polymerase	ORF63 (*L. plantarum* phage LP65) (90/323; 28)	1.0E−13	434	YP_164698
71	52780	51737	347	39.2	5.9	AGGAGTGAGAGTATAaaa**ATG**	ATP/GTP binding protein	yorG (*Bacillus* phage SPBc2) (81/335; 24)	3.0E−18	323	NP_046680
73	54148	53165	327	36.0	4.8	AACTAGGAGGAATTTgta**ATG**	Replication protein	ORF29 (*L. casei* phage phiAT3) (25/64; 39)	2.4E−2	185	YP_25056
74	54555	54220	111	13.1	4.4	CACGATAATGTGAATt**ATG**		BsubsN3_22549 (*Bacillus subtilis* sNCIB 3610) (27/92; 30)	7.3E−2	344	ZP_03598300
75	55051	54527	174	20.4	9.2	TAAAATTAAAAATACaa**ATG**		LRU_02117 (*Lactobacillus ruminis* SPM0211) (68/143; 48)	1.0E−38	151	ZP_08564332
82	57966	58499	177	20.5	6.3	GAAGTGGAGTTGAGCgaat**ATG**		Sca_0483 (*Streptococcus carnosus* TM300) (32/67; 48)	1.0E−9	158	YP_002633582

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TABLE 3 (Continued)

ORF[a] or tRNA	Start (bp)	Stop (bp)	Predicted protein: Size (aa)	Predicted protein: Molecular mass (kDa)	Predicted protein: pI	Putative RBS and start codon[b]	Predicted function	Best matches (extent[c]; % amino acid identity)	E value	Aligned protein: Size (aa)	Aligned protein: GenBank accession no.
83	58517	59224	235	27.2	4.8	AATTAGGAGGAAAAAta**TTG**	Deoxyguanosine kinase	ORF73 (*L. paracasei* phage Lb338-1) (80/239; 33)	2.0E−42	240	YP_002790752
85	59491	59961	156	18.2	9.1	AAACAGGAGGTTAAAacca**ATG**		yorH (*Bacillus subtilis* subsp. *natto*) (49/162; 31)	3.0E−12	162	YP_004243622
86	59954	61543	529	60.3	4.9	TTAGTGGAGATGATTtac**TTG**	DNA helicase	yorI (*Bacillus subtilis* subsp. *natto*) (186/517; 36)	2.0E−89	530	YP_004243623
87	61745	62590	281	32.1	8.6	CTCAAACTGTGGTTCa**ATG**	DNA primase	ORF61 (*Lc. lactis* phage 949) (49/162; 31)	2.0E−6	330	ADM73619
88	62587	64332	581	65.6	5.3	ACAAGGAAGGTAATGtc**TTG**	Single-stranded DNA exonuclease	ORF62 (*Lc. lactis* phage 949) (141/572; 25)	2.0E−18	593	YP_004306222
91	64909	65292	127	14.4	6.6	CATTAGGAGGAAAAAgcg**ATG**		ORF14 (*L. plantarum* phage LP65) (53/118; 45)	2.0E−22	120	YP_164649
98	67529	67873	114	13.5	4.8	TAAAATGACGAAAGAacta**ATG**		ORF13 (*L. plantarum* phage LP65) (33/106; 31)	7.0E−9	124	YP_164648
99	67870	68175	101	11.7	5.3	TGGAATGGAGAGAGC**ATA**	DNA binding protein	ORF97 (*L. plantarum* phage phiJL-1) (66/97; 68)	3.0E−41	97	YP_223877
100	68172	68573	133	15.6	4.4	AAAGAGGAGGATAAGaagct**ATG**		ORF5 (*L. plantarum* phage LP65) (26/71; 37)	1.0E−1	182	YP_164640
101	68896	69588	230	26.4	6.7	GGAGAAGAGGAGTTTaaat**ATG**		ORF93 (*L. plantarum* phage phiJL-1) (35/85; 41)	1.0E−08	93	YP_223879
102	69578	70021	147	16.9	5.9	AGAAAGGTGACAACG**ATG**		ORF157 (*L. plantarum* phage LP65) (55/156; 35)	4.0E−10	146	YP_164792
								ORF142 (*L. plantarum* phage phiJL-1) (52/149; 35)	2.0E−10	142	YP_223880
105	70682	71113	143	16.3	4.4	AAAACGGAGGTGGCAacg**ATG**	DNA replication protein	ORF15 (*L. plantarum* phage LP65) (55/161; 34)	2.0E−07	149	YP_164650
								ORF115 (*L. plantarum* phage phig1e) (49/144; 34)	2.0E−10	115	NP_695176
108	71911	72354	147	16.6	4.9	TCGATGGTAGTGACGat**ATG**		ORF8 (*L. plantarum* phage Sha1) (49/106; 46)	3.0E−18	140	ADW01289
110	72510	72953	147	17.4	5.7	AGGAAGGCAGTGGTAatc**ATG**		ORF93 (*L. plantarum* phage phiJL-1) (54/91; 59)	4.0E−21	93	YP_223879
111	72964	73206	80	9.5	4.7	TGTTAGGGGGAATAAt**ATG**		phig1ep44 (*L. plantarum* phage phig1e) (16/50; 32)	2.0E−3	73	NP_695175
114	73964	74617	217	25.0	5.0	AGTAAGAAGGGAAAAa**ATG**	Thymidine kinase	tk (enterobacterial phage RB69) (56/196; 29)	1.0E−12	193	NP_861801
123	77549	77731	60	7.0	9.3	TAAAAGGGGGTGTTGag**ATG**		ORF40 (*Staphylococcus* phage 2638A) (33/56; 59)	9.0E−12	93	YP_239845
124	77757	78140	127	14.5	10.6	AGAATAGAGGCTTATtaaa**ATG**		ORF165 (*L. plantarum* phage LP65) (85/122; 70)	5.0E−58	135	YP_164800
127	79214	79630	138	15.8	4.3	AAATAAGGGTTGCAAttaa**GTG**		gp24 (*Brochothrix* phage A9) (37/125; 30)	2.0E−2	198	YP_004301357

[a] Only the ORFs with significant hits to those of other proteins in the database are included.
[b] Abbreviation and presentation are as for Table 2.
[c] Extent is as defined for Table 2.
[d] C.D., conserved domains.

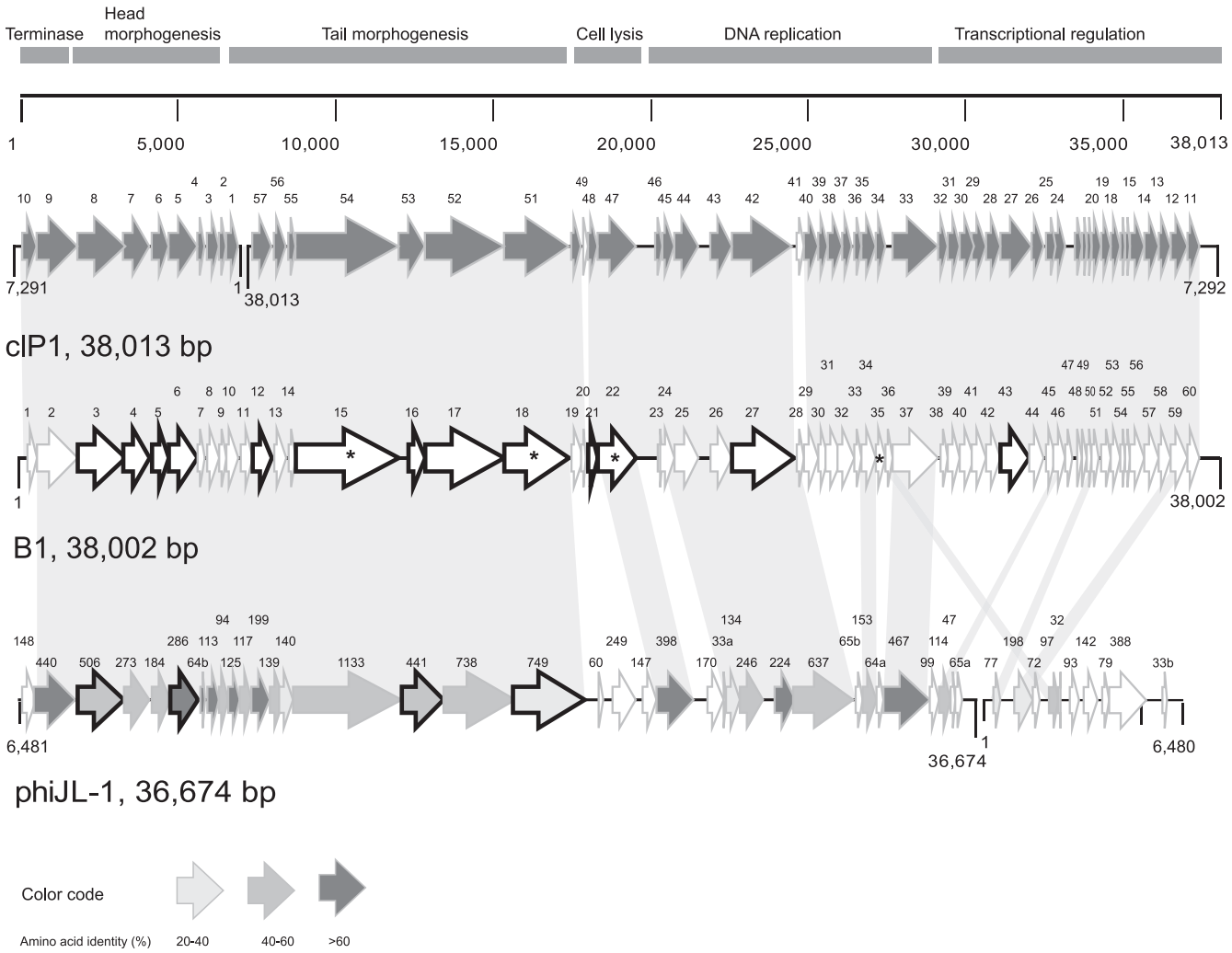


FIG 2 Genomic organizations of *L. plantarum* phages B1 and phiJL-1 as well as *P. damnosus* phage clP1. The scales above the genomes are in base pairs. Each arrow represents an ORF, and the numbering refers to Table 2 (for B1) and to the locus tags from phiJL-1 (accession number AY236756) and clP1 (accession number JN051154). The modules were based on the B1 organization. Genes coding for structural proteins experimentally determined by SDS-PAGE are indicated by thick outlines. Products of ORFs from phiJL-1 and clP1 sharing amino acid identity with those from B1 were drawn in a shade of gray according to the color code, and were linked by a shadow. White arrows represent products of ORFs sharing no identity. Phage phiJL-1 and clP1 genomes were split and reorganized in order to facilitate the alignment. ORFs sharing identity (>20%) with those of phage B2 are indicated by asterisks.

tion, and its identity with B2 ORF36 agrees with the observation that both phages infect the same host strain.

Presence of tRNA in the B2 genome. Six tRNAs were found in genome of phage B2 (Table 3) but none in B1. These six tRNAs deliver the amino acids asparagine (Asn, AAC), leucine (Leu, CTA), methionine (Met, ATG), glycine (Gly, GGA), and arginine (Arg, AGG and AGA). They were located in two genomic regions (6246 to 7814 and 42308 to 42522) of phage B2. Among *L. plantarum* phages for which the genomes are available, only the myophage LP65 contained tRNAs (14 tRNAs). The presence of tRNAs is often linked to large phage genomes (62).

The frequency of codon usage was then investigated for phages B1 and B2 (Table 4). The anticodons of some tRNAs found in the genome of phage B2 did not correspond to the codons most frequently used by the phage. For example, one tRNA matched the CTA codon, encoded a leucine, and had a frequency of 21.1% in the whole genome, whereas the most frequently used leucine codon was TTA, which had a frequency of 42.5%. However, the CCT and TCT codons, which encoded arginine, were used more by phage B2 than other possible codons.

The codon usage of phage B2 was also compared to that of *L. plantarum* WCFS1 because no bacterial host strain for phage B2 has been sequenced yet (Table 4). Our results agreed with others (3) who suggested that phages encode tRNAs corresponding to codons that are less used by the host bacteria to increase specific phage protein expression (Table 4). The presence of tRNAs was reported for some *Lactococcus* phages: P087 (5 tRNAs) (69), KSY1 (3 tRNAs) (11), and 949 (6 tRNAs) (62). In contrast to the results observed here, the frequencies of codon usage by phage 949 tRNAs were similar for the phage and its host *Lactococcus lactis* IL1403.

Function assignment and genomic organization of phages B1 and B2. The ORF functions were assigned based on comparison with sequences in public databases (NCBI, InterProScan). Only the ORFs with the highest identity with those encoding other

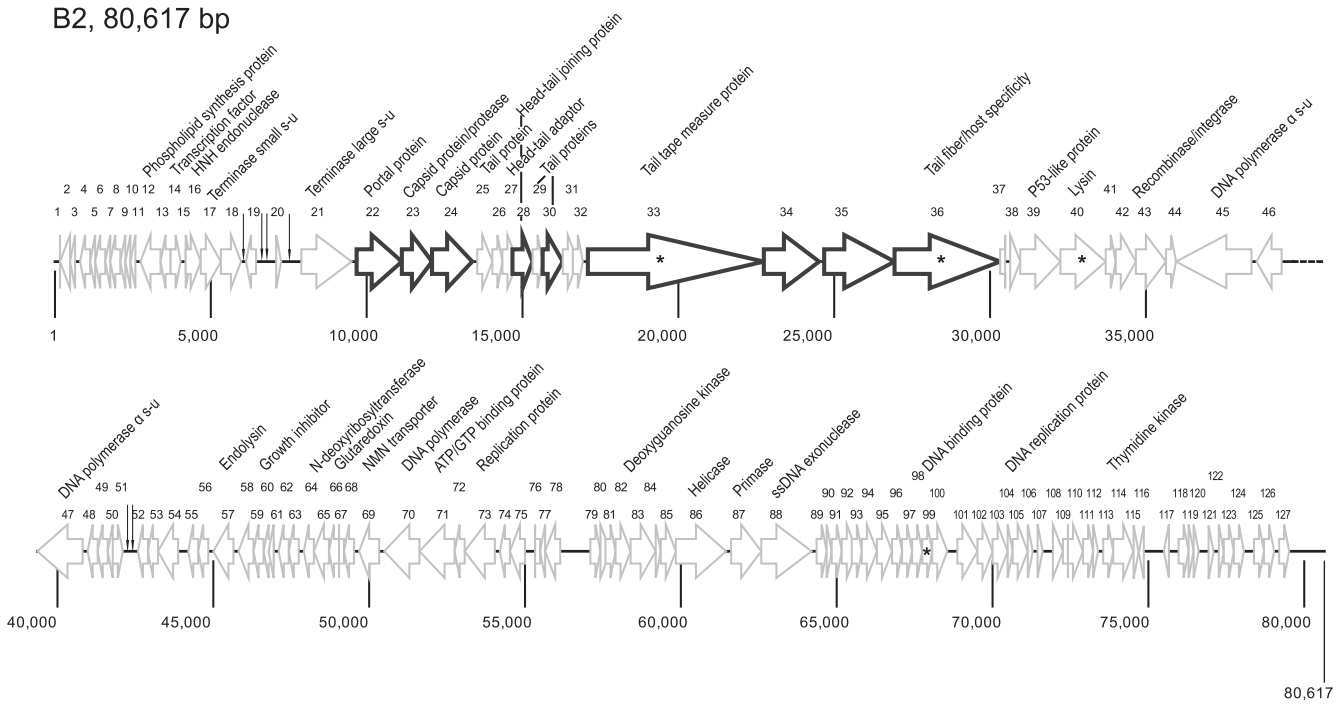


FIG 3 Genomic organization of *L. plantarum* phage B2. The scale under the genome is in base pairs. Each arrow represents an ORF, with its putative function, and the numbering refers to Table 3. Genes coding for structural proteins experimentally determined by SDS-PAGE are indicated by thick outlines. tRNAs are indicated by vertical arrows. ORFs sharing identity (>20%) with those of phage B2 are indicated by asterisks.

proteins in the database are shown in Tables 2 and 3. Although phages B1 and B2, isolated from corn silage and anaerobic sewage sludge, respectively, were similar according to morphological observations, genome sequencing confirmed wide differences between the phages. Diversity among *Lactobacillus* phages, due possibly to the high number of species in the *Lactobacillus* genus, was reported previously (74). However, a relatively conserved genome organization among them was evidenced (74). Yet, *L. plantarum* phages appear to be among the most diverse *Lactobacillus* phages. Distinct ecological niches and unrelated host strains may explain such diversity.

TABLE 4 Codon usage of *L. plantarum* strains and phage B2 for amino acids encoded by the B2 tRNAs[a]

Amino acid	Anticodon	Codon	Frequency of codon usage (%) for: Phage B1	Phage B2	*L. plantarum* WCSF1
Asn	**GTT**	**AAC**	**16.9**	**19.3**	**17.5**
		AAT	36.8	25.9	26.6
Leu	**TAG**	**CTA**	**14.3**	**21.1**	**11.5**
		TTA	17.6	42.5	33.4
		TTG	14.1	41.0	25.3
		CTT	9.4	13.6	8.9
		CTC	5.1	5.3	8.7
		CTG	11.2	21.9	12.3
Met	**CAT**	**ATG**	**32.0**	**38.4**	**26.1**
Gly	**TCC**	**GGA**	**12.9**	**8.5**	**10.0**
		GGT	22.7	12.4	26.4
		GGC	25.2	5.3	17.3
		GGG	11.9	6.8	12.3
Arg	**TCT**	**AGA**	**3.7**	**20.7**	**1.7**
	CCT	**AGG**	**2.9**	**11.3**	**0.8**
		CGT	12.6	8.7	11.7
		CGC	12.3	4.1	8.8
		CGA	6.6	6.6	7.1
		CGG	11.2	7.1	12.9

[a] Codons indicated in boldface are those encoded by the tRNAs in the phage B2 genome.

As for many siphophages, the genome of phage B1 is organized into the following functional clusters: DNA packaging, morphogenesis, lysis, and DNA replication (Fig. 2). No genes/proteins related to lysogeny were found, confirming its virulent nature. Interestingly, a high level of identity (97%) with the genome of phage clP1, infecting *Pediococcus damnosus*, followed by 77% identity with the genome of *L. plantarum* phage JL-1, was found. Of note, the genome of phage clP1 showed a GC content of 47.6%, which is much higher than those reported for pediococci (37.8 to 41.2%) (35). When each ORF was analyzed, high levels of identity with phage clP1 deduced proteins (65 to 100%) were also observed, while the levels of identity with proteins of phage JL-1 were always lower (29 to 80%) (Table 2). *Pediococcus* and *L. plantarum* strains are often found in the same ecological niches (cucumber fermentation, silage inoculants) (34, 73); thus, these comparative analyses support the notion that coexistence in the same environment can lead to the exchange of genetic elements (45). Others have shown that phages of *L. plantarum* were able to infect strains of other bacterial species isolated from the same habitat (10, 20, 45), although this was not tested here. *L. plantarum* myophage LP65 unexpectedly infected *Carnobacterium* strains associated with fermented meat (10), and some *L. plantarum* phages isolated from silage and sauerkraut were able to infect *Lactobacillus pentosus* and *Lactobacillus brevis* strains (20, 45). On the other hand,

phages B1 and B2 have a narrow host range, as reported for other *L. plantarum* phages (11, 44, 69).

The genomic organization of phage B2 was also similar to those of other siphophages (Fig. 3). Some ORFs exhibited homology with *L. plantarum* myophage LP65. However, most were similar to ORFs of *Bacillus* and *Lactobacillus* strains and their phages (Table 3). Few proteins (Orf39, Orf43, and Orf105) were linked to prophage proteins, but phage B2 had the growth characteristics of a virulent phage. This observation was also reported for *L. plantarum* phages LP65 (10), g1e (70), and Sha1 (72). Overall, the genome assemblage of phage B2 was rather unique and appears to be made from parts of other characterized phages.

Phage DNA packaging. The deduced B1 proteins Orf1 and Orf2 share high similarity with the putative small and large terminase subunits from various phages, including *P. damnosus* clP1 and *L. plantarum* g1e and phiJL-1. Phage B2 Orf17 and Orf21 exhibited sequence similarities to the small and large subunits of the terminases from *Bacillus subtilis* subsp. *natto* and *Lactobacillus delbrueckii* phage c5, respectively. Of note, this B2 genomic region was interrupted by 4 tRNAs. In tailed phages, the small terminase subunit is responsible for specific DNA binding whereas the large terminase subunit mediates the cleavage of concatameric phage DNA into genome units as well as prohead binding (26). In particular, the large subunit usually provides the endonuclease and ATPase activities for packaging (38).

The Orf59 gene product of phage B1 was associated with endonuclease function due to its homology with Orf12 of *Pediococcus* phage clP1 and Orf51 of *Lactobacillus casei* phage phiAT3. Taking into account the position of the gene in the phage B1 genome, this protein might also be involved in the DNA packaging or replication (43). In phage B2, Orf16 was identified as an HNH endonuclease, which could be involved in DNA packaging since it precedes the small terminase subunit. The HNH family of proteins is associated with DNA binding and cutting functions and includes some phage packaging proteins (47).

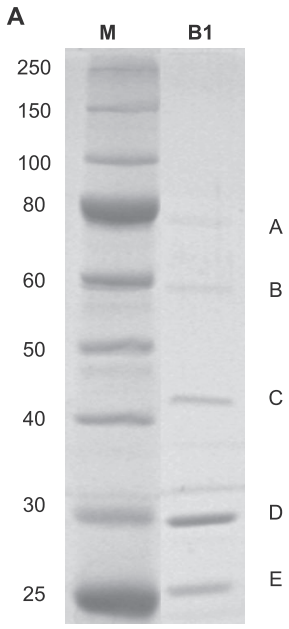
Phage DNA replication. Orf24 and Orf26 of phage B1 have several characteristics in common with endonucleases and helicases (NTP binding). Orf27 exhibited homology to DNA primases, Orf31 to replication proteins, Orf34 to replicases, and Orf35 to DNA binding proteins. A helicase function was also attributed to Orf37 since it shared 99% identity with the putative helicase from phage clP1 (*P. damnosus*). These seven proteins may be involved in DNA replication. The phage B2 proteins Orf45 and Orf47 exhibited similarities to the DNA polymerase III protein (α subunit) from *Bacillus* phage SPBc2 (42). A DNA polymerase function was also attributed to Orf70. It is tempting to speculate that phage B2 encodes its own DNA polymerase instead of relying on its host. Helicase and DNA primase functions were attributed to Orf86 and Orf87, respectively. The protein product of ORF88 may be an exonuclease, and Orf71 may be linked to ATP/GTP binding proteins. Other B2 proteins may have roles in nucleotide modification (Orf65, Orf69, Orf83, and Orf114).

Host lysis. A key step of the phage infection process is the release of new virions at the end of the lytic cycle. Orf21 of phage B1 has similarities with the holins of *P. damnosus* phage clP1 and of *L. casei* phage AT3. It has a transmembrane domain in the N-terminal part similar to holins of *Lactobacillus rhamnosus* phages Lc-Nu and Lmr1 (24, 66). Orf22 exhibited sequence similarity to the endolysins from various phages and was classified an endo-*N*-acetylmuramidase (muramidase). For phage B2, the endolysin function was attributed to ORF40 (muramidase-like endolysin) as well as Orf57 (transglycosylase). Similarly, two endolysins were encoded by the *L. plantarum* myophage LP65 genome (Orf88 and Orf121) (10). No recognizable gene encoding a holin was found for phage B2. Of the four classes of bacterial endolysins recognized (muramidase, tranglycosylase, amidase, and peptidase), two are commonly found in *Lactobacillus* phages (muramidase and amidase) (70). Moreover, similarities found among lysins of phages infecting several bacterial species could suggest a common evolutionary origin. Endolysins from phages LL-H (*Lactobacillus delbrueckii* subsp. *lactis*) and 0303 (*Lactobacillus helveticus*) were able to hydrolyze the cell walls of some species from *Lactobacillus* and *Pediococcus* (17, 67).

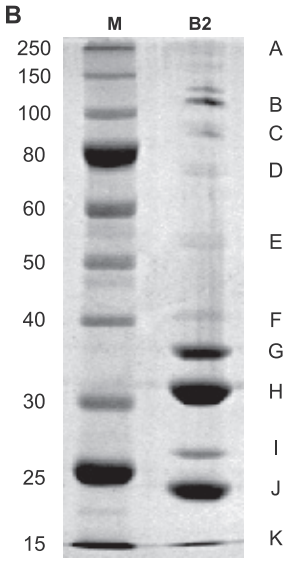
Structural proteins of phages B1 and B2. Analysis of phage B1 using SDS-PAGE revealed at least five protein bands (Fig. 4A). Band B was associated with one phage protein (Orf3, portal), whereas two phage proteins were identified in the other four bands. Band A contained a minor tail protein (Orf18) and, surprisingly, a putative DNA primase (Orf27). Band C was made of Orf43 and Orf22 (endolysin). Band D contained two capsid proteins (Orf4 and Orf6). Finally, bands B and E contained two tail proteins, Orf12 and Orf16. Orf27 (primase) and Orf22 (endolysin) are likely nonstructural proteins that were carried over despite the phage purification procedure. Overall, the observed molecular masses of the phage proteins matched the theoretical values (Fig. 4A). Proteomic analysis of the complete phage particle revealed four other proteins (Orf5, Orf15, Orf17, and Orf21). Orf5 and Orf15 likely correspond to the scaffold and the tape measure proteins, respectively.

For phage B2, significantly more protein bands were observed by SDS-PAGE (Fig. 4B). Except for protein band G, which contained two phage capsid proteins (Orf23 and Orf24), all Coomassie-stained bands contained only one phage protein. Orf24 (major capsid protein), with a calculated molecular mass of 45.8 kDa, was associated with three protein bands (F, G, and H), with estimated molecular masses of 45, 35, and 30 kDa, respectively. In fact, when the peptides from Orf24 in bands G and H were analyzed, it was found that the N-terminal peptides of the protein were missing. This suggested that the B2 major capsid protein was processed, a phenomenon observed for other phages (28, 40). Orf23, found in band G, shared homology with a major capsid protein from *Bacillus* and peptidase U35, which can be fused with capsid proteins (28). This putative peptidase activity may be involved in cleavage of Orf24. Orf36, associated with band B, showed homology with the tail fiber protein of phage Sha1 (*L. plantarum*). However, tail fibers were not observed in the morphology of phage B2 by electron microscopy (72). In total, nine structural proteins were identified for phage B2 (Fig. 4B). Analysis of the complete phage B2 particles did not reveal any additional structural proteins.

Conclusions. *Lactobacillus* phages are understudied compared to other industrially relevant lactic acid bacteria (18, 29). One possible reason is that there are fewer reports of *Lactobacillus* phage infections than of *Lactococcus lactis* and *Streptococcus thermophilus* infections in the food industry. It is unclear if this lack of reported *Lactobacillus* phage infections is due their specific uses or due to their intrinsic properties. Understanding this paucity of *Lactobacillus* phage infections in industrial settings may provide novel tools to control phage populations in other susceptible environments. Still, phages infecting several *Lactobacillus* species represent a risk for industrial users (6, 7, 10, 58, 70). Knowledge of



Phage band	Molecular mass (KDa)		ORF	Putative function
	SDS-PAGE	Calculated		
A	80	75.7	18	Minor tail protein
		72.6	27	DNA Primase
B	60	58.3	3	Portal protein
C	40	45.5	22	Endolysin
		36.8	43	Structural protein
D	30	30	6	Major capsid protein
		31	4	Capsid protein
E	25	22.7	12	Major tail protein
		19	16	Minor tail protein



Phage band	Molecular mass (KDa)		ORF	Putative function
	SDS-PAGE	Calculated		
A	200	199.4	33	Tail tape measure protein
B	130	118.7	36	Tail fiber
C	90	88	35	Structural protein
D	75	66	34	Structural protein
E	55	47.9	22	Portal protein
F	45	45.8	24	Major capsid protein
G	35	45.8	24	Major capsid protein
		40.8	23	Major capsid protein /protease
H	30	45.8	24	Major capsid protein
I	28	21.5	30	Major tail protein
J	20	21.5	30	Major tail protein
K	17	18.4	28	Head-tail joining protein

FIG 4 Migration of the phage B1 (A) and B2 (B) proteins on a 12% SDS-PAGE gel followed by Coomassie blue staining. The numbers on the left indicate the molecular masses of the ladder (protein ladder, 10 to 250 kDa; New England BioLabs). Letters on the right indicate bands cut out of the gel and identified by LC-MS/MS. Tables show the analysis of phage B1 and B2 structural proteins by LC-MS/MS.

their diversity is necessary to devise adapted control strategies. *L. plantarum* phages seem to have a relatively narrow host range, suggesting that strain rotation could be, whenever possible, an approach to limit phage multiplication. Moreover, some *L. plantarum* strains carry phage resistance mechanisms, which may be taken into account during the strain selection process. Comparative analysis of the phage B1 genome indicated that it is related to that of *L. plantarum* phage JL-1, suggesting that they form a phage group. On the other hand, analysis of the phage B2 genome suggested that this phage is currently unique among *L. plantarum* phages. The ever-increasing number of complete phage genome sequences has greatly improved our knowledge about phage diversity. The characterization of additional *L. plantarum* phages will help to determine the extent of their diversity.

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