Original Article

Killing Efficiency and Full Genome Sequencing of Bacteriophage BAG1 Targeting Endodontic Clinical Strain of *Enterococcus faecalis*

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Abstract

Objectives: Bacterial biofilms can increase the survival of bacteria through providing resistance to conventional disinfectants as well as antibiotics, thus biofilm considered a serious risk to human health with hug loss. Consequently, novel approaches to deal with bacterial biofilms are required. Viruses that may eradicate bacteria are known as bacteriophages, they are also known as "bacteria eaters." Due to their activity on bacteria, bacteriophages are distinct from other organisms and do not pose a threat to human. Consequently, they are regarded as secure substitutes for antibiotics in the management of bacterial infections. **Aim of the Study:** In this study, we report the full genome sequencing of bacteriophage, namely BAG1 that targeting an endodontic clinical strain of *Enterococcus faecalis* (K3). Furthermore, we evaluate it killing efficiency to eliminate *E. faecalis* in both planktonic media and dentine slabs. **Materials and Methods:** We used the previously isolated endodontic *E. faecalis* clinical strain, namely K3 and enterococcus bacteriophage BAG1 in this study by spotting on the double layer agar technique. DNA extracted proceeded with NORGEN phage DNA isolation kit. Phage full genome sequencing was performed using Illumina nova Seq 600, 150 bp PE. In addition, the killing capacity of BAG1 phage against *E. faecalis* K3 was measured by infecting 5×10^6 *E. faecalis* with BAG1 phage at multiplicity of infection of 0.1. Furthermore, the biofilm fraction and the number of *E. faecalis* colony-forming unit in planktonic media were measured after infecting 24 dentine slabs with K3 bacteria and BAG1 phage. SYTO® 9 green LIVE/DEAD® BacLight Bacterial Viability Kits were used to visualize K3 biofilm on dentine slabs using fluorescent microscope. **Results:** Full Genome Organization of BAG1 phage was circular composed of 68 open reading frame with GC percentage of 34.56%. An efficient killer reflecting by completely eliminating K3 strain within 180 min in killing test. In addition, the result revealed that BAG1 highly significant to eliminate K3 bacteria in both planktonic broth and biofilm fraction obtained from dentine slabs infection with the endodontic K3 clinical strain. **Conclusion:** Our results support that anti *E. faecalis* bacteriophage BAG1 significantly eliminate biofilm of *E. faecalis* K3 on dentine slabs with a lytic properties due to the absence of lysogenic genes which make it a suitable substitute to adjunctive anti *E. faecalis* therapy.

Keywords: Bacteriophage, biofilm, dentin slabs, *Enterococcus faecalis*, endodontic infection, full genome sequencing

Introduction

Antimicrobial agents, known as antibiotics, have the power to either stop or eliminate germs, they are frequently employed in both medical and nonmedical applications to treat bacterial infections in both people and animals.[1]

During the previous 10 years, there has been an increase in interest in how bacterial resistance is spreading in the environment, and this interest has been sparked by the rapidly rising number of germs that are resistant to antibiotics, which is causing great concern among the medical and scientific communities, includes resistance to last-resort medications and a new generation of antibiotics.[2]

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The enterococci, such as *Enterococcus faecalis*, have become common hospital-acquired pathogens and are becoming more and more virulent and antibiotic resistant.[3] Last-resort medications such as vancomycin and daptomycin are ineffective against antibiotic resistance in *E. faecalis*.[4] In order to control enterococcal populations, new therapeutic approaches are required, such as bacteriophage therapy.[5]

Endodontic infections are polymicrobial including a mixture of Gram-positive, Gram-negative, strict anaerobic bacteria, and facultative anaerobes.[6] *E. faecalis* is among the bacteria that are still present in the root canals.[7] The biomechanical methods may have allowed *E. faecalis* to persist, or it may have entered the canal through the coronal leakage of the root filling. One of the most prevalent bacteria found in root canals after endodontic treatment is *E. faecalis* according to the bacterial cultures and molecular analyses.[8,9]

Phage is a green alternate toward antibiotics extracted from the natural environment. Phages have a variety of qualities, including being environmentally friendly and secure.[10] Phages are now again being studied for their potential as bactericidal agents due to the urgent need for innovative therapies in the fight against multidrug-resistant bacteria.[5]

To make better use of bacteriophages' special capabilities, which are essential for eradicating bacterial biofilm and ensuring biological safety in all spheres of life, it is essential to comprehend how bacterial biofilm is formed and how they infect bacteria.[11]

In this study, we report the full genome sequencing of bacteriophage, namely BAG1 that targeting an endodontic clinical strain of *E. faecalis* (K3). Furthermore, we evaluate it killing efficiency to eliminate *E. faecalis* in both planktonic media and dentine slabs.

Materials and Methods

Bacteria and phage used in this study

In this study, we used the previously isolated endodontic clinical strain, namely K3 Figure 1 and enterococcus bacteriophage BAG1. BAG1 was isolated from cow dung targeting K3 (endodontic clinical strain) by spotting on the double layer agar technique and it showed clear plaques of 3 mm in diameter in the double layer agar Figure 2. In addition, K3 was register at NCBI 16Sr RNA gene partial sequence under accession number OM250466.

Phage DNA extraction

5 µg/ml DNase and RNase were added to remove contaminated DNA and RNA from the phage stock. The mixture was incubated at 37°C for 30 min. A final concentration of 100 µg/ml of proteinase K was added to the mixture to degrade the enzymes and incubated at 50°C for 45 min. 600 µl of isopropanol was added to further ensure the deactivation of any remaining RNase activity. DNA extracted proceeded with NORGEN phage DNA isolation kit (NORGEN BIOTEK CROP, Canada) and according to manufacture instructions and the resulted phage DNA was stored at −20°C for further use. The isolated DNA was sent to (Humanizing Genomic Macrogen, Korea) for full genome sequencing of BAG1 bacteriophage. Phage DNA sequencing steps were performed as following.

Phage DNA sequencing

Phage full genome sequencing was performed using Illumina nova Seq 600, 150 bp PE as following:

1. Sample preparation: BAG1 phage DNA was proceeded with the library construction. After quality control, passing
2. Library construction: The sequenced library is prepared by random fragmentation of the DNA or cDNA sample, followed by 5' and 3' adapter ligation. Alternatively, "tagmentation" combines the fragmentation and ligation reactions into a single step that greatly increases the efficiency of the library preparation process. Adapter-ligated fragments are then polymerase chain reaction amplified and gel purified
3. Sequencing for cluster generation: The library was loaded onto a flow cell where fragments were caught on a lawn of surface-bound oligos complementary to the library adapters. Then, each piece is magnified to create distinct, clonal clusters through bridge amplification (or exclusion amplification). As soon as cluster generation was finished, sequencing of the templates was available.

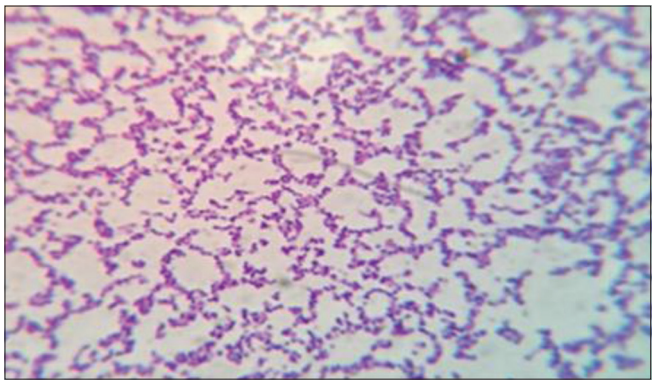

Figure 1: *Enterococcus faecalis* under microscope

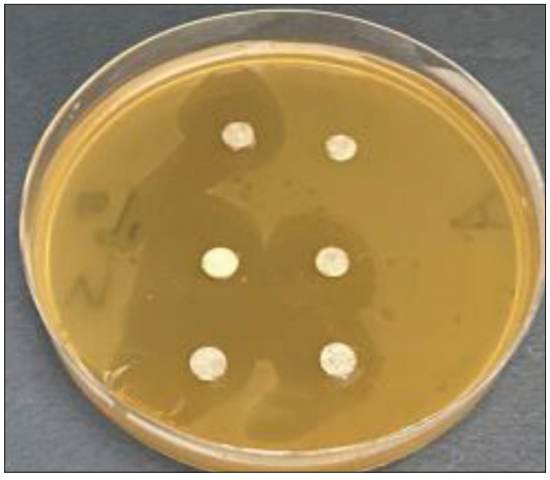

Figure 2: Spotting on double layer agar

In order to detect single bases as they were incorporated into DNA template strands, Illumina sequencing by synthesis technology uses a proprietary, terminator based, reversible technique. Natural competition significantly lowers raw error rates compared to other technologies and minimizes the incorporation bias because all four reversible, terminator bound dNTPs are present during each sequencing cycle. The end result is extremely accurate base-by-base sequencing that almost completely eradicates sequence-context-specific mistakes, including inside repeating sequence areas and homopolymers

4. Raw data after sequencing: For the analysis, recognized bases and the expected quality of each base were transformed into raw data. Next, the cBCL/BCL file was transformed to FASTQ file using bcl2fastq from Illumina set.

Analysis

1. Filtered read *de novo* assembly: *De novo* assemblage was done. A top k-mer is designated using several stats by assemblage outcomes (numeral of contigs, totality of contigs, N50, and etcetra.) and a preeminent accumulated sequence set was determined
2. Assembly validation: The accumulated genome was validated using self-mapping plan. Filtered reads were affiliated against accumulated genome and their insert size was assessed for validation.

The data were received in FASTA format. Annotation was performed using phaster program (WWW.PHASTER.CA). Phaster program is a free online annotation tool used with phage sequence in FASTA format.

Killing curve measurement

Exponentially growing cultures of 5×10^6 *E. faecalis* (K3) strain were inoculated with the BAG1 phage at multiplicity of infection of 0.1. Incubated the mixtures at 37°C and monitored the changes in the bacterial culture over time by measuring OD600 nm every 30 min with spectrophotometer (Optima spectrophotometer, Japan). The similar method was done with the *E. faecalis* K3 only as the negative control of infection.

Biofilm assay on dentin slabs

Samples Preparation

Samples were arranged in according with (Rosen, E., *et al*., (2016)[12] with modifications. In brief, twenty freshly extracted single rooted and fully developed intact human teeth were stored in 0.05% sodium hypochlorite solution.

The crowns of the chosen teeth were removed to obtain the multiple root specimens of 13 mm length, and the apical 3 mm of the root end was resected without a bevel using Zakaria high speed bur (Maillefer, Ballaigues, Switzerland).

The root canal lumen was then widen to a min of 1 mm using low speed burs (Gates Glidden Drills, Dentsply Maillefer, Tulsa, OK, USA). The roots were implanted in self cure acrylic repair material (UNIFAST Trad, GC America). To prepare the dentin slabs, the roots were cut perpendicular to its long axis under water cooling by using a diamond cutting wheel attach to prosthetic microengine handpiece with speed (40,000 RPM). To ensure perpendicular cutting of dentine slabs, the handpiece of microengine was attached to the vertical arm of the paratherm dental surveyor (REF 097-270-00, Dentarum GmbH and Co. KG, Germany) with zip ties. For cooling, normal saline was used with IV set which was also attached with the headpiece to vertical arm of the surveyor. Two dentin slabs of 1 mm thickness each were obtained from each root. The specimens were then placed in little bowls and sterilized by autoclaved for 15 min at 121°C and 15 psi.[13]

Biofilm formation on dentin slabs

5×10^6 K3 bacterial strain was inoculated into dentine slabs Eppendorf tubes at 37°C for 24 h. After 24 h, the broth was removed completely and replace with 1 ml fresh brain heart infusion broth. 25 µl of BAG1 phage was inoculated into 5 Eppendorf tubes while the 5 control tubes were inoculated with 25 µl SM buffer only. After 4 h, the broth was removed from all samples and analyses for colony-forming units (CFUs)/1 ml. Furthermore, the dentine slabs were washed with phosphate-buffered saline (PBS) and tested for biofilm fraction. The biofilm fraction was obtained by three washes of the dentin disks with phosphate buffer to the final volume of the suspension solution. To determine the number of live cells, cells were serially diluted in PBS, plated on brain infusion plates, and CFUs were counted after incubation at 37°C overnight. This test was repeated in duplicate.

LIVE/DEAD® BacLight Bacterial Viability Kits

The LIVE/DEAD BacLight Bacterial Viability Kits (Invitrogen by thermo fisher scientific, USA and Canada) was used to visualize *E. faecalis* biofilm on dentin slabs, it utilize of SYTO® 9 green, These stains differ both in their spectral features and in their ability to enter healthy bacterial cells, fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide.

An equal volumes of components A and B were mixed in an Eppendorf tube, then mix thoroughly. Each one ml of bacterial suspension was mixed with 3 µl of the dye mixture. Then, it mixed carefully and incubated for 15 min at the room temperature in the dark. Five µl of the stained bacterial suspension has been trapped between a slide and an 18 mm square coverslip. Ultimately, it observed in a fluorescence microscope: Olympus, Japan.

Statistical analysis

The statistical analysis was performed using GraphPad Prism version 6 for Windows (GraphPad Software, La Jolla, CA, USA).

Results

Full Genome Organization of BAG1

The sequenced results were taken in a basal formats, namely: FASTA (include the sequence of nucleotide in one or several nodes). One node was received with size of 41.7 kbp.

The nucleotide sequence of the FASTA node of BAG1 was 97% and 99% resemble phage SHEF10 (OL799256.1) and SHEF2 (NC_042021), respectively, when blasted at NCBI BLASTN (WWW. BLASTN. COM) Figure 3.

The FASTA sequence was furthermore analyzed use the online web server: PHASTER phage searching tool (WWW. PHASTER. CA). The results of the analysis revealed that BAG1 genome harbor 68 ORF with a GC percentage of around 34.56% as illustrated in Tables 1 and 2 and Figure 4.

Killing curve

Infection cycles of phage were performed at multiplicity of infection of 1.0 with the K3 host. BAG1 was capable of K3 eradication within 180 min [Figures 5 and 6].

Biofilm eradication of K3 from human dentin slabs

We tested the efficiency of BAG1 to eliminate the biofilm of K3 created on natural mineralized tooth root surfaces and we determined it through counting a CFU of the broth and biofilm fraction. Furthermore, K3 biofilm was visualize on dentin slabs using live and dead fluorescent microscope.

The results revealed that BAG1 significantly eliminated *E. faecalis* K3 of the dentin slabs broth. The result was highly significant ($P < 0.008$), as shown in Table 3. After counting the CFU of *E. faecalis* K3 obtained from biofilm fragment, it showed that K3 was capable of eliminating *E. faecalis* biofilm. The result was highly significant ($P < 0.008$), as shown in Table 4 and Figure 7.

Discussion

E. faecalis is a commensal microorganism; Gram-positive is found in the digestive system. However, it can result in life-threatening infections such meningitis, bacteremia, endocarditis, and urinary

Table 1: BAG1 characterization of the main nucleotide sequence of phage BAG1 was analyzed by Phaster online program

Phage	Region length (kbp)	Open reading frame	Region position	GC (%)	DNA phage
BAG1	41.7	68	8-41705	34.56	Circular

ORF: Open reading frame, GC: Guanine-cytosine

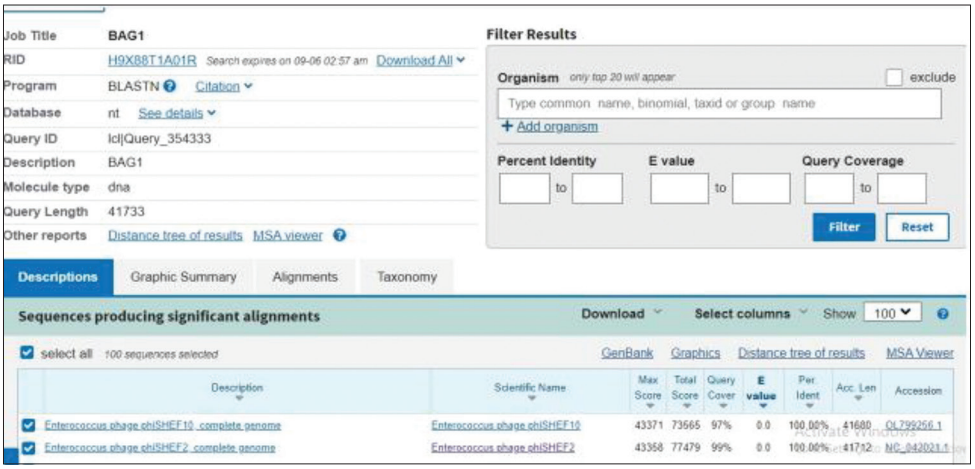

Figure 3: BAG1 nucleotide sequence BlastN result

Figure 4: Genome organization *Enterococcus faecalis* lytic phage BAG1. Image produced by phaster program

Table 2: Names and proposed function of BAG1 genes blast hits. hits against virus and prophage database, hits against bacterial database or genbank file

CDS position	Blast hit
complement (8.232)	PP_00001; phage (gi100025) hypothetical protein;
complement (303.1010)	PP_00002; phage (gi100026) hypothetical protein;
complement (1088.1339)	PP_00003; phage (gi100027) hypothetical protein;
complement (1340.1636)	PP_00004; phage (gi100028) hypothetical protein;
complement (1637.2455)	PP_00005; phage (gi100029) hypothetical protein;
complement (2445.2633)	PP_00006; phage (gi100030) hypothetical protein;
complement (2609.3385)	PP_00007; phage (gi100031) hypothetical protein;
complement (3396.3875)	PHAGE_Entero_phiSHEF2_NC_042021: u-spanin; PP_00008; phage (gi100032)
complement (3872.4093)	PHAGE_Entero_phiSHEF2_NC_042021: endolysin; PP_00009; phage (gi100033)
complement (4095.4694)	PHAGE_Entero_phiSHEF2_NC_042021: holin; PP_00010; phage (gi100034)
complement (4851.5042)	PP_00011; phage (gi100035) hypothetical protein;
complement (5050.5568)	PP_00012; phage (gi100036) hypothetical protein;
complement (5522.6262)	PHAGE_Entero_phiSHEF2_NC_042021: putative phosphoesterase; PP_00013; phage (gi100037)
complement (6275.6472)	PP_00014; phage (gi100038) hypothetical protein;
complement (6535.6711)	PP_00015; phage (gi100039) hypothetical protein;
complement (6708.8003)	PP_00016; phage (gi100040) hypothetical protein;
complement (7996.8385)	PHAGE_Entero_phiSHEF2_NC_042021: DNA adenine methyltransferase; PP_00017; phage (gi100041)
complement (8369.8605)	PP_00018; phage (gi100042) hypothetical protein;
complement (8608.8817)	PP_00019; phage (gi100043) hypothetical protein;
complement (8820.9029)	PHAGE_Entero_phiSHEF2_NC_042021: DNA helicase; PP_00020; phage (gi100044)
complement (9031.9192)	PHAGE_Entero_phiSHEF2_NC_042021: putative transcriptional regulator; PP_00021; phage (gi100045)
complement (9194.9466)	PHAGE_Entero_phiSHEF2_NC_042021: HNH endonuclease; PP_00022; phage (gi100046)
complement (9515.9958)	PHAGE_Entero_phiSHEF2_NC_042021: primase; PP_00023; phage (gi100047)
complement (10031.10219)	PHAGE_Entero_phiSHEF2_NC_042021: exonuclease; PP_00024; phage (gi100048)
complement (10308.11888)	PP_00025; phage (gi100049) recombination protein;
complement (11983.12180)	PHAGE_Entero_phiSHEF2_NC_042021: putative single-stranded DNA-binding protein; PP_00026; phage (gi100050)
complement (12177.12416)	PP_00027; phage (gi100051) hypothetical protein;
complement (12413.12628)	PP_00028; phage (gi100052) tail fiber protein;

Contd...

Table 2: Contd...

CDS position	Blast hit
complement (12625.12852)	PP_00029; phage (gi100053) tail assembly protein;
complement (12849.13067)	PP_00030; phage (gi100054) tail tip protein;
complement (13067.13288)	PP_00031; phage (gi100055) minor tail protein;
complement (13288.13428)	PP_00032; phage (gi100056) minor tail protein;
complement (13425.13586)	PHAGE_Entero_phiSHEF2_NC_042021: tail length tape-measure protein; PP_00033; phage (gi100057)
complement (13742.14452)	PP_00034; phage (gi100058) hypothetical protein;
complement (14529.14663)	PP_00035; phage (gi100059) hypothetical protein;
complement (14966.15229)	PP_00036; phage (gi100060) hypothetical protein;
complement (15226.15411)	PP_00037; phage (gi100061) hypothetical protein;
complement (15411.15818)	PP_00038; phage (gi100062) hypothetical protein;
complement (15815.16027)	PP_00039; phage (gi100063) hypothetical protein;
complement (16065.16430)	PP_00040; phage (gi100064) hypothetical protein;
complement (16444.16761)	PP_00041; phage (gi100065) hypothetical protein;
17265.17468	PP_00042; phage (gi100066) capsid decoration protein;
17551.17757	PP_00043; phage (gi100067) capsid maturation protease;
17757.18128	PP_00044; phage (gi100068) head morphogenesis protein;
18520.18672	PHAGE_Entero_phiSHEF2_NC_042021: methyltransferase type 11; PP_00045; phage (gi100001)
18687.19160	PP_00046; phage (gi100002) hypothetical protein;
19697.21421	PP_00047; phage (gi100003) hypothetical protein;
21491.21655	PP_00048; phage (gi100004) hypothetical protein;
21660.22811	PP_00049; phage (gi100005) hypothetical protein;
22798.23361	PP_00050; phage (gi100007) hypothetical protein;
23431.24681	PP_00051; phage (gi100007) hypothetical protein;
24807.25007	PP_00052; phage (gi100008) hypothetical protein;
25051.25347	PP_00053; phage (gi100009) hypothetical protein;
25319.25654	PP_00054; phage (gi100009) hypothetical protein;
25651.26058	PP_00055; phage (gi100011) hypothetical protein;
26055.26420	PP_00056; phage (gi100012) hypothetical protein;
26496.27062	PP_00057; phage (gi100013) hypothetical protein;
27257.27568	PP_00058; phage (gi100014) hypothetical protein;
27825.32195	PP_00059; phage (gi100015) hypothetical protein;
32278.34359	PP_00060; phage (gi100016) hypothetical protein;
34430.36487	PP_00061; phage (gi100017) hypothetical protein;
36502.36789	PP_00062; phage (gi100018) hypothetical protein;
36967.37212	PP_00063; phage (gi100019) hypothetical protein;
37227.37463	PP_00064; phage (gi100020) hypothetical protein;
37460.38446	PP_00065; phage (gi100021) hypothetical protein;
complement (38528.38713)	PP_00066; phage (gi100022) hypothetical protein;

Contd...

Table 2: Contd...

CDS position	Blast hit
complement (38752.39351)	PP_00067; phage (gi100023) hypothetical protein;
complement (39414.41705)	PP_00068; phage (gi100024) hypothetical protein;

CDS: is a sequence of nucleotides that corresponds with the sequence of amino acids in a protein. Atypical CDS starts with ATG and ends with a stop codon. CDS can be a subset of an open reading fram (ORF).

Table 3: Number of *Enterococcus faecalis* on dentin slabs (planktonic broth)

Infected	Treated
512×10^{19}	28×10^{4}
768×10^{19}	32×10^{4}
665×10^{19}	28×10^{4}
716×10^{19}	24×10^{4}
819×10^{19}	20×10^{4}

Table 4: Number of *Enterococcus faecalis* on dentin slabs (biofilm fraction)

Infected	Treated
2×10^{10}	32×10^{8}
2×10^{10}	48×10^{8}
24×10^{10}	48×10^{8}
24×10^{10}	28×10^{8}
18×10^{10}	24×10^{8}

tract infections,[14] and this is particularly harmful in hospitals where antibiotic resistance raised. In addition, *E. faecalis* is frequently collected from persistent infections associated with failure endodontic treatment[15] and may cause abscesses to develop as a result of chronic or acute inflammation, tissue loss, and inflammation of the tooth-root tip.[16]

We choose K3 clinical strain because it is isolated directly from root canal infection and it showed high resistance to vancomycin (result not shown).

To further analysis, the genome of the isolated BAG1, i.e., genome modules distribution, genome size, and the most important to realize the nature of phage. BAG1 phage DNA was subjected to full genome sequencing. The BAG1 genome came in one node with 41.7 kbp. The genome composed of 68 ORF distributed as the genome of BAG1 coding areas are divided into two halves reversed transcription Figure 3.

Like the majority of Enterococcus phages, the genomes are organized in modules, which include replication and regulatory modules (left ward transcribed genes) and modules for structural components, DNA packaging, cell lysis (right ward transcribed genes) and a module as shown in Figure 3.

There was no putative integrase-encoding gene, which indicates BAG1 phage probable to be lytic in nature, which favorable it uses as suitable candidate for therapy.

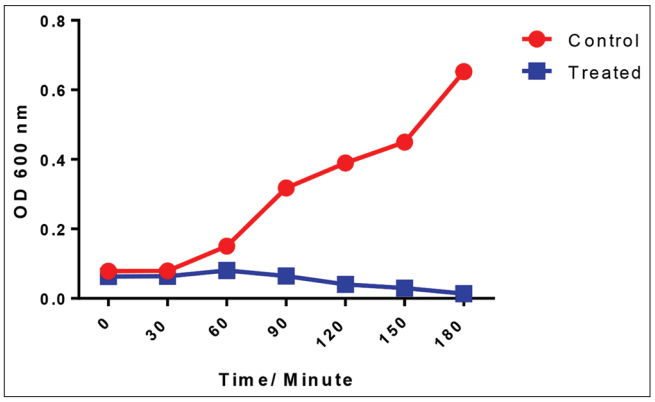

Figure 5: Killing curve. BAG1 was capable of K3 elimination within 180 min

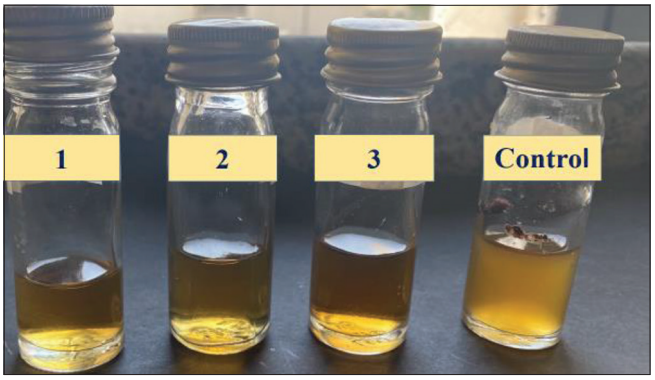

Figure 6: Elimination of K3 by BAG1 represented by the broth clearance. Tubes 1, 2, and 3 represent treated tubes with BAG1, tube 4 represent control tube of K3 only

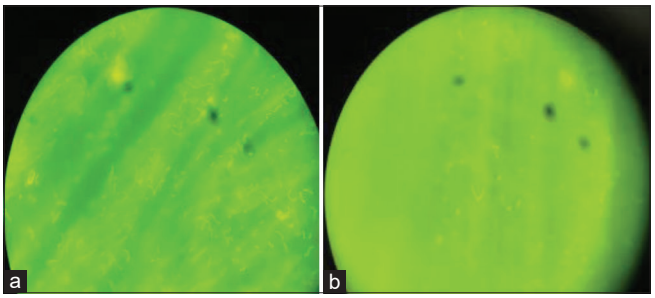

Figure 7: Image of *Enterococcus faecalis* biofilm grown on dentine slabes and stained using Live/Dead® viability stain with the green colour indicating live cells. (a) As shown in this image high number of *Enterococcus faecalis* grown on dentine slabs. (b) As shown in this image few number of *Enterococcus faecalis* K3 was bserved on treated dentine slabs

In addition, BAG1 phage was capable of complete eliminating K3 endodontic clinical strain in planktonic test within 180 min Figures 5 and 6, that makes it an efficient and suitable candidate for the treatment of *E. faecalis* infection.

Even after careful mechanical and chemical cleaning during root canal therapy, infection may still exist,[17] in the majority of filled and treated root canals, and in some circumstances, might cause fail of the treatment and lead to new complications.

Currently, there are few therapeutic methods available to eliminate *E. faecalis* biofilm infection that persist within root canal.[18] A significant health risk is posed by bacterial biofilms, since bacteria within biofilm are both inaccessible to body's immune system and anti-bacterial agents.[19] Several causes may be connected to the penetration failure, including the development of bacteria's multidrug resistance within the biofilm. Furthermore, biofilm cells and its extracellular matrix.[20]

Biofilms can consist of either mono-or poly-microbial organisms, and they are usually associated with persistent illnesses.[21]

In our study, biofilm assay on dentin slabs, BAG1 can significantly reduce monomicrobial biofilm formation of K3 endodontic clinical strain reflected by its capability to eliminate *E. faecalis* in both dentin slabs broth and biofilm fraction.

In addition, several studies reported significant reduction of clinical and laboratory strains biofilm after treatment with phage on human dentin slabs. Paisano *et al*., 2004, infect human dentin roots with *E. faecalis* ATCC 29212 (isolated from human urine) in the presence of the phage lysate that result substantial reduction in bacterial cell viability.[22]

Moreover, Khalifa *et al*., 2015[14] showed that EFDG1 phage eliminates *E. faecalis* (V583) significantly that results in the reduction in bacterial leakage in *ex-vivo* model of human teeth. Similarly, Al-Zubidi *et al*., 2019[23] studied the ability of SHEF2 bacteriophages to eliminate biofilm formed by *E. faecalis* Ef 54 (nonoral human isolate) on the surfaces of natural tooth roots and result in significant reduction in bacterial numbers.

However, none of them use the clinical strain of *E. faecalis* that was identified directly from a root canal infection.

Phages are naturally occurring bacterial killers that often only affect the isolated bacterial species and have little impact on the commensal flora. Phages can break down the biofilm matrix by producing depolymerases or they can pierce biofilms through water channels.[21]

In the present study we sequence the full genome of anti *E. faecalis* bacteriophage BAG1. In addition we tested its efficiency to eliminate the endodontic clinical strain K3 in planktonic media and assess its capability to eradicate *E. faecalis* K3 in both biofilm and its broth media. Bacterial viability obtain from biofilm fraction that has been grown on root dentine model.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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