Original Article

Avicenna Journal of Medical Biotechnology
Volume 14, Issue no. 3, 196-205
https://doi.org/10.18502/ajmb.v14i3.9826

Check for updates

# Genome Analysis of an Enterococcal Prophage, Entfac.MY

Maryam Yazdanizad [1], Ramin Mazaheri Nezhad Fard [2,3], Golshid Javdani Shahedin [4], Mohammadreza Salehi [5], Mahsa Dumanloo [6], and Ali Akbar Saboor Yaraghi [2*]

*1. Department of Medical Biotechnology, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*
*2. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
*3. Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran*
*4. Pasteur Institute of Iran, Tehran, Iran*
*5. Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*
*6. Department of Clinical Pathobiology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran*

## Abstract

**Background:** Bacteriophages are bacterial parasites. Unlike lytic bacteriophages, lysogenic bacteriophages do not multiply immediately after entering the host cells and may integrate their genomes into the bacterial genomes as prophages. Prophages can include various phenotypic and genotypic effects on the host bacteria. *Enterococcus* spp. are Gram-positive bacteria that cause infections in humans and animals. In recent decades, these bacteria have become resistant to various antimicrobials, including vancomycin. The aim of this study was to analyze genome of an enterococcal prophage.

**Methods:** In this study, *Enterococcus faecium* EntfacYE was isolated from biological samples and its genome was analyzed using next-generation sequencing method.

**Results:** Overall, 254 prophage genes were identified in the bacterial genome. The prophage included 39 housekeeping, 41 replication and regulation, 80 structural and packaging, and 48 lysis genes. Moreover, 46 genes with unknown functions were identified. All genes were annotated in DNA Data Bank of Japan.

**Conclusion:** In general, most prophage genes were linked to packaging and structure (31.5%) gene group. However, genes with unknown functions included a high proportion (18.11%), which indicated necessity of further analyses. Genomic analysis of the prophages can be effective in better understanding of their roles in development of bacterial resistance to antibiotics. Moreover, identification and study of prophages can help researchers develop genetic engineering tools and novel infection therapies.

*** Corresponding author:**
Ali Akbar Saboor Yaraghi, Ph.D., Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
**Tel:** +98 21 42933168
**Fax:** +98 21 88984913
**E-mail:** asaboor@tums.ac.ir
**Received:** 10 Nov 2021
**Accepted:** 30 Apr 2022

*Avicenna J Med Biotech 2022; 14(3): 196-205*

**Keywords:** *Enterococcus faecium*, Genomic analysis, Prophage

## Introduction

Enterococci are Gram-positive bacteria found in soil, water, plants and dairy products. Furthermore, they are naturally found in regular gastrointestinal microbiota of the vertebrates. These bacteria may be detected on the skin and mucosa as well as in the mouth and vagina [1,2]. In addition, enterococci are commonly found in wastewater or fecal contaminated water [3]. Of the enterococcal species, *Enterococcus faecalis (E. faecalis)* and *Enterococcus faecium* (*E. faecium*) majorly infect humans [4]. Nowadays, antimicrobial resistance of *E. faecium* is a serious health threat according to the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC). Enterococci increasingly show resistance to various antimicrobial drugs and disinfectants, including important antibiotic of vancomycin. This resistance prolongs the hospital stay and increases overall medical costs as well as risks of secondary infections and mortalities in patients [2,5]. Despite widespread phages in nature and their critical roles in medicine and industries, current knowledge of the biodiversity of these viruses is surprisingly small [4].

Bacteriophages generally include two infection cycles of lytic and lysogenic cycles. In lytic cycle, phages

use host replication machinery to reproduce and eventually lyse the bacterial cells. In lysogenic cycle, phages integrate their genomes to the host genomes as prophages. Prophages may be conserved in the bacterial genomes for several years. Lysogenic phages enter the lytic phase (conversion) only when they are induced by natural factors (*e.g.* high temperatures) or laboratory agents (*e.g.* mitomycin C, hydrogen peroxide and UV radiation). In fact, conversion frequency of the prophages by natural inducers is low [6]. In general, study on the phages can help development of genetic engineering tools and novel infection therapies. Therefore, the major aim of the present study was to genetically analyze Entfac.MY prophage in a clinical isolate of *E. faecium*.

## Materials and Methods

### *Bacterial strain*

*E. faecium* was isolated from the biological sample of a hospitalized patient in Tehran, Iran, using routine microbiological methods. Bacterial isolation was verified using phenotypic methods such as Gram staining and biochemical methods such as catalase, oxidase, arabinose, bile esculin, NaCl, and PYR hydrolysis tests [7]. Additionally, the bacterial strain was genetically verified via amplification of the enterococcal *tuf* gene using PCR technique and specific primers of forward Ent1: 5'-TACTGACAAACCATTCATGATG-3' and reverse Ent2: 5'-AACTTCGTCACCAACGCGAAC-3' [8]. The PCR cycling conditions were as follows: initial denaturation for 5 *min* at 95°*C*, followed by 30 cycles of denaturation at 95°*C* for 30 *s*, annealing at 54°*C* for 30 *s* and extension at 72°*C* for 45 *s*. Final extension was carried out at 72°*C* for 7 *min* (modified from Mazaheri Nezhad Fard *et al*, 2010). The PCR product was sequenced using Sanger method (Kowsar Biotech, Iran). Furthermore, antimicrobial susceptibility assessment of the isolate was carried out using disk diffusion method for vancomycin, erythromycin, clindamycin, ceftriaxone, and cefoxitin antibiotics. Characterized enterococcal strain was then used to isolate bacteriophages.

### *Complete genome sequencing*

In a previous study by Elahi *et al*, wastewater samples were used as the bacteriophage sources. After mixing 360 $\mu l$ of each wastewater sample with 160 $\mu l$ of the bacteria, suspension was poured onto a plate using two-layer agar method. Incubation was carried out at 37°*C* for 24 *hr*. After phage isolation on *E. faecium* strains, these bacterial strains were used for genome extraction [9]. A small quantity of agar (containing bacteria and phages) was dissolved in 1 *ml* of Saline Magnesium (SM) buffer. After 10 *min* of centrifugation at 4480 *g*, supernatant was separated and used for the bacterial genome extraction. Genome extraction was carried out using High Pure Viral Nucleic Acid Kit (Roche, Switzerland) as follows: 200 $\mu l$ of working solution were added to a sterile microtube and mixed with 50 $\mu l$ of proteinase K. After addition of supernatant to the microtube, solution was mixed well using vortex and incubated at 72°*C* for 10 *min*. Then, 100 $\mu l$ of binding buffer were added to the solution and mixed well. After transferring filter column into the microtube, 600 $\mu l$ of the solution were added to the filter. Solution was centrifuged at 8000 *g* for 1 *min* at 4°*C*. Filter was transferred into a new microtube and 500 $\mu l$ of inhibitor removal buffer were added to the filter. Solution was centrifuged at 8000 *g* for 1 *min* at 4°*C*. After transferring filter to a new microtube, 450 $\mu l$ of wash buffer were added to the filter. Solution was centrifuged at 8000 *g* for 1 *min* at 4°*C*. Then, filter was transferred into a fresh microtube and the last two steps were repeated. To dry the filter, it was centrifuged at 13000 *g* for 10 *s* at 4°*C*. Filter was then transferred into a new sterile microtube and 30 $\mu l$ of the elution buffer were added to the filter. After 1 *min* of setting at room temperature, centrifuge was carried out at 8000 *g* for 1 *min* at 4°*C*. The extracted bacterial genome was wholly sequenced using Illumina HiSeq Platform (Novogene, China) and analyzed using SPAdes *de novo* and reference assembly technologies. Technically, Illumina is a Next-Generation Sequencing (NGS) technology, which uses a proprietary reversible terminator-based technique that detects single bases as they are incorporated into the DNA template strands. In this study, prophage was analyzed using RAST online sequence analysis service and results were publicly deposited in DNA Data Bank of Japan (DDBJ).

## Results

Results of phenotypic and biochemical tests verified the primary characteristics of the enterococcal isolate. Gram-staining results showed that the isolate was a Gram-positive coccus. Results of the biochemical tests were reported as follows: negative catalase, negative oxidase, negative arabinose, positive bile esculin and positive PYR hydrolysis. Results of Sanger sequencing for the bacterial *tuf* gene also verified molecular characteristics of the enterococcal isolate (DDBJ accession numbers of LC580430 and LC580431). Antimicrobial susceptibility assessment results showed that *E. faecium* isolate was resistant to commonly used antibiotics, including vancomycin, erythromycin, clindamycin, ceftriaxone and cefoxitin. In general, Entfac.MY prophage included 130241 nucleotides with 35.74% A, 27.08% T, 16.02% C and 21.15% G. In total, 254 prophage genes were analyzed and grouped based on their functions (Table 1). These included 39 housekeeping genes, 41 replication and regulation genes, 80 packaging and structural genes, and 48 lysis group genes. Moreover, 46 genes with unknown functions were analyzed (Figure 1).

## Discussion

In general, bacteriophages isolated by exposing a clinical strain of *E. faecium* to wastewater samples

Table 1. Genomic analysis of the Enfac.MY prophage and its similarity to other analyses from GenBank

| CDS | Protein | Group | *bp* | DDBJ | GenBank | Cv | Id |
|---|---|---|---|---|---|---|---|
| 1 | Tape measure protein | PS | 3849 | LC644811 | WP_002350712.1 | 99 | 100 |
| 2 | YhgE/Pip domain-containing protein | H | 2706 | LC644652 | WP_002296556.1 | 99 | 100 |
| 3 | CHAP domain-containing protein | H | 2316 | LC644748 | HAP5428988.1 | 98 | 99.21 |
| 4 | DNA primase | RR | 2313 | LC644886 | WP_187171400.1 | 99 | 99.87 |
| 5 | Phage tail tape measure protein | PS | 2310 | LC644882 | WP_002303051.1 | 99 | 100 |
| 6 | Phage tail protein | H | 1911 | LC644883 | BCR31617.1 | 100 | 99.84 |
| 7 | Terminase large subunit | PS | 1728 | LC644732 | WP_212463399.1 | 99 | 99.83 |
| 8 | Phage major capsid protein | PS | 1365 | LC644734 | MBJ1280756.1 | 99 | 99.78 |
| 9 | Hypothetical protein | N/A | 1359 | LC644891 | HAP4612701.1 | 100 | 99.78 |
| 10 | Phage terminase large subunit | PS | 1350 | LC644750 | WP_201689995.1 | 99 | 99.11 |
| 11 | Terminase large subunit | PS | 1320 | LC644814 | WP_010729419.1 | 99 | 100 |
| 12 | Hypothetical protein | N/A | 1296 | LC644889 | BCT02878.1 | 99 | 100 |
| 13 | PcfJ family protein | N/A | 1260 | LC644747 | WP_033782414.1 | 99 | 99.76 |
| 14 | Phage portal protein | PS | 1230 | LC644881 | AGS74854.1 | 99 | 99.76 |
| 15 | Site-specific integrase | L | 1227 | LC644809 | HAP7047036.1 | 99 | 99.75 |
| 16 | Phage portal protein | PS | 1221 | LC644813 | WP_002350718.1 | 99 | 100 |
| 17 | Site-specific integrase | L | 1215 | LC644749 | WP_002379954.1 | 99 | 100 |
| 18 | Phage major capsid protein | PS | 1194 | LC644739 | WP_219277563.1 | 99 | 99.75 |
| 19 | Site-specific integrase | L | 1182 | LC644890 | WP_002287705.1 | 96 | 100 |
| 20 | Site-specific integrase | L | 1179 | LC644950 | WP_002288969.1 | 99 | 100 |
| 21 | Site-specific integrase | L | 1143 | LC631094 | MBK8154746.1 | 73.56 | 0.0 |
| 22 | Site-specific integrase | L | 1140 | LC644714 | WP_073470529.1 | 99 | 99.74 |
| 23 | Site-specific integrase | L | 1137 | LC644817 | WP_012197643.1 | 96 | 100 |
| 24 | Site-specific integrase | L | 1128 | LC644951 | WP_002321493.1 | 98 | 100 |
| 25 | Site-specific integrase | L | 1122 | LC644744 | HBB6776700.1 | 98 | 100 |
| 26 | Portal protein | H | 1035 | LC631113 | WP_105116343.1 | 97 | 52.66 |
| 27 | DNA polymerase | RR | 1022 | LC644897 | HAP4621056.1 | 99 | 99.71 |
| 28 | N-acetylmuramoyl-L-alanine amidase | L | 1017 | LC644737 | WP_020944989.1 | 96 | 99.69 |
| 29 | DNA polymerase | RR | 1008 | LC644820 | NSU25233.1 | 92 | 100 |
| 30 | Phage tail protein | H | 972 | LC644761 | WP_165548432.1 | 100 | 100 |
| 31 | YqaJ viral recombinase family protein | RR | 942 | LC644745 | WP_033646797.1 | 99 | 100 |
| 32 | DUF2800 domain-containing protein | N/A | 924 | LC644831 | HAP3631774.1 | 76 | 99.15 |
| 33 | Phage baseplate upper protein | PS | 906 | LC644824 | WP_048946725.1 | 99 | 100 |
| 34 | Deoxyribonuclease IV | RR | 903 | LC644819 | MBR8696505.1 | 99 | 99.67 |
| 35 | Hypothetical protein | N/A | 894 | LC644752 | HAP5478159.1 | 98 | 96.99 |
| 36 | Recombinase RecT | RR | 891 | LC644746 | WP_002301573.1 | 99 | 100 |
| 37 | Hypothetical protein | N/A | 840 | LC644899 | HBI1605865.1 | 100 | 99.64 |
| 38 | DEAD/DEAH box helicase family protein | RR | 837 | LC644756 | WP_185933038.1 | 100 | 100 |
| 39 | Small subunit of terminase | PS | 834 | LC644888 | WP_048946579.1 | 99 | 100 |
| 40 | N-acetylmuramoyl-L-alanine amidase | L | 825 | LC644836 | HBA0027879.1 | 100 | 100 |
| 41 | LysM peptidoglycan-binding domain-containing protein | H | 813 | LC644828 | HAP5669678.1 | 94 | 100 |
| 42 | YhgE/Pip domain-containing protein | H | 810 | LC644765 | WP_195947705.1 | 91 | 100 |
| 43 | Hypothetical protein | N/A | 789 | LC644770 | EHU5028596.1 | 77 | 95.51 |
| 44 | Phage antirepressor | RR | 777 | LC644806 | WP_002290310.1 | 99 | 100 |
| 45 | Helix-turn-helix domain-containing protein | RR | 750 | LC644730 | WP_130017038.1 | 99 | 100 |
| 46 | Phage major capsid protein | PS | 738 | LC644900 | WP_153883634.1 | 100 | 100 |
| 47 | Phage portal protein | PS | 732 | LC644829 | WP_195960918.1 | 99 | 97.94 |
| 48 | YhgE/Pip domain-containing protein | H | 726 | LC644841 | WP_074934422.1 | 90 | 100 |
| 49 | Phage regulatory protein | RR | 723 | LC644953 | WP_002403884.1 | 99 | 98.75 |
| 50 | Phage tail tape measure protein | PS | 723 | LC644832 | HAP3877100.1 | 100 | 99.59 |
| 51 | DUF4145 domain-containing protein | N/A | 714 | LC631111 | WP_033585566.1 | 96 | 57.33 |
| 52 | ORF6C domain-containing protein | H | 698 | LC644715 | WP_213386100.1 | 98 | 97.25 |
| 53 | Phage tail tape measure protein | PS | 696 | LC644843 | WP_010773843.1 | 100 | 100 |
| 54 | Deoxyribonuclease IV | RR | 693 | LC644912 | MBS6508197.1 | 100 | 100 |
| 55 | AAA family ATPase | H | 687 | LC631112 | WP_000704947.1 | 95 | 52.47 |
| 56 | Phage tail protein | H | 681 | LC644913 | WP_198765169.1 | 100 | 98.68 |
| 57 | YhgE/Pip domain-containing protein | H | 666 | LC644769 | EOI23661.1 | 84 | 97.34 |
| 58 | Antireceptor | H | 660 | LC631105 | APC45840.1 | 59 | 47.47 |
| 59 | Hypothetical protein | N/A | 657 | LC644755 | MBS5814550.1 | 99 | 100 |
| 60 | YhgE/Pip domain-containing protein | H | 657 | LC644846 | WP_053832241.1 | 100 | 100 |
| 61 | Hypothetical protein | N/A | 648 | LC631102 | WP_044981200.1 | 82 | 64.25 |
| 62 | Peptidoglycan recognition protein | RR | 639 | LC644844 | MBJ1172703.1 | 100 | 100 |
| 63 | Phage tail protein | H | 627 | LC644736 | MBG7868557.1 | 99 | 99.04 |
| 64 | Site-specific integrase | L | 627 | LC644847 | WP_137663438.1 | 100 | 100 |
| 65 | YhgE/Pip domain-containing protein | H | 624 | LC644840 | RDC50037.1 | 100 | 100 |
| 66 | Tail tape measure protein | PS | 615 | LC644916 | EGO8897385.1 | 100 | 99.02 |
| 67 | Lysin | L | 609 | LC644835 | EGO8925797.1 | 99 | 100 |
| 68 | YhgE/Pip domain-containing protein | H | 606 | LC644848 | WP_192380427.1 | 100 | 99.01 |
| 69 | Tape measure protein | PS | 600 | LC644918 | HAP2857181.1 | 100 | 100 |
| 70 | Recombinase family protein | RR | 594 | LC644774 | EHV0135166.1 | 100 | 100 |

Contd. Table 1. Genomic analysis of the Enfac.MY prophage and its similarity to other analyses from GenBank

| CDS | Protein | Group | *bp* | DDBJ | GenBank | Cv | Id |
|---|---|---|---|---|---|---|---|
| **71** | Tape measure protein | PS | 591 | LC644902 | HBC4147789.1 | 98 | 97.95 |
| **72** | YhgE/Pip domain-containing protein | H | 591 | LC644909 | WP_142424143.1 | 100 | 100 |
| **73** | Phage tail tape measure protein | PS | 585 | LC644778 | WP_010773806.1 | 100 | 99.49 |
| **74** | Thermonuclease family protein | RR | 585 | LC644821 | WP_002368095.1 | 99 | 100 |
| **75** | Structural protein | PS | 585 | LC644919 | HAP4242704.1 | 97 | 97.89 |
| **76** | Recombinase family protein | RR | 582 | LC644651 | WP_002294320.1 | 99 | 100 |
| **77** | DUF2815 family protein | N/A | 576 | LC631101 | WP_199363988.1 | 88 | 60.95 |
| **78** | HK97 family phage prohead protease | PS | 570 | LC644733 | WP_002303035.1 | 99 | 100 |
| **79** | Endonuclease | L | 570 | LC644911 | EGO6070115.1 | 99 | 100 |
| **80** | Deoxyribonuclease IV | RR | 570 | LC644920 | WP_048952159.1 | 100 | 100 |
| **81** | Phage tail tape measure protein | PS | 564 | LC644921 | HAP3877078.1 | 100 | 100 |
| **82** | Phage tail protein | H | 561 | LC644812 | WP_113998124.1 | 98 | 99.46 |
| **83** | Phage scaffolding protein | PS | 558 | LC631103 | WP_171943061.1 | 77 | 41.67 |
| **84** | Tyrosine-type recombinase/integrase | L | 555 | LC644818 | WP_204357435.1 | 99 | 100 |
| **85** | DEAD/DEAH box helicase | RR | 534 | LC644898 | HAP5399412.1 | 99 | 100 |
| **86** | Hypothetical protein | N/A | 525 | LC631114 | WP_123159857.1 | 92 | 69.57 |
| **87** | Phage major capsid protein | PS | 516 | LC644772 | EGO2693906.1 | 100 | 100 |
| **88** | Phage portal protein | PS | 513 | LC644833 | MRI70555.1 | 100 | 95.91 |
| **89** | HK97 family phage prohead protease | PS | 510 | LC644775 | WP_086295712.1 | 99 | 98.82 |
| **90** | Terminase large subunit | PS | 495 | LC644924 | HBI1611120.1 | 100 | 100 |
| **91** | Recombinase RecT | RR | 480 | LC644763 | WP_142974208.1 | 99 | 100 |
| **92** | Terminase large subunit | PS | 474 | LC644741 | WP_154059379.1 | 93 | 100 |
| **93** | Tyrosine-type recombinase/integrase | L | 474 | LC631100 | WP_153046652.1 | 60 | 56.84 |
| **94** | Terminase large subunit | PS | 474 | LC644915 | NSP35552.1 | 99 | 100 |
| **95** | Recombinase family protein | RR | 471 | LC644766 | WP_035006379.1 | 99 | 99.36 |
| **96** | Helix-turn-helix transcriptional regulator | RR | 471 | LC644839 | HAP4862478.1 | 99 | 100 |
| **97** | Hypothetical protein | N/A | 459 | LC644764 | WP_077143720.1 | 100 | 100 |
| **98** | Site-specific integrase | L | 459 | LC644925 | WP_194193259.1 | 100 | 100 |
| **99** | Tyrosine-type recombinase/integrase | L | 456 | LC644781 | WP_086295839.1 | 98 | 100 |
| **100** | P27 family phage terminase small subunit | PS | 453 | LC644731 | HAQ5377436.1 | 99 | 94.67 |
| **101** | Phage tail family protein | H | 453 | LC644782 | WP_142968761.1 | 100 | 100 |
| **102** | Hypothetical protein | N/A | 450 | LC644751 | WP_002298034.1 | 99 | 100 |
| **103** | Peptidoglycan endopeptidase EnpA | L | 441 | LC644856 | WP_010706628.1 | 100 | 100 |
| **104** | Phage major capsid protein | PS | 438 | LC644857 | WP_216442317.1 | 100 | 99.32 |
| **105** | Hypothetical protein | N/A | 435 | LC644849 | WP_002386667.1 | 100 | 100 |
| **106** | Site-specific integrase | L | 429 | LC644773 | EGO5860053.1 | 100 | 97.90 |
| **107** | Phage tail tape measure protein | PS | 429 | LC644784 | EHV2895864.1 | 100 | 100 |
| **108** | Siphovirus Gp157 family protein | N/A | 420 | LC631099 | WP_048784131.1 | 99 | 50.00 |
| **109** | Autolysin | L | 414 | LC644815 | GER95770.1 | 99 | 100 |
| **110** | Hypothetical protein | N/A | 414 | LC644930 | WP_195960930.1 | 86 | 98.33 |
| **111** | Phage major capsid protein | PS | 405 | LC631108 | WP_096649614.1 | 97 | 55.30 |
| **112** | Site-specific integrase | L | 402 | LC644859 | WP_165710100.1 | 100 | 98.51 |
| **113** | Phage tail protein | H | 396 | LC644787 | EHR4559110.1 | 100 | 98.48 |
| **114** | Hypothetical protein | N/A | 396 | LC644838 | WP_048943143.1 | 99 | 99.24 |
| **115** | Hypothetical protein | N/A | 396 | LC644885 | WP_002350714.1 | 99 | 100 |
| **116** | Phage tail tape measure protein | PS | 390 | LC644861 | EHV2895864.1 | 100 | 96.92 |
| **117** | Recombinase RecT | RR | 387 | LC644780 | WP_002380492.1 | 99 | 100 |
| **118** | Hypothetical protein | N/A | 387 | LC631109 | WP_102570448.1 | 40 | 50.00 |
| **119** | RloB domain-containing protein | H | 387 | LC631116 | WP_003011523.1 | 88 | 37.72 |
| **120** | Baseplate J/gp47 family protein | PS | 381 | LC644788 | EGS7987863.1 | 100 | 100 |
| **121** | HNH endonuclease | L | 381 | LC644880 | HBC4262174.1 | 99 | 100 |
| **122** | Tail tape measure protein | PS | 378 | LC644789 | HAP4230866.1 | 100 | 100 |
| **123** | Helix-turn-helix transcriptional regulator | RR | 375 | LC644743 | HAQ4376708.1 | 99 | 99.19 |
| **124** | Phage tail tape measure protein | PS | 375 | LC644757 | EGO8777387.1 | 99 | 99.19 |
| **125** | Hypothetical protein | N/A | 375 | LC644952 | WP_048943142.1 | 99 | 100 |
| **126** | Toxin | N/A | 375 | LC644826 | NSU57868.1 | 99 | 85.48 |
| **127** | HNH endonuclease | L | 366 | LC644767 | WP_086269578.1 | 99 | 99.17 |
| **128** | Phage tail tape measure protein | PS | 363 | LC644790 | MRI75296.1 | 100 | 99.17 |
| **129** | Deoxyribonuclease IV | RR | 363 | LC644791 | WP_201688942.1 | 100 | 100 |
| **130** | YhgE/Pip domain-containing protein | H | 363 | LC644792 | WP_167822678.1 | 100 | 97.52 |
| **131** | DUF1073 domain-containing protein | N/A | 363 | LC644863 | WP_010706623.1 | 100 | 100 |
| **132** | Terminase large subunit | PS | 363 | LC644908 | WP_086295714.1 | 99 | 100 |
| **133** | Phage major capsid protein | PS | 360 | LC644933 | WP_216442317.1 | 100 | 100 |
| **134** | Terminase large subunit | PS | 357 | LC644932 | WP_086295714.1 | 100 | 97.48 |
| **135** | Phage tail family protein | H | 351 | LC644934 | WP_048948486.1 | 100 | 100 |
| **136** | PBSX family phage terminase large subunit | PS | 345 | LC644868 | HBD0933441.1 | 100 | 100 |
| **137** | Phage tail protein | H | 342 | LC644768 | HBD0803692.1 | 100 | 100 |
| **138** | Siphovirus ReqiPepy6 Gp37-like family protein | N/A | 342 | LC644928 | WP_048943146.1 | 100 | 100 |
| **139** | Hypothetical protein | N/A | 339 | LC644808 | WP_010706753.1 | 100 | 100 |
| **140** | Hypothetical protein | N/A | 339 | LC644822 | WP_002303043.1 | 99 | 100 |

Contd. Table 1. Genomic analysis of the Enfac.MY prophage and its similarity to other analyses from GenBank

| CDS | Protein | Group | *bp* | DDBJ | GenBank | Cv | Id |
|---|---|---|---|---|---|---|---|
| **141** | Nucleoid-associated protein | RR | 330 | LC644870 | WP_021428623.1 | 87 | 100 |
| **142** | Toxin | N/A | 321 | LC644910 | HAP4862477.1 | 100 | 100 |
| **143** | Site-specific integrase | L | 321 | LC631118 | MBK8154746.1 | 97 | 91.35 |
| **144** | VRR-NUC domain-containing protein | H | 318 | LC644816 | WP_002350665.1 | 99 | 99.05 |
| **145** | Lysozyme family protein | L | 318 | LC644937 | WP_156233373.1 | 93 | 100 |
| **146** | MazG-like family protein | RR | 315 | LC644742 | WP_012197627.1 | 99 | 100 |
| **147** | Helix-turn-helix domain-containing protein | RR | 312 | LC631107 | WP_118138944.1 | 99 | 52.38 |
| **148** | DNA methyltransferase | RR | 309 | LC644794 | WP_195960921.1 | 100 | 99.03 |
| **149** | Hypothetical protein | N/A | 309 | LC644866 | WP_002369984.1 | 100 | 100 |
| **150** | Phage portal protein | PS | 309 | LC644896 | WP_201689987.1 | 100 | 99.03 |
| **151** | Phage tail tape measure protein | PS | 306 | LC644871 | WP_153841946.1 | 100 | 98.04 |
| **152** | Hypothetical protein | N/A | 303 | LC644807 | HAQ9461605.1 | 99 | 99 |
| **153** | Phage tail tape measure protein | PS | 300 | LC644867 | EGO8777387.1 | 80 | 100 |
| **154** | Hypothetical protein | N/A | 300 | LC644895 | EGO5085241.1 | 99 | 96.97 |
| **155** | Hypothetical protein | N/A | 300 | LC644938 | WP_010748256.1 | 85 | 100 |
| **156** | Tyrosine-type recombinase/integrase | L | 297 | LC644939 | WP_216442824.1 | 100 | 100 |
| **157** | Terminase large subunit | PS | 297 | LC631119 | MBF1717610.1 | 100 | 77.78 |
| **158** | Hypothetical protein | N/A | 294 | LC644907 | WP_096709598.1 | 98 | 78.35 |
| **159** | HK97 family phage prohead protease | PS | 293 | LC644837 | WP_137192767.1 | 99 | 100 |
| **160** | Nucleoid-associated protein | RR | 291 | LC644777 | WP_035114676.1 | 100 | 100 |
| **161** | Site-specific integrase | L | 291 | LC644796 | EGO9032817.1 | 100 | 100 |
| **162** | Peptidoglycan endopeptidase EnpA | L | 291 | LC644922 | WP_002400013.1 | 88 | 100 |
| **163** | Site-specific integrase | L | 285 | LC631096 | WP_128080223.1 | 86 | 71.95 |
| **164** | AAA family ATPase | H | 285 | LC644873 | WP_010828176.1 | 98 | 100 |
| **165** | Phage gp6-like head-tail connector protein | PS | 282 | LC644735 | WP_002303039.1 | 98 | 100 |
| **166** | YhgE/Pip domain-containing protein | H | 282 | LC644834 | WP_142966911.1 | 98 | 100 |
| **167** | Phage gp6-like head-tail connector protein | PS | 282 | LC644851 | WP_161843549.1 | 98 | 95.70 |
| **168** | Tyrosine-type recombinase/integrase | L | 282 | LC644865 | WP_201705518.1 | 100 | 97.87 |
| **169** | Phage terminase large subunit | PS | 282 | LC644940 | EGO6069701.1 | 100 | 100 |
| **170** | Site-specific integrase | L | 279 | LC644771 | WP_192201846.1 | 100 | 100 |
| **171** | Terminase large subunit | PS | 276 | LC644799 | EHU8855151.1 | 100 | 100 |
| **172** | Minor capsid protein | PS | 276 | LC644850 | WP_142425073.1 | 98 | 100 |
| **173** | Phage portal protein | PS | 273 | LC631097 | APD22584.1 | 100 | 54.95 |
| **174** | Structural protein | PS | 267 | LC644759 | EGO2667974.1 | 98 | 100 |
| **175** | Phage tail tape measure protein | PS | 264 | LC644800 | HAP4877189.1 | 90 | 98.75 |
| **176** | YhgE/Pip domain-containing protein | H | 264 | LC644923 | HAP5737370.1 | 100 | 100 |
| **177** | Phage tail tape measure protein | PS | 261 | LC644874 | EHB6454364.1 | 100 | 89.66 |
| **178** | Phage terminase large subunit | PS | 261 | LC644936 | HAP5353043.1 | 98 | 97.67 |
| **179** | Phage portal protein | PS | 258 | LC644802 | WP_194943338.1 | 100 | 100 |
| **180** | Phage major capsid protein | PS | 258 | LC644935 | WP_002417392.1 | 98 | 100 |
| **181** | Hemolysin XhlA family protein | L | 255 | LC644758 | WP_048948902.1 | 98 | 92.86 |
| **182** | Hypothetical protein | N/A | 255 | LC631098 | WP_018165267.1 | 100 | 44.94 |
| **183** | MazG-like family protein | RR | 255 | LC644901 | WP_010717138.1 | 100 | 100 |
| **184** | Site-specific integrase | L | 252 | LC644853 | HAP4918633.1 | 100 | 100 |
| **185** | Hypothetical protein | N/A | 249 | LC644893 | WP_010748256.1 | 100 | 96.39 |
| **186** | Phage tail protein | H | 249 | LC644825 | MRJ04801.1 | 91 | 82.89 |
| **187** | Hypothetical protein | N/A | 249 | LC644803 | HAP2781243.1 | 98 | 100 |
| **188** | AAA family ATPase | H | 246 | LC644943 | WP_127341875.1 | 75 | 100 |
| **189** | Phage tail tape measure protein | PS | 246 | LC644944 | WP_118215595.1 | 100 | 95.12 |
| **190** | Hemolysin XhlA family protein | L | 243 | LC644810 | WP_002332427.1 | 98 | 100 |
| **191** | Phage tail tape measure protein | PS | 243 | LC644876 | WP_085406750.1 | 100 | 100 |
| **192** | Hypothetical protein | N/A | 243 | LC644929 | MRJ04805.1 | 98 | 100 |
| **193** | Helix-turn-helix transcriptional regulator | RR | 240 | LC644779 | HAP3299532.1 | 100 | 100 |
| **194** | Phage tail tape measure protein | PS | 237 | LC644947 | MBR8697128.1 | 68 | 100 |
| **195** | Phage tail tape measure protein | PS | 237 | LC644804 | MRI62301.1 | 100 | 98.73 |
| **196** | Hemolysin XhlA family protein | L | 234 | LC644753 | WP_214144888.1 | 98 | 96.10 |
| **197** | Collagen-like protein | PS | 234 | LC644776 | WP_207656718.1 | 98 | 97.40 |
| **198** | Phage tail protein | H | 234 | LC644786 | EHB6443012.1 | 98 | 100 |
| **199** | Peptidoglycan endopeptidase EnpA | L | 234 | LC644872 | WP_138218550.1 | 100 | 100 |
| **200** | Phage terminase large subunit | PS | 234 | LC644877 | HBD0837071.1 | 100 | 100 |
| **201** | Terminase large subunit | PS | 234 | LC631117 | MBF0843203.1 | 98 | 59.74 |
| **202** | Hypothetical protein | N/A | 231 | LC631115 | WP_125852315.1 | 100 | 41.56 |
| **203** | Terminase small subunit | PS | 231 | LC644926 | WP_070363956.1 | 98 | 100 |
| **204** | Site-specific integrase | L | 228 | LC631110 | WP_044761187.1 | 98 | 60.00 |
| **205** | DUF2213 domain-containing protein | N/A | 228 | LC644879 | WP_115252339.1 | 100 | 100 |
| **206** | Site-specific integrase | L | 228 | LC644949 | EGT2099792.1 | 100 | 100 |
| **207** | YhgE/Pip domain-containing protein | H | 225 | LC644805 | RDC49008.1 | 100 | 100 |
| **208** | Phage holin | RR | 225 | LC631106 | WP_155198660.1 | 69 | 98.08 |
| **209** | DUF3383 family protein | N/A | 225 | LC644878 | WP_002377529.1 | 100 | 100 |
| **210** | IS607 family transposase | RR | 225 | LC644887 | WP_104845943.1 | 90 | 54.17 |

Contd. Table 1. Genomic analysis of the Enfac.MY prophage and its similarity to other analyses from GenBank

| CDS | Protein | Group | *bp* | DDBJ | GenBank | Cv | Id |
|---|---|---|---|---|---|---|---|
| **211** | Phage tail protein | H | 225 | LC644931 | WP_198765584.1 | 100 | 98.67 |
| **212** | Terminase large subunit | PS | 222 | LC644855 | HBI1611155.1 | 100 | 100 |
| **213** | Phage portal protein | PS | 216 | LC644842 | EOI11687.1 | 100 | 98.61 |
| **214** | Phage holin | RR | 216 | LC644903 | WP_033626583.1 | 98 | 100 |
| **215** | Holin | RR | 207 | LC644754 | WP_002369080.1 | 97 | 100 |
| **216** | Phage major tail protein | PS | 207 | LC644858 | EFT89608.1 | 100 | 100 |
| **217** | Baseplate J/gp47 family protein | PS | 207 | LC644864 | HAP3865876.1 | 100 | 98.55 |
| **218** | Phage holin | RR | 198 | LC644738 | WP_002333030.1 | 98 | 98.46 |
| **219** | Cupin domain-containing protein | H | 192 | LC644845 | WP_168932514.1 | 98 | 98.41 |
| **220** | HNH endonuclease | L | 192 | LC644927 | EHU8855159.1 | 100 | 100 |
| **221** | AAA family ATPase | H | 189 | LC644860 | WP_127341875.1 | 100 | 98.41 |
| **222** | Phage major capsid protein | PS | 186 | LC644795 | NSR35780.1 | 95 | 96.61 |
| **223** | Hypothetical protein | N/A | 186 | LC644894 | APS16315.1 | 98 | 100 |
| **224** | 1,4-β-N-acetylmuramidase | L | 186 | LC644942 | HAP4707744.1 | 100 | 100 |
| **225** | Ig domain-containing protein | H | 183 | LC644905 | WP_010773805.1 | 98 | 95.00 |
| **226** | Structural protein | PS | 174 | LC644760 | MBO6404911.1 | 100 | 98.28 |
| **227** | Hypothetical protein | N/A | 168 | LC631104 | WP_050285512.1 | 83 | 53.19 |
| **228** | Site-specific integrase | L | 165 | LC644948 | EGO8320093.1 | 98 | 100 |
| **229** | Phage holin | RR | 162 | LC644827 | WP_002379919.1 | 100 | 100 |
| **230** | Ribbon-helix-helix domain-containing protein | RR | 159 | LC644740 | WP_010729417.1 | 98 | 100 |
| **231** | DUF2800 domain-containing protein | N/A | 159 | LC644892 | WP_198765184.1 | 98 | 100 |
| **232** | Deoxyribonuclease IV | RR | 153 | LC644801 | WP_144330580.1 | 98 | 100 |
| **233** | Phage tail tape measure protein | PS | 147 | LC644906 | HAP2938854.1 | 100 | 100 |
| **234** | Terminase large subunit | PS | 144 | LC644946 | HBI1611197.1 | 97 | 100 |
| **235** | DUF4065 domain-containing protein | N/A | 141 | LC644793 | HAP4215483.1 | 97 | 100 |
| **236** | XkdX family protein | L | 138 | LC644862 | WP_071974452.1 | 97 | 100 |
| **237** | XkdX family protein | L | 138 | LC644884 | WP_002332428.1 | 97 | 100 |
| **238** | Phage tail tape measure protein | PS | 138 | LC644904 | MBO6395993.1 | 97 | 100 |
| **239** | N-acetylmuramoyl-L-alanine amidase | L | 135 | LC644798 | WP_192400940.1 | 91 | 90.24 |
| **240** | Nucleoid-associated protein | RR | 132 | LC644783 | WP_114543333.1 | 95 | 95.24 |
| **241** | Hypothetical protein | N/A | 126 | LC644762 | WP_086295717.1 | 100 | 97.62 |
| **242** | Ig domain-containing protein | H | 120 | LC644785 | WP_048943144.1 | 100 | 100 |
| **243** | HNH endonuclease | L | 114 | LC644914 | NSR35697.1 | 97 | 100 |
| **244** | DUF2184 domain-containing protein | N/A | 108 | LC644854 | WP_002386753.1 | 100 | 100 |
| **245** | Phage major capsid protein | PS | 102 | LC644917 | WP_086295711.1 | 100 | 100 |
| **246** | DUF2184 domain-containing protein | N/A | 96 | LC644797 | WP_010706626.1 | 96 | 100 |
| **247** | Tyrosine-type recombinase/integrase | L | 84 | LC644852 | WP_153841893.1 | 96 | 100 |
| **248** | Helix-turn-helix domain-containing protein | RR | 81 | LC631095 | WP_048782931.1 | 100 | 85.19 |
| **249** | BppU family phage baseplate upper protein | PS | 78 | LC644830 | HAP6528242.1 | 100 | 100 |
| **250** | Phage head-tail connector protein | H | 78 | LC644869 | WP_214144901.1 | 100 | 100 |
| **251** | Phage portal protein | PS | 78 | LC644945 | WP_114679410.1 | 100 | 96.15 |
| **252** | XkdX family protein | L | 75 | LC644823 | WP_074394623.1 | 100 | 100 |
| **253** | Phage major capsid protein | PS | 75 | LC644941 | HAP2938884.1 | 100 | 100 |
| **254** | Holin | RR | 69 | LC644875 | HBC4422148.1 | 95 | 100 |

CDS: Coding Sequence, *bp*: Base Pair, DDBJ: DNA Data Bank of Japan, GenBank: GenBank Protein ID for this study, Cv: Coverage, Id: Identity, N/A: Not Applicable or Announced, L: Lysis, PS: Packaging and structural, H: Housekeeping, RR: Replication and regulation.

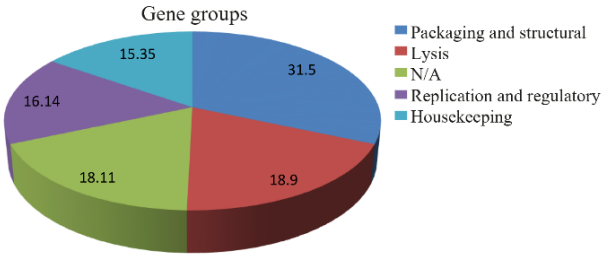


Figure 1. Major gene groups of Enfac.MY prophage.

included lysogenic phages. After complete genomic sequencing of Entfac.MY prophage, 254 genes were functionally analyzed. The housekeeping group of genes included YhgE/Pip domain-containing protein (12 copies), CHAP domain-containing protein, phage tail protein (ten copies), RloB domain-containing protein, antireceptor, LysM peptidoglycan-binding domain-containing protein, ORF6C domain-containing protein, VRR-NUC domain-containing protein, Ig domain-containing protein (two copies), portal protein (ten copies), AAA family ATPase (four copies), phage tail family protein (two copies) and phage head-tail connector protein. Portal protein forms a channel for two-way passes of the viral DNA. The EFRM31 phage genome also included this gene [10]. The functional mechanisms of AAA family ATPase, including cell cycle regulation, proteolysis and protein breakdown and intracellular transport, were investigated in 2002 [11].

The phage tail family protein encodes tail components. The antireceptor gene is involved in identifying and binding phage to its bacterial hosts. Function of this gene was previously reported by Vegge *et al* [12] and Duplessis and Moineau [13]. The phage head-tail connector protein gene is involved in the assembly of virions by connecting their heads and tails.

The replication and regulation group of genes included IS607 family transposase, ribbon-helix-helix domain-containing protein, recombinase RecT (three copies), phage regulatory protein, thermonuclease family protein, helix-turn-helix transcriptional regulator (three copies), MazG-like family protein (two copies), DNA polymerase (two copies), DNA primase, deoxyribonuclease IV (five copies), recombinase family protein (four copies), DEAD/DEAH box helicase family protein, phage antirepressor, helix-turn-helix domain-containing protein (three copies), peptidoglycan recognition protein, nucleoid-associated protein (three copies), DNA methyltransferase and phage holin (six copies). In fact, DNA polymerase gene produces enzymes that act in pairs to produce two identical DNA strands from an original DNA molecule and are essential for DNA replication. Krzywkowski *et al* studied this gene in 2018 [14]. Naturally, DNA primase is an enzyme involved in DNA replication and is linked to RNA polymerases. Structure and mechanism of this gene have been studied by O'Brien *et al* in 2018 [15] and Lee *et al* in 2010 [16]. When cells are in repairing process of the nucleotide cleavage pathway, deoxyribonuclease IV is active in aqueous or aporin-apyrimidinic sites. The gene function has been explained by Enriquez *et al* [17]. Recombinase family protein produces enzymes involved in repairing DNA damages. Voziyanova *et al* investigated function and evolution of this gene [18]. The DEAD/DEAD box helicase family protein produces enzymes that bind and hydrolyze NTP to double-stranded (ds) RNA molecules or regenerate RNA protein complexes. The phage antirepressor gene prevents suppressor proteins from binding to their operators. This mechanism has recently been investigated by Silpe *et al* in 2020 [19]. The helix-turn-helix domain-containing protein can bind to DNA molecules. The peptidoglycan recognition protein gene detects peptidoglycan in the bacterial cell walls; as described by Skerry *et al* [20] and Jiang *et al* [21]. Nucleoid-associated proteins naturally include abundant polypeptides with Low-Molecular Weights (LMW) that bind DNA molecules and change their shapes and abilities to participate in processes such as transcription. Sometimes, these proteins can bind RNA molecules and post-transcriptionally affect the gene expression of cells; as described by Stojkova *et al* in 2019 [22]. DNA methyltransferases are a family of enzymes involved in production and maintenance of CpG methylation in the genome; as described by Furuta *et al* in 2014 [23] and Stoddard *et al* in 2019 [24]. Holins include diverse groups of small proteins from dsDNA phages that destruct the host cell walls in the virus lytic cycle. Functional mechanisms of the holins were previously described by Bardina *et al* [25], Stamereilers *et al* [26] and Jacobs *et al* [27].

The packaging and structure group of genes included tail tape measure protein (24 copies), P27 family phage terminase small subunit, baseplate J/gp47 family protein (two copies), PBSX family phage, phage gp6-like head-tail connector protein (two copies), collagen-like protein, phage major tail protein, BppU family phage baseplate upper protein, HK97 family phage prohead protease (three copies), phage scaffolding protein, terminase large subunit (17 copies), phage major capsid protein (11 copies), minor capsid protein, phage baseplate upper protein, structural protein (three copies) and small subunit of terminase (two copies). The terminase large subunit gene is involved in viral DNA transfer and packaging termination. The phage major capsid protein gene encodes capsid proteins and was previously reported in the phage genome of EFRM31 in 2010 [10]. Stamereilers *et al* reported this gene as well [26]. Another gene involved in encoding capsid proteins is the minor capsid protein gene. In 2015, Pawlowski *et al* studied functions of this gene in P23-77 phage [28]. The phage baseplate upper protein gene was also reported by Li *et al* in ϕ11 genome [29]. Structural protein gene plays an important role in shaping structure of the virus. In 2015, McNulty *et al* studied terminase small subunit gene, which is responsible for binding of the encoded protein to several identifying elements at the beginning of viral packaging [30]. In addition, Roy *et al* studied this gene in 2011 [31].

The lysis group of genes included hemolysin XhlA family protein (three copies), 1,4-β-N-acetylmuramidase, XkdX family protein (three copies), site-specific integrase (21 copies), N-acetylmuramoyl-L-alanine amidase (three copies), lysin, endonuclease, tyrosine-type recombinase/integrase (six copies), peptidoglycan endopeptidase EnpA (three copies), autolysin, HNH endonuclease (four copies) and lysozyme family protein. Site-specific integrase gene is responsible for rearranging DNA fragments; as previously identified by Petersen *et al* in the genome of TPW22 phage [32]. This gene was also identified in TP901-1 phage genome [33]. The N-acetylmuramoyl-L-alanine amidase gene produces an enzyme that catalyzes a chemical reaction and cleaves the link between N-acetylmuramoyl and L-amino acid residues in the cell-wall glycopeptides. Bierbaum *et al* have described functional mechanisms of this gene [34]. Lysines are hydrolytic phage enzymes produced to separate the host cell walls in lytic cycles. In 2015, LeBlanc *et al* reported this gene in their phage genome [35]. The endonuclease gene produces enzymes that break down DNA molecules at specific locations. The tyrosine-type recombinase/integrase gene is involved in DNA binding and recombination. In a similar study in 2021, Malecki *et al* reported peptidoglycan endopeptidase EnpA gene in an enterococcal prophage

[36]. The autolysin gene is an enzyme that breaks down the components of peptidoglycans in cells and separates daughter cells after cell division. Ju *et al* in 2012 [37] and Kohler *et al* in 2014 studied this gene [38]. The HNH endonuclease gene plays various roles in the phage life cycle as the major component of the phage DNA packaging machinery. In 2017, Zhang *et al* studied the mechanism of this gene in GVE2 phage genome [39]. Lysozyme is a glycoside hydrolase that catalyzes the hydrolysis of 1,4-β bonds between N-acetylmuramic and N-acetyl-D-glucosamine residues of the peptidoglycan. Previously, Irwin studied this gene in 2014 [40].

The unknown function group of genes included PcfJ family protein, DUF2800 domain-containing protein, DUF4145 domain-containing protein, DUF2815 family protein, siphovirus Gp157 family protein, toxin (two copies), DUF1073 domain-containing protein, siphovirus ReqiPepy6 Gp37-like family protein, DUF2213 domain-containing protein, DUF3383 family protein, DUF4065 domain-containing protein, DUF2184 domain-containing protein (two copies) and hypothetical protein (31 copies). Hypothetical proteins were previously addressed by Mazaheri *et al* in EFRM31 [10] and Tang *et al* in ϕNJ2 [41]. Based on the complete genomic analysis of an enterococcal phage by Mazaheri Nezhad Fard *et al* in 2010, genes similar to the genes of this study were reported, including hypothetical proteins, portal proteins, major capsid proteins, and holins [10]. In another study by Tan *et al* in 2007, genes similar to the genes of the current study were reported as well, including terminase large subunit and portal protein genes [42]. In a study by O'Flaherty *et al* on staphylococcal phage genomes (2004), 63 hypothetical protein genes were identified [43] while 31 hypothetical protein genes were identified in the present study. In the present study, AAA family ATPase, holin, and major capsid protein genes were successfully characterized.

## Conclusion

In general, bacteriophages are present in all environments with alive bacteria, especially in wastewaters. Bacteriophages can insert their genomes into the bacterial genomes to become prophages. Prophages can regulate bacterial populations by changing the bacterial gene expression rates and are involved in bacterial resistance by transferring antibiotic resistance genes to their bacterial hosts. Up-to-date, pathogenic bacteria have developed multiple resistance to available antibiotics, creating serious problems with costly infection treatments and unwanted mortalities. Bacteriophages can be used as novel solutions for these problems. Therefore, genomic analysis of the prophages can improve better understanding of their effects on bacteria. Furthermore, study of prophages can help researchers develop novel genetic engineering tools and effective medical therapies.

## Acknowledgement

The authors thank staff within the Microbiology Laboratory, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences.

## Conflict of Interest

The authors declare no conflict of interest.

## Ethics Statement

The current study was carried out based on the methods approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC. 1397.139).

## References

1. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology 2009;155(Pt 6):1749-57.
2. Garcia-Solache M, Rice LB. The Enterococcus: a model of adaptability to its environment Clin Microbiol Rev 2019;32(2):e00058-18.
3. Zaheer R, Cook SR, Barbieri R, Goji N, Cameron A, Petkau A, et al. Surveillance of Enterococcus spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. Sci Rep 2020;10(1): 3937.
4. Topka-Bielecka G, Bloch S, Nejman-Falenczyk B, Grabski M, Jurczak-Kurek A, Gorniak M, et al. Characterization of the bacteriophage vB_EfaS-271 infecting Enterococcus faecalis. Int J Mol Sci 2020;21(17):6345.
5. Revtovich AV, Tjahjono E, Singh KV, Hanson BM, Murray BE, Kirienko NV. Development and characterization of high-throughput Caenorhabditis elegans–Enterococcus faecium infection model. Front Cell Infect Microbiol 2021;11:667327.
6. Abdulrahman Ashy R, Agusti S. Low host abundance and high temperature determine switching from lytic to lysogenic cycles in planktonic microbial communities in a tropical sea (Red Sea). Viruses 2020;12(7):761.
7. Stepien-Pysniak D, Hauschild T, Dec M, Marek A, Urban-Chmiel R, Kosikowska U. Phenotypic and genotypic characterization of Enterococcus spp. from yolk sac infections in broiler chicks with a focus on virulence factors. Poult Sci 2021;100(4):100985.
8. Nasiri M, Hanifian S. Enterococcus faecalis and Enterococcus faecium in pasteurized milk: Prevalence, genotyping, and characterization of virulence traits. LWT 2022; 153:112452.
9. Elahi Y, Nowroozi J, Fard RMN. Isolation and characterization of bacteriophages from wastewater sources on Enterococcus spp. isolated from clinical samples. Iran J Microbiol 2021;13(5):671-7.
10. Fard RM, Barton MD, Arthur JL, Heuzenroeder MW. Whole-genome sequencing and gene mapping of a newly isolated lytic enterococcal bacteriophage EFRM31. Arch Virol 2010;155(11):1887-91.
11. Elderkin S, Jones S, Schumacher J, Studholme D, Buck

M. Mechanism of action of the Escherichia coli phage shock protein PspA in repression of the AAA family transcription factor PspF. J Mol Biol 2002;320(1):23-37.

12. Vegge CS, Vogensen FK, Mc Grath S, Neve H, van Sinderen D, Brøndsted L. Identification of the lower baseplate protein as the antireceptor of the temperate lactococcal bacteriophages TP901-1 and Tuc2009. J Bacteriol 2006;188(1):55-63.
13. Duplessis M, Moineau S. Identification of a genetic determinant responsible for host specificity in Streptococcus thermophilus bacteriophages. Mol Microbiol 2001;41 (2):325-36.
14. Krzywkowski T, Kuhnemund M, Wu D, Nilsson M. Limited reverse transcriptase activity of phi29 DNA polymerase. Nucleic Acids Res 2018;46(7):3625-32.
15. O'Brien E, Holt ME, Salay LE, Chazin WJ, Barton JK. Substrate binding regulates redox signaling in human DNA primase. J Am Chem Soc 2018;140(49):17153-62.
16. Lee SJ, Zhu B, Hamdan SM, Richardson CC. Mechanism of sequence-specific template binding by the DNA primase of bacteriophage T7. Nucleic Acids Res 2010; 38(13):4372-83.
17. Enriquez A, Chu IW, Mellis C, Lin WY. Nebulised deoxyribonuclease for viral bronchiolitis in children younger than 24 months. Cochrane Database Syst Rev 2012; (11): 11(11):CD008395.
18. Voziyanova E, Li F, Shah R, Voziyanov Y. Genome targeting by hybrid Flp-TAL recombinases. Sci Rep 2020;10(1):17479.
19. Silpe JE, Bridges AA, Huang X, Coronado DR, Duddy OP, Bassler BL. Separating functions of the phage-encoded quorum-sensing-activated antirepressor Qtip. Cell Host Microbe 2020;27(4):629-41.
20. Skerry C, Goldman WE, Carbonetti NH. Peptidoglycan recognition protein 4 suppresses early inflammatory responses to Bordetella pertussis and contributes to sphingosine-1-phosphate receptor agonist-mediated disease attenuation. Infect Immun 2019;87(2):e00601-18.
21. Jiang L, Liu W, Guo H, Dang Y, Cheng T, Yang W, et al. Distinct functions of Bombyx mori peptidoglycan recognition protein 2 in immune responses to bacteria and viruses. Front Immunol 2019;10:776.
22. Stojkova P, Spidlova P, Stulik J. Nucleoid-associated protein HU: A lilliputian in gene regulation of bacterial virulence. Front Cell Infect Microbiol 2019;9:159.
23. Furuta Y, Namba-Fukuyo H, Shibata TF, Nishiyama T, Shigenobu S, Suzuki Y, et al. Methylome diversification through changes in DNA methyltransferase sequence specificity. PLoS Genet 2014;10(4):e1004272.
24. Stoddard CI, Feng S, Campbell MG, Liu W, Wang H, Zhong X, et al. A nucleosome bridging mechanism for activation of a maintenance DNA methyltransferase. Mol Cell 2019;73(1):73-83.
25. Bardina C, Colom J, Spricigo DA, Otero J, Ssnchez-Osuna M, Cortes P, et al. Genomics of three new bacteriophages useful in the biocontrol of Salmonella. Front Microbiol 2016;7:545.
26. Stamereilers C, Fajardo CP, Walker JK, Mendez KN, Castro-Nallar E, Grose JH, et al. Genomic analysis of 48 Paenibacillus larvae bacteriophages. Viruses 2018;10 (7):377.
27. Jacobs-Sera D, Abad LA, Alvey RM, Anders KR, Aull HG, Bhalla SS, et al. Genomic diversity of bacteriophages infecting Microbacterium spp. PLoS One 2020;15 (6):e0234636.
28. Pawlowski A, Moilanen AM, Rissanen IA, Määttä JA, Hytonen VP, Ihalainen JA, et al. The minor capsid protein VP11 of thermophilic bacteriophage P23-77 facilitates virus assembly by using lipid-protein interactions. J Virol 2015;89(15):7593-603.
29. Li X, Koc C, Kuhner P, Stierhof YD, Krismer B, Enright MC, et al. An essential role for the baseplate protein Gp45 in phage adsorption to Staphylococcus aureus. Sci Rep 2016;6:26455.
30. McNulty R, Lokareddy RK, Roy A, Yang Y, Lander GC, Heck AJ, et al. Architecture of the complex formed by large and small terminase subunits from bacteriophage P22. J Mol Biol 2015;427(20):3285-99.
31. Roy A, Bhardwaj A, Cingolani G. Crystallization of the nonameric small terminase subunit of bacteriophage P22. Acta Crystallogr Sect F Struct Biol Cryst Commun 2011; 67(Pt 1):104-10.
32. Petersen A, Josephsen J, Johnsen MG. TPW22, a lactococcal temperate phage with a site-specific integrase closely related to Streptococcus thermophilus phage integrases. J Bacteriol 1999;181(22):7034-42.
33. Stoll SM, Ginsburg DS, Calos MP. Phage TP901-1 site-specific integrase functions in human cells. J Bacteriol 2002;184(13):3657-63.
34. Bierbaum G, Sahl HG. Autolytic system of Staphylococcus simulans 22: influence of cationic peptides on activity of N-acetylmuramoyl-L-alanine amidase. J Bacteriol 1987;169(12):5452-8.
35. LeBlanc L, Nezami S, Yost D, Tsourkas P, Amy PS. Isolation and characterization of a novel phage lysin active against Paenibacillus larvae, a honeybee pathogen. Bacteriophage 2015;5(4):e1080787.
36. Malecki PH, Mitkowski P, Jagielska E, Trochimiak K, Mesnage S, Sabala I. Structural characterization of EnpA D, L-endopeptidase from Enterococcus faecalis prophage provides insights into substrate specificity of M23 peptidases. Int J Mol Sci 2021;22(13):7136.
37. Ju CX, Gu HW, Lu CP. Characterization and functional analysis of atl, a novel gene encoding autolysin in Streptococcus suis. J Bacteriol 2012;194(6):1464-73.
38. Kohler TP, Gisch N, Binsker U, Schlag M, Darm K, Volker U, et al. Repeating structures of the major staphylococcal autolysin are essential for the interaction with human thrombospondin 1 and vitronectin. J Biol Chem 2014;289(7):4070-82.
39. Zhang L, Xu D, Huang Y, Zhu X, Rui M, Wan T, et al. Structural and functional characterization of deep-sea thermophilic bacteriophage GVE2 HNH endonuclease. Sci Rep 2017;7:42542.

40. Irwin DM. Evolution of the vertebrate goose-type lysozyme gene family. BMC Evol Biol 2014;14:188.

41. Tang F, Bossers A, Harders F, Lu C, Smith H. Complete genome sequence of the Streptococcus suis temperate bacteriophage ϕNJ2. Genome Announc 2013;1(1):e 00008-12.

42. Tan Y, Zhang K, Rao X, Jin X, Huang J, Zhu J et al. Whole genome sequencing of a novel temperate bacteriophage of P. aeruginosa: evidence of tRNA gene mediating integration of the phage genome into the host bacterial chromosome. Cell Microbiol 2007;9(2):479-91.

43. O'Flaherty S, Coffey A, Edwards R, Meaney W, Fitzgerald GF, Ross RP. Genome of staphylococcal phage K: a new lineage of Myoviridae infecting gram-positive bacteria with a low G+C content. J Bacteriol 2004;186(9): 2862-71.