Genome Analysis of the *Enterococcus faecium* Entfac.YE Prophage

Yara Elahi 1, Ramin Mazaheri Nezhad Fard 2, Arash Seifi 3, Saeideh Mahfouzi 4, and Ali Akbar Saboor Yaraghi 2*

1. Department of Genetics, Faculty of Life Sciences, Islamic Azad University Tehran North Branch, Tehran, Iran
2. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Infectious Diseases, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Abstract

**Background:** Bacteriophages are viruses that infect bacteria. Bacteriophages are widely distributed in various environments. The prevalence of bacteriophages in water sources, especially wastewaters, is naturally high. These viruses affect evolution of most bacterial species. Bacteriophages are able to integrate their genomes into the chromosomes of their hosts as prophages and hence transfer resistance genes to the bacterial genomes. Enterococci are commensal bacteria that show high resistance to common antibiotics. For example, prevalence of vancomycin-resistant enterococci has increased within the last decades.

**Methods:** Enterococcal isolates were isolated from clinical samples and morphological, phenotypical, biochemical, and molecular methods were used to identify and confirm their identity. Bacteriophages extracted from water sources were then applied to isolated *Enterococcus faecium* (*E. faecium*). In the next step, the bacterial genome was completely sequenced and the existing prophage genome in the bacterial genome was analyzed.

**Results:** In this study, *E. faecium* EntfacYE was isolated from a clinical sample. The EntfacYE genome was analyzed and 88 prophage genes were identified. The prophage content included four housekeeping genes, 29 genes in the group of genes related to replication and regulation, 25 genes in the group of genes related to lysis, 18 genes in the group of genes related to structure and packaging, and four genes belonging to the group of genes associated with lysis. Moreover, 26 genes were identified with unknown functions.

**Conclusion:** In conclusion, genome analysis of prophages can lead to a better understanding of their roles in the rapid evolution of bacteria.

*Avicenna J Med Biotech 2022; 14(1): 54-60*

**Keywords:** Anti-bacterial agents, Bacteriophages, *Enterococcus faecium*, Genome analysis, Prophages

Introduction

Two important species of commensal enterococci, *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*), are one of the leading causes of medical conditions, causing various hospital infections such as endocarditis and sepsis 1. Due to the increased antibiotic resistance properties of these bacteria, their infections are often difficult to treat 2. For example, the prevalence of Vancomycin-Resistant Enterococci (VRE) has increased inducing complexities in hospitalized patients in the last two decades 3. Since one of the most important health concerns is to find novel solutions to fight these multidrug-resistant bacterial infections, the use of novel strategies seems urgently necessary. In this regard, the use of phages can hopefully be promising 4. Bacteriophages (Phages) are prokaryotic viruses detected in various environments within their bacterial hosts or in large numbers of free virions 5. Currently, these bacterial viruses are under the spotlight as appropriate substitutes for the available antibiotics. Phages can effectively infect and kill antibiotic-resistant bacteria regardless of the resistance patterns of these bacteria 6. In addition, phages have several advantages over other antimicrobial agents with no serious or irreversible side effects 7. Therefore, the characterization of novel phages and understanding their evolutionary ecology can greatly help scientists improve the process of chemical antimicrobial replacement. Novel genome analyzing methods, including next-generation sequenc-
ing methods, and established genome databases have relatively facilitated the development of phage knowledge. Therefore, the purpose of the current study was to analyze the Entfac.YE prophage.

Materials and Methods

**Bacterial strain**

*E. faecium* was isolated from clinical samples in a university teaching hospital in Tehran, Iran, using routine culture methods as well as phenotypic and genotypic methods. The isolate was verified using morphological, biochemical, and molecular techniques such as catalase test, arabinose fermentation, salt tolerance, optochin susceptibility, CAMP, and PYR tests. Enterococcal *tuf* gene was amplified using PCR (Polymerase chain reaction) and partially sequenced using the Sanger sequencing platform (Kawar Biotech, Iran). The bacterial strain was also used for the isolation of enteroococcal phages.

**Whole-genome sequencing**

After isolation of phages on *E. faecium*, the bacterial strains challenged by the phages were used to extract their genome. Briefly, a small volume of two-layer agar-containing bacteria with phages was removed and dissolved in Saline Magnesium (SM) buffer. This was centrifuged at 4480 g for 10 min. After centrifugation, the supernatant was filtered through 0.45-μm syringe filters and mixed with DNase 1 and RNase A. The mixture was stored at 37°C for 30 min. Then, the bacterial genome was extracted using the precipitation method with ethanol and propanol. The extracted bacterial genome was completely sequenced using the Illumina Hiseq platform (Novogene, China) (Table 1). SPAdes algorithm was used in the de novo technology of genome assembling. Furthermore, the reference assembly method was used for the raw data. The prophage was analyzed using Regulatory Sequence Analysis Tools (RAST) (https://rast.nmpdr.org/) and then DDBJ Nucleotide Sequence Submission System (NSSS) (https://www.ddbj.nig.ac.jp).

Results

Sanger sequencing results of the bacterial *tuf* gene verified the initial characteristics of the isolated bacteria (DDBJ accession numbers: LC580430 and LC580431). The bacterial genome was completely sequenced and information obtained through Novaseq 6000 platform (Illumina, USA) are demonstrated in table 1. The Entfac.YE prophage included 69,990 nucleotides, consisting of 31.13% A, 31.60% T, 18.94% C, and 18.29% G nucleotides. In total, 88 prophage genes were analyzed for their functions (Table 2). The prophage content included four housekeeping genes, 29 genes in the group of replication and regulation, 25 genes in the group of structure and packaging, and four genes in the group of lysis. The functions of other 26 genes were unknown (Figure 1). Bacteriophages isolated in a relative phase of the current study included three lytic members. Two tailed phages included isometric shapes (*Siphoviridae* and *Myoviridae*) and the other one was filamentous (*Inoviridae*).

Discussion

In general, phages isolated from the clinical strain of *E. faecium* using sources of wastewaters were lysogenic phages with the suggestion of further prophages in the bacterial genome. Therefore, whole-genome sequencing of the enterococcal strain was carried out. The selected prophage of *E. faecium* was named Entfac.YE, including 88 genes. Of the identified genes, four genes were in the group of housekeeping genes, 29 genes were in the group of replication and regulation genes, 25 genes were in the group of packaging and structure genes, four genes were in the group of lysis genes, and 26 genes were in no groups due to their unknown functions. In this study, 14 *E. faecium* were identified in 25 enterococcal isolates. In 2019, Karna et al identified four *E. faecium* in five enterococcal isolates. Although *E. faecium* is a large-intestine symbiotic bacterium of humans and animals,
| CDS                      | Protein                                    | Gg     | bp     | DDBJ       | NCBI       | Cv. | Id. |
|--------------------------|--------------------------------------------|--------|--------|------------|------------|-----|-----|
| Tape measure protein     | PS                                         | 3848   | LC606177 | WP_002350712 | 99 | 100 |
| Phage tail tape measure protein | PS                                         | 3320   | LC606192 | WP_010776531 | 99 | 100 |
| Yhgl/E/Pp domain-containing protein | H                                         | 2705   | LC603693 | WP_002296556 | 99 | 100 |
| Phage/plasmid primase P4 family domain-containing protein | RR                                         | 2312   | LC606170 | WP_012197635 | 99 | 100 |
| Phage tail tape measure protein | PS                                         | 2309   | LC602689 | WP_002303051 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 2003   | LC602243 | HAQ643042   | 95 | 99.8 |
| Terminase large subunit  | PS                                         | 1727   | LC603684 | WP_002303033 | 99 | 100 |
| Terminase large subunit  | PS                                         | 1694   | LC606188 | WP_002286533 | 99 | 100 |
| Terminase large subunit  | PS                                         | 1391   | LC606195 | MUA1326607  | 94 | 100 |
| Phage major capsid protein | PS                                         | 1364   | LC602693 | WP_002303037 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 1319   | LC606183 | EOG13061   | 99 | 100 |
| Hypothetical protein     | N/A                                        | 1295   | LC606163 | HAQ9917245  | 99 | 100 |
| Phage portal protein     | PS                                         | 1229   | LC603683 | WP_002303034 | 99 | 99.7 |
| Site-specific integrase  | RR                                         | 1226   | LC603694 | HAP7047036  | 99 | 99.75 |
| Phage portal protein     | PS                                         | 1220   | LC606182 | WP_002350718 | 99 | 100 |
| Phage major capsid protein | PS                                         | 1193   | LC606180 | WP_002350716 | 99 | 100 |
| Virulence-associated protein E | N/A                                       | 1190   | LC606216 | EOH59192   | 91 | 95.6 |
| Site-specific integrase  | RR                                         | 1178   | LC606176 | WP_002288969 | 99 | 100 |
| Phage portal protein     | PS                                         | 1178   | LC606189 | WP_002317387 | 99 | 100 |
| Phage portal protein     | PS                                         | 1175   | LC606197 | WP_002296486 | 99 | 100 |
| Tyrosine-type recombinase/integrase | RR                                         | 1142   | LC606186 | WP_010729348 | 99 | 100 |
| Site-specific integrase  | RR                                         | 1142   | LC606193 | WP_002287705 | 99 | 100 |
| Site-specific integrase  | RR                                         | 1139   | LC603692 | WP_002286587 | 99 | 100 |
| Site-specific integrase, phage integrase family | RR                                         | 1136   | LC606165 | EIX5302   | 96 | 100 |
| Site-specific integrase  | RR                                         | 1127   | LC606187 | WP_002288357 | 99 | 100 |
| Site-specific integrase  | RR                                         | 1121   | LC606158 | WP_033647077 | 99 | 100 |
| Site-specific integrase  | RR                                         | 1034   | LC606210 | HAQ1208098  | 95 | 99.7 |
| YqA viral recombinase family protein | RR                                         | 941    | LC606160 | WP_033646797 | 99 | 100 |
| Recombinase RecT         | RR                                         | 890    | LC606161 | WP_002301573 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 875    | LC606191 | WP_129984688 | 99 | 99.6 |
| BppU family phage baseplate upper protein | PS                                         | 854    | LC606214 | WP_192183965 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 800    | LC606162 | WP_048946585 | 99 | 100 |
| Tyrosine-type recombinase/integrase | RR                                         | 785    | LC606215 | WP_074399919 | 94 | 98.7 |
| Phage antirepressor      | RR                                         | 776    | LC603691 | WP_002290310 | 99 | 100 |
| Helix-turn-helix domain-containing protein | RR                                         | 749    | LC603689 | WP_130017038 | 99 | 100 |
| Phage antirepressor      | RR                                         | 746    | LC606211 | WP_047641521 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 713    | LC603687 | AGS74848   | 99 | 100 |
| Hypothetical protein     | N/A                                        | 698    | LC603690 | KH16430   | 99 | 100 |
| AAA family ATPase        | H                                          | 686    | LC606169 | WP_012197638 | 99 | 100 |
| Clp protease ClpP        | PS                                         | 686    | LC606190 | WP_002286527 | 99 | 100 |
| Clp protease ClpP        | PS                                         | 677    | LC606181 | WP_002350717 | 99 | 100 |
| Site-specific integrase  | RR                                         | 638    | LC606202 | WP_148905844 | 97 | 100 |
| Tail protein             | PS                                         | 626    | LC602690 | WP_002303047 | 99 | 99.5 |
| Hypothetical protein     | N/A                                        | 599    | LC606224 | PW70842   | 36 | 55.5 |
| Recombinase family protein | RR                                         | 581    | LC600466 | WP_002294320 | 99 | 100 |
| HK97 family phage prohead protease | PS                                         | 569    | LC602694 | WP_002349220 | 99 | 100 |
| Phi13 family phage major tail protein | PS                                         | 560    | LC606178 | WP_002332434 | 99 | 100 |
| Hypothetical protein     | N/A                                        | 554    | LC606174 | ELB35873  | 98 | 100 |
| Hypothetical protein     | N/A                                        | 518    | LC606164 | HAQ9543613  | 97 | 100 |
| Hypothetical protein     | N/A                                        | 485    | LC606204 | KKS73631   | 95 | 99.3 |
| Hypothetical protein     | N/A                                        | 479    | LC606223 | WP_168472540 | 66 | 96.2 |
| Tyrosine-type recombinase/integrase | RR                                         | 473    | LC606173 | WP_002290382 | 99 | 100 |
| Phage terminase-like protein, large subunit | PS                                         | 473    | LC606185 | AGS76423   | 99 | 100 |
| Phage terminase small subunit P27 family | PS                                         | 473    | LC606194 | WP_002296488 | 99 | 100 |
it is listed by the World Health Organization as a global priority for multidrug-resistant pathogens. In the present study, *E. faecium* isolates were resistant to vancomycin, erythromycin, clindamycin, ceftriaxone, and cefoxitin. Based on the published studies, 70% of isolated *E. faecium* strains from Tehran hospitals were resistant to vancomycin and erythromycin. In a similar study by Rahbar et al. on clinical samples in Tehran, antibiotic resistance patterns of enterococcal isolates included 79% resistance to erythromycin and 51% to vancomycin. In this study, the prophage was identified in the *E. faecium* genome as reported in other studies.

In the present study, two recombinase family protein genes were reported. This protein catalyzes sensitive DNA exchange reactions between short target sequences (30–40 nucleotides). In 2010, Lopes et al. reported the gene in the phage genome. Other genes identified in this study were hypothetical protein genes. The exact functions of these genes are unknown. In total, 25 hypothetical protein genes were reported in Entfac.YE prophage. Similar genes were identified in the EFRM31 phage genome by Mazaheri et al. Another gene was the tail tape measure protein, which determines the tail length and facilitates DNA transfer to the cell cytoplasm during cell infection. In the current study, two copies of this gene were identified. This gene was reported in 2016 in phage TP901-1 as well. In the present study, two copies of the tail protein gene were reported. This gene encodes proteins linked to the

### Table 2. An overview of the genes from Entfac.YE prophage

| CDS   | Protein                                      | Gg | bp | DDBJ         | NCBI          | Cv. | Id. |
|-------|----------------------------------------------|----|----|---------------|---------------|-----|-----|
| 55    | P27 family phage terminase small subunit     | PS | 452| LC603685     | HAQ3737436    | 99  | 99.3|
| 56    | Hypothetical protein                         | N/A| 449| LC606213     | WP_002341785  | 99  | 100 |
| 57    | Hypothetical protein                         | N/A| 425| LC606221     | MBA1326600    | 69  | 100 |
| 58    | ImmA/IrI family metallo-endopeptidase        | RR | 422| LC606166     | WP_00230678   | 99  | 100 |
| 59    | Hypothetical protein                         | N/A| 419| LC606168     | EGPS556401    | 99  | 100 |
| 60    | Helix-turn-helix transcriptional regulator   | RR | 410| LC606159     | WP_00297387   | 99  | 100 |
| 61    | Hypothetical protein                         | N/A| 395| LC606179     | WP_00235071   | 99  | 100 |
| 62    | HNH endonuclease                             | L  | 380| LC603866     | WP_00234435   | 99  | 100 |
| 63    | Helix-turn-helix transcriptional regulator   | RR | 374| LC606167     | WP_00231592   | 99  | 100 |
| 64    | Hypothetical protein                         | N/A| 338| LC602691     | WP_00230343   | 99  | 100 |
| 65    | VRR-NUC domain-containing protein            | H  | 317| LC606171     | WP_00235066   | 99  | 99.0|
| 66    | MazG-like family protein                     | RR | 314| LC606172     | WP_01219762   | 99  | 100 |
| 67    | Hypothetical protein                         | N/A| 308| LC606218     | WP_18075398   | 99  | 100 |
| 68    | MazG-like family protein                     | RR | 302| LC603688     | WP_00230317   | 99  | 100 |
| 69    | Hypothetical protein                         | N/A| 299| LC601696     | KKJ73220      | 99  | 100 |
| 70    | Phage gp6-like head-tail connector protein   | PS | 281| LC602692     | WP_00230303   | 99  | 100 |
| 71    | Glucosaminidase domain-containing protein    | H  | 278| LC606175     | WP_19697536   | 88  | 100 |
| 72    | Phage gp6-like head-tail connector protein   | PS | 268| LC601698     | HAQ420964     | 79  | 87.8|
| 73    | Hemolysin XhA family protein                 | L  | 242| LC606200     | WP_00233242   | 98  | 100 |
| 74    | Hemolysin XhA family protein                 | L  | 242| LC606207     | WP_00299182   | 98  | 98.7|
| 75    | Hypothetical protein                         | N/A| 239| LC606205     | WP_00296498   | 98  | 100 |
| 76    | Hypothetical protein                         | N/A| 230| LC606203     | RB538180      | 98  | 100 |
| 77    | Hypothetical protein                         | N/A| 224| LC606157     | WP_00230487   | 98  | 98.6|
| 78    | Phage holin                                  | RR | 224| LC606222     | WP_00238663   | 98  | 100 |
| 79    | Tyrosine-type recombinase/integrase          | RR | 221| LC606220     | WP_148779155  | 83  | 95.1|
| 80    | Phage holin                                  | RR | 197| LC606199     | WP_00230212   | 98  | 100 |
| 81    | Phage holin                                  | RR | 197| LC606206     | WP_00587682   | 98  | 100 |
| 82    | Ribbon-helix-helix domain-containing protein| RR | 158| LC606184     | WP_01072947   | 98  | 100 |
| 83    | XkdX family protein                          | L  | 137| LC606201     | WP_00233242   | 97  | 100 |
| 84    | Hypothetical protein                         | N/A| 128| LC606208     | HAP976180     | 97  | 100 |
| 85    | Hypothetical protein                         | N/A| 125| LC606219     | PZM702929     | 96  | 93.1|
| 86    | Phage gp5-like head-tail connector protein   | PS | 119| LC606209     | WP_00296484   | 97  | 100 |
| 87    | Hypothetical protein                         | N/A| 95 | LC606212     | KKJ66783      | 81  | 96.3|
| 88    | Phage baseplate upper protein                | PS | 89 | LC606217     | HAQ6822550    | 90  | 100 |

CDS: Coding sequence; Gg: Gene group; bp: base pair; DDBJ: DNA Data Bank of Japan; NCBI: NCBI reference sequence; Cv: Coverage; Id: Identity; N/A: Not applicable; L: Lysis; RR: Replication and regulation; PS: Packaging and structural; H: Housekeeping.
The present study, have a variety of roles such as cell cycle regulation, proteolysis and protein breakdown, and intracellular transport. The mechanisms of action of these genes were also investigated before. The function of the primase P4 family domain-containing gene includes nucleotide binding. The molecular function of the VRR-NUC gene includes domain-containing hydrolyase activity that affects ester bonds. Based on previous studies, the exact function of this gene in phages is unknown. The tyrosine-type recombinase/integrase gene is involved in DNA binding and recombination, of which four copies were identified in the present study.

A domain version of the glucosaminidase domain-containing protein was reported in this study, which is responsible for the structural determination of peptides and glycans during vegetative growth. A copy of the phi13 family phage major tail gene was identified in Entfac.YE prophage and its molecular function was also investigated before. Two copies of ClpP protease gene were detected in the present study. This gene includes ATP-dependent peptidase and serine endopeptidase activities. Naturally, the ribbon-helix-helix domain-containing protein gene is involved in transcriptional regulation, a copy of which was identified in this study. Three copies of holin genes were identified in Entfac.YE prophage. Holins are a diverse group of small proteins produced by dsDNA phages to stimulate and control the destruction of the host cell walls at the end of the lytic cycle. Holins have been suggested as the clock proteins of phage infections. Two copies of the hemolysin XhIA family protein gene were identified in this study. XhIA is a cell surface-associated hemolysin that breaks down two common types of insect immune cells (Glanulocytes and plasmocytes) as well as rabbit and horse red blood cells. A copy of XkDX family protein gene reported in this study is detected in phage genomes close to choline and endolysin genes. The BppU family phage baseplate upper gene detected in the present study has been identified in the S. aureus phage genome. The virulence-associated protein E gene has also been identified in Streptococcus spp.

In the current study, a copy of the baseplate upper protein gene was reported. Based on the complete genomic analysis of an enteroococcal phage by Mazaheri et al., genes similar to those of the present study were found, including genes of hypothetical protein, HNH endonuclease, tape measure protein, terminase small subunit, portal protein, prohead protease, major capsid protein, and major tail protein. In a study by Tan et al. in 2007, genes similar to those of this study were reported, including terminase large subunit and portal protein genes. In the present study, 25 genes of hypothetical proteins were analyzed. In a study by O’Flaherty et al. on the phage genome, 63 hypothetical protein genes were identified. Furthermore, they identified one AAA family ATPase, one endonuclease and one P27 family gene as well as
one holin and one major capsid protein gene 31.

**Conclusion**

Bacteriophages are naturally prevalent in the environment, especially in wastewaters. Nowadays, clinical bacterial isolates generally show resistance to antibiotics, which has created many problems for health professionals and patients. Prophages can be considered as mobile genetic elements in the transfer of antibiotic resistance genes to genomes of their host bacteria. Furthermore, genome analysis of prophages can help researchers better understand their roles in bacterial ecology and evolution.

**Acknowledgement**

The authors thank all the staff within the Microbiology Laboratory, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran for their help.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**

1. Monstein H-I, Quednau M, Samuelsson A, Ahrens S, Isaksen B, Jonasson J. Division of the genus Enterococcus into species groups using PCR-based molecular typing methods. Microbiology 1998;144:1171-9.

2. Tsakris A, Woodford N, Pournaras S, Kaufmann M, Doubovas J. Apparent increased prevalence of high-level aminoglycoside-resistant Enterococcus durans resulting from false identification by a semiautomatic software system. J Clin Microbiol 1998;36:1419-21.

3. Tünger A, Aydemir S, Uluer S, Cilli F. In vitro activity of linezolid & quinupristin/dalfopristin against Gram-positive cocci. Indian J Med Res 2004;120(6):546-52.

4. Alavidze Z, Aminov R, Betts A, Bardiau M, Bretaudeau L, Caplin J, et al. Silk route to the acceptance and re-implementation of bacteriophage therapy: new applications for old resources. Biotechnol. J 2016;11(5):595-600.

5. Sime-Ngando T. Environmental bacteriophages: viruses of microbes in aquatic ecosystems. Front Microbiol 2014;24:5:355.

6. Nobrega FL, Costa AR, Kluskens LD, Azeredo J. Revisiting phage therapy: new applications for old resources. Trends Microbiol 2015;23(4):185-91.

7. Pirnay JP, Blasdel BG, Bretaudeau L, Buckling A, Chandhivili N, Clark JR, et al. Quality and safety requirements for sustainable phage therapy products. Pharm Res 2015;32(7):2173-9.

8. Al-Rammahi SA, Hussein JM, Kadhem EJ, Al-Hadad AS. Bacteriological study of Enterococcus bacteria isolated from different diseases in the female cows. Biomed Pharmacol J 2020;13(2):989-98.

9. Karna A, Baral R, Khanal B. Characterization of clinical isolates of enterococci with special reference to glycopeptide susceptibility at a tertiary care center of Eastern Nepal. Int J Microbiol 2019:2019.

10. Chatterjee A, Willett JL, Nguyen UT, Monogue B, Palmer KL, Dunny GM, et al. Parallel genomics uncover novel enteroococcal-bacteriophage interactions. MBio 2020;11(2):e03120-19.

11. Arbabi L, Boustanshenas M, Adabi M, Fatihazadeh S, Rasouli Kooohi S, Afshar M, et al. Isolation and antibiotic susceptibility pattern among vancomycin-resistant enterococci isolated from clinical samples of different parts of Rasoul-E-Akram Hospital. J Ardabil University of Medical Sciences (JAUoMS) 2016;15(4):404-13.

12. Samadi H, Pirhajati Mahabadi R, Pournajaf A, Omidi S, Moghimyan S, Alyasian N. An investigation of the vanA and vanB genes in Enterococcus faecalis and Enterococcus faecium strains isolated from the hospitalized patients in Shariati Hospital and evaluation of their antibiotic susceptibility. Qom Univ Med Sci J 2015;9(3):32-8.

13. Bhardwaj SB, Mehta M, Sood S, Sharma J. Isolation of a novel phage and targeting biofilms of drug-resistant oral enterococci. J Glob Infect Dis 2020;12(1):11.

14. Lopes A, Amarir-Bouhram J, Faure G, Petit MA, Gueiros R. Detection of novel recombinases in bacteriophage genomes unveils Rad52, Rad51 and Gp2: 5 remote homologs. Nucleic Acids Res 2010;38(12):3952-62.

15. Fard RM, Barton MD, Arthur JL, Heuzenroeder MW. Whole-genome sequencing and gene mapping of a newly isolated lytic enteroococcal bacteriophage EFRM31. Arch Virol 2010;155(11):1887-91.

16. Mahony J, Alqarni M, Stockdale S, Spinelli S, Feyereisen M, Cambillau C, et al. Functional and structural dissection of the tape measure protein of lactococcal phage TP901-1. Sci Rep 2016;6(1):36667.

17. Chang Y, Lee JH, Shin H, Heu S, Ryu S. Characterization and complete genome sequence analysis of Staphylococcus aureus bacteriophage SA12. Virus Genes 2013;47(2):389-93.

18. Duda RL, Oh B, Hendrix RW. Functional domains of the HK97 capsid maturation protease and the mechanisms of protein encapsidation. J Mol Biol 2013;425(15):2765-81.

19. Kahlon AK, Darokar MP, Sharma A. Comparative Analysis of Common and Unique Targets in Drug-Resistant Strains of Staphylococcus aureus. In: Shukla P, ed. Frontier Discoveries and Innovations in Interdisciplinary Microbiology. New Delhi: Springer; 2016. p.193-205.

20. Goncalves AM, De Sanctis D, McSweeney SM. Structural and functional insights into DR2231 protein, the MazG-like nucleoside triphosphate pyrophosphohydrolase from Deinococcus radiodurans. J Biol Chem 2011;286(35):30691-705.

21. Suskkind MM, Botstein D. Mechanism of action of Salmonella phage P22 antirepressor. J Mol Biol 1975;98(2):413-24.

22. Stoll SM, Ginsburg DS, Calos MP. Phage TP901-1 site-specific integrase functions in human cells. J Bacteriol 2002;184(13):3657-63.
23. Sun Z, Deng A, Hu T, Wu J, Sun Q, Bai H, et al. A high-efficiency recombineering system with PCR-based ssDNA in Bacillus subtilis mediated by the native phage recombinase GP35. Appl Microbiol Biotechnol 2015;99 (12):5151-62.

24. 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.

25. Pennell S, Déclais AC, Li J, Haire LF, Berg W, Saldanha JW, et al. FAN1 activity on asymmetric repair intermediates is mediated by an atypical monomeric virus-type replication-repair nuclease domain. Cell Rep 2014;8 (1):84-93.

26. Fokine A, Rossmann MG. Molecular architecture of tailed double-stranded DNA phages. Bacteriophage 2014;4 (2):e28281.

27. Laachouch JE, Desmet L, Geuskens V, Grimaud R, Toussaint A. Bacteriophage Mu repressor as a target for the Escherichia coli ATP-dependent Clp Protease. EMBO J 1996;15(2):437-44.

28. Cowles KN, Goodrich-Blair H. Expression and activity of a Xenorhabdus nematophilus haemolysin required for full virulence towards Manduca sexta insects. Cell Microbiol 2005;7(2):209-19.

29. Ji X, Sun Y, Liu J, Zhu L, Guo X, Lang X, et al. A novel virulence-associated protein, vapE, in Streptococcus suis serotype 2. Mol Med Rep 2016;13(3):2871-7.

30. 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.

31. 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.