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Characterization of 1706, a virulent phage from *Lactococcus lactis* with similarities to prophages from other Firmicutes

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Abstract

The virulent lactococcal phage 1706, isolated in 1995 from a failed cheese production in France, represents a new lactococcal phage species of the *Siphoviridae* family. This phage has a burst size of 160 and a latent period of 85 min. Its linear double-stranded DNA genome was composed of 55,597 bp with a 33.7% G+C content. Its deduced proteome (76 ORFs) shared limited similarities to other known phage proteins. SDS-PAGE coupled with LC-MS/MS analyses led to the identification of 15 structural proteins. The most striking feature of the 1706 proteome was that 22 ORFs shared similarities with proteins deduced from the genome of either *Ruminococcus torques* and/or *Clostridium leptum*. Both are Firmicutes bacteria found in the gut flora of humans. We also identified a four-gene module in phage 1706, most likely involved in host recognition that shared similarities with lactococcal prophages. We propose that the virulent phage 1706 infected another bacterial genus before picking up a lactococcal host recognition module.

Keywords: Bacteriophage; *Lactococcus lactis*; *Siphoviridae*; Genome sequence; *Ruminococcus torques*; *Clostridium leptum*; Firmicutes; Evolution

Introduction

Lactococcus lactis is a mesophilic, low G+C Gram-positive bacterium that is added to milk as part of a starter culture to produce an array of fermented dairy products such as cheeses. However, the same starter culture cannot be used on a daily basis because the bacterial strains may become infected by virulent phages that are ubiquitous in dairy factories (Moineau et al., 2002). Rotating starter cultures and using phage-resistant strains have significantly reduced the problems associated with dairy phages. Nonetheless, virulent phages are still responsible for most milk fermentation failures and lower cheese quality (Moineau and Lévesque, 2005). This biotechnological problem is mainly due to the diversity of phage populations and the evolution of phages in the dairy plants where they encounter high concentrations of diverse and rapidly growing host cells.

All lactococcal phages that have been characterized to date have a double-stranded DNA genome and a tail and thus belong to the *Caudovirales* order. Only *Siphoviridae* (long non-contractile tail) and *Podoviridae* (short tail) phages are known to regularly infect *L. lactis* strains.

A new classification scheme was recently proposed for lactococcal phages (Deveau et al., 2006). This grouping was mainly based on stringent DNA–DNA hybridizations, electron microscopic observations, and comparative genome analyses. Ten lactococcal phage species have been proposed, namely 936, c2, P335, KSY1, Q54, 1358, P087, 949, P034, and 1706. Phages belonging to the 936, c2, and P335 species have been the most studied because they are by far the most common in dairy plants (Josephsen et al., 1994). Complete genome sequences are available for 20 wild-type lactococcal phages. Eighteen of them belong to one of the three main groups, including eleven P335-like phages (Blatny et al., 2004; Brøndsted et al., 2001; Chopin et al., 2001; Labrie and Moineau, 2002, 2007; Mahanivong et al., 2001; Seegers et al., 2004; Trotter et al., 2006; van Sinderen et al., 1996), five from the 936 species (Chandry et al., 1997; Crutz-Le Coq et al., 2002; Mahony et al., 2006), and two from

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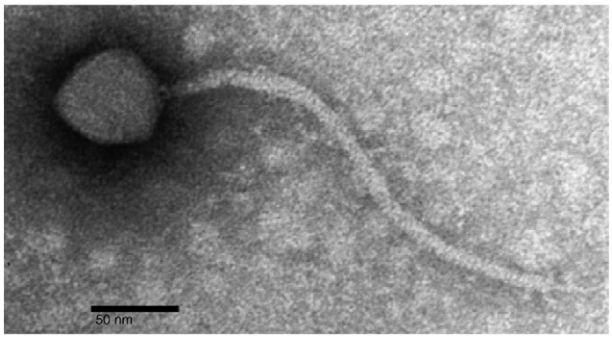


Fig. 1. Electron micrograph of phage 1706. Staining was done with 2% phosphotungstic acid. 250,000× magnification. Scale bar = 50 nm.

the c2 species (Lubbers et al., 1995; Schouler et al., 1994). The genomes of virulent phages Q54 (Fortier et al., 2006) and KSY1 (Chopin et al., 2007), both of which are reference phages for the lactococcal phage species that bears their names, make up the other two published sequences. Thus, the complete genome sequences are available for at least one member of five of the 10 lactococcal phage species.

The characterization of these lactococcal phage genomes has already led to a more accurate assessment of their natural diversity and has shed light on their origins, evolution, and relationships with other phages (Chopin et al., 2007). For example, members of the 936 and c2 species display great intra-species homogeneity but are genetically distinct from each other. It is worth mentioning that these two lactococcal phage species only contain virulent members. On the other hand, the P335 group is also genetically distinct but its members exhibit a very high degree of genomic mosaicism (Labrie and Moineau, 2002). This genetic diversity is likely due to the presence of both virulent and temperate phages in this group, which increases the recombination possibilities between the genomes of incoming virulent phages and prophage DNA in infected cells (Labrie and Moineau, 2007). This genomic plasticity is a way for the phages to adapt to a new host environment, including to natural resistance mechanisms found in certain cells (Bouchard and Moineau, 2000; Durmaz and Klaenhammer, 2000; Hill et al., 1991; Moineau et al., 1994).

Similarly, the characterization of rare lactococcal phages has provided additional information on their overall diversity. The recent genome characterization of the only member of the lactococcal phage species Q54 exemplifies such diversity as it contains an unusual configuration of modules that points to past recombination events between c2 and 936-like phages (Fortier et al., 2006). A recent analysis of phage KSY1 has shown a rare morphology that is conferred by an uncommon genome organization coupled to a transcriptional system similar to coliphage T7 (Chopin et al., 2007). Comparative genome analysis has suggested that KSY1 is the result of genetic exchanges between phages and bacteria from diverse environments (Chopin et al., 2007).

We report here the microbiological and molecular characterization of lactococcal phage 1706. This virulent phage was first isolated in 1995 in France from the failed manufacture of a soft cheese and has rarely been isolated since. Previous stringent DNA–DNA hybridizations revealed that this phage shares almost no identity with members of the nine other species (Deveau et al., 2006). It also has a broader host range than other reference lactococcal phages. Lastly, 1706 has a novel genome architecture and is surprisingly similar to the putative prophages found in the genomes of *Ruminococcus torques* and *Clostridium leptum*, two bacteria found in the human gut flora. This study provides new insights into lactococcal phage diversity.

Results

Characteristics of phage 1706 and its lytic cycle

According to Deveau et al. (2006), the virulent phage 1706 is currently the only member of its species. Transmission electron microscopic observations of 1706 (Fig. 1) revealed a distinctive, long, non-contractile tail (276 × 11 nm) with an icosahedral capsid (58 nm). Like the majority of lactococcal phages, 1706 thus belongs to the *Siphoviridae* family of the *Caudovirales* order (Ackermann, 1998).

Then, we determined the host range of phage 1706 on 57 *L. lactis* strains obtained from various sources (Table 1). Seven of the 57 *L. lactis* strains were sensitive to 1706, including its original host strain *L. lactis* SMQ-450 as well as lactococcal strains NCK203, SMQ-86, SMQ-384, SMQ-385, SMQ-451, and SMQ-562. Interestingly, these strains are also sensitive to other reference lactococcal phages. For example, *L. lactis* NCK203 and SMQ-86 are sensitive to many P335-like phages (Durmaz and Klaenhammer, 2000) such as ul36 (Labrie and Moineau, 2002), while *L. lactis* SMQ-384 and SMQ-385 are sensitive to phages P087 (P087 species) and 949 (949 species), *L. lactis* SMQ-450 to phage 1138 (P034 species), *L. lactis* SMQ-451 to 1007 (P335 species), and *L. lactis* SMQ-562 to phage Q54 (Q54 species). Taken altogether, these host range results indicate that the virulent phage 1706 can infect the host strains of five different lactococcal phage species (Table 1).

A single-step growth curve assay of phage 1706 was performed on its host *L. lactis* SMQ-450. The latent period of phage 1706 was estimated at 85 ± 2 min and its burst size at 160 ± 27 plaque forming units (PFU) released per infected cell. The sensitivity of 1706 to three abortive infection mechanisms (Abis), namely AbiK (Émond et al., 1997), AbiQ (Émond et al., 1998), and AbiT (Bouchard et al., 2002), was also determined. Numerous phage resistance mechanisms are currently used by

Table 1
Host range of the phage 1706 and other lactococcal phages

Phage	1706	ul36	P087	949	1138	1007	Q54	ΦSMQ86
Species / Strain[a]	1706	P335	P087	949	P034	P335	Q54	P335
NCK203	X	X				X		
SMQ384	X		X	X				
SMQ385	X		X	X				
SMQ450	X				X			
SMQ451	X					X		
SMQ562	X						X	
SMQ86	X			X				X

[a]Grey shadows represent *L. lactis* host strains.

Table 2
Putative ORFs deduced from 1706 genome sequence and their predicted functions

ORF [a]	Size (a.a.)	MM [b] (kDa)	pI	Putative RBS [c] and start codon [d] AGAAAGGAGGT------ATG	Predicted function [e]	Best match (No of identical residues/size of the alignment; % a.a. identity) [f]	E-value	Size (a.a.) [g]	Accession number
1	149	17.3	7.7	ACAAAGGAGGTtaatattATG	-	No significant hit			
2	103	11.6	5.5	AGTCAGGAGCGtgataATG	-	No significant hit			
3	134	14.3	5.9	AGAAGGGAAAAattaaTTG	**Holin**	C.D.: Phage holin LL-H			
4	116	13.5	5.9	AGAAAGGAAAAtATG	-	RUMTOR_01353 *R. torques* ATCC 27756 (40/104; 38%)	9.0E-9	112	EDK24614
5	131	15.5	5.5	TTTGAGGAAATgtgATG	-	CLOLEP_01405 *C. leptum* DSM 753 (51/135; 38%) RUMTOR_01352 *R. torques* ATCC 27756 (50/135; 37%)	1.0E-15 3.0E-13	138 137	EDO61897 EDK24613
6	121	13.5	4.5	AGAAAGGAG1AAacactTTG	Structural protein	RUMTOR_01351 *R. torques* ATCC 27756 (38/100; 38%) CLOLEP_01407 *C. leptum* DSM 753 (36/113; 32%)	3.0E-4 8.0E-4	166 111	ZP01967794 EDO61899
7	462	51.3	9.9	GGAGTGGAGTTtgatatcttATG	Structural protein	No significant hit			
8	574	65.4	5.0	AGAAATTAGGTttttagtaaaggaataaaATG	**Terminase** large subunit	CLOLEP_01409 *C. leptum* DSM 753 (344/578; 60%) RUMTOR_01349 *R. torques* ATCC 27756 (335/578; 58%)	0.0 0.0	581 581	EDK24610 EDO61901
9	447	49.4	5.3	AGAAATCAGGAggcattcatgtaaATG	Portal protein	RUMTOR_01348 *R. torques* ATCC 27756 (163/427; 38%) CLOLEP_01411 *C. leptum* DSM 753 (151/380; 40%)	1.0E-79 7.0E-78	446 429	EDK24609 EDO61903
10	686	74.9	4.7	CAGATGGAGGTtcctattaaATG	Capsid protein / **protease**	CLOLEP_01412 *C. leptum* DSM 753 (226/653; 35%) RUMTOR_01346 *R. torques* ATCC 27756 (211/671; 31%)	5.0E-81 1.0E-73	621 612	EDO61904.1 EDK24607
11	109	12.1	5.1	CAAAATGAGGTggtaatATG	Structural protein	CLOLEP_01413 *C. leptum* DSM 753 (23/96; 24%) RUMTOR_01344 *R. torques* ATCC 27756 (23/91; 25%)	3.2E-2 2.7E-1	107 104	EDO61905 EDK24605
12	114	13.2	5.5	TTAGGGGAGGTctttgttgctaATG	Structural protein	CLOLEP_01414 *C. leptum* DSM 753 (23/95; 24%) RUMTOR_01343 *R. torques* ATCC 27756 (21/84; 25%)	6.0E-1 1.3	101 108	EDO61906 EDK24604
13	245	26.3	5.2	TCCTTGGAGGAaaataaataATG	Major structural protein	CLOLEP_01415 *C. leptum* DSM 753 (104/229; 45%) RUMTOR_01342 *R. torques* ATCC 27756 (103/228; 45%)	9.0E-53 5.0E-51	237 233	EDO61907 EDK24603
14	283	32.9	5.0	ATTAGGGAGAAttgtATG	-	ORF45 *L. lactis* phage bIL285 (165/284; 58%)	4.0E-95	285	NP 076617
15	136	15.5	7.8	TTAAAGGAGAAtatcATG	-	RUMTOR_01340 *R. torques* ATCC 27756 (29/113; 26%) CLOLEP_01416 *C. leptum* DSM 753 (26/127; 20%)	2.0E-4 7.9E-1	146 130	EDK24601 EDO61908.
16	176	20.8	5.4	TTAAATGAGGTagatATG	-	CLOLEP_01417 *C. leptum* DSM 753 (53/176; 30%) RUMTOR_01339 *R. torques* ATCC 27756 (48/157; 31%)	4.0E-15 2.0E-14	180 180	EDO61909 EDK24600
17	128	14.1	9.6	CGAAAGGATGAtatATG	Structural protein	RUMTOR_01336 *R. torques* ATCC 27756 (29/117; 25%) CLOLEP_01421 *C. leptum* DSM 753 (28/122; 23%)	9.0E-5 2.0E-4	125 125	EDO61913.1 EDK24597
18	1995	205.5	9.1	TGAAAGGAGGAtaaagtcaaATG	Tape measure protein	CLOLEP_01422 *C. leptum* DSM 753 (279/1230; 23%) RUMTOR_01335 *R. torques* ATCC 27756 (261/1067; 24%)	3.0E-60 3.0E-59	2074 2082	EDO61914 EDK24596
19	547	59.8	5.2	ATATAGGAGGTattcaacaaaacttATG	**Tail protein**	ORF53 *L. lactis* phage bIL286 (156/535; 29%)	4.0E-53	511	NP 076687
20	587	65.9	5.1	ATAAAGAAGGAgagatacatATG	Structural protein	ORF54 *L. lactis* phage bIL286 (273/594; 46%)	1.0E-139	595	NP 076688
21	457	48.2	5.7	TAATAGGAGGTataaaatATG	Receptor binding protein	ORF46 *L. lactis* phage phismq86 (320/450; 71%)	0.0	1027	ABD63894

22	78	8.4	4.2	TAAAAGGAGGAaaaa**ATG**	-	ORF47 *L. lactis* phage phismq86 (69/78; 88%)	2.0E-33	78	ABD63895
23 -29				Variable **ATG**	-	No significant hit			
30	162	19.6	9.1	ACTAAGGAGAAtaaat**ATG**	-	EFP_gp172 *E. faecalis* phage phiEF24C (32/115; 28%)	1.0E-5	146	YP001504281
31-42				Variable **ATG**	-	No significant hit			
43	215	24.3	9.2	TATAAGGAGGCaaaa**ATG**	-	RUMTOR_02027 *R. torques* ATCC 27756 (56/189; 30%) CLOLEP_01365 *C. leptum* DSM 753 (58/210; 28%)	5.0E-14 2.0E-12	200 201	EDK23725 EDO61858
44	115	13.4	4.0	ATAAATAAGGAggaatc**ATG**	-	No significant hit			
45	80	9.3	4.4	AGAAAGAGAGActt**ATG**	-	ORF26 *L. lactis* phage 4268 (19/52; 37%)	1.4E-2	90	NP 839917
46	214	24.2	8.5	AATAAGGAGAAtaaaaa**ATG**	-	No significant hit			
47	218	24.9	8.4	TTTAAGGAGGAcaaa**ATG**	**Positive regulator of sigma**	C.D.: Positive regulator of sigma			
48	172	19.6	6.7	CTTAAGGAGGAcaaacata**ATG**	-	No significant hit			
49	111	12.7	5.4	TAAAAGGAGAAcctc**ATG**	-	No significant hit			
50	187	20.6	9.1	AAATAGGAGAAtaaaa**ATG**	Repressor	ORF5 *L. lactis* phage bIL286 (91/181; 50%)	3.0E-39	169	NP 076659
51	273	30.2	5.8	TTAAAGGAGGAatccaaa**ATG**	-	RUMTOR_02029 *R. torques* ATCC 27756 (63/252; 25%) CLOLEP_01368 *C. leptum* DSM 753 (63/288; 22%)	4.0E-8 4.0E-8	319 305	EDK23727 EDO61861
52	130	14.8	9.7	TATAAGGAGGAcatataa**ATG**	-	No significant hit			
53	373	42.8	4.4	GCATAGGAGATtata**ATG**	-	No significant hit			
54	101	12.1	7.9	CAGAAGGAGGCtttg**ATG**	-	No significant hit			
55	1317	151.5	5.3	AGAAAGGATAAacttat**ATG**	**Helicase**	RUMTOR_02036 *R. torques* ATCC 27756 (528/1349; 39%) CLOLEP_01375 *C. leptum* DSM 753 (522/1351; 39%)	0.0	1354 1383	EDK23734 EDO61868
56	96	11.1	5.1	GTAAAGGAGAAtataa**ATG**	-	No significant hit			
57	120	13.5	9.1	AGAAAGAAGGTatataaat**ATG**	-	No significant hit			
58	94	10.9	9.3	AGAAAGTAAATaca**ATG**	-	No significant hit			
59	191	21.7	5.3	ACAAAGGAGATtaaata**ATG**	-	ORF21 *L. lactis* phage phismq86 (75/78; 96%)	5.0E-38	180	ABD63718
60	181	21.4	4.9	ACAAAGGAGATtaaata**ATG**	-	No significant hit			
61	88	9.9	9.7	AGAAATTAAATggagaataaatagt**ATG**	-	No significant hit			
62	92	10.7	4.8	GGAAAGGAGGTtggaaata**ATG**	Structural protein	No significant hit			
63	118	13.5	9.4	AATAAGGAGAActa**ATG**	-	gp38 *L. monocytogenes* phage P35 (35/70; 50%)	4.0E-12	194	YP001468822
64	80	9.2	9.6	GGTAAGGAAATtaaatag**ATG**	**Transcriptionnal regulator**	No significant hit			
65	159	18.4	4.9	AGAAAGAAGGAttaatcaa**ATG**	-	gp140 *L. monocytogenes* phage A511 (55/119; 46%)	6.0E-20	115	YP001468591
66	159	18.1	4.6	TCTCAGGAGGAcaaaacaa**ATG**	-	CLOLEP_01377 *C. leptum* DSM 753 (55/149; 37%) RUMTOR_01384 *R. torques* ATCC 27756 (55/157; 35%)	2.0E-19 1.0E-18	160 154	EDO61870 EDK24645
67	418	18.6	8.8	ACTAAGGAGAGaat**ATG**	**Helicase**	RUMTOR_01381 *R. torques* ATCC 27756 (163/415; 39%) CLOLEP_01379 *C. leptum* DSM 753 (154/409; 38%)	2.0E-80 2.0E-75	412 412	EDK24642 EDO61872
68	99	11.6	5.7	CAAAAGGAACAattag**ATG**	-	CLOLEP_01398 *C. leptum* DSM 753 (43/97; 44%) RUMTOR_01358 *R. torques* ATCC 27756 (40/97; 41%)	3.0E-15 7.0E-13	96 96	EDO61891 EDK24619
69	191	22.6	6.2	TAAAAGGAGAAtaat**ATG**	-	RUMTOR_01357 *R. torques* ATCC 27756 (81/185; 44%) CLOLEP_01399 *C. leptum* DSM 753 (82/185; 44%)	1.0E-38 4.0E-37	185 185	EDK24618 EDO61892
70	106	12	5.0	ATAAAGGAATTactg**ATG**	-	No significant hit			
71	117	13	8.6	GAAAAGGAGCGtaaatattaat**ATG**	Structural protein	CLI_2417 *C. botulinum* (35/79; 44%)	2.0E-9	122	YP001391659

(*continued on next page*)

Table 2 (*continued*)

ORF [a]	Size (a.a.)	MM [b] (kDa)	pI	Putative RBS [c] and start codon [d] AGAAAGGAGGT------ATG	Predicted function [e]	Best match (No of identical residues/size of the alignment; % a.a. identity) [f]	E-value	Size (a.a.) [g]	Accession number
72	115	13.5	5.5	CGAAGGCAGGTagacac**ATG**	-	No significant hit			
73	256	28.5	5.1	ATATAGGAGGAtaagt**GTG**	**Amidase**	lbd0225 *L. delbrueckii* ATCC 11842 (43/145; 30%)	1.0E-14	536	YP618401
74	70	8.4	9.9	AAAAATCAGCTaagttgtaa**ATG**	-	No significant hit			
75	1129	113.9	9.3	AGGATTAAGGAataat**ATG**	Structural protein	RUMTOR_01356 *R. torques* ATCC 27756 (390/987; 40%) CLOLEP_01400 *C. leptum* DSM 753 (392/1001; 39%)	1.0E-170 1.0E-161	915 906	EDK24617 EDO61893
76	86	10	3.9	AGAGAGGAGAGtaacca**ATG**	-	CLOLEP_01401 *C. leptum* DSM 753 (29/80; 36%) RUMTOR_01355 *R. torques* ATCC 27756 (24/75; 32%)	6.0E-7 5.0E-6	87 88	EDO61894 EDK24616

[a]Grey shadows represent structural proteins.

[b]MM, molecular mass.

[c]RBS, ribosomal binding site.

[d]Underline indicates nucleotides identical to the RBS consensus; lowercase indicates spacer nucleotides between the RBS and start codon; boldface indicates the start codon.

[e]ORF predicted function in bold characters shows the functions attributed according to their conserved domains.

[f]C.D., conserved domain, RUMTOR/*R. torques*: *Ruminococcus torques*; CLOLEP/*C. leptum*: *Clostridium leptum*; *L. lactis*: *Lactococcus lactis*; *C. botulinum*: *Clostridium botulinum*; *E. faecalis*: *Enterococcus faecalis*; *L. monocytogenes*: *Listeria monocytogenes*; *L. delbrueckii*: *Lactobacillus delbrueckii*.

[g]Total size of the aligned protein.

the dairy industry to control members of the three main phage species (936, c2, and P335). We were interested in determining whether these Abis could inhibit phage 1706. High-copy number plasmids expressing the three Abi systems were transferred into *L. lactis* SMQ-450 and the resulting transformants were challenged with 1706. AbiK and AbiT were effective against the virulent phage 1706, while AbiQ was not.

Distinctive features of the genome of phage 1706

As the next step to further characterize this unique phage, we determined its complete nucleotide sequence. Phage 1706 has a linear double-stranded, 55,597-bp DNA genome, which makes it the second largest lactococcal phage genome, after KSY1 (Chopin et al., 2007). Its 33.7% G+C content is similar to that of *L. lactis* strains and other lactococcal phages (Bolotin et al., 2001; Chandry et al., 1997; Labrie and Moineau, 2002; Lubbers et al., 1995). No redundant sequences were observed at the ends of the genome suggesting the presence of cohesive termini. This was further confirmed by sequencing the ligated genome and the *cos* site was found to be 9-nucleotide long (5′-GCCC-TGTCT-3′). This *cos* site is one of the shortest found for lactococcal phage genomes (Fortier et al., 2006).

Bioinformatic analyses revealed that the genome of 1706 had 76 open reading frames (*orfs*) of 40 or more codons. Most (63/76) of these *orfs* were preceded by a suitable ribosome binding site (RBS) complementary to the 3′ end of the 16S rRNA of *L. lactis*. The gene organization was very different from that of other lactococcal phages. For example, the replication, morphology, and lysis modules of 1706 were not adjacent to each other. In addition, the lysis module overlapped the *cos* site, and the putative holin (*orf3*) and endolysin (amidase-type, *orf73*) genes were separated by 5 kb. The holin (ORF3) of phage 1706 was predicted by the presence of conserved domain (InterProScan). No other ORF in the genome of phage 1706 had the general features of holins.

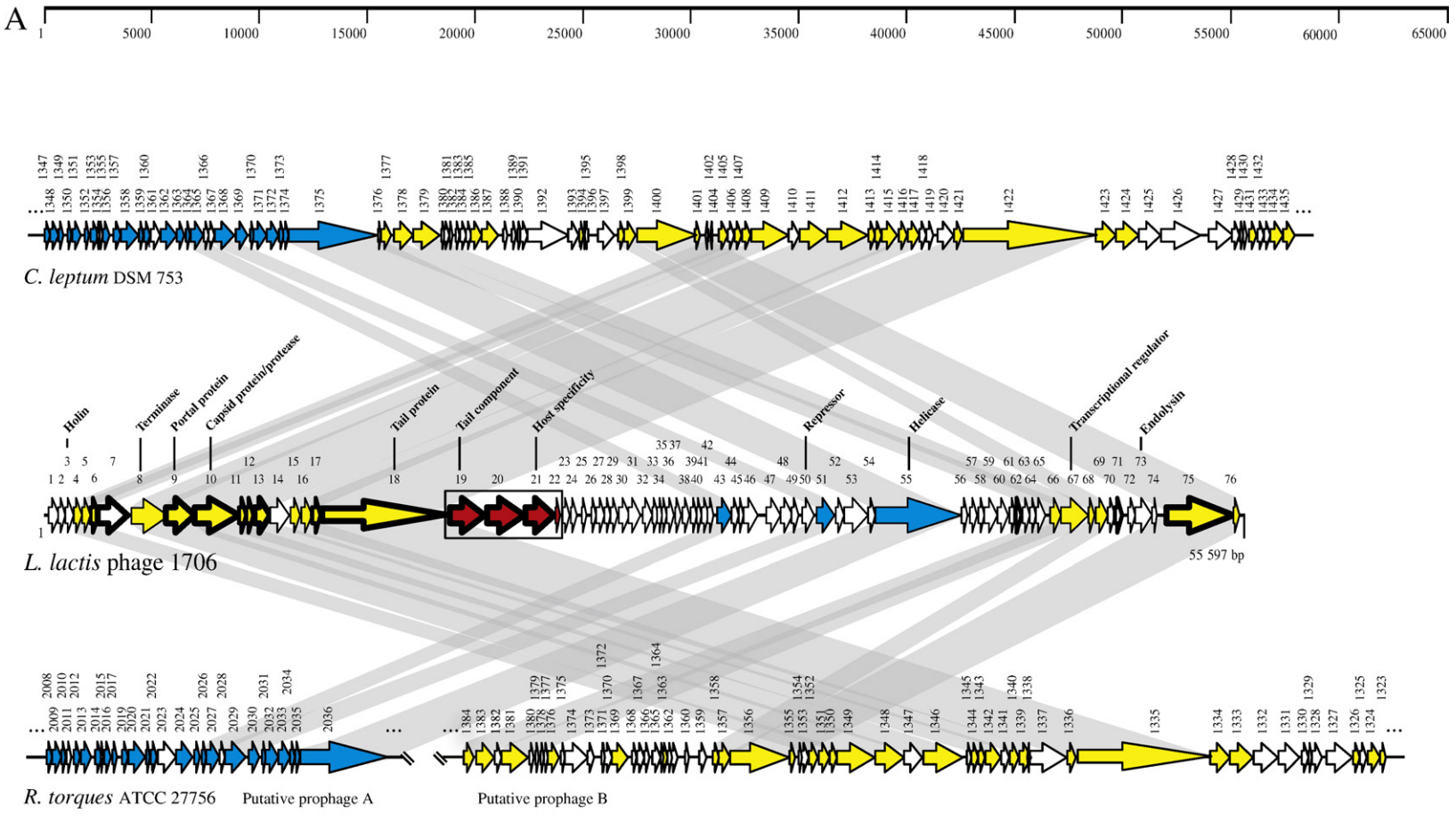
Limited similarities were found with other known phage genomes available in GenBank. No phage group was close to 1706, confirming its status as a distinct lactococcal phage species. Nonetheless, eight of the 76 ORFs shared identity with deduced proteins from P335-like phages. ORF14 of 1706 shared 58% identity with a protein of unknown function (ORF45) in prophage bIL285 (Table 2). Similarly, ORF19, ORF20, and ORF50 of 1706 shared identity with three ORFs of *L. lactis* prophage bIL286 (P335 species) (Table 2). ORF19 is likely a tail protein and was found in the structure of 1706 (see below) while ORF50 might be a repressor (Table 2). Three other ORFs of 1706 (ORF21, ORF22, and ORF59) also shared identity (Table 2) with deduced proteins of phismq86 (P335 species), a mitomycin C-inducible prophage of *L. lactis* SMQ-86 (Labrie and Moineau, 2007). Interestingly, ORF21 of 1706 is likely involved in host recognition, which is in agreement with the observation that 1706 infects *L. lactis* SMQ-86. Lastly, ORF45 of 1706 shared identity with ORF26 of phage 4268, a virulent P335-like phage.

The most striking feature we observed following the computational analyses of the 1706 proteome was the high level of identity with several ORFs found in the genomes of *R. torques* ATCC 27756 as well as ORFs retrieved from *C. leptum* DSM 753. Both Firmicutes anaerobic strains were isolated from human feces (Moore and Holdeman, 1974, 1976) and their genome is currently being sequenced at Washington University School of Medicine (http://genome.wustl.edu/). In fact, 21 of the 76 ORFs (28%) from lactococcal phage 1706 shared identity with ORFs found in both *R. torques* and *C. leptum* genomes, while a 22nd ORF was only similar to *R. torques* ATCC 27756, suggesting the presence of prophages in these two genomes. Computational analyses were also performed with specific genomic regions of *R. torques* ATCC 27756 and *C. leptum* DSM 753 to uncover putative prophages. Our results showed that in a region containing 89 *C. leptum* consecutive ORFs, 54 of them shared similarities (average 59%) with one of the two *R. torques* putative prophages (Fig. 2). Most of the shared ORFs were clustered together in both bacterial genomes, suggesting that large pieces of DNA may have been acquired through recombination, possibly following an unsuccessful phage infection. The genus *Ruminococcus* of the phylum Firmicutes, is also found in the rumen of mammals (cattle, sheep) as it is a cellulolytic microorganism (Bryant, 1959). Large numbers of phage-like particles were found in the rumen and it was demonstrated that most of those phages originated from lysogenic bacteria (Klieve et al., 1989). The *C. leptum* subgroup is also a constituent of the human fecal microbiota. Many strains of *C. leptum* are fibrolytic and produce butyrate, which may influence colonic health (Saunier et al., 2005).

It appears that the entire genome of *C. leptum* DSM 753 had a G+C content of approximately 50.2% while its putative prophage (*orf1347* to *orf1435*, 58 145 bp) possessed a slightly lower G+C content at 45.1%. On the other hand, the whole genome of *R. torques* ATCC 27756 had a G+C content of 41%, while prophage A (*orf2008* to *orf2036*, 42 710 bp) had G+C of 38.6% and prophage B (*orf1323* to *orf1384*, 15 816 bp) of 40.1%. All these G+C values were higher than those for the genomes of phage 1706 (33.7%) and *L. lactis* strains (36.0%) (Wegmann et al., 2007).

Structural proteome of phage 1706

The protein composition of 1706 was characterized by SDS-PAGE coupled with liquid chromatography-mass spectrometry (LC-MS/MS). The SDS-PAGE analysis of 1706 gave rise to ten Coomassie-stained bands of structural proteins (Fig. 3, bands A to J). Seven of the bands contained only one protein, each of which was associated with an ORF. Three bands (D, I, and J) contained more than one protein. Band D contained several peptides (coverage between 49 to 57%) that were found in the deduced ORF20 and ORF21 of 1706. These two structural proteins were only found in band D. Band I also contained two structural proteins (ORF7 and ORF17), although in this case, part of ORF7 was also found in band H (see below). Lastly, band J contained at least five structural proteins (ORF6, ORF11, ORF12, ORF62, ORF71) that were only found in this band. Thus, 15 of the 76 ORFs could be assigned as structural proteins.



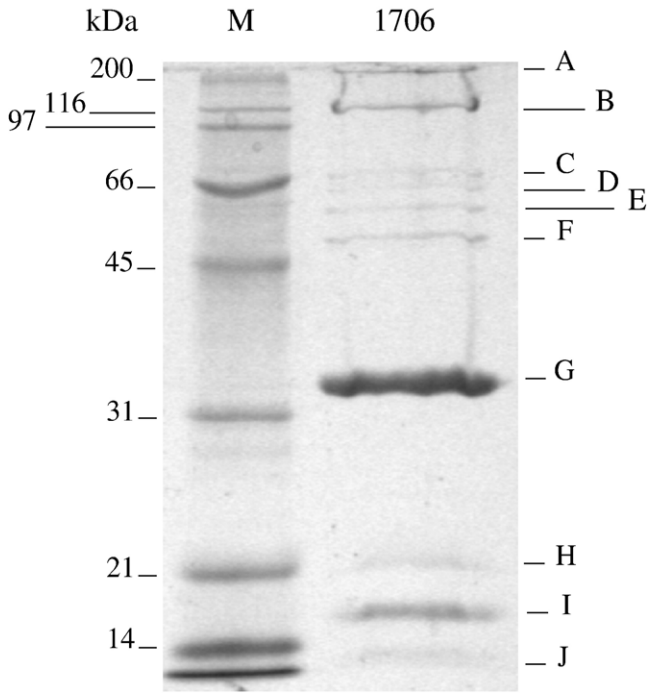
B

ORF7

```
  1 MIKIEDIVLD GKESNSSGRQ MPAMTEEEKD ILQHRGVIGM RWGKGRKTKA
 51 PKQTVAQKQK GVDEAAREKQ FKNEYVNRDK MSTRSLQNRV KRLQAEQQFK
101 ELVNKPAAER AKAAAAIAEK KRKRNKMLLR TALGIYGNVP MDSFGKKGKD
151 LKEILGPSQA IAKAASSSDA LKNSEVLDNI LLHKGVKGMK WGLKNAALQD
201 DTGNDAAVNQ QVDTLNQQIA VEKDPKKKAF LEVSLSKYKK TNAENANAEI
251 TRSPQETRDA LVKGVDTIKK NIAKETDPSK LAELKKSLKV YNSNLAKVNT
301 QMGADKSLAH SFEKQQGKNM TINKNDVILH SSTGIGLHEL HRGFKYKSKH
351 QNKKGKWIYN YDDAKEAITG LTDKDATVKI SKKVNTDYTG KDLSNAIKSI
401 EKSANKSISG NSYDKSQTYT KSIDSIKPIG KGLTKKITGT VNVRRKGTIH
451 KTYDSVLKKL GF
```

Fig. 2. Complete genome of phage 1706 (55,597 bp). (A) Genomic organization of the virulent phage 1706 (middle) as well as the putative prophages of *Clostridium leptum* (top) and *Ruminococcus torques* (bottom). The scale above the map is in base pairs. Each arrow represents a putative ORF, and the numbering for phage 1706 refers to Table 2. The putative functions that could be inferred from the bioinformatic or structural analyses are indicated above the ORFs. White arrows represent ORFs for which no putative function can be attributed. For the 1706 genome, arrows with heavy outlines represent gene products detected by LC-MS/MS analyses (Fig. 3). Yellow and blue arrows represent ORFs sharing identity with one or many (pro)phages shown here. Grey shadows linking ORFs with the same color indicate more than 23% amino acid identity. ORFs in red and in the box are the genes potentially acquired from lactococcal phages (see Table 2 for details). (B) Amino acid sequence of ORF7. Peptides underlined are the N-terminal sequences of each cleaved product.

A



B

Band	ORF 1706	Molecular mass SDS-PAGE	Molecular mass Calculated [a]	Coverage	Function
A	ORF10	>200	74.9	50%	Major capsid protein
B	ORF75	117	113.8	87%	Unknown
C	ORF18 truncated	70	73.8 for the first 696 a.a.	27% of the complete ORF	Tail protein
D	ORF20	66	65.9	57%	Unknown
	ORF21		48.2	49%	
E	ORF19	55	59.8	37%	Tail protein
F	ORF9	48	49.4	35%	Portal protein
G	ORF13	32	26.3	77%	Unknown
H	ORF7	20	51.3	69%	Unknown
I	ORF7	18	51.3	47%	Unknown
	ORF17		14.1	50%	
J	ORF12	10	13.2	100%	Unknown
	ORF62		10.7	97%	Unknown
	ORF6		13.5	64%	Unknown
	ORF71		13.0	60%	Unknown
	ORF11		12.1	41%	Unknown

Fig. 3. LC-MS/MS analysis of structural proteins of phage 1706. (A) Coomassie blue staining of a 12% SDS-polyacrylamide gel showing structural proteins of phage 1706. Letters on the right indicate bands cut out of the gel and identified by LC-MS/MS. The sizes (in kDa) of the proteins in the broad-range molecular mass standard (M) are indicated on the left. (B) Identification of 1706 proteins from corresponding bands shown in panel A. Letters on the right indicate bands cut out from the gel and identified by LC-MS/MS. [a]Calculated from the gene sequence.

The molecular masses estimated by SDS-PAGE were in agreement with those calculated from the gene sequences coding for most of the proteins. Some discrepancies were observed for proteins in bands A, C, H, and I. The molecular mass of band A could not be properly estimated as it is above 200 kDa. The molecular mass calculated from the gene sequence of *orf10* was 74.9 kDa, indicating that ORF10 forms multimers in the phage structure. Interestingly, ORF10 shared identity with the major capsid protein (MCP) of lactococcal phage Q54, which also assembles as multimers (Fortier et al., 2006). The bioinformatic analysis of the ORF10 also revealed a peptidase U35 motif at the N-terminal. These proteases have an unknown catalytic mechanism and are often fused with capsid protein (Duda et al., 1995). If ORF10 is indeed the MCP, its concentration is rather low as compared to other siphophages, which contain several hundred independent copies of their MCP (Duda, 1998). It should be noted that large protein complexes were also found at the top of the SDS-PAGE gel (data not shown). Taken altogether, these findings suggest that ORF10 might be the MCP and is largely found in a covalently cross-linked form in the structure of phage 1706, a phenomenon known as the protein chainmail (Duda, 1998).

Band C was unquestionably assigned to ORF18 of 1706, which was the largest ORF in the genome and had a deduced size of 205.5 kDa. However, according to the migration pattern on the SDS-PAGE gel, this protein had a molecular mass of approximately 70 kDa. Analysis of the peptides covering the protein suggested that ORF18 might have undergone a post-translational cleavage around position 696, removing the C-terminal part of the protein. The cleavage would generate a truncated 74-kDa protein, which corresponds to the molecular mass observed for band C (Fig. 3). Alternatively, the truncated protein could be the result of a frameshift (−1) at nucleotide 2088 leading to a stop codon (UAA) (Xu et al., 2004). However, no slippery sequence was found in the region.

Finally, bands H (20 kDa) and I (18 kDa) were intriguing as they both contained proteins linked to ORF7. Moreover, the theoretical molecular mass of ORF7 (462 amino acids) was calculated to be 51.3 kDa. The N-terminal sequencing of the proteins extracted from bands H and I shed further light on their identity. The ORF7 protein in band H began at amino acid 35 while the protein in band I began at amino acid 343. LC/MS-MS analysis also confirmed these findings, except that the last amino acid in the peptides of band H associated with ORF7 was predicted to be at position 297, suggesting that a third cleavage had occurred. These findings indicate that ORF7 underwent multiple post-translational cleavages.

Discussion

Phages are the most abundant organisms in the biosphere, exceeding bacteria by at least one order of magnitude (Chibani-

Chennoufi et al., 2004). It is thus not surprising to find virulent lactococcal phages in dairy plants worldwide. Milk is produced on a daily basis on numerous farms, each with its own farming practices, and then often transported to large milk processing facilities. It is well known that milk naturally contains phages and is one of the main reservoirs of new phages entering cheese manufacturing plants (Madera et al., 2004). The use of selected strains of *L. lactis* to transform milk into diverse fermented products determines which phages are amplified within the dairy environment (Moineau and Lévesque, 2005). Lactococcal phages of the 936, c2, and P335 species appear to be the most able to multiply in this specialized niche as they are isolated on a regular basis. In fact, the industry has developed a plethora of strategies to interfere with the proliferation of these bacterial viruses (Moineau et al., 2002).

While seven other lactococcal phage species have been described in the literature (Deveau et al., 2006), they are isolated much less frequently and more often from raw milk rather than from failed fermentations (Deveau et al., 2006). However, given the right conditions, these rare phages may propagate efficiently in dairy processing plants. Phage 1706 represents one such phage, and was responsible for a failed manufacture of soft cheese. The long latent period of 1706 likely explains why this phage is uncommon in large dairy processing plants, which often manufacture cheese at faster rates. However, the large burst size of 1706 indicates that a slower manufacturing process might allow this phage to multiply in high numbers. The characterization of these rare phages is not only important from a diversity standpoint but also from ecological and economical perspectives. For example, the isolation frequency of members of the c2 and P335 species has dropped lately due to the adoption of multiple anti-phage strategies. It has been argued that rare lactococcal phages may evolve and become more common (Deveau et al., 2006). The sensitivity of 1706 to AbiK and AbiT indicates that tools are already available to limit its propagation in the short-term.

The genomic analyses of 1706 confirmed its uniqueness. Its genome was roughly twice the size of most lactococcal phages for which the sequence is available, explaining its larger capsid size. Its gene organization was also novel for a *L. lactis* phage. Only 20 complete genomes of wild-type lactococcal phages have been sequenced to date, which limits comparative analyses. Nonetheless, members of the P355 species seem to be the most related to 1706, albeit at a limited level (8 ORFs). It should be remembered that the P335 species is a heterologous group of virulent and temperate phages that are prone to frequent genetic rearrangements (Bouchard and Moineau, 2000; Durmaz and Klaenhammer, 2000; Labrie and Moineau, 2002, 2007). Considering that 1706 can replicate in at least three *L. lactis* hosts that are sensitive to virulent P335 phages, it is conceivable that recombination events may have occurred during co-infection. Recombination between the incoming genome of 1706 and prophages of the P335 species is also a possibility. Of particular interest was the identification of a four-gene module (*orf19* to *orf22*) in phage 1706 that encodes for putative tail and host recognition proteins, which shares similarities with P335 prophages. It is reasonable to assume that the acquisition of this module by 1706 is responsible for its ability to multiply in several *L. lactis* strains.

The most remarkable characteristic of 1706 is without a doubt its similarity with deduced proteins of *R. torques* ATCC 27756 and *C. leptum* DSM 753. Indeed, 21 ORFs of phage 1706 share identity with *R. torques* and *C. leptum* proteins. Interestingly, the identity score of 1706 proteins is approximately the same with the corresponding ORFs in both Firmicutes, while the ORFs of *R. torques* and *C. leptum* share less than 60% identity between each other. Of note, many of these shared ORFs of 1706 were structural proteins and thus functional. To our knowledge, the existence of an inducible or a defective prophage in either *R. torques* ATCC 27756 or *C. leptum* DSM 753 has yet to be demonstrated. However, inducible *Ruminococcus* prophages have been reported in the literature and they have the same general morphology as the lactococcal phage 1706 (Klieve et al., 1989). In all likelihood, *R. torques* ATCC 27756 and *C. leptum* DSM 753 harbor such a prophage, which most likely had a common ancestor. It is tempting to speculate that 1706 might be derived from an ancient Firmicute prophage that had acquired the host recognition module of a lactococcal phage. This would explain why 1706 is so different from other known lactococcal phages. A similar hypothesis was put forward by Chopin et al. (2007) to describe the rare lactococcal phage KSY1. The genome of KSY1 shares homology with some marine microorganisms and with the transcriptional machinery of T7-like phages. Interestingly, KSY1 also appears to have acquired a 5-kb region from P335 phages that codes for the distal tail structure and for host recognition.

The analysis of the structural proteome of 1706 also unveiled some interesting differences. For example, two cases of post-translational cleavages were observed. It appears that ORF7 was cleaved at least twice, and possibly three times, generating distinct structural proteins. ORF18 is also most likely cleaved to remove the C-terminal region of this structural protein. Such post-translational cleavages are uncommon in the lactococcal phages studied to date. The putative peptidase activity of ORF10 might be involved in these cleavages. This protein has also been designated as the major capsid protein (MCP) due to its similarity with the MCP of the rare lactococcal phage Q54 (Fortier et al., 2006), which is largely found in a covalently cross-linked form (Duda, 1998).

In summary, we demonstrated that 1706 is a member of a new lactococcal phage species with limited similarities to other *L. lactis* phages. The proteomic similarities with the putative prophages from *R. torques* and *C. leptum* coupled with the possible acquisition of a host recognition module from a lactococcal P335 phage suggest a possible mechanism for the emergence of 1706 and possibly other new lactococcal phage species. These new phages may have infected another bacterial genus or species before picking up a lactococcal host recognition module. They may not have had the time to develop the fitness to rapidly multiply in the dairy ecosystem like the predominant lactococcal phages (936, c2, and P335). Given the remarkable diversity of phages, it is very likely that other novel lactococcal phages will emerge in the future. Widespread dairy phage control programs may help to detect these newly emerging phages early on.

Materials and methods

Microbial strains and culture conditions

L. lactis SMQ-450 was grown at 30 °C in M17 broth (Terzaghi and Sandine, 1975) (Oxoid) supplemented with 0.5% glucose (GM17). Lactococcal phage 1706 was routinely amplified at 21 °C in GM17 broth supplemented with 10 mM calcium chloride (GM17-Ca) and stored at 4 °C until used. When needed, 0.5% glycine was added to GM17-Ca agar to facilitate plaque counting (Lillehaug, 1997).

Microbiological assays

One-step growth curve assays were performed in triplicate as reported elsewhere (Moineau et al., 1993) with a multiplicity of infection (M.O.I.) of 0.1 and at a temperature of 30 °C. The burst size was calculated by dividing the average phage titer after the exponential phase by the average titer before the infected cells began to release virions (Moineau et al., 1993). Efficiency of plaquing (EOP) was calculated by dividing the phage titer on the test *L. lactis* strain by the titer on *L. lactis* SMQ-450. The test strains included *L. lactis* SMQ-450 transformed with the vector pNZ123 (De Vos, 1987) containing the phage defense mechanism AbiK (Émond et al., 1997), AbiQ (Émond et al., 1998), or AbiT (Bouchard et al., 2002). The host range of 1706 was assessed by spotting 10 μl of a 10^{-2} dilution of the high-titer lysate (10^9 pfu/ml) on a top agar containing a pure culture of an *L. lactis* strain. A total of 57 industrial and laboratory *L. lactis* strains were tested.

Phage DNA analysis

Phage 1706 genomic DNA was isolated using Maxi Lambda DNA purification kit (Qiagen) with previously described modifications (Deveau et al., 2002). The restriction profile of the isolated DNA was compared to the published profile of 1706 to confirm its identity (Deveau et al., 2006). Genome sequencing was first performed using a shotgun cloning strategy (Integrated Genomics Inc., Chicago, IL) followed by direct sequencing of the phage DNA with primers using an ABI Prism 3100 apparatus from the genomic platform at the Centre Hospitalier de l'Université Laval. The *cos* site was determined by direct sequencing of the phage DNA and by sequencing a PCR product encompassing the T4-ligated cohesive termini. Standard PCR and ligation procedures were used (Sambrook and Russell, 2001).

Bioinformatics analysis

DNA sequence analysis, contig assembly, and editing were done using the Staden (Staden et al., 2000) package 2003.0b1 (http://staden.sourceforge.net/). Some editing was also done using BioEdit 7.0.1 (Hall, 1999) (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Open reading frame (ORF) prediction was performed using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and Heuristic GeneMark (Besemer and Borodovsky, 1999). The prediction was verified and further improved by visual inspection using criteria such as the presence of a ribosome-binding site, the possible existence of short ORFs and non-AUG start codons, as well as codon usage analysis. The translated ORF products were compared with known protein sequences using BLASTP (Altschul et al., 1997). Conserved domains were searched with InterProScan databases (http://www.ebi.ac.uk/InterProScan/). The complete genomic sequence of phage 1706 is available under GenBank accession number EU081845.

Analysis of phage 1706 structural proteins

One liter of phage lysate was PEG-concentrated and purified on two CsCl gradients (Sambrook and Russell, 2001). Ultracentrifugation was performed using a Beckman SW41 Ti rotor at 35,000 rpm for 3 h. A second ultracentrifugation was performed using a Beckman NVT65 rotor at 60,000 rpm for 18 h. The phage preparation (8×10^{11} PFU/ml) was then dialyzed and analyzed for structural proteins by standard Tris–glycine 12% SDS-polyacrylamide gel electrophoresis (PAGE) (Laemmli, 1970). Samples were mixed with 4× sample loading buffer and boiled for 5 min before loading. Protein bands were detected by Coomassie blue staining. The bands were cut out of the gel, digested with trypsin, and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Genome Quebec Innovation Centre, McGill University, and the Centre Protéomique de l'Est du Québec). Following another SDS-PAGE separation, the structural proteins of 1706 were transferred to a PVDF membrane assembled on a blotting apparatus and electroeluted overnight at 4 °C at 450 mA in transfer buffer. The PVDF membrane was then washed with deionized water for 5 min, stained with 0.1% Coomassie blue for 5 min, destained with 40% methanol:10% acetic acid for 7 min, rinsed with deionized water for 5 min, and air-dried. Two bands were cut out of the membrane for the N-terminal sequencing using an automated Edman degradation chemistry capillary sequencer (NRC Biotechnology Research Institute).

Acknowledgments

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References

Ackermann, H.W., 1998. Tailed bacteriophages: the order Caudovirales. Adv. Virus Res. 51, 135–201.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Besemer, J., Borodovsky, M., 1999. Heuristic approach to deriving models for gene finding. Nucleic Acids Res. 27, 3911–3920.

Blatny, J.M., Godager, L., Lunde, M., Nes, I.F., 2004. Complete genome sequence of the *Lactococcus lactis* temperate phage phiLC3: comparative analysis of phiLC3 and its relatives in lactococci and streptococci. Virology 318, 231–244.

Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S.D., Sorokin, A., 2001. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. lactis IL1403. Genome Res. 11, 731–753.

Bouchard, J.D., Moineau, S., 2000. Homologous recombination between a lactococcal bacteriophage and the chromosome of its host strain. Virology 270, 65–75.

Bouchard, J.D., Dion, É., Bissonnette, F., Moineau, S., 2002. Characterization of the two-component abortive phage infection mechanism AbiT from *Lactococcus lactis*. J. Bacteriol. 184, 6325–6332.

Brøndsted, L., Østergaard, S., Pedersen, M., Hammer, K., Vogensen, F.K., 2001. Analysis of the complete DNA sequence of the temperate bacteriophage TP901-1: evolution, structure, and genome organization of lactococcal bacteriophages. Virology 283, 93–109.

Bryant, M.P., 1959. Bacterial species of the rumen. Bacteriol. Rev. 23, 125–153.

Chandry, P.S., Moore, S.C., Boyce, J.D., Davidson, B.E., Hillier, A.J., 1997. Analysis of the DNA sequence, gene expression, origin of replication and modular structure of the *Lactococcus lactis* lytic bacteriophage sk1. Mol. Microbiol. 26, 49–64.

Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.L., Brussow, H., 2004. Phage-host interaction: an ecological perspective. J. Bacteriol. 186, 3677–3686.

Chopin, A., Bolotin, A., Sorokin, A., Ehrlich, S.D., Chopin, M.-C., 2001. Analysis of six prophages in *Lactococcus lactis* IL1403: different genetic structure of temperate and virulent phage populations. Nucleic Acids Res. 29, 644–651.

Chopin, A., Deveau, H., Ehrlich, S.D., Moineau, S., Chopin, M.-C., 2007. KSY1, a lactococcal phage with a T7-like transcription. Virology 365, 1–9.

Crutz-Le Coq, A.M., Cesselin, B., Commissaire, J., Anba, J., 2002. Sequence analysis of the lactococcal bacteriophage bIL170: insights into structural proteins and HNH endonucleases in dairy phages. Microbiology 148, 985–1001.

De Vos, W.M., 1987. Gene cloning and expression in lactic streptococci. FEMS Microbiol. Rev. 46, 281–295.

Deveau, H., Van Calsteren, M.R., Moineau, S., 2002. Effect of exopolysaccharides on phage-host interactions in *Lactococcus lactis*. Appl. Environ. Microbiol. 68, 4364–4369.

Deveau, H., Labrie, S.J., Chopin, M.-C., Moineau, S., 2006. Biodiversity and classification of lactococcal phages. Appl. Environ. Microbiol. 72, 4338–4346.

Duda, R.L., 1998. Protein chainmail: catenated protein in viral capsids. Cell 94, 55–60.

Duda, R.L., Martincic, K., Xie, Z., Hendrix, R.W., 1995. Bacteriophage HK97 head assembly. FEMS Microbiol. Rev. 17, 41–46.

Durmaz, E., Klaenhammer, T.R., 2000. Genetic analysis of chromosomal regions of *Lactococcus lactis* acquired by recombinant lytic phages. Appl. Environ. Microbiol. 66, 895–903.

Émond, É., Holler, B.J., Boucher, I., Vandenbergh, P.A., Vedamuthu, E.R., Kondo, J.K., Moineau, S., 1997. Phenotypic and genetic characterization of the bacteriophage abortive infection mechanism AbiK from *Lactococcus lactis*. Appl. Environ. Microbiol. 63, 1274–1283.

Émond, É., Dion, É., Walker, S.A., Vedamuthu, E.R., Kondo, J.K., Moineau, S., 1998. AbiQ, an abortive infection mechanism from *Lactococcus lactis*. Appl. Environ. Microbiol. 64, 4748–4756.

Fortier, L.C., Bransi, A., Moineau, S., 2006. Genome sequence and global gene expression of Q54, a new phage species linking the 936 and c2 phage species of *Lactococcus lactis*. J. Bacteriol. 188, 6101–6114.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hill, C., Miller, L.A., Klaenhammer, T.R., 1991. In vivo genetic exchange of a functional domain from a type II A methylase between lactococcal plasmid pTR2030 and a virulent bacteriophage. J. Bacteriol. 173, 4363–4370.

Josephsen, J., Andersen, N., Behrndt, H., Brandsborg, E., Christiansen, G., Hansen, M.B., Hansen, S., Nielsen, E.W., Vogensen, F.K., 1994. An ecological study of lytic bacteriophages of *Lactococcus lactis* subsp. cremoris isolated in a cheese plant over a five year period. Int. Dairy J. 4, 123–140.

Klieve, A.V., Hudman, J.F., Bauchop, T., 1989. Inducible bacteriophages from ruminal bacteria. Appl. Environ. Microbiol. 55, 1630–1634.

Labrie, S., Moineau, S., 2002. Complete genomic sequence of bacteriophage ul36: demonstration of phage heterogeneity within the P335 quasi-species of lactococcal phages. Virology 296, 308–320.

Labrie, S.J., Moineau, S., 2007. Abortive infection mechanisms and prophage sequences significantly influence the genetic makeup of emerging lytic lactococcal phages. J. Bacteriol. 189, 1482–1487.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lillehaug, D., 1997. An improved plaque assay for poor plaque-producing temperate lactococcal bacteriophages. J. Appl. Microbiol. 83, 85–90.

Lubbers, M.W., Waterfield, N.R., Beresford, T.P., Le Page, R.W., Jarvis, A.W., 1995. Sequencing and analysis of the prolate-headed lactococcal bacteriophage c2 genome and identification of the structural genes. Appl. Environ. Microbiol. 61, 4348–4356.

Madera, C., Monjardin, C., Suarez, J.E., 2004. Milk contamination and resistance to processing conditions determine the fate of *Lactococcus lactis* bacteriophages in dairies. Appl. Environ. Microbiol. 70, 7365–7371.

Mahanivong, C., Boyce, J.D., Davidson, B.E., Hillier, A.J., 2001. Sequence analysis and molecular characterization of the *Lactococcus lactis* temperate bacteriophage BK5-T. Appl. Environ. Microbiol. 67, 3564–3576.

Mahony, J., Deveau, H., Mc Grath, S., Ventura, M., Canchaya, C., Moineau, S., Fitzgerald, G.F., van Sinderen, D., 2006. Sequence and comparative genomic analysis of lactococcal bacteriophages jj50, 712, and P008: evolutionary insights into the 936 phage species. FEMS Microbiol. Lett. 261, 253–261.

Moineau, S., Durmaz, E., Pandian, S., Klaenhammer, T., 1993. Differentiation of two abortive mechanisms by using monoclonal antibodies directed toward lactococcal bacteriophage capsid proteins. Appl. Environ. Microbiol. 59, 208–212.

Moineau, S., Pandian, S., Klaenhammer, T., 1994. Evolution of a lytic bacteriophage via DNA acquisition from the *Lactococcus lactis* Chromosome. Appl. Environ. Microbiol. 60, 1832–1841.

Moineau, S., Tremblay, D., Labrie, S., 2002. Phages of lactic acid bacteria: from genomics to industrial applications. ASM News 68, 288–393.

Moineau, S., Lévesque, C., 2005. Control of bacteriophages in industrial fermentations. In: Kutter, E., Sulakvelidze, A. (Eds.), Bacteriophages: Biology and Applications, pp. 285–296.

Moore, W.E., Holdeman, L.V., 1974. Special problems associated with the isolation and identification of intestinal bacteria in fecal flora studies. Am. J. Clin. Nutr. 27, 1450–1455.

Moore, W.E., Holdeman, L.V., 1976. Emendation of Bacteroidaceae and Butyrivibrio and descriptions of Desulfomonas gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium*, and *Ruminococcus*. Int. J. Syst. Bacteriol. 26, 238–252.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, New York.

Saunier, K., Rouge, C., Lay, C., Rigottier-Gois, L., Dore, J., 2005. Enumeration of bacteria from the *Clostridium leptum* subgroup in human faecal microbiota using Clep1156 16S rRNA probe in combination with helper and competitor oligonucleotides. Syst. Appl. Microbiol. 5, 454–464.

Schouler, C., Ehrlich, S.D., Chopin, M.-C., 1994. Sequence and organization of the lactococcal prolate-headed bIL67 phage genome. Microbiology 140, 3061–3069.

Seegers, J.F., Mc Grath, S., O'Connell-Motherway, M., Arendt, E.K., van de Guchte, M., Creaven, M., Fitzgerald, G.F., van Sinderen, D., 2004. Molecular and transcriptional analysis of the temperate lactococcal bacteriophage Tuc2009. Virology 329, 40–52.

Staden, R., Beal, K.F., Bonfield, J.K., 2000. The Staden package, 1998. Methods Mol. Biol. 132, 115–130.

Terzaghi, B.E., Sandine, W.E., 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29, 807–813.

Trotter, M., McAuliffe, O., Callanan, M., Edwards, R., Fitzgerald, G.F., Coffey, A., Ross, R.P., 2006. Genome analysis of the obligately lytic bacteriophage 4268 of *Lactococcus lactis* provides insight into its adaptable nature. Gene 366, 189–199.

van Sinderen, D., Karsens, H., Kok, J., Terpstra, P., Ruiters, M.H., Venema, G., Nauta, A., 1996. Sequence analysis and molecular characterization of the temperate lactococcal bacteriophage r1t. Mol. Microbiol. 19, 1343–1355.

Wegmann, U., O'Connell-Motherway, M., Zomer, A., Buist, G., Shearman, C., Canchaya, C., Ventura, M., Goesmann, A., Gassonm, M.J., Kuipers, O.P., van Sinderen, D., Kok, J., 2007. Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. cremoris MG1363. J. Bacteriol. 189, 3256–3270.

Xu, J., Hendrix, R.W., Duda, R.L., 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. Mol. Cell 16, 11–21.