Genome Characterization of the Novel Lytic Genome Sequence of Phage YUEEL01 of the Myoviridae Family

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Research Article

Keywords: Enterobacteria phage, Multi-drug resistance, Genome YUEEL01, Phage therapy, Comparative genomics, Homing endonuclease, nrdB and nrdD introns

Posted Date: July 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-734852/v1

Version of Record: A version of this preprint was published at Virus Research on December 1st, 2021. See the published version at https://doi.org/10.1016/j.virusres.2021.198670.

Abstract

Antimicrobial resistance is a global concern due to its rapid emergence in the environment and the associated high risk to human and animal health. Municipal wastewater, including urban, hospital, and pharmaceutical effluent, is the main source of antibiotic and antibiotic resistant bacteria (ARB) contamination, and biological processes are commonly used for wastewater treatment. Biologically based strategies seem to be a promising approach to effective integrated ARB control since they can focus on the bases of antibiotic resistance. To develop an effective bacteriophage against multi-drug resistance (MDR) microbes in municipal wastewater, phage YUEEL01 was isolated from a livestock farm, and its genome sequenced. Phenotypic characterization suggested that the phage is a member of the *Myoviridae* family, and that it encodes 168,266 bp with an overall GC content of 35.4% had 259 putative protein-coding genes, 11 tRNA and six hypothetical genes. A number of putative DNA replication and regulation, DNA packing and structure and host lysis genes were identified. Further, whole genome phylogenetic analysis demonstrated that phage YUEEL01 is closely related to phage slur03. Elucidation of the YUEEL01 phage might be helpful when developing antibacterial tools for controlling MDR bacteria in wastewater treatment systems.

Introduction

Most bacteria are resistant to certain antibiotics, and they can acquire resistance to antibiotics via mutations in chromosomal genes or by horizontal gene transfer [4]. Antibiotic-resistant bacteria (ARB) can occur in several areas including municipal sewers and farm soil fertilized with manure or sewage sludge [1]. In addition to antibiotics being present in wastewater, they may also have long-term permanence in water systems. Genetic selection pressures on micro-organisms at sub-inhibitory concentrations can result in the development of ARB and antibiotic resistant genes [5, 17, 18].

Wastewater comprises high levels of organic and inorganic matter, as well as high concentrations of microorganisms that can convert sewage into an ecological niche that is especially adapted to the growth and spread of ARB. Recent studies suggest that there is a high level of multidrug-resistant (MDR) bacteria present in wastewater effluent [18, 24, 29]. Bacteriophages are viruses that contain a long, contractile, proteinaceous tail that enables specific host recognition and subsequent adsorption to a receptor at the surface of the host bacterium [13]. Recent studies have reported on bacteriophage applications in various fields including medicine [11], diabetic wound infections (foot ulcers), topical cleaning and disinfection [7], foodborne pathogen control and detection [3, 8, 12, 23, 28], plant diseases [15], water-well clog prevention [10], foam formation reduction [31], and slime and biofilm control [2]. Based on the potential use of phages as antibacterial tools, it can be proposed that during wastewater treatment, inoculation of wastewater with specific lytic phages active against specific bacteria will be effective. To develop a specific lytic phage application for the control of ARB bacteria during wastewater treatment, it is first necessary to understand the genomic features of that phage.

In the present study, a newly identified phage named YUEEL01 was isolated and characterized. The phage genome was sequenced and annotated and phylogenetic analysis showed that YUEEL01 is belongs to the member of Myoviridae family. These results might be helpful to understand the interaction of phage with their bacterial hosts.

A Gram-negative bacterial isolate, ART2, was isolated from a local municipal wastewater sample in South Korea and used as a source of lytic phages. Briefly, the bacterial mixture was first sequentially filtered through 0.45 and 0.2 mm filter papers to remove bacteria and other debris in order to isolate phage-like particles. The filtrate containing the phage-like particles was then mixed with soft TSB agar, applied on an agar plate containing a bacterial lawn, and incubated overnight at 37°C. The plaque that had formed on the plate was gently picked and the bacteria lawn again infected with the phage particles. Eventually, the lytic phage was stored in salt magnesium gelatin (SMG) buffer until further use.

Genomic DNA was extracted by following a standard method [19]. The obtained high-quality DNA was sequenced by using an Illumina NextSeq 500 (LabGenomics, South Korea). The genome sequence of phage YUEEL01 was assembled by applying a *de nova* method using a SSAKE assembler (. A total of 6,099,132 paired-end reads with 1,835,838,732 total bases (84.98%, >Q30) were generated with a read length of ~300 bp by using Illumina MiSeq (LabGenomics, South Korea). Sequence trimming, assembly were performed with SSAKE assembler v4.0 [32]. The resulting 125 contigs (>350 bp) were scaffolded with SSAKE using pair-end library reads. Further, assembly gaps and polishing were carried with Racon v1.4.20 (https://github.com/lbcb-sci/racon). In addition, the complete genome (Supplementary Fig. 1) and boundary regions (Supplementary Fig. 2) were mapped using the Bowtie2 v2.4.4 [21] to accomplish the genome is circular or not. The average depth coverage of the assembled sequence is 10,319x. The resulting consensus sequence was annaotated using RAST server v2.0 [25] and deposited in the GenBank database under accession number KY2900975.

The phage genome of YUEEL01 is a circular molecule, that contains 168, 266 bp in length with a G+C content of 35.4%. Approximately 277 open reading frames (ORFs) were predicted by RAST server v2.0 [25]. The YUEEL01 genome contains 266 protein-coding genes and 11 tRNAs ($tRNA^{Arg}$, $tRNA^{Asn}$, $tRNA^{Gln}$, $tRNA^{Gly}$, $tRNA^{His}$, $tRNA^{Leu}$, $tRNA^{Met}$, $tRNA^{Pro}$, $tRNA^{Ser}$, $tRNA^{Thr}$ and $tRNA^{Tyr}$ (Supplementary Table S1). Approximately 64.75% of the protein-coding genes are located on the plus strand with the remaining 35.25% protein-coding genes present on the minus strand. The capsid and tail genes, as well as representatives of the DNA replication, packaging, and lysis protein-coding genes, were also identified in the genome of YUEEL01 (Fig. 1).

The phage YUEEL01 genome can be divided into three functional modules, including a phage structure and DNA packaging module , a DNA replication/recombination module, and a host lysis module.

The YUEEL01 genome encodes at least 83 genes associated with nucleotide metabolism. These include DNA polymerase, DNA repair mechanism, DNA helicase, restriction endonuclease activity, replicative helicase-primase, single strand DNA-binding protein-coding, sigma factor for transcription, ribonuclease

H, DNA topoisomerase, DNA ligase, 3' phosphatase-5'-polynucleotide kinase, adenine-specific methyltransferase, dihydrofolate reductase, and endoribonuclease translational repressor and recombination-related genes. When compared with other closely related phage genomes, most of the genes in those genomes are present in the YUEEL01 genome.

The YUEEL01 genome also encodes at least 18 cell-lysis cassette genes such as holins, spanin Rz, and other phage-encoded proteins. The lysis-related genes were located in the upstream region of DNA metabolism genes. Lyase and hydrolase phage-encoding genes, which are responsible for degradation of carbohydrate-containing polymers on the surface of bacterial cells, are also present in the genome. Due to the high specificity to host receptors by phage particles, these proteins might be useful in medical, food, plant, and animal industries. Previous studies have reported that lysis cassette genes have been effectively applied as detectors of potential foodborne pathogens in the food industry, as a clinical diagnostic tool in the medical industry, as a natural antibacterial for food product preservation, in animal feeding, and in plant cultivation [6, 9, 12, 14, 20, 22, 26, 27].

The YUEEL01 genome encodes 114 structural assembly protein genes and 45 DNA packaging-related genes. These genes include phage head, neck, tail fibers, baseplate, major capsid and capsid assembly protein, head-to tail joining protein. host range protein, DNA maturation protein, DNA packaging protein and different types of internal virion protein genes. Most of these genes are homologous to those in its closely related RB3, RB68, and T4 genomes.

In the present study, phage genomes were selected based on closely related genomes. A molecular phylogenetic tree was constructed to evaluate the phylogenetic relationships of *Enterobacteria* phages by using a complete 20 phage genome sequences. The genome of *Staphylococcus* phage K was set as the outgroup. Genome sequences were aligned by using MAFFT v7.475 [16]. The aligned genome sequences were used to generate a phylogenetic tree. Phylogenetic analysis was conducted based on maximum likelihood (ML) analysis with rapid bootstrapping via the general time-reversible model and gamma model site heterogeneity (GTRGAMMA) nucleotide substitution model, with default parameters, in RAxML v8.2.12 [30]. The bootstrap probability of each branch was calculated by performing 1000 replications. The resulting phylogenetic tree was divided into two clades. Two *Salmonella* and one *Serratia* phages formed one clade and the other clade was formed by the *Enterobacteria*, S*higella* and *Klebsiella* phages. Interestingly, *Enterobacteria* phage dhaeg and Bp7 diverged from the other *Enterobacteria* phages and formed a sister clade with the *Salmonella* phages. Also notable was that *Serratia* phage PS2 and *Enterobacteria* phage GEC-3S formed a single subclade with the other major *Enterobacteria* phages with a 100% BS value. Within this clade, the phage YUEEL01 formed a sister clade with phage slur03 with a 100% BS value (Fig. 2). Based on morphological and phylogenetic analysis, we deduce that phage YUEEL01 is a novel member of the family *Myoviridae*.

Nucleotide sequence accession number

The GenBank accession number for Escherichia phage YUEEL01 is KY2900975 (https://www.ncbi.nlm.nih.gov/nuccore/KY290975).

Declarations

Acknowledgments

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2020R1I1A305481611).

Author contributions

G.R., A.S., S.P., and Y.A., conceived and designed the study. Y.A. provided the materials. S.P. and Y.A. supervised the work and critically revised the manuscript. A.S performed the isolation as well as the phenotypic characterization of the phage. G.R performed the bioinformatic analysis, the genome annotations, analyzed the data, and drafted the manuscript.

Competing interests

The authors read and approved the manuscript and declare no competing financial interests.

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Figures

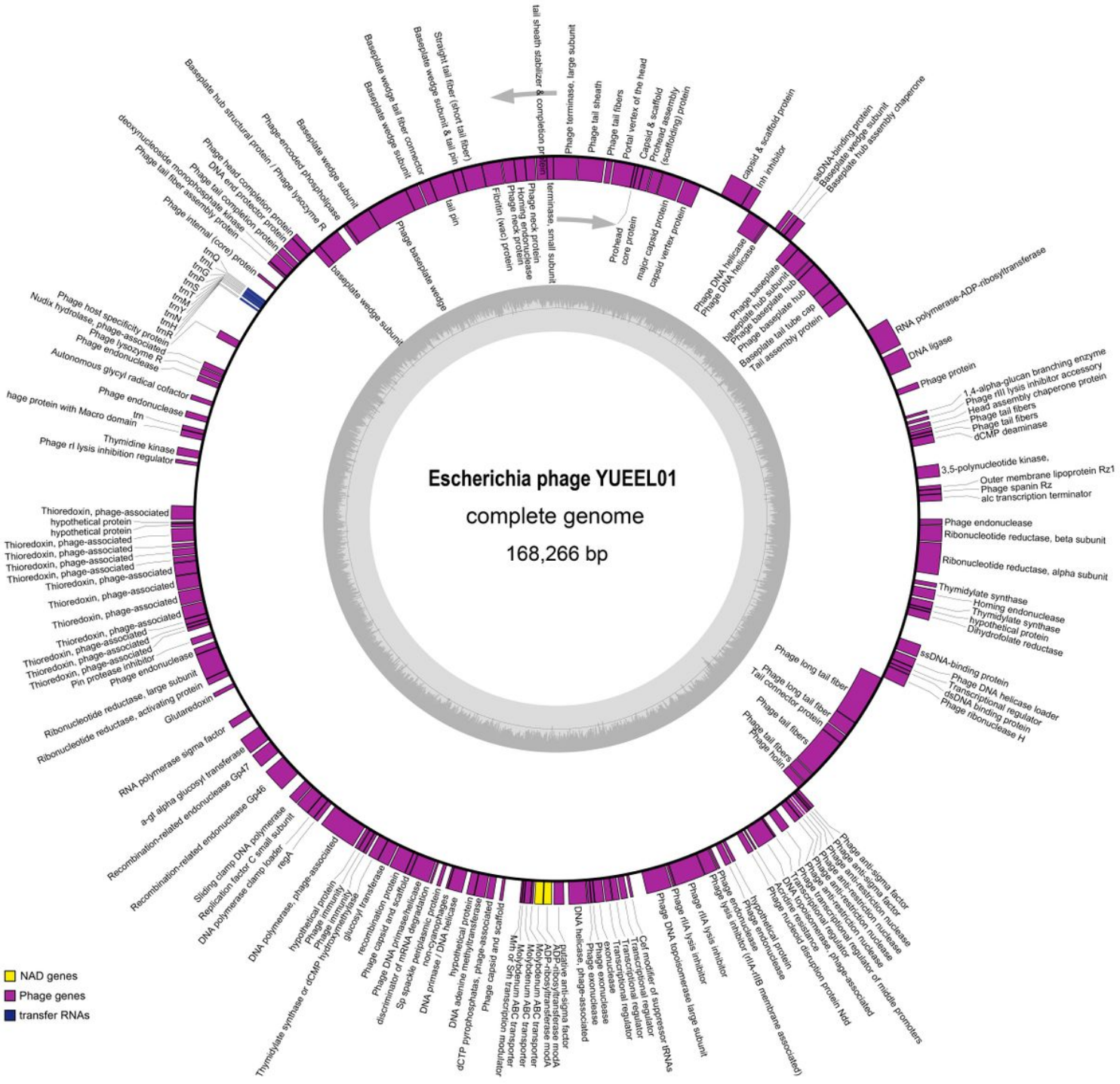

Figure 1

Gene map of Enterobacteria phage YUEEL01. Genes located on the outside of the outermost layer of the circle are transcribed in a counterclockwise direction, whereas genes located on the inside of the circle are transcribed in a clockwise direction. The colored bars indicate known protein-coding genes and transfer RNA (tRNA) genes. The dashed, darker gray area in the inner circle denotes the guanine-cytosine (GC) content of the genome, whereas the lighter gray area in the inner circle indicates the adenosine-thymine (AT) content of the genome.

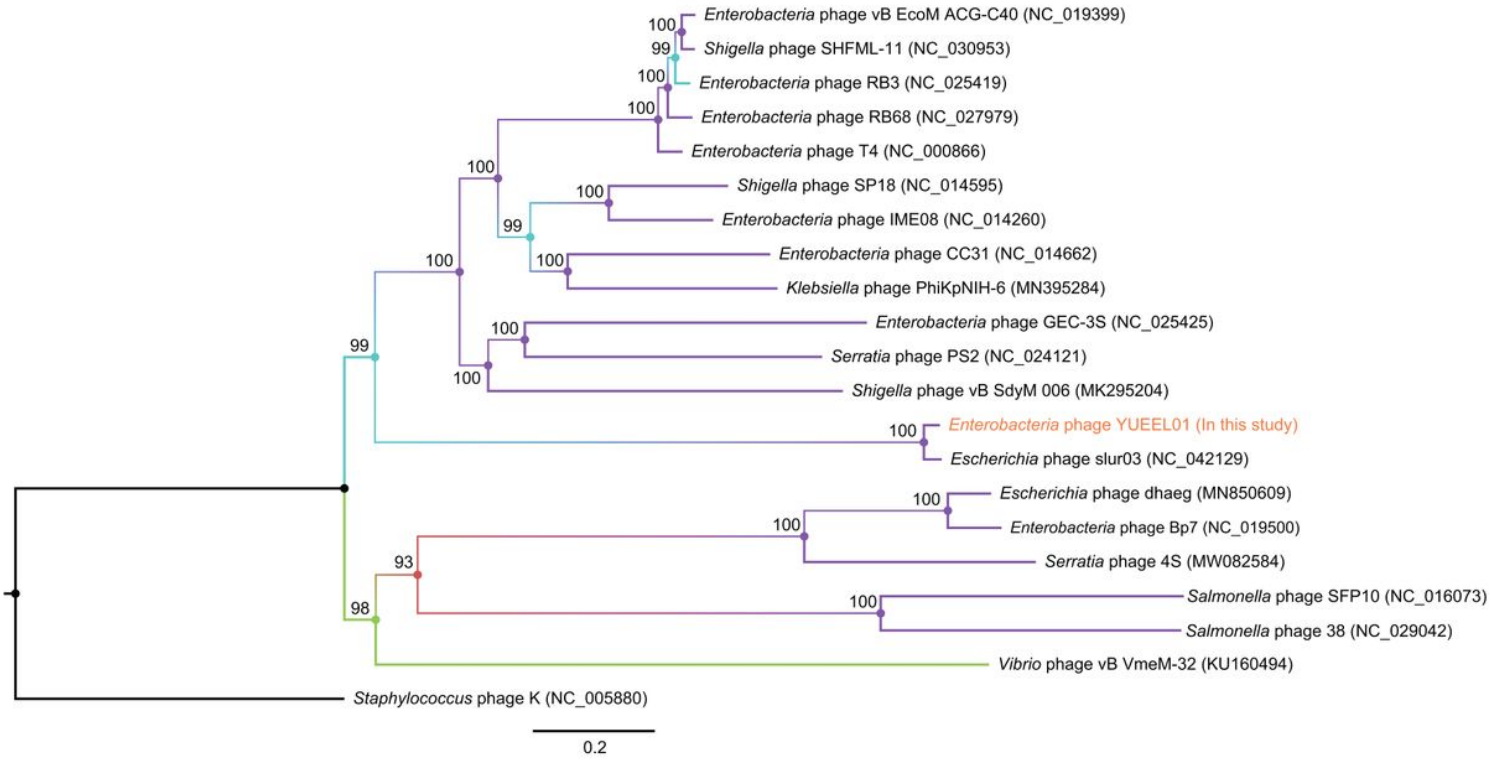

Figure 2

Molecular phylogenetic analysis of whole genome sequences of the Enterobacteriaceae family. The tree was constructed by performing maximum likelihood (ML) analysis using RaxML software and the GTRGAMMA nucleotide model. The stability of each tree node was tested by undertaking bootstrap analysis with 1000 replicates. Staphylococcus phage K was set as the outgroup. Rectangular red box shows the position of Enterobacteria phage YUEEL01.

Supplementary Files

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