





Taylor & Francis

ISSN: (Print) 2159-7081 (Online) Journal homepage: informahealthcare.com/journals/kbac20

Structural proteins of Enterococcus faecalis bacteriophage *\phi*Ef11

Roy H. Stevens, Hongming Zhang, Chaiwing Hsiao, Scott Kachlany, Eduardo M. B. Tinoco, Jessica DePew & Derrick E. Fouts

To cite this article: Roy H. Stevens, Hongming Zhang, Chaiwing Hsiao, Scott Kachlany, Eduardo M. B. Tinoco, Jessica DePew & Derrick E. Fouts (2016) Structural proteins of Enterococcus faecalis bacteriophage *(Ef11, Bacteriophage, 6:4, e1251381, DOI: 10.1080/21597081.2016.1251381)*

To link to this article: https://doi.org/10.1080/21597081.2016.1251381

6 © 2016 The Author(s). Published with license by Taylor & Francis© Roy H. Stevens, Hongming Zhang, Chaiwing Hsiao, Scott Kachlany, Eduardo M. B. Tinoco, Jessica DePew, and Derrick E. Fouts

đ	1	(1
Е			
Е			
_			_

Published online: 28 Nov 2016.



🕼 Submit your article to this journal 🗗

Article views: 2454

0 View related articles 🗹



View Crossmark data 🗹



Citing articles: 1 View citing articles

BRIEF REPORT

Taylor & Francis

∂ OPEN ACCESS

Structural proteins of Enterococcus faecalis bacteriophage *ø*Ef11

Roy H. Stevens^{a,b,1}, Hongming Zhang^{a,b,1}, Chaiwing Hsiao^{a,#}, Scott Kachlany^c, Eduardo M. B. Tinoco^d, Jessica DePew^{e,##}, and Derrick E. Fouts^e

^aLaboratory of Oral Infectious Diseases, Temple University Kornberg School of Dentistry, Philadelphia, PA, USA; ^bDepartment of Endodontics, Temple University Kornberg School of Dentistry, Philadelphia, PA, USA; ^cDepartment of Oral Biology, Rutgers School of Dental Medicine, Rutgers University, Newark, NJ, USA; ^dDepartment of Periodontology, UERJ/Unigranrio, Rio de Janeiro, Brazil; ^eDepartment of Genomic Medicine, J Craig Venter Institute, Rockville, MD, USA

ABSTRACT

 ϕ Ef11, a temperate *Siphoviridae* bacteriophage, was isolated by induction from a root canal isolate of *Enterococcus faecalis*. Sequence analysis suggested that the ϕ Ef11 genome included a contiguous 8 gene module whose function was related to head structure assembly and another module of 10 contiguous genes whose products were responsible for tail structure assembly. SDS-PAGE analysis of virions of a ϕ Ef11 derivative revealed 11 well-resolved protein bands. To unify the deduced functional gene assignments emanating from the DNA sequence data, with the structural protein analysis of the purified virus, 6 of the SDS-PAGE bands were subjected to mass spectrometry analysis. 5 of the 6 protein bands analyzed by mass spectrometry displayed identical amino acid sequences to those predicted to be specified by 4 of the ORFs identified in the ϕ Ef11 genome. These included: ORF8 (predicted scaffold protein), ORF10 (predicted major head protein), ORF15 (predicted major tail protein), and ORF23 (presumptive antireceptor).

ARTICLE HISTORY

Received 7 September 2016 Revised 11 October 2016 Accepted 18 October 2016

KEYWORDS bacteriophage; *Enterococcus faecalis*; phage ϕ Ef11

Introduction

Viruses that infect Enterococci have been known for nearly 90 y (Clark & Clark,¹ Evans²). Since then, more than 40 different enterococcal phages have been identified (for a review see reference,³ and refs.⁴⁻⁹). Among these, 22 have genome sequences available. These include: virulent Siphoviridae phages OSH-56, IME-EF1, SAP6, BC-611, and EFRM-1 (see refs.^{6,8,10-12}), temperate Siphoviridae phages EFC-1, F4, V1/7, pp1pp6, FL1A, FL1B, FL1C, FL2A, FL2B, FL3A, FC1 and ϕ Ef11 (see refs.^{9,13-17}), and virulent *Myoviridae* phages ϕ EF24C and EFDG1 (see refs.^{18,19}). From these sequence data, the functions of many of the gene products of these phage genomes have been predicted. However, there has been little experimental evidence confirming these predicted functions. Here we present SDS-PAGE and mass spectrometry analysis of virion structural proteins in support of our previous annotation of the genome of *Enterococcus faecalis* bacteriophage ϕ Ef11.¹⁷

φEf11 is a temperate, *Siphoviridae* morphotype bacteriophage that was originally isolated by induction of a lysogenic E. faecalis strain recovered from an infected root canal.²⁰ Its DNA genome is 42,822 bp in length encoding 65 ORFs.¹⁷ The functions of many of the ϕ Ef11 open reading frames (ORFs) were predicted based upon sequence similarity with gene products of known function reported for previously characterized bacteriophages. Similar to numerous other phages of low GC Gram-positive bacteria, the genes appear to be arranged in functional modules. Among these 8 functional modules, module 2 (containing ORFs 4 through 10) was predicted to encode the proteins of the virion head structure assembly, and module 3 (containing ORFs 11-20) was predicted to be responsible for tail structure assembly. To extend our

CONTACT Roy H. Stevens 🖾 roy.stevens@temple.edu 💽 Laboratory of Oral Infectious Diseases and; Department of Endodontics, Temple University Kornberg School of Dentistry, 3223 N. Broad St., Philadelphia, PA 19140, USA.

[#]Current Address: 2240 Pine Road, Huntington Valley, PA 10006, USA.

¹Both authors contributed equally

^{##} Current Address: 2827 W. Canyon Ave., San Diego, CA 92123, USA.

^{© 2016} Roy H. Stevens, Hongming Zhang, Chaiwing Hsiao, Scott Kachlany, Eduardo M. B. Tinoco, Jessica DePew, and Derrick E. Fouts. Published with license by Taylor & Francis. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

knowledge of the biology of this phage and to confirm the identity of the genes whose products comprise the phage capsid proteins, we identified the structural proteins of the purified phage virions by SDS-PAGE, and compared their amino acid sequences, as determined by MALDI/TOF/TOF mass spectrometry (MS) analysis, with the predicted amino acid sequences of the proteins encoded by the genes of the head and tail structure assembly modules.

Results and discussion

SDS-PAGE analysis of the dissociated purified phage disclosed 11 well-resolved protein bands ranging in size from 27 to 85 kDa, in addition to several less resolved bands (Fig. 1, Table 1). Of these, the proteins in the 6 most prominent bands (bands 2, 6, 8, 9, 10 and 11) were subjected to MALDI/TOF/TOF MS analysis. The amino acid sequences of the proteins in each of these bands, as determined by the MS analysis, was compared with the amino acid sequences of the



Figure 1. SDS-PAGE analysis of proteins from purified bacteriophage ϕ Ef11(Δ 61-65, FL1C39-44). Lane 1, molecular weight markers (Fermentas Broad Range Protein Ladder; Lanes 2 and 3, dissociated, purified bacteriophage ϕ Ef11(Δ 61-65, FL1C39-44).

Table 1.	Summary o	of phag	ge ϕ Ef1	I1(∆61-65,	ϕ FL1C	39-44) vi	rion
proteins	predicted	from	DNA	sequence	and	detected	by
SDS-PAG	E.						

Band No./ ORFNo.*	Predicted function of ORF product	MW predicted from DNA sequence	MW determined by SDS-PAGE analysis
2 / 23 6 / 10 8 / 10 9 /	Anti-receptor Major head protein Major head protein	63,273 37,324 37,324	73,000 - 74,000 41,000 - 43,000 38,000 - 39,000
10 / 15 11 / 8	Major Tail protein Scaffold protein	21,542 23,446	30,000 – 28,000 27,000 – 27,000

*ORF assignment of the protein in each band made by comparing the deduced gene product amino acid sequence with the amino acid sequences of the peptides detected in each band by MS.

gene products deduced from the genome of ϕ Ef11.¹⁷ It can be seen in Fig. 2A–D, that the amino acid sequences of 5 of the phage ϕ Ef11(Δ 61-65, FL1C 39-44) virion protein bands (bands 2, 6, 8, 10 and 11) exactly matched the predicted amino acid sequences of the deduced gene products of phage ϕ Ef11 ORFs 8, 10, 15 and 23 (corresponding to GenBank accession GQ452243.1 loci PHIEF11_0008, PHIEF11_0010, PHIEF11_0015, and PHIEF11_0023, respectively). However, the proteins of both bands 6 and 8 had amino acid sequences corresponding to the predicted gene product of ϕ Ef11 ORF 10 (locus PHIEF11_0010).

The peptides detected from the protein seen as band 2 of the SDS-PAGE gel had amino acid sequences that were identical to those predicted from the deduced amino acid sequence of the ORF 23 (locus PHIEF11_0023) gene product (Fig 2A), although the observed MW of this protein (ca. 73 kDa) was somewhat larger than that calculated (63.273 kDa) from its predicted amino acid sequence (Table 1). Previously, it was not possible to predict the function of the ORF 23 gene product from its deduced amino acid sequence since there was little sequence similarity to any characterized protein of known function.¹⁷ Furthermore, ORF 23 is located downstream of what we predicted to be the terminal gene (ORF20) of the tail morphogenesis gene module (ORFs 11 through 20). Therefore, in our previous annotation of the ϕ Ef11 genome, we characterized the ORF 23 gene product as a "hypothetical protein."¹⁷ Subsequently, based on bioinformatics inference to a characterized lactococcal phage tail²¹ we initiated infection inhibition studies using the product of the cloned ORF 23, expressed in Escherichia coli. These studies, which will be presented in a subsequent report, clearly demonstrated that the ORF 23 gene product blocked





Figure 2. Comparison of deduced amino acid sequences of bacteriophage ϕ Ef11 gene products with amino acid sequences of peptides detected in MS analysis of SDS-PAGE-separated bacteriophage ϕ Ef11(Δ 61-65, FL1C39-44) virion proteins. Black lettering is the ORF DNA base sequence, Blue lettering is the deduced gene product amino acid sequence, Red or Green lettering is the amino acid sequence of the peptides detected by MS analysis of the material in the bands seen in the SDS-PAGE-separated ϕ Ef11(Δ 61-65, FL1C39-44) virion proteins. (A) ORF23/SDS-PAGE Band 2 protein. (B) ORF10/Bands 6 and 8 proteins (Green is SDS-PAGE band 6 material, Red is SDS-PAGE band 8 material). (C) ORF15/Band 10 protein. (D) ORF8/Band 11 protein.

infection by phage ϕ Ef11(Δ 61-65, FL1C 39-44), and suggested that this protein represents the receptorbinding apparatus of this phage (data not shown). These findings, along with the fact that we now find this protein (band 2) in the SDS-PAGE analysis of the dissociated, purified virion, suggest that ORF 23 codes

1

61

121

181

241

301

361

for a tail structure protein, notably, the receptor-binding protein. Considering the modular nature of the phage genome, these results suggest that the ϕ Ef11 tail morphogenesis gene module should be extended to ORFs PHIEF11_0021include 21 - 23(loci PHIEF11_0023) (Fig 3).

900 300

960 320

60

20

120

180

240

300

360

420

480

540 180

600

643 215



Figure 3. Map of head and tail morphogenesis region of bacteriophage ϕ Ef11 genome.

Bands 6 (ca. 41 kDa) and 8 (ca. 38 kDa) seen in the SDS-PAGE gels displayed polypeptide fragments with identical amino acid sequences, as detected by mass spectroscopic analysis (Fig. 2B, Table 1). Furthermore, the amino acid sequence of the proteins comprising bands 6 and 8 both precisely matched the deduced amino acid sequence of the gene product of ORF 10 (locus PHIEF11_0010), a protein with a predicted mass of 37.324 kDa, and the predicted function as a major component of the phage head structure (Fig. 3).¹⁷ These data suggest that the 2 proteins seen as band 6 and band 8 in the SDS-PAGE gels are both encoded by the same gene, ORF 10, although one protein (from band 6) exceeded the mass of the other protein (from band 8) by ca. 3 kDa. A similar circumstance has been reported for several other phages (Bacillus subtilis phage SPP1,²² Listeria monocytogenes phage PSA,²³ Lactococcus lactis phage Q54²⁴) in which 2 structural proteins of different masses, detected by SDS-PAGE analysis, were encoded by a single gene. For example, in the case of phage SPP1, 2 bands seen in SDS-PAGE analysis of the phage virion proteins were shown to be gene products of the same ORF; however, one had an observed additional mass of 9.1 kDa.²² In all these cases, the production of the larger version of each pair of proteins was seen to be due to a programmed translational frameshift resulting in the elimination

of a stop codon and the consequent extension of the translated product of the reading frame.^{22,23,24} The conditions known to promote a translational reading frame-shift near the 3' terminus of phage mRNA include a region of overlapping synonymous or identical codons just upstream from the stop codon, and the presence of an RNA secondary structure (e.g., a stem-loop) adjacent to the site of The the reading frame-shift. overlapping synonymous or identical codons, forming what is known as a "slippery sequence," allow one base backward slippage of the ribosome in the 5' direction as it moves along the mRNA. The slippery sequence takes the form of a heptanucleotide with the sequence of X XXY YYZ, where Y is either A or T²⁵. If there is any destabilization of the 2 ribosome-bound tRNAs from their codons in the mRNA, the slippery sequence permits bond reformation of the tRNAs to the mRNA, one nucleotide upstream (in the 5' direction), resulting in a frameshift for all subsequent downstream codons of the ORF. As a consequence, a downstream stop codon may be converted to an amino acyl-tRNA-specifying sequence, resulting in translational readthrough past the original stop codon, and the Cterminal extension of the protein product.^{25,26} Such a mechanism was originally reported for retroviruses by Jacks et al,²⁷ and has been shown to be a common feature in the synthesis of phage major



Figure 4. Region of 3' end of ORF 10. The 0 reading frame is shown in the upper nucleotide sequence. Proposed slippery sequence (AAAAAAA) is indicated by a solid line box. The ORF stop codon is indicated by *. A potential -1 frameshift would result in the nucleotide sequence shown below. In the shifted reading frame, the original stop codon would be converted into 2 amino acid-specifying codons (ATA and GAA), and the protein translation product would be extended by 21 amino acids.

tail proteins of the tailed phages^{22,23,26,29,30} as well as in phage capsid head proteins.³¹ The RNA secondary structure is thought to positively influence frame-shifting by impeding the progress of the ribosome along the mRNA, thereby allowing more time for realignment of codon and anticodon.³²

In the case of ϕ Ef11 ORF10, it can be seen that there are 2 tandem identical (AAA) lysine codons immediately upstream to the ORF10 TAG stop codon (Fig 4). Other phages having slippery sequences composed of AAAAAA include *Mycobacterium thermoautotrophicum* phage ψ m2, *Lactococcus* phage c2, *Listeria monocytogenes* phage PSA, *Lactobacillus oenos* phage L10, and *Haemophilus influenza* phage HP1.²⁸

There is also the potential for the formation of a stem-loop structure immediately downstream of the stop codon of ORF 10 and the adjacent/overlapping lysine codons, as determined by MFOLD analysis³³ (Fig 5, $\Delta G = -20.10$). These observations are consistent with a potential for a translational frame-shift that would extend the translated gene product by 21 amino acids. As illustrated in Fig. 4, a -1 frameshift would result in a read-through past the original in-frame stop codon, and the extension of the resulting protein by 21 amino acids, increasing the protein mass by 2,106 Daltons. If this occurs in phage ϕ Ef11 infection during expression of ORF 10, it would explain the observation of the 2 protein bands (bands 6 and 8) seen in the SDS-PAGE analysis differing in apparent mass, but composed of a similar amino acid sequence. However, it should be noted that the MS analysis failed to detect any peptides of the larger version of the protein (band 6) with amino acid sequences that would be expected to be present as a

result of the extension of the protein. Therefore, until confirmatory information is available, this interpretation of our results should be considered speculative.

The protein comprising band 10 of the SDS-PA GE-separated ϕ Ef11 virion proteins had an amino acid sequence that matched that of the deduced gene product of ORF 15 (locus PHIEF11_0015, Fig 2C). Based upon sequence similarities, the ORF 15 gene product was predicted to be a major component of the phage tail structure. ¹⁷ The present findings are consistent with this prediction; however, the mobility of the band10 protein seen in the SDS-PAGE gel corresponded to a molecular mass of 28 kDa, which is somewhat larger than the molecular mass predicted

UA
GU
U-A
G-C
A C
A A
A A
G-C
U-A
U-A
G¯C
A ⁻ U
A G
A-A
G-C
C ~ G
C ~ G
C ⁻ G
C⁻G
G¯C
A C
A_U
5' ·····UCAAAAAAAAAAA

Figure 5. Potential secondary structure of mRNA in region immediately downstream of the proposed slippery sequence and terminal codon of ORF 10, as determined by MFOLD analysis. Initial $\Delta G = -20.10$ kcal/mol. Proposed slippery sequence is indicated by solid line box and 0 frame stop codon is indicated by dotted line box.

for the ORF 15 gene product (21.542 kDa), as determined from the deduced amino acid sequence. Furthermore, the deduced mass of the ORF 15 gene product (21.542 kDa) was less than that deduced for the ORF 8 gene product (23.446 kDa), and yet the putative ORF 15 gene product (band 10) clearly migrated in SDS-PAGE at a rate corresponding to a protein of greater mass than the putative ORF 8 gene product (i.e., band 11, see below). This suggests that either the ORF 15 gene product is posttranscriptionally processed in such a way that it is increased in mass, or the ORF 8 gene product is post-translationally cleaved to result in a decrease in molecular mass. Since both bands 10 and 11 migrated at rates corresponding to greater masses than that predicted from their deduced amino acid sequences, the former alternative seems more likely. There are several examples of phage structural proteins displaying mobilities in SDS-PAGE that correspond to molecular masses that are greater than what would be predicted from their deduced amino acid sequences. For example, the lactococcal phage Tuc2009 scaffold protein (ORF36 gene product Msp2) migrates to a position in SDS-PAGE corresponding to a molecular mass of 40 kDa, although the predicted mass of the ORF36 gene product is 24.5 kDa.²¹ Also in Tuc2009, the major head protein (MP4) is encoded by a fusion of portions of ORFs 37 and 39, resulting in an observed molecular mass of MP4 greater than that predicted for either the ORF 37 or ORF 39 gene product alone.³⁴ Similarly, the computationally-determined masses of the major head and tail proteins (mhp and mtp) of lactococal phage TP901-1 (28.9 and 18.6 kDa respectively) were not in agreement with the masses of these proteins determined by SDS-PAGE (31 and 23 kDa, respectively).³⁵ It was proposed that the discrepancy between the observed and computationally-calculated masses of each of these proteins was due to a fusion of the genes encoding the mhp and mtp proteins with overlapping genes in different reading frames, resulting in an altered mass and charge in the fused protein.³⁵ Examination of the alternative reading frames possible within ϕ Ef11 ORF 15 failed to disclose an overlapping ORF that would permit readthrough of an altered ORF 15 stop codon, resulting in an enlarged gene product. Consequently the reason for the apparent increased size of the ORF 15 gene product compared to that predicted from its deduced amino acid sequence may be due to a posttranslational modification; however, this remains to be determined.

The protein comprising band 11 displayed an amino acid sequence that was identical to that predicted for the gene product of ϕ Ef11 ORF 8 (Fig. 2D). Moreover, the molecular mass of this protein as determined by SDS-PAGE analysis (27 kDa) was close to that predicted for the deduced protein gene product of ORF 8 (23.446 kDa). Based upon sequence similarity to deduced characterized proteins in several genomic data base repositories, the ORF 8 gene product was predicted to function as a scaffold protein for phage head structure assembly.¹⁷ The present findings appear to support this contention. Viral scaffold proteins are typically hydrophilic, acidic (pI 4.2-5.0), and small (11.3 kDa-34.0 kDa) in size.³⁶ The ORF 8 gene product (213 amino acids) has a predicted pI of 5.01, and molecular mass of 23.446 kDa (predicted)-27.0 kDa (observed). Viral scaffold proteins are characterized as proteins that are required for the proper assembly of the viral capsid, but are not present in the mature virion.36,37 The scaffold proteins are responsible for the correct arrangement of the structural proteins in order to form a procapsid. Following completion of procapsid assembly, the scaffold proteins are typically removed or cleaved prior to DNA packaging and completion of the mature capsid. Therefore, the presence of a scaffold protein in the ϕ Ef11 virion is somewhat unusual, although there are previous reports of other phages (e.g., Streptococcus pneumoniae phage Cp-1 and Lactococcal phage Tuc2009) whose nucleocapsids include at least portions of scaffold protiens.^{21,38}

The peptides detected by MS in the SDS-PAGE band 9 material did not display any amino acid sequence homology to any of the deduced ϕ Ef11 gene products. They were, however, found to be identical in sequence to a protein ("hypothetical protein EF1463") predicted to be a gene product of *E. faecalis* V583. Although the function of this protein (Accession number NP_815184.1) could not be predicted based upon sequence homology, it did possess a conserved domain found in membrane proteins of other bacterial species (*Corynebacterium ulcerans* and *C. pseudotuberculosis*). It is likely that this protein was a contaminant that co-purified along with the *E. faecalis* virions.

In conclusion, peptides having amino acid sequences identical to those of the deduced gene products of phage ϕ Ef11 ORFs 8, 10, 15 and 23, were detected in the bands produced by SDS-PAGE analysis of purified phage ϕ Ef11(δ 61-65, FL1C 39-44).

These findings support and strengthen our previous gene function assignments for phage ϕ Ef11 structural proteins, with the exception that it appears that ORFs 21-23 should now be included in the module of tail structure morphogenesis genes. Specifically, they suggest that ORFs 8, 10, 15 and 23 code for the major head protein, major tail protein, scaffold protein, and the receptor-binding protein, respectively.

Materials and methods

Growth and purification of phage

Phage were grown in 10 Ls of a log phase brain heart infusion broth culture of E. faecalis JH2-2. To increase the obtainable phage titer, we used a more virulent recombinant variant of ϕ Ef11: Phage ϕ Ef11(Δ 61-65, FL1C39-44), which contains 5 genes of an E. faecalis phage FL1C-like phage replacing 6 ϕ Ef11 genes whose predicted functions were related to DNA replication.³⁹ After incubation at 37°C for 6 h, DNAse $(1\mu g/ml)$ was added and the culture was incubated at room temperature for an additional hour. The resulting lysate was clarified by centrifugation (GSA rotor at $16,000 \times g$ for 10 min) and the phage was pelleted from the clarified lysate by a series of ultracentrifugations (45 Ti rotor at 150,000 \times g for 1 h). After decanting the supernatants, the phage pellets were resuspended overnight at 4°C, in a total volume of 5 ml of SM buffer (0.58% NaCl, 0.2% MgS04, 0.788% Tris HCl pH7.5, 0.01% gelatin).40 Portions of the phage suspension were layered onto CsCl (in SM buffer) step gradients having density layers of 1.70, 1.50, 1.40, and 1.15 g/ml. The gradients were centrifuged (SW41 rotor at 110,000 \times g for 2 h), and the phage bands, which formed at the 1.40/1.50 g/ml interface were collected and dialyzed against SM buffer at 4°C overnight. Finally, the phage was pelleted (45 Ti rotor at 150,000 \times g for 1 h), and resuspended in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

SDS-PAGE

The phage was dissociated in SDS-PAGE sample buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.025% bromphenol blue), and the phage proteins were separated by SDS-PAGE in 13% polyacrylamide gels.⁴¹ Electrophoresis was carried out for at 200 V for 1h, and the phage proteins were visualized by staining with Coomassie Brilliant Blue.

TOF/TOF mass spectrometry

Selected, well-resolved bands were excised from the polyacrylamide gel. The protein in each excised band was eluted in distilled water and subjected to mass spectrometry analysis following procedures described by Tibrewal et al.42 The eluted proteins were extracted with 30 μ l of 1% trifluoroacetic acid followed by C₁₈ZipTip desalting. After mixing with 7 mg/ml α -cyano-4-hydroxy-cinnamic acid matrix in a 1:1 ratio, the peptides were spotted onto a MALDI plate. Peptide analysis was carried out with a 4800 Protomics Analyzer tandem mass spectrometer (Applied Biosystems, ABI, Framingham, MA) in positive reflector mode for acquiring the mass spectra (m/z 800-3,000). Subsequent MS/MS sequencing analysis was conducted on the most intense ions. The identification of the peptides detected in the MS/MS sequencing was accomplished by comparing the combined MS and MS/MS spectra with the sequences in the swissprot database using a MASCOT search engine.

Abbreviations

GC	guanine/cytosine
MALDI/TOF/TOF	matrix-assisted laser desorption
	ionization-time of flight-time of
	flight
MS	mass spectrometry
ORF	open reading frame
SDS-PAGE	sodium dodecyl sulfate poly-
	acrylamide gel electrophoresis

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

- Clark PF, Clark AS. A bacteriophage active against a virulent hemolytic Streptococcus. Proc Soc Exp Biol Med 1927; 24(7):635-9; http://dx.doi.org/10.3181/00379727-24-3498
- [2] Evans AC. Prevalence of Streptococcus bacteriophage. Science 1934; 80(2063):40-1; PMID:17818639; http://dx. doi.org/10.1126/science.80.2063.40

e1251381-8 👄 R. H. STEVENS ET AL.

- [3] Duerkop BA, Palmer KL, Horsburgh MJ. Enterococcal Bacteriophages and Genome Defense. IN: Enterococci from Commensals to leading causes of drug resistance. Gilmore MS, Clewell DB, Yasuyoshi I, Shankar N (EDs). Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014-. Available from: http://www.ncbi.nlm.nih.gov/books/NBK190419/
- [4] Jarvis AW, Collins LJ, Ackermann H-W. A study of five bacteriophages of the Myoviridae family which replicate on different gram-positive bacteria. Arch Virol 1993; 133:75-84; PMID:8340019; http://dx.doi.org/10.1007/BF01309745
- [5] Yang H-Y, Kim Y-W, Chang H-I. Construction of an integration-proficient vector based on the sitespecific recombination mechanism of Enterococcal temperate phage ϕ FC1. J Bacteriol 2002; 184 (7):1859-64; PMID:11889091; http://dx.doi.org/ 10.1128/JB.184.7.1859-1864.2002
- [6] Vinodkumar C, Srinivasa H, Basavarajappa KGeethalakshmi S, Bandekar N. Isolation of bacteriophages to multi-drug resistant Enterococci obtained from diabetic foot: A novel antimicrobial agent waiting in the shelf? Ind J Pathol Microbiol 2011; 54.1:90; PMID:21393885; http://dx.doi.org/ 10.4103/0377-4929.77333
- [7] Rigvava S, Tchgkonia I, Jgenti D, Dvalidze T, Caprino J, Goderdzishvili M. Comparative analysis of the biological and physical properties of Enterococcus faecalis bacteriophage vB_SmM_GEC-SmitisM_2. Can J Microbiol 2012; 59:18-21; PMID:23391224; http://dx.doi.org/10.1139/ cjm-2012-0385
- [8] Zhang W, Mi Z, Yin X, Fan H, An W, Zhang Z, Chen J, Tong Y. Characterization of Enterococcus faecalis phage IME-EF1 and its endolysin. PLoS One 2013; 8(11): e80435; PMID:2436180
- [9] Yoon BH, Chang H-I. Genomic annotation for the temperature phage EFC-1, isolated from Enterococcus faecalis KBL101. Arch Virol 2015; 160:601-4; PMID:25359106; http://dx.doi.org/10.1007/s00705-014-2263-4
- [10] Lee Y-D, Park J-H. Complete genome sequence of enterococcal bacteriophage SAP6. J Virol 2012; 86(9):5402-5403; PMID:22492926; http://dx.doi.org/10.1128/JVI.00321-12
- [11] Horiuchi T, Sakka M, Hayashi A, Shimada T, Kimura T, Sakka K. Complete genome sequence of bacteriophage BC-611 specifcially infecting Enterococcus faecalis strain NP-10011. J. Virol 2012; 86(17):9538-9; PMID:22870611; http://dx.doi.org/10.1128/JVI.01424-12
- [12] Fard RMN, Barton MD, Arthur JL, Heuzenroeder MW. Whole-genome sequencing and gene mapping of a newly isolated lytic enterococcal bacteriophage EFRM31. Arch Virol 2010; 155:1887-91; PMID:20844906; http://dx.doi. org/10.1007/s00705-010-0800-3
- [13] Nigutova K, Styriak I, Javorsky P, Prestas P. Partial characterization of Enterococcus faecalis bacteriophage F4.
 Folia Microbiol 2008; 53(3):234-6; PMID:18661299; http://dx.doi.org/10.1007/s12223-008-0033-y

- [14] Duerkop BA, Clements CV, Rollins D, Rodrigues JLM, Hooper LV. A composite bacteriophage alters colonization by an intestinal commensal bacterium. PNAS 2012; 109(43):17621-6; PMID:23045666; http://dx.doi.org/ 10.1073/pnas.1206136109
- [15] Matos RC, Lapaque N, Rigottier-Gois L, Debarbieux L, Meylheuc T, Gonzalez-Zorn B, Repoila F, Lopes Mde F, Serror P. Enterococcus faecalis prophage dynamics and contributions to pathogenic traits. Plos One 2013; 9(6): e1003539. PMID:23754962
- [16] Yasmin A, Kenny JG, Shankar J, Darby AC, Hall N, Edwards C, Horsburgh MJ. Comparative genomics and transduction potential of Enterococccus faecalis temperate bacteriophages. J Bacteriol 2010; 192(4):1122-30; PMID:20008075; http://dx.doi.org/10.1128/JB.01293-09
- [17] Stevens RH, Ektefaie MR, Fouts DE. The annotated complete DNA sequence of Enterococcus faecalis bacterio-phage φEf11 and its comparison to all available phage and prophage genomes. FEMS Microbiol Lett 2011; 317:9-26; PMID:21204936; http://dx.doi.org/10.1111/j.1574-6968.2010.02203.x
- [18] Uchiyama J, Rashel M, Takemura I, Wakiguchi H, Matsuzaki S. In Silico and In vitro evaluation of bacteriophage¢Ef24C, a candidate for treatment of Enterococcus faecalis infections. Appl Environ Microbiol 2008; 74(13):4149-63; PMID:18456848; http://dx. doi.org/10.1128/AEM.02371-07
- [19] Khalifa L, Brosh Y, Gelman D, Coppenhagen-Glazer S, Beyth S, Poradosu-Cohen R, Que Y-A, Beyth N, Hazan R. Targeting Enteococcus faecalis biofilms with phage therapy. Appl. Environ. Microbiol 2015; 81:2696-705; PMID:25662974. http://dx.doi.org/10.1128/AEM.00096-15
- Stevens RH, Porras O, Delisle A. Bacteriophages induced from lysogenic root canal isolates of Enterococcus faecalis. Oral Microbiol Immunol 2009; 24:278-84; PMID:19572888; http://dx.doi.org/10.1111/j.1399-302X.2009.00506.x
- [21] McGrath S, Neve H, Seegers JFML, Eijlander R, Vegg CS, Brøndsted L, Heller KJ, Fitzgerald GF, Vogensen FK, van Sinderen D. Anatomy of a Lactococcal phage tail. J Bacteriol 2006; 188(11):3972-82; PMID:16707689; http://dx. doi.org/10.1128/JB.00024-06
- [22] Auzat I, Dröge A, Weise F, Lurz R, Tavares P. Origin and function of the two major tail proteins of bacteriophage SPP1. Mol Microbiol 2008; 70(3):557-69; PMID:18786146; http://dx.doi.org/10.1111/j.1365-2958. 2008.06435.x
- [23] Zimmer M, Sattelberger E, Inman RB, Calendar R, Loessner MJ. Genome and proteome of Listeria monocytogenes phage PSA: an unusual case for programmed +1 translational frameshifting in structural protein synthesis. Mol Microbiol 2003; 50(1):303-17; PMID:14507382; http://dx.doi.org/10.1046/j.1365-2958.2003.03684.x
- [24] Fortier LC, Bransi A, Moineau S. Genome sequence and global gene expression of Q54, a new phage species linking the 936 and c2 phage species of Lactococcus lactis. J

Bacteriol 2006; 188:6101-14; PMID:16923877; http://dx. doi.org/10.1128/JB.00581-06

- [25] Giedroc DP, Theimer CA, Nixon PL. Structure, stability and function of RNA pseudoknots involved in stimulating ribosomal frameshifting. J Mol Biol 2000; 298:167-85; PMID:10764589; http://dx.doi.org/10.1006/jmbi.2000.3668
- [26] Levin ME, Hendrix RW, Casjens SR. A programmed translational frameshift is required for the synthesis of bacteriophage λ tail assembly protein. J Mol Biol 1993; 234:124-39; PMID:8230192; http://dx.doi.org/10.1006/ jmbi.1993.1568
- [27] Jacks T, Madhani HD, Masiarz FRT, Varmus HE. Signals for ribosomal frameshifting in the rous sarcoma virus gag-pol region. Cell 1988; 55:447-58; PMID:2846182; http://dx.doi.org/10.1016/0092-8674(88)90031-1
- [28] Xu J, Hendrix RW, Duda RL. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. Molecular Cell 2004; 16:11-21; PMID:15469818; http:// dx.doi.org/10.1016/j.molcel.2004.09.006
- [29] Fraser JS, Yu Z, Maxwell KL, Davidson AR. Ig-like domains on bacteriophages: A tale of promiscuity and deceit. J Mol Biol 2006; 359:496-507; PMID:16631788; http://dx.doi.org/10.1016/j.jmb.2006.03.043
- [30] Rodriguez I, Garcia P, Suarez JE. A second case of -1 ribosomal frameshifting affecting a major viron protein of the Lactobacillus bacteriophage A2. J Bacteriol 2005; 187(23):8201-4; PMID:16291695; http://dx.doi.org/ 10.1128/JB.187.23.8201-8204.2005
- [31] Garcia P, Rodriguez I, Suarez JE. A –1 ribosomal frameshift in the transcript that encodes the major head protein of bacteriophage A2 mediates biosynthesis of a second essential component of the capsid. J Bacteriol 2004; 186(6):1714-9; PMID:14996802; http://dx.doi.org/ 10.1128/JB.186.6.1714-1719.2004
- [32] Alam SL, Atkins JF, Gesteland RF. Programmed ribosomal frameshifting: much ado about knotting! Proc Natl Acad Sci USA 1999; 96:14,177-14,179. PMID:10588670; http://dx.doi.org/10.1073/pnas.96.25.14177
- [33] Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nuc Acid Res 2003; 31 (13):3406-15; PMID:12824337. http://mfold.rna.albany. edu; http://dx.doi.org/10.1093/nar/gkg595

- [34] Seegers JFML, McGrath S, O'Connell-Motherway M, Arendt EK, van de Guchte M, Creaven M, Fitzgerald GF, van Sinderen D. Molecular and transcriptional analysis of the temperate lactococcal bacteriophage Tuc2009. Virology 2004; 329:40-52; PMID:15476873; http://dx.doi. org/10.1016/j.virol.2004.07.003
- [35] Johnsen MG, Appel KF, Madsen PL, Vogensen FK, Hammer K, Arnau J. A genomic region of lactococcal temperate bacteriophage TP901-1 encoding major virion proteins. Virology 1996; 218:306-16; PMID:8610457; http://dx.doi.org/10.1006/viro.1996.0199
- [36] Dokland T. Scaffolding proteins and their role in viral assembly. Cell Mol Life Sci 1999; 56:580-603; PMID:11212308; http://dx.doi.org/10.1007/s000180050455
- [37] Chang JR, Polakov A, Prevelige PE, Mobley JA, Dokland T. Incorporation of scaffolding protein gpO in bacteriophages P2 and P4. Virology 2008; 370 (2):352-61; PMID:17931675; http://dx.doi.org/10.1016/ j.virol.2007.08.039
- [38] Häuser R, Sabri M, Moineau S, Uetz P. The proteome and interactome of Streptococcus pneumoniae phage Cp-1. J Bacteriol 2011; 193(12):3135-8; PMID:21515781; http://dx.doi.org/10.1128/JB.01481-10
- [39] Zhang H, Fouts DE, DePew J, Stevens RH. Genetic modifications to temperate Enterococcus faecalis phage ϕ Ef11 that abolish the establishment of lysogeny and sensitivity to repressor, and increase host range and productivity of lytic infection. Microbiol 2013; 159(6):1023-35; PMID:23579685; http://dx.doi. org/10.1099/mic.0.067116-0
- [40] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
- [41] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227:680-5; PMID:5432063; http://dx.doi.org/10.1038/ 227680a0
- [42] Tibrewal N, Liu T, LI H, Birge RB. Characterization of the biochemical and biophysical properties of the phosphatidylserine receptor (PS-R) gene product. Mol Cell Biochem 2007; 304:119-25; PMID:17534701; http://dx. doi.org/10.1007/s11010-007-9492-8