P087, a lactococcal phage with a morphogenesis module similar to an *Enterococcus faecalis* prophage

Manuela Villion[1,2], Marie-Christine Chopin[3], Hélène Deveau[1,2], S. Dusko Ehrlich[3], Sylvain Moineau[1,2,4*], and Alain Chopin[3].

[1]*Département de Biochimie et de Microbiologie, Faculté des Sciences et de Génie,*

[2]*Groupe de Recherche en Ecologie Buccale (GREB), Faculté de Médecine Dentaire, 2420 rue de la Terrasse, Université Laval, Québec, Québec G1V 0A6, Canada,*

[3]*Laboratoire de Génétique Microbienne, INRA, 78352 Jouy-en-Josas, France*

[4]*Félix d'Hérelle Reference Center for Bacterial Viruses, 2420 rue de la Terrasse, Université Laval, Québec, Québec, G1V 0A6*

[*]* Corresponding author. Mailing address: Groupe de recherche en écologie buccale, Faculté de médecine dentaire, 2420 rue de la Terrasse, Université Laval, Quebec City, Quebec, Canada, G1V 0A6

Phone: 418-656-3712. Fax: 418-656-2861. E-mail: Sylvain.Moineau@bcm.ulaval.ca..

Abstract

The virulent lactococcal phage P087 was isolated from a dairy environment in 1978. This phage was then recognized as the reference member for one of the ten phage groups currently known to infect *Lactococcus lactis* strains. The double-stranded DNA genome of this *Siphoviridae* phage is composed of 66,074 bp and is circularly permuted. Five tRNA and 88 ORFs were found within an uncommon genome architecture. Eleven structural proteins were also identified through SDS-PAGE and LC-MS/MS analyses. Of note, 11 translated ORFs from the structural module of phage P087 have identities to gene products found in a prophage located in the genome of *Enterococcus faecalis* V583. The alignment of both genomic sequences suggests that DNA exchanges could occurr between these two phages which are infecting low G+C bacteria found in similar ecological niches.

Introduction

Bacteriophages are now widely recognized as the most abundant microorganisms on the planet and they are arguably also the most diverse (Whitman et al., 1998; Wommack and Colwell, 2000). Such natural variation is a reflection of the array of hosts available to them as well as their high rate of adaptive evolution when facing selective pressure. In fact, in most environments, a large pool of phages and their respective bacterial hosts are involved in continuous cycles of co-evolution where phage-resistant host mutants help to preserve bacterial cell lineages while counter-resistant phage mutants emerge and threaten the new bacterial strains (Emond and Moineau, 2007). Genome recombination is one of the processes by which novel phages with unique characteristics are developed (Labrie and Moineau, 2007). In fact, it is the field of large-scale genome sequencing that led to unprecedented insights into phage and bacterial co-evolution (Ackermann and Kropinski, 2007). Viral metagenomic studies have also revealed that the overall gene pool of viruses is still vastly untapped (Edwards and Rohwer, 2005).

Lactococcus lactis is one of the relatively few Gram-positive bacterial species that has been domesticated by humans and extensively used for food fermentation processes (Moineau et al., 2002). Unfortunately, these bacteria are often susceptible to phage attacks, particularly during large-scale milk fermentation (Emond and Moineau, 2007). Due to their negative impacts on food bioprocesses and on the quality of fermented products, lactococcal phages are among the most studied bacterial viruses. Over 700 hundred lactococcal phage isolates have been reported in the literature while several other isolates are stored in company and university laboratories (Ackermann and Kropinski, 2007).

Basic research on lactococcal phages led to the development of practical phage classification schemes which were used to design effective strategies to limit phage propagation within manufacturing factories (Jarvis et al., 1991). The latest classification of *L. lactis* phages recognized 10 morphologically and genetically distinct groups (Deveau et al., 2006), including 8 belonging to the *Siphoviridae* family (non-contractile tail) and 2 to the *Podoviridae* family (short tail). This grouping was based on electron microscopic observations, DNA-DNA hybridizations, and comparative genome analyses. Siphophages belonging to three groups (936, c2, and P335) have been the most scrutinized because they are the main causes of milk fermentation failures worldwide (Moineau et al., 1992; Josephsen et al., 1994; Bissonnette et al., 2000). For example, among the 25 wild-type lactococcal phage genomes for which a complete sequence is currently available, 13 belong to P335-like phage group, 6 belong to the 936 and 2 belong to the c2.

Members of the seven other lactococcal phage groups are infrequently found in the dairy industry or in ecological niches associated with lactococci. Nonetheless, members of these phage groups can still lead to fermentation collapses. With the aims of understanding how phage genomes are related to each other and what evolutionary mechanisms shaped the lactococcal phage population (Hatfull, 2008), the genome of phage members representing 4 of the less studied *L. lactis* phage groups were recently analyzed. This included the virulent siphophages Q54 (Fortier et al., 2006) and 1706 (Garneau et al., 2008) as well as the virulent podophages KSY1 (Chopin et al., 2007) and asscphi28, a member of the P034 group (Kotsonis et al., 2008). Thus, at least one complete genome sequence is available for 7 of the 10 recognized lactococcal phage groups. Here, we report the complete genome sequence and analysis of phage P087, a virulent representative of the lactococcal phage group that bears its name.

Results and Discussion

Phage P087 was isolated in Germany from a dairy environment in 1978 (Braun et al., 1989). Electron microscopy revealed that it belonged to the *Siphoviridae* family as for the majority of the lactococcal phages. Its noncontractile tail is 163 nm in length and 14-16 nm in width while its isometric capsid is approximately 59 nm in diameter (Fig. 1). It differs from other lactococcal phages through its complex baseplate with no terminal fiber (Deveau et al., 2006).

Microbiological characterization

We performed a host range analysis and phage P087 was able to infect the following *L. lactis* strains: SMQ-384 (also named C10), SMQ-385 (also named ML8 strain), NCK203, and SMQ-86. The latter two *L. lactis* strains are also sensitive to several lactococcal phages belonging to the P335 group (Hill et al., 1990; Moineau et al., 1992; Emond et al., 1997). Interestingly, it was recently shown that phage 1706 can also infect the same four strains (Garneau et al., 2008). Phage P087 was not able to infect a set of 42 industrial *L. lactis* strains. Taken altogether, the host range of P087 is limited, but it can infect *L. lactis* strains that are sensitive to phages from at least two other genetically-distinct groups.

To determine whether P087 was sensitive to abortive infection mechanisms (Abi), we introduced a high copy vector (pNZ123) expressing AbiK (Emond et al., 1997), AbiQ (Emond et al., 1998) and AbiT (Bouchard et al., 2002) into *L. lactis* SMQ-384, and the resulting transformants were challenged with P087. Of the three mechanisms, only the AbiQ system was very effective against P087 (EOP of $\leq 10^{-8}$). Thus, natural means are already available to curtail the multiplication of this virulent phage.

Genome sequence

A single contig with overlapping ends was obtained, suggesting that P087 extremities are circularly permuted. After removing the duplicated sequence, a sequence of 60,074 bp was obtained. This gives the second largest sequenced genome for a lactococcal phage, after KSY1 (Chopin et al., 2007). Most known *Siphoviridae* phages with genome of more than 60 kb and infecting Gram-positive bacteria are mycophages, but their genome have a GC content of 57.3% to 69% (Pedulla et al., 2003). The GC content of the lactococcal P087 genome is 34.4 % which is within the range of its *L. lactis* hosts (35.3 %) (Bolotin et al., 2001; Makarova et al., 2006; Wegmann et al., 2007), and other lactococcal phages (Hejnowicz et al., 2008).

The circular permutation of P087 genome was further analyzed by cloning and sequencing the ends. The sequences obtained were apparently random, with no common features (not shown). The terminal redundancy length could not be determined from the sequencing data. The positions of the ends were uniformly scattered throughout the phage genome, indicating a highly circularly permuted genome, characteristic of *pac*-type phages.

Bioinformatic analysis and P087 genome organization

Eighty-eight open reading frames were deduced from the complete genomic sequence. They were all oriented in the same direction and 22 of them were overlapping (Table 1 and Fig. 2). The smallest gene preceded by an adequate ribosome binding site (RBS) complementary to the end of *L. lactis* 16S rRNA would encode a protein of only 29 amino acids (gp44). The sizes of the remaining gene products varied from 33 (gp39) to 1309 amino acids (gp73). As observed for other phages, the P087 genome was highly compact, with only 5% of the genome having no

coding function. The longest non-coding region (about 600 bp) was found upstream of *orf1*. Globally, P087 genes were clustered in a typical temporal expression pattern. As seen for other dairy phages, the leftward half of the genome (*orf1*-*orf55*) contained genes involved in DNA metabolism, and expected to be expressed early in the phage infection cycle. The rightward half of the genome (*orf56*-*orf88*) contained genes encoding either structural proteins, or proteins involved in phage assembly or cell lysis (Fig. 2). These genes would likely be expressed later in the phage lytic cycle. No lysogeny module (or remnant) was found in the P087 genome, confirming its virulent nature. We could not find another siphophage with an identical genome architecture in databases. However, some similarities were observed with the recently described *Pseudomonas* phage YuA (Ceyssens et al., 2008). Bioinformatic analyses revealed that 32 gene products out of 88 (36%) were similar to known proteins in the GenBank database (Altschul et al., 1997) and in the ACLAME database, which is specific to viruses (Leplae et al., 2004). Of those, ten P087 gene products share significant homology to other lactococcal phage or host genome proteins available in public databases, although not necessarily giving the best hits.

P087 DNA replication and metabolism

Gp2 was found to contain a conserved domain from the helicase superfamily (HELICc: helicase superfamily C-terminal domain), a domain associated with DEXDc, DEAD-, and DEAH-boxes. Thus, gp2/helicase probably plays a role in DNA replication or transcription. The DNA polymerase of phage P087 can be encoded by *orf4*, as it deduced gene product has 27% identity with the polymerase of *Lactobacillus salivarius* phage SalI. Gp15 could encode a methylase homologous to the C-5 cytosine-specific DNA methylase family (Cheng, 1995) and be involved in the methylation of P087 genome to avoid the negative effect of host R/M systems.

153 Gp19 contains the motif CXXC (CMQC) and would be involved in reducing ribonucleotides
154 while Gp23 possesses a peptidase domain (peptidase T family). We also identified a cysteine
155 synthase (gp31, family CysK) involved in the amino acid transport and metabolism suggesting
156 that P087 acquired a host pyrimidine biosynthesis function, as was the case for the myophage T4
157 (Drake and Kreuzer, 1994), and the large phages infecting *Pseudomonas aeruginosa* (Hertveldt et
158 al., 2005). It was shown for coliphage T4 that the presence of such a gene helps phage growth
159 (Drake and Kreuzer, 1994). To our knowledge, this is the first time that such a gene has been
160 found in a lactococcal phage. Gp53, which could be a DNA polymerase, shares 45% identity with
161 a putative polymerase found in the virulent lactococcal phage Q54 (Fortier et al., 2006) and gp77
162 from *Listeria* phage A511 (Klumpp et al., 2008). Gp54 contains a ParB-like nuclease domain in
163 its N-terminus and may be involved in DNA binding and cleavage of single-stranded DNA
164 (Johnson et al., 1999). gp54 has also 40% identity with a methyltransferase from the temperate
165 streptococcal phage EJ-1. The identity was limited to the N-terminal part, which corresponds to
166 the ParB-like domain (data not shown). Gp62 is likely a protease/scaffold protein because it
167 shows 34% identity with the N-terminus of the CLP-protease of *Lactobacillus reuteri* F275.
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169 *The structural proteome of P087 and other genes coding for morphogenetic proteins*
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171 Proteomic analysis of phage P087 led to the identification of eleven structural proteins
172 (Fig. 3). The three most abundant (major) structural proteins were easily identifiable by SDS-
173 PAGE and were in agreement with a previously published protein profile of P087 (Braun et al.,
174 1989). The smallest of these three bands represented a protein containing a bacterial Ig-like
175 domain found in cellular surface proteins (gp59), while the other two, identified as gp63 and
176 gp70 are likely the major capsid (MCP) and the major tail (MTP) proteins. Gp73 has homology

177 to the tail tape measure protein of staphylococcal phage phiNM3, while a segment of gp86 shares
178 similarity with the studied neck passage structure (NPS) of *L. lactis* phage TP901-1 (Johnsen et
179 al., 1995; Vegge et al., 2006). A 547-bp region in the middle part of the *nps* gene was highly
180 conserved (82% identity) between TP901-1 (P335 group) and P087. However, it is worth
181 mentioning that gp86 is 274 amino acids larger than the NPS protein of TP901-1. The receptor
182 binding protein (RBP) of phage P087 could be gp78 as it shares 26% identity with the RBP from
183 *L. lactis* phage SL3 (936 group). The identity was limited to the C-terminus, which corresponds
184 to the head domain involved in host recognition (Spinelli et al., 2006; Tremblay et al., 2006).
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186 *P087 lysis genes*
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188 Gp85 has the hallmarks of an endolysin as it contains an amidase domain and is highly
189 homologous to several lactococcal phage endolysins. Interestingly, gp75 may also be an
190 endolysin as it is 29% identical to an endolysin found in *Enterococcus faecalis*. The presence of
191 two endolysins was also predicted in the lactococcal phage KSY1 (Chopin et al., 2007) and in
192 other double-stranded DNA phages (Wang et al., 2000). As reported for KSY1 and asccphi28
193 (lactococcal P034 group), no holin gene could be detected in the genome of P087. However,
194 gp83 possesses several holin structural features such as two transmembrane domains (MEMSAT
195 program; Jones, 2007), a short hydrophobic N-terminus, and a highly charged C-terminus (Young
196 and Blasi, 1995). Usually, the holin gene directly precedes an endolysin gene in siphophage
197 genomes. However, a short *orf* is found between *orf83* (holin gene) and *orf85* (endolysin gene).
198 Interestingly, the lysis module was located within the morphogenesis module of P087. This
199 organization was previously only observed in the genome of lactococcal phages 1706 (Garneau et
200 al., 2008) and KSY1 (Chopin et al., 2007) as well as in mycophages (Hatfull, 2008).

Presence of tRNA in P087 genome

Bioinformatic searches revealed the presence of five tRNAs clustered together at the 3' end of the P087 genome, namely $tRNA^{Asn}$ (recognizing the codon AAC), $tRNA^{Asp}$ (GAC), $tRNA^{Cys}$ (UGC), $tRNA^{Pro}$ (CCA), and $tRNA^{Thr}$ (ACA). Transfer RNAs are the only translation-associated genes usually found in phages, particularly those with a large genome (Bailly-Bechet et al., 2007). Three tRNAs were also identified in lactococcal phage KSY1 but they were separated from each other by several short *orfs* (Chopin et al., 2007). To date, this is the highest number of tRNA genes found in a lactococcal phage genome. However, up to 14 tRNA genes were recognized in the genome (131,573 bp) of the virulent *Lactobacillus plantarum* myophage LP65 (Chibani-Chennoufi et al., 2004). These tRNA genes were likely acquired by recombination with other phages or host DNA (Weinbauer, 2004). Transfer RNAs found in virulent phage genomes tend to correspond to highly used codons, leading to an enhanced translational efficiency (Bailly-Bechet et al., 2007). Indeed, the frequency of usage per thousand codons corresponding to four (of the five) tRNAs was significantly higher in phage P087 than in *L. lactis* strains (Table 2). Moreover, the genes coding for three most abundant structural proteins of P087 (gp59, gp63, and gp70) appears to have more codons recognized by those tRNA found in P087 genome (at least 2/5). However, it should be noted that the genomic sequence is not available for any of P087 host strains thereby, limiting the interpretation.

Similarity to the sequence of a putative Enterococcus faecalis *V583 prophage*

Many structural proteins of P087 shows identities with proteins deduced from the genome of *Enterococcus faecalis* V583, a clinical vancomycin-resistant isolate (Paulsen et al., 2003).

225 Analysis of the complete genomic sequence of strain V583 revealed seven mobile regions that
226 could be linked to prophages (Paulsen et al., 2003). One of these regions (coordinates EF2084 to
227 EF2145) codes for several proteins sharing between 20 to 45% identities with P087 structural
228 proteins (gp77 to gp54, Table 1, Fig. 2). This specific V583 region corresponds to the
229 enterococcal prophage 05 (Lepage et al., 2006), which is located downstream of the 3'end of
230 tRNA-Thr2 and flanked by a 15-bp repeat corresponding to its *attL*/*R* attachment site (Fig. 2).
231 Interestingly, *E. faecalis* prophage 05 is present only in clinical isolates and is apparently absent
232 in *E. faecalis* strains isolated from dairy foods. This enterococcal prophage could contribute to
233 the adaptation of some *E. faecalis* strains to a specific ecological niche (Lepage *et al*. 2006).

234 *E. faecalis* is a low GC Gram positive bacterium found in the mammalian gastrointestinal
235 tract but also in soil, water, and foods (Klare et al., 2001). It is tempting to speculate that the
236 virulent *Lactococcus lactis* phage P087 is derived, at least in part, from a phage infecting another
237 low GC Gram positive bacteria. Alternatively, the *E. faecalis* prophage 05 could be derived from
238 a dairy phage. In fact, DNA exchanges between *Lactococcus* and *Enterococcus* have been
239 observed previously. For example, a multi-antibiotic resistance plasmid (pK214) isolated from a
240 *Lactococcus* strain found in raw milk cheese could be transferred to an *E. faecalis* strain (Perreten
241 et al., 1997). Similarly, another plasmid (pRE25) could be transferred by conjugation from an *E.*
242 *faecalis* strain to a *L. lactis* strain, confirming a molecular communication between these two
243 bacterial species (Teuber et al., 2003).

244 In conclusion, we have described the reference member of the 8th lactococcal phage
245 group. Genome analysis of P087 revealed a mosaic structure made up of modules that come from
246 disparate origins. The proteomic similarities with an enterococcal prophage coupled with the
247 possible acquisition of a receptor-binding protein (gp78) from another lactococcal phage (936),
248 suggests a mechanism for the emergence of P087.

Materials and Methods

Microbiological assays

Phage P087 and its host were obtained from the Félix d'Hérelle Reference Center for Bacterial Viruses (www.phage.ulaval.ca). The host, *Lactococcus lactis* C10, was grown at 30°C in M17 broth supplemented with 0.5% glucose (GM17). For phage amplification, phage and host were incubated at room temperature in the presence of 10 mM $CaCl_2$. When needed, glycine (0.5%) was added to the top agar to increase plaque size and facilitate phage enumeration (Lillehaug, 1997). Phage lysates were concentrated with polyethylene glycol (PEG) and purified on a discontinuous-step CsCl gradient (Sambrook and Russell, 2001). Phages were stained with 2% uranyl acetate and observed using a JEOL 1230 transmission electron microscope at 80 kV as described elsewhere (Deveau et al., 2006). To measure the efficacy of phage defense mechanisms, the EOP was calculated by dividing the phage titer for the tested *L. lactis* strain by the titer for the sensitive strain (*L. lactis* SMQ384 containing pNZ123; De Vos, 1987). The tested strains were SMQ-384 transformed with pNZ123 containing, either *abiK* (pSRQ823; Emond et al., 1997), *abiT* (pED209; Bouchard et al., 2002) or *abiQ* (pSRQ928; Emond et al., 1998).

DNA sequencing

The DNA of phage P087 was isolated from CsCl-purified phages as reported elsewhere (Chibani Azaiez et al., 1998). The complete genomic sequence was determined as previously described (Chopin et al., 2007). Approximately 1000 reads were assembled, achieving 8.6-fold coverage and resulting in a single circular contig. To identify genome ends, terminal genome

fragments were cloned and sequenced. P087 genomic DNA was treated with T4 polymerase (New England Biolabs). Blunted genomes were ligated into the SmaI-digested cloning vector pUC19. The genomic DNA/pUC19 ligation reaction was then digested with EcoRI and HindIII and self-ligated. pUC19 has unique EcoRI and HindIII sites while these enzymes have 0 and 30 restriction sites in the genome of P087, respectively. The ligation mixture was transformed into *Escherichia coli* TG1 competent cells. Transformants were picked at random and P087 DNA cloned fragments were PCR-amplified using oligonucleotides complementary to pUC19 sequence. The sequence of fragments cloned into 17 independent end clones was determined.

Orf prediction and annotation

Open reading frame and tRNA searches were carried out as described elsewhere (Chopin et al., 2007; Garneau et al., 2008). Predictions were visually inspected and the putative ribosome-binding sites were verified using the 3'-end of *L. lactis* IL1403 16S rRNA (Bolotin et al., 2001). The presence of conserved domains was investigated on NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), within the CDD database. The isoelectric point and molecular mass were determined using http://ca.expasy.org/tools/pi_tool.html. The translated ORF products were compared with known protein sequences using BLASTP (Altschul et al., 1997) and the non-redundant public GenBank database. Blast searches were also done using the ACLAME database of clustered viral proteins maintained at Service de Conformation de Macromolécules Biologiques et de Bioinformatique, Université Libre de Bruxelles (http://aclame.ulb.ac.be/) (Leplae et al., 2004). The frequency usage was calculated with the countcodon program from the web site: http://www.kazusa.or.jp/codon/cgi-bin/countcodon.cgi.

297 *Analysis of phage P087 structural proteins*

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299 One liter of phage lysate was PEG-concentrated, purified on a discontinuous CsCl
300 gradient, and on a one-step CsCl gradient. Ultracentrifugation was performed using a Beckman
301 SW41 Ti rotor at 35,000 rpm for 3 h. The second ultracentrifugation was performed using a
302 Beckman NVT65 rotor at 60,000 rpm for 17 h. The phage preparation (8×10^{11} PFU/ml) was
303 then dialyzed against phage buffer (20 mM Tris-HCl pH 7.4, 100mM NaCl, 10mM $MgSO_4$) and
304 analyzed for structural proteins by standard Tris-glycine 12% SDS-polyacrylamide gel
305 electrophoresis (PAGE). Samples were mixed with 4X sample loading buffer and boiled for 5
306 min before loading. Protein bands were detected by Coomassie blue staining. The bands were cut
307 out of the gel, digested with trypsin, and identified by liquid chromatography-tandem mass
308 spectrometry (LC-MS/MS) (Genome Quebec Innovation Centre, McGill University).

309

310 *Accession number*

311

312 The sequence data was deposited in EMBL/Genbank/DDBJ databases under accession number
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314

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470

Legends of the Figures

Figure 1: Electron micrograph of lactococcal phage P087.

Figure 2: Genomic organization of the virulent *Lactococcus lactis* phage P087 compared to *Enterococcus faecalis* V583 prophage 05. Prophage 05 was previously identified by Paulsen et al. (2003) and the *att* sites by Lepage et al. (2006). Each putative ORF is represented by an arrow. The putative functions of the corresponding gene products are indicated above (for P087) or below (for prophage 05) the arrows. Bold arrows indicate structural proteins identified by LC-MS/MS (see Fig 3). Blue arrows linked by grey shadows indicate ORFs for which translated products share identity. The percentage of identity is also indicated. The scale above the map is in base pairs.

Figure 3: Analysis of structural proteins of lactococcal phage P087. Panel A, Coomassie blue staining of a 12% SDS-PAGE gel showing the structural proteins of phage P087. The bands extracted and identified by LC-MS/MS are numbered on the right side of the gel. On the left side, the 7-175 kDa Broad Range Marker (New England BioLabs) was used to estimate protein molecular mass. Panel B, Identification of the structural proteins of P087 identified in Panel A.

Table 1

TABLE 1. ORFs deduced from P087 genome sequence for which gene products present a putative function or have homologs in databases

ORF	Position Start	Position End	Size (a.a.)	MM (KDa)	pI	Putative RBS and start codon TTAGAAAGGAGGTGATCC	Predicted function (or domain)	Best homologs in databases	Identical/overall (%)	Size (a.a.)	E-value	Accession number
gp2	3416	4942	508	57.2	8.1	GGATAGATTTCTTGCTTAAGGAGGTaat**ATG**	Helicase	Hypothetical protein alr7157 *[Nostoc* sp. PCC 7120]	107/359 (29%)	629	1.0E-30	NP_490263.1
								Putative helicase [*Lactobacillus* phage phiJL-1]	101/353 (28%)	467	6.0E-29	YP_223915.1
gp4	5675	7642	655	75.4	5.2	TAAGAGTTTACTCTTAAGGAGGTacaat**ATG**	DNA polymerase	Phage DNA polymerase [*Lactobacillus salivarius* phage Sal1]	140/501 (27%)	565	2.0E-25	YP_535651.1
								ACLAME: protein:vir:78051 *Listeria monocytogenes* phage P35 # gp33	146/520 (28%)	635	7.0E-25	YP_001468817
gp6	8479	9174	231	26.5	5.7	TGAAGCTCTCCTTGCAGGAGGTcaagctta**ATG**		Metallo-β-lactamase superfamily hydrolase [*Bacteroides vulgatus* 8482]	64/228 (28%)	267	4.0E-08	YP_001300327.1
								ACLAME: protein:vir:78081 *Listeria monocytogenes* phage P35 # gp30	60/217 (27%)	190	1.0E-06	YP_001468814
gp8	9463	9876	137	16.3	5.6	CTACAAATCGTAAGGAGGTaggtt**ATG**		Hypothetical protein CLOLEP_01819 [*Clostridium leptum* DSM 753]	41/122 (33%)	124	4.0E-07	ZP_02080366.1
								Prophage Lp2 protein 24 [*Lactobacillus plantarum* WCFS1]	37/125 (29%)	126	4.0E-07	NP_785890.1
gp15	11474	12112	212	24.5	8.9	CTTATAGAAATGAAGGAGGTaacagaat**ATG**	DNA methylase	Putative C5 methylase MarMP *[Mycoplasma* phage MAV1]	72/215 (33%)	259	4.0E-15	NP_047260.1
gp18	13128	13487	119	14.2	4.4	TTTACATGGGGAGGTaaataat**ATG**		Hypothetical protein EF0327 [*Enterococcus faecalis* V583]	31/104 (29%)	100	1.0E-04	NP_814119.1
gp19	13484	13720	78	9.1	5.5	GAGAAGCAAAGGGGTacaaata**TTG**	NrdH-redoxin family	Glutaredoxin related protein [*Lactococcus lactis* SK11]	30/63 (47%)	72	3.0E-12	YP_809022.1;
								Glutaredoxin-like protein nrdH [*Lactococcus lactis* MG1363]				YP_001032827.1
								ACLAME: protein:vir:81265 [*Brevibacterium flavum*] phage BFK20 gp53	22/73 (30%)	88	9.0E-09	YP_001456783
gp23	14652	15698	348	39.6	4.4	AACAGAAAGAAA GAGGaactatcaat**ATG**	Peptidase	Hypothetical BACCAP_03129 [*Bacteroides capillosus* ATCC29799]	73/221 (33%)	313	4.0E-16	ZP_02037512.1
gp31	17772	18707	311	33.1	5.3	GGAAAATCACTTAAAGAAATCATGGAGGacaaata**ATG**	Cysteine synthase	Cysteine synthase A [*Enterococcus faecalis* V583]	154/304 (50%)	310	4.0E-74	NP_815300.1
gp32	18718	19011	97	11.6	6.3	GAAGGCTATAAATATTAAGGAGGTcacc**ATG**		p12 putative *[Lactococcus* phage c2]	29/75 (38%)	122	3.0E-05	NP_043538.1
gp42	22330	23439	369	43.4	9.7	TCAAATAAAAGGAGtttagaaa**ATG**		Viral A-type inclusion protein, putative [*Trichomonas vaginalis* G3]	59/270 (21%); N-part	4045	1.0E-06	XP_001304893.1
gp48	26300	26791	163	18.6	4.3	AATTAAGGAGGTagtaaat**ATG**		Isoleucyl-tRNA synthetase [*Methanobrevibacter smithii* ATCC 35061]	34/112 (30%)	1090	1.7E-02	YP_001273914.1
gp53	28080	29324	414	47.1	5.3	TAGAAAAGGGAGGTcaat**ATG**	DNA polymerase	Putative DNA polymerase [*Lactococcus* phage Q54]	179/391 (45%)	387	4.0E-88	YP_762586.1
gp54	29324	29848	174	19.9	5.2	GCTTTAGGAGGaattaacta**ATG**	ParB-like nuclease	Hypothetical BACSTE_02196 [*Bacteroides stercoris* ATCC43183]	40/98 (40%); N-part	371	1.0E-09	ZP_02435943.1
									37/92 (40%)	421	1.0E-09	NP_945276
								ACLAME: protein:vir:102949 *Streptococcus* phage EJ-1 methyltransferase				
gp56	30577	32313	578	66.3	5.7	TTAAAACTCTTAGAGAAAGGtgact**ATG**		Hypothetical protein EF2112 [*Enterococcus faecalis* V583]	146/554 (26%)	519	6.0E-37	NP_815775.1
gp57	32310	33788	492	56.4	5.1	TTAGAATTTCAAAAGGAGttacataa**ATG**	Structure	Hypothetical protein EF2111 [*Enterococcus faecalis* V583]	84/403 (20%)	426	2.0E-09	NP_815774.1
gp59	34440	34952	170	18.2	8.9	TTATAAGGAGaataaattaaa**ATG**	Tail (Ig-like domains)	Contains cell adhesion domain [*Clostridium acetobutylicum* ATCC 824]	35/101 (34%)	439	7.0E-05	NP_348726.1
gp62	35488	36528	346	38.2	4.2	TTATCCCTTATTCATGGAGGTgaaata**ATG**	Scaffolding protein	Phage clp-protease [*Lactobacillus reuteri* F275]	59/173 (34%) N-part	244	1.0E-18	YP_001842056.1
								ACLAME: protein:vir:102115 *Clostridium perfringens* SM101 phage phiSM101# Clp protease domain protein	55/184 (29%)	243	2.0E-13	YP_699942
gp63	36543	37679	378	42.1	5.0	ATGATATCTAAGGAGaattaaaaaa**ATG**	Major capsid protein	Hypothetical protein EF2104 [*Enterococcus faecalis* V583]	80/399 (20%)	392	5.0E-04	NP_815767.1
gp64	37692	38414	240	28.2	4.9	TCCCACGTTAATAGGAGGTttaa**ATG**	Structure	Hypothetical protein EF2103 [*Enterococcus faecalis* V583]	45/158 (28%)	235	6.0E-07	NP_815766.1
gp70	40256	41596	446	48.5	4.7	GTAGCTAAATCACTTAAGGAGGacaaata**ATG**	Structure	Hypothetical protein EF2099 [*Enterococcus faecalis* V583]	120/436 (27%)	444	9.0E-27	NP_815762.1
gp71	41708	42334	208	23.8	4.5	CATAACGTACTAGAAACATTTGGAGGaatgttaa**ATG**		Sensory box histidine kinase [*Shewanella benthica* KT99]	46/175 (26%)	867	7.5E-02	ZP_02159271.1
gp72	42379	42669	96	11.5	7.9	AGACGATAAGG GTtatagtcgagaagac**ATG**		Hypothetical protein EF2097 [*Enterococcus faecalis* V583]	23/86 (26%)	101	6.4E-02	NP_815760.1
gp73	42674	46603	1309	136.4	9.4	TGAAATGAAGAATAAAGGAaactaattaa**ATG**	Tail tape measure	Tail protein [*Enterococcus faecalis* V583]	56/196 (28%); C-part	1720	5.0E-06	NP_815759.1
								ACLAME: protein:vir:101308 *Staphylococcus aureus* phage phiNM3 # phage tail tape measure protein	53/237 (22%) (N-term part of both proteins)	1509	3.0E-05	YP_908841
gp74	46607	47737	376	42.7	4.4	TAAATCATATATCAAAGGAGtttaattc**ATG**		Hypothetical protein EF2095 [*Enterococcus faecalis* V583]	88/328 (26%)	371	3.0E-30	NP_815758.1
gp75	47737	48969	410	47.0	5.0	TAACAAATTGGAAAGGActtatta**ATG**		Endolysin domain protein [*Enterococcus faecalis* V583]	111/376 (29%)	858	1.0E-39	NP_815756.1
gp76	48969	49793	274	30.9	5.2	AGCAGGAGTATATGGAAATGGAGTaaatta**ATG**	Structure	NSS: hypothetical protein EF2092 [*Enterococcus faecalis* V583]	17/55 (30%)	229	0.65	NP_815755.1
gp77	49768	50253	161	18.2	4.5	AATAGAACAAATGGAGGaattacaaa**ATG**		Hypothetical protein EF2091 [*Enterococcus faecalis* V583]	74/161 (45%)	162	2.0E-35	NP_815754.1
gp78	50243	50896	217	24.4	8.2	ACGTCCAAGGAGGataatttag**ATG**	Receptor binding	Putative receptor binding protein [*Lactococcus* bacteriophage SL3]	50/189 (26%)	273	8.0E-08	AAT81485.1
gp79	50908	51681	257	29.2	4.5	TCCAACAACTAATTAAAGGAGaattaa**ATG**		Hypothetical ANASTE_01764 [*Anaerofustis stercorihominis* DSM17244]	45/149 (30%)	556	2.0E-03	ZP_02862545.1
gp80	51694	52509	271	29.1	5.0	GTAAGAAAGTACTATAAGGAGattaatca**ATG**	Structure	NSS				
gp82	52911	53231	106	11.9	5.9	GAACATTAGTATTATAGAAGGataacact**ATG**		ACLAME: *Clostridium botulinum* C phage C-St, phage virion protein	21/69 (30%)	523	7.0E-03	YP 398596
gp83	53234	53572	112	12.1	4.6	AGTAAGCTGGGTAAATTCGACCAAGGTaactaaat**ATG**	Holin	NSS				
gp85	53855	54568	237	26.8	7.8	TACCGTTGCACAAAGTACAGGAGGTcaaata**GTG**	Endolysin	gp22 putative lysin [*Lactococcus* phage P008]	114/227 (50%)	233	1.0E-58	YP_762533.1
gp86	54663	57488	941	103.2	5.3	GGTGGTATTTTAATAAGGAGGactat**ATG**	Neck Passage	NPS [*Lactococcus* phage TP901-1]	140/214 (65%)	667	3.0E-71	NP_112714.1
gp87	57497	58330	277	30.9	5.0	ATTGGATTAACAGATAGGAGTttaa**ATG**	Structure	NSS				
gp88	58345	59274	309	34.2	5.9	TGCCAAGTTAAGATAAGGAGataca**ATG**	Structure	NSS				

pI and MM taken from ExPASy home page: Compute pI/Mw (**http://ca.expasy.org/tools/pi_tool.html)**
Conserved domains had been found on NCBI (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), within the CDD database, with an evalue of 0.01.
NSS: No significant hits
MM: Molecular Mass
pI: isoelectric point

Table 2

Table 2. Frequency usage per thousand codons

tRNA	Codon	Amino Acid	P087	*L. lactis* MG1363	*L. lactis* IL1403	*L. lactis* SK11	*E. faecalis* V583	*E. faecalis* V583 prophage 05
Pro	CCA	P	17.2	15.0	15.5	14.5	16.2	13.4
Thr	ACA	T	28.6	22.1	22.9	22.1	24.8	23.5
Asn	AAC	N	20.3	10.7	10.6	11.1	14.0	14.9
Asp	GAC	D	23.4	14.7	13.9	14.4	12.5	13.3
Cys	UGC	C	1.7	1.1	1.0	1.1	1.7	3.0
Total number of codons			18442	739646	667611	696252	963629	13623

The putative prophage 05 codons from E. faecalis V583 were calculated from positions 2004910 (start of EF2084) to 2048146 (stop of EF2145).

Figure 1

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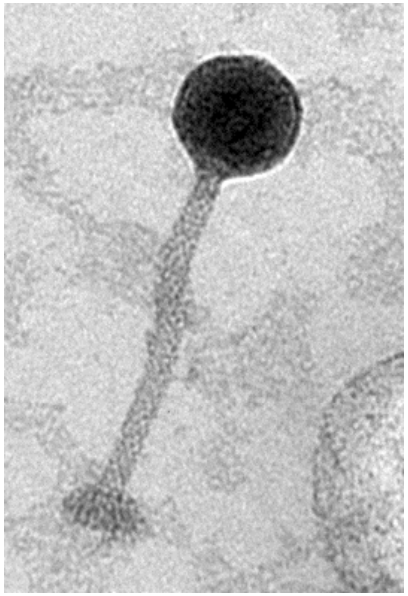

Figure 1

Figure 2

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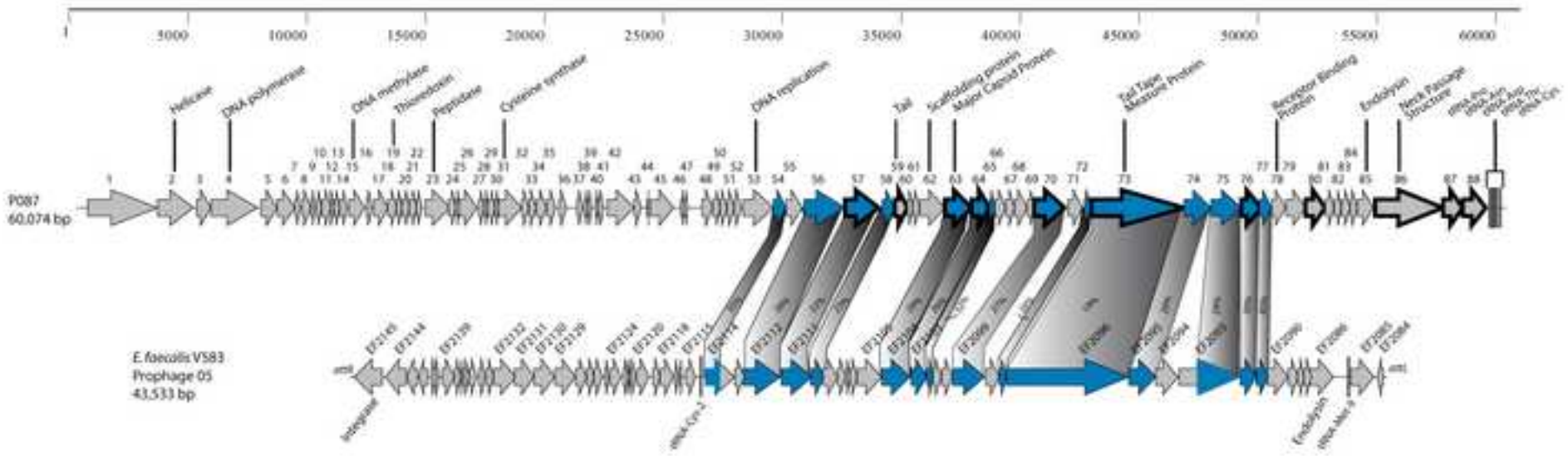

Figure 3
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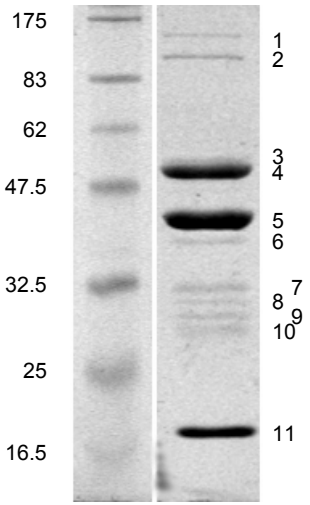
A



B

Band	Gene product	Molecular mass (KDa) SDS-PAGE	Calculated
1	gp73	132	136.3
2	gp86	110	103.2
3	gp57	54	56.4
4	gp70	53	48.5
5	gp63	42	42.5
6	gp88	40	34.2
7	gp87	32	31.0
8	gp76	31	30.9
9	gp80	29	29.1
10	gp64	28	28.8
11	gp59	18	18.2

Figure 3