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Genome analysis of novel *Salmonella*-infecting bacteriophage ϕSen-NF

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Abstract. *Febrianti RA, Narulita E, Savitri FA, Widianto AH, Addy HS, Sulistyaningsih E, Kuswati K, Utami BR. 2025. Genome analysis of novel* Salmonella*-infecting bacteriophage ϕSen-NF. Biodiversitas 26: 2011-2019.* Genome mapping of bacteriophages that infect *Salmonella* is an important step in understanding the interactions between these pathogenic bacteria and viruses. *Salmonella* is one of the leading causes of food poisoning worldwide, with multiple serotypes that can cause serious infections in humans. This study aimed to identify and analyze bacteriophage genomes that can be used as therapeutic agents to control *Salmonella* infections. The study began with isolating bacteriophages from food samples, followed by morphological characterization, molecular and genomic analysis, and phylogenetics. The successfully isolated bacteriophage ϕSen-NF can only infect *Salmonella enterica* ser. Typhi, which has a diameter of ±1mm with a clear plaque. Bacteriophage ϕSen-NF, which infects *Salmonella*, is a linear double-stranded DNA virus with a genome size of 108,537 base pairs and a G+C content of 46.8%. Comparative genomic analysis revealed similarities with Myoviridae, such as ϕSE24 and ϕSw2. Phylogenetic reconstruction showed a close evolutionary relationship with phages such as ϕSE24 and ϕSw2. The results from this study are expected to make significant contributions to the development of new strategies to address food poisoning caused by *Salmonella*, as well as improve overall food safety.

Keywords: Bacteriophage, foodborne disease, genome mapping, *Salmonella*

INTRODUCTION

Foodborne diseases or diseases caused by the infection of foodstuffs by microbes, are one of the health problems that occur in all parts of the world. There are more than 600 million cases of foodborne diseases (nearly one in ten cases) caused by contaminated food (Faour-Klingbeil et al. 2019). It is recorded that the most common cause of foodborne disease is caused by pathogenic bacteria such as *Salmonella, Vibrio parahaemolyticus, Listeria monocytogenes, Staphylococcus aureus*, alongside several other pathogens (He et al. 2023). *Salmonella* is one of the pathogenic bacteria that have a major contribution in infecting humans to cause disease, and they have caused significant health and economic impacts worldwide (Baptista et al. 2018). Every year, about 200 million to 1 billion cases of *Salmonella* infection are recorded worldwide with 93 million cases of gastroenteritis and 155,000 deaths, and about 85% of these cases are related to the consumption of contaminated food (Chlebicz and Śliżewska 2018).

Efforts to treat *Salmonella* infections usually use therapy and treatment using antibiotics, but, currently, this treatment has a weakness in bacterial resistance to antibiotics. Some *Salmonella* serovars are resistant to several antibiotics such as ceftriaxone, chloramphenicol, ampicillin, and the fluoroquinolone class. Southeast Asian countries including Malaysia, Thailand, Indonesia, Vietnam, Cambodia, Singapore, and the Philippines have reported that *Salmonella enterica* serovar Typhimurium is resistant to three or more groups of antibiotics (Multidrug Resistant or MDR) (Herrera-Sánchez et al. 2021; Patra et al. 2021; Wain et al. 2021) with one study conducted in Indonesia reporting that 27 isolates of *Salmonella enterica* serovar Typhi showed that 3.7% led to MDR (Jamilah et al. 2020). Antibiotic resistance in *Salmonella* is mediated by several mechanisms including drug inactivation, which is the most common cause of resistance. In this mechanism, the antibiotic agent is destroyed or inactivated through chemical modification using enzymes that catalyze reactions such as acetylation, phosphorylation, and adenylation (Castro-Vargas et al. 2020).

Another treatment that can be used to reduce the infection rate of *Salmonella* is to use bacteriophages. Bacteriophages are viruses that infect specific bacteria by lysing bacterial cells. Bacteriophage research has grown rapidly since Edward Twort discovered them in 1917. Bacteriophages have been widely used as biocontrol of pathogenic bacteria since 1970 because they offer many

advantages (Elois et al. 2023). Bacteriophages have the ability to disrupt bacterial metabolism and cause cell lysis, and they also bind to specific receptors on bacteria, thus minimizing side effects on host cells. This specificity provides an advantage in the treatment of bacterial infectious diseases (Hadimli et al. 2021). Research conducted by Bao et al. (2019) using *Salmonella* phage vB_SenM-PA13076 showed the effectiveness and safety of bacteriophage application in mice, and this is reinforced by data on bacteriophage application to overcome bacterial infections in China. Animals infected with bacteria at a minimum lethal dose survived when injected with bacteriophages after 15 minutes of infection (Liang et al. 2023).

Several studies have been successfully isolated bacteriophages in foodborne disease, including ϕGKR1a, ϕGKR1b, ϕGKR2, ϕGPT1a, ϕGPT1b, and ϕGMJ1b infecting *Escherichia coli* (Narulita et al. 2018); ϕSZUT, ϕSZIP1, and ϕSZIP2 infecting *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli* (Iqbal et al. 2020), as well as finding that phage-antibiotic synergy (ϕPT1b with tetracycline/amoxicilline) can reduce the level of antibiotic resistance in *Escherichia coli* (Narulita et al. 2020). For further utilization of these bacteriophages, complete genomic information is needed, so this research aimed to analyze the bacteriophage genome that will be used as the basis for developing bacteriophage-based drugs/therapies as an alternative solution in order to overcome cases of *Salmonella* bacterial infections. The goal of sequencing the entire bacteriophage genome is to understand the genetic makeup and interaction of the bacteriophage with its bacterial host. This information can be used to develop new treatments for bacterial infections, as well as to understand the evolution of bacteriophages better.

MATERIALS AND METHODS

Research materials

This research was conducted in the laboratory of molecular medicine, CDAST, University of Jember, Jember, Indonesia. The experiment was carried out using materials including *Salmonella* bacteria (laboratory collection), Luria Bertani (LB) medium (HIMEDIA), DNAse and RNAse (Thermo Scientific), phenol (MERCK), chloroform (MERCK), isoamyl alcohol (MERCK).

Procedures

Bacterial rejuvenation

Bacterial rejuvenation uses *Salmonella* isolates as the primary host bacteria of bacteriophages cultured on Luria Bertani (LB) medium. Rejuvenation was carried out by taking pure isolates of bacteria from glycerol to LB medium using a micropipette up to 250 µL. The medium tube containing the bacterial isolate was given a cotton swab and plastic wrap. The bacterial culture was then incubated for 24 hours at 37°C to obtain stock bacteria. Stock bacteria were then cultured on LB medium, which would then be used as working isolates (Narulita et al. 2018).

Bacteriophage propagation

Calculation of bacteriophage particles was carried out based on the plaque assay method on LB medium to determine the number of bacteriophage particles (Narulita et al. 2018). The plaque assay method began with rejuvenating *Salmonella* in LB medium, then after inoculating the medium, the bacteria were incubated until they reached the logarithmic phase (±4 hours). After reaching the logarithmic phase, 300 µL of bacterial suspension and 10 µL of bacteriophage were mixed in the top agar medium (0.45% agar), which was still warm (±50°C). The mixture of bacterial suspension was homogenized and poured over LB medium, which became the bottom medium (double layer), before being incubated for 24 hours at 37°C. Then, the number of plaques formed was counted.

Host range test

The host range test was conducted using *Salmonella enterica* serovar Enteriditis, *Salmonella enterica* serovar Typhi, and *Salmonella enterica* serovar Typhimurium, using the spot test method. The bacteria to be used were cultured in LB media and then incubated at 37°C for five hours, which represented the initial exponential phase. The incubated bacterial culture was then dissolved in up to 300 µL of top agar (0.45% agar) and poured into LB media, which began to solidify. The double layer medium was allowed to stand for about 30 minutes and then plotted with 3 µL bacteriophage samples tested to determine the host range. Differences in the type of plaque that appeared in the host range test indicated differences in infection interactions between bacteriophages and their host bacteria. Cloudy or turbid plaque indicated that the infection that occurred in the host bacteria belongs to the lysogenic group (Muthu and Durairaj 2016; Hashish et al. 2022; Narulita et al. 2023).

Identification of bacteriophage nucleic acid types

There are two types of bacteriophage nucleic acid, namely DNA and RNA. The nucleic acid type identification stage is important in bacteriophage characterization, because the nucleic acid type can be the basis for bacteriophage classification. The method used to determine the type of bacteriophage nucleic acid refers to Narulita et al. (2016), which starts with extracting genetic material from bacteriophage particles.

Whole genome sequencing

Phage genomes were manually extracted from phage stocks using phenol, chloroform, and isoamyl alcohol according to the method described in Choi and Kim (2021), following DNase (1 mg/mL) and RNase (0.001 mg/mL) treatments to remove external genomic contaminants. Genome-extracted phages were sequenced using Illumina MiSeq (Illumina, USA) and reads were assembled de novo with SPAdes v.3.14.1.

Data analysis

This research employed a combination of experimental and bioinformatics methods to characterize its biological and genetic properties; and was conducted using a double-

layer agar method to confirm lytic activity. DNase/RNase treatments followed by gel electrophoresis confirmed ϕSen-NF contains double-stranded DNA. Illumina MiSeq generated short reads, assembled de novo using SPAdes v3.14.1. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), HMMER (https://www.ebi.ac.uk/Tools/hmmer/), and InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/) identified: Class I, Class II, and Class III. MEGA X for phylogenetic tree.

RESULTS AND DISCUSSION

Morphological characteristics of bacteriophages

The multiplication of bacteriophage particles was carried out by the plaque assay method, and the formation of a clear zone indicates the presence of active bacteriophage particles (Figure 1). Based on the level of plaque turbidity, the plaque results show a lytic-type bacteriophage, because the plaque produced is very clear. The plaque formed is ±1mm in size. A good bacteriophage has a lytic cycle because it can cause complete lysis of bacterial cells and can produce mature bacteriophages.

Bacteriophage was isolated using the initial hosts of the three bacterial serovars, namely, *Salmonella enterica* serovar Enteriditis, *Salmonella enterica* serovar Typhi, and *Salmonella enterica* serovar Typhimurium. ϕSen-NF can only infect bacteriophages of *Salmonella enterica* ser. Typhi. The results of bacteriophage isolation using the hosts of the three *Salmonella enterica* serovars are shown in Figure 2.

Molecular characterization of bacteriophages

After the bacteriophage was propagated, the bacteriophage genome was isolated by DNAse and RNAse treatment. The marker lane contains a DNA ladder (1kbp), which serves as a molecular weight marker to estimate the size of the nucleic acids in other lanes. RNase specifically degrades RNA. The presence of visible bands suggests that DNA remains intact while RNA has been digested. Bacteriophages obtained have a type of nucleic acid in the form of DNA (Figure 3).

Genomic and phylogenetic analysis of ϕSen-NF

We performed comprehensive genomic and phylogenetic analyses to elucidate the evolutionary relationships and genetic diversity among the bacteriophages studied. The outer circle shows various coding sequences and hypothetical proteins (Figure 4). These are regions of the genome that code for proteins or are predicted but not yet confirmed. Each gene or protein is labeled with its respective function or name, which provides a quick reference to important genomic features. The circular map provides a detailed visual representation of the genome structure highlighting important genes and regions in the genome. The outermost labels identify specific genes and functional elements within the genome. Genes are categorized into different functional groups, such as structural proteins, virion structural proteins, tail proteins, and capsid proteins; replication and repair enzymes, DNA polymerases, DNA helicases, and DNA ligases; regulatory and accessory proteins, transposases, recombinases, and CRISPR-associated proteins; and hypothetical proteins, genes predicted from genome analysis but with unknown functions. The blue segments in the outer ring represent Coding Sequences (CDS), which are the protein-coding genes. These segments vary in size, indicating different gene lengths. The black plot in the inner circle represents GC content (Guanine-Cytosine percentage) across different regions of the genome.

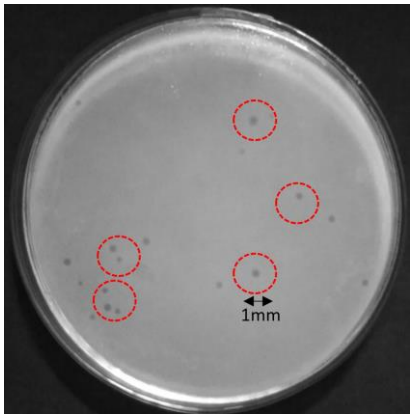

Figure 1. Plaque assay ϕSen-NF

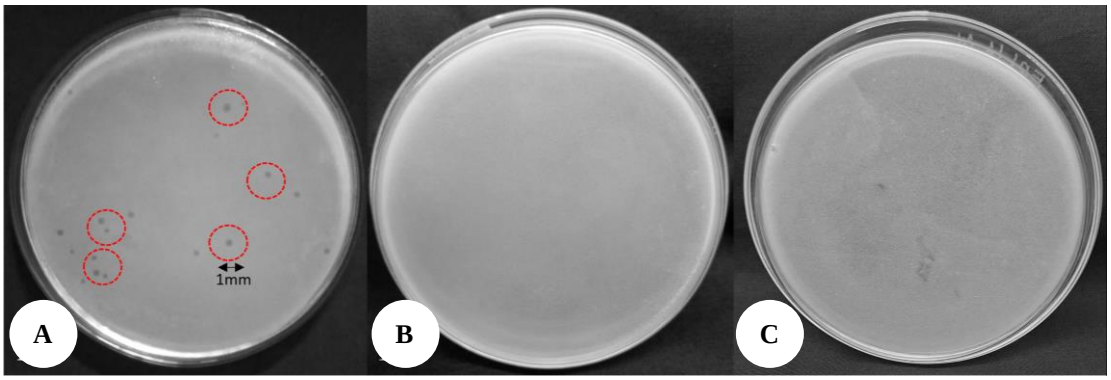

Figure 2. Host range test of ϕSen-NF: A. ser. Typhi; B. ser. Typhimurium; C. ser. Enteriditis

A comparison of genes and synthesis among various bacteriophages divided into three classes was obtained: Class I, Class II, and Class III (Figure 5). T7 genome length: 39,936 bp; SeaKAS2: 61,803 bp; SeaALZ2: 154,311 bp; S1: 53,949 bp; S1DCP: 85,395 bp; S144: 50,879 bp; Sen-NF: 108,537 bp. This analysis is profound as it visually illustrates the conservation and variation of gene organization among these bacteriophages, providing insight into their evolutionary relationships and functional similarities.

Each tree represents an evolutionary relationship among different entities. The trees are titled RNAP, DNAP, ligase, MCP, and lys, and the entities are grouped in clusters based on the evolutionary relationships (Figure 6). Each tree shows evolutionary distances and relationships between Sen-NF and other bacteriophages, including *Escherichia* phage T7, S1, S144, S19cd, SenALZ1, and SenASZ3. The placement of Sen-NF varies across trees, indicating that different genes have undergone divergent evolutionary pathways. In some cases (DNAP, Lys), Sen-NF is closely related to *Escherichia* phage T7, while in others (RNAP, Lig), it is more distant, suggesting possible gene-specific evolutionary pressures.

The phylogenetic tree represents the evolutionary relationships among various phages (Figure 7). Each branch represents different phages, with labels indicating the names of the phages, their accession numbers, and their genome sizes in base pairs (bp). The numbers on the branches indicate the evolutionary distances between the phages, while the numbers at the nodes represent bootstrap values, which indicate the reliability of the branching points. High bootstrap values (e.g., 100) indicate strong support for certain branches. The genomes of *Salmonella* phage NC_049439.1 and NC_049440.1 are very close to each other, suggesting that they may have originated from a common evolutionary ancestor or have very high genomic similarity.

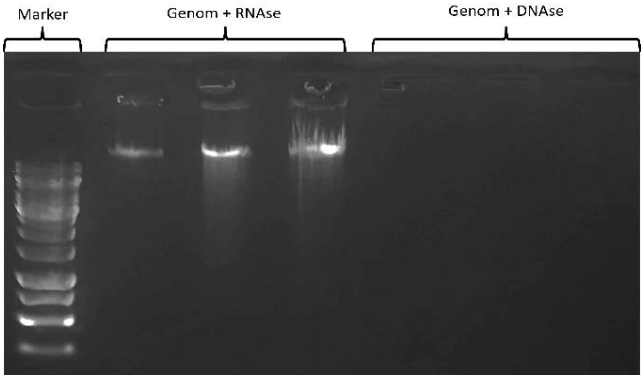

Figure 3. Nucleic acid type of ϕSen-NF

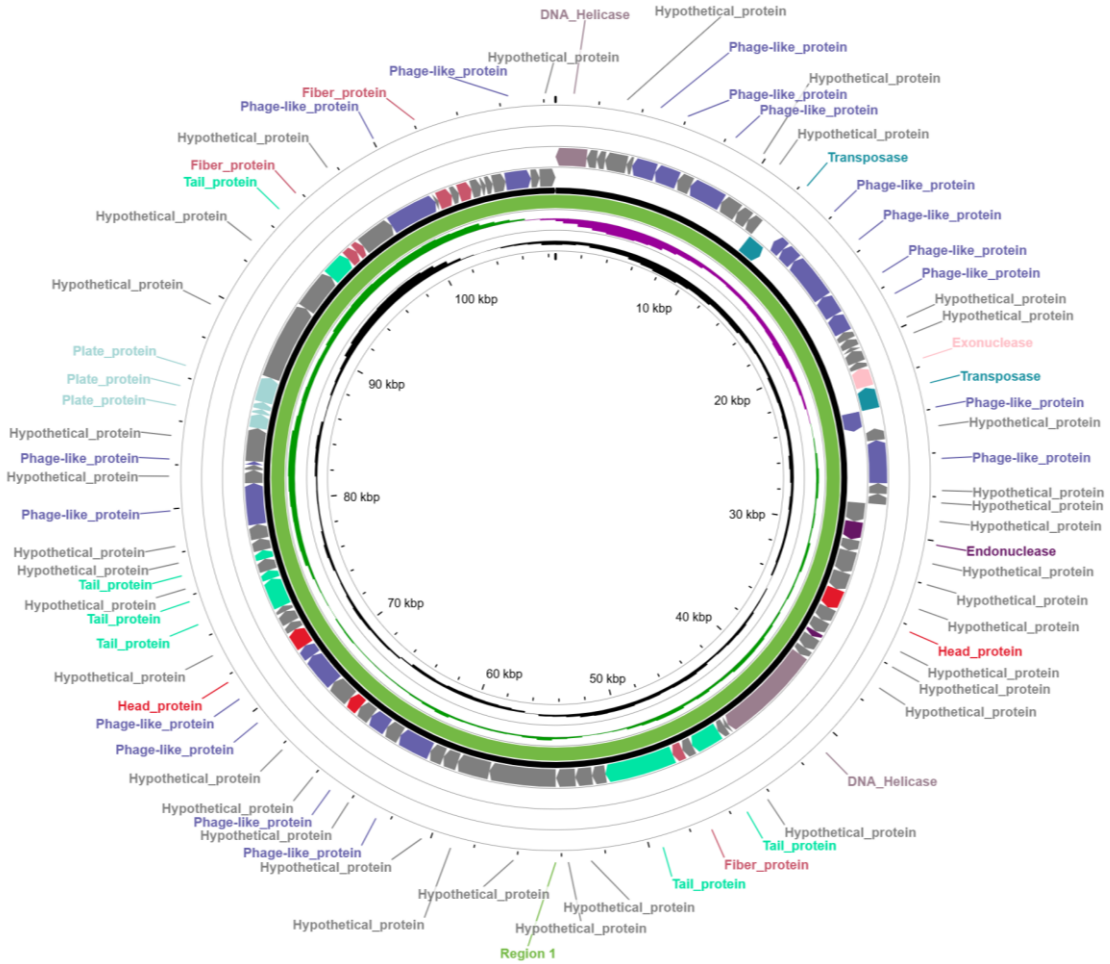

Figure 4. Circular map of ϕSen-NF

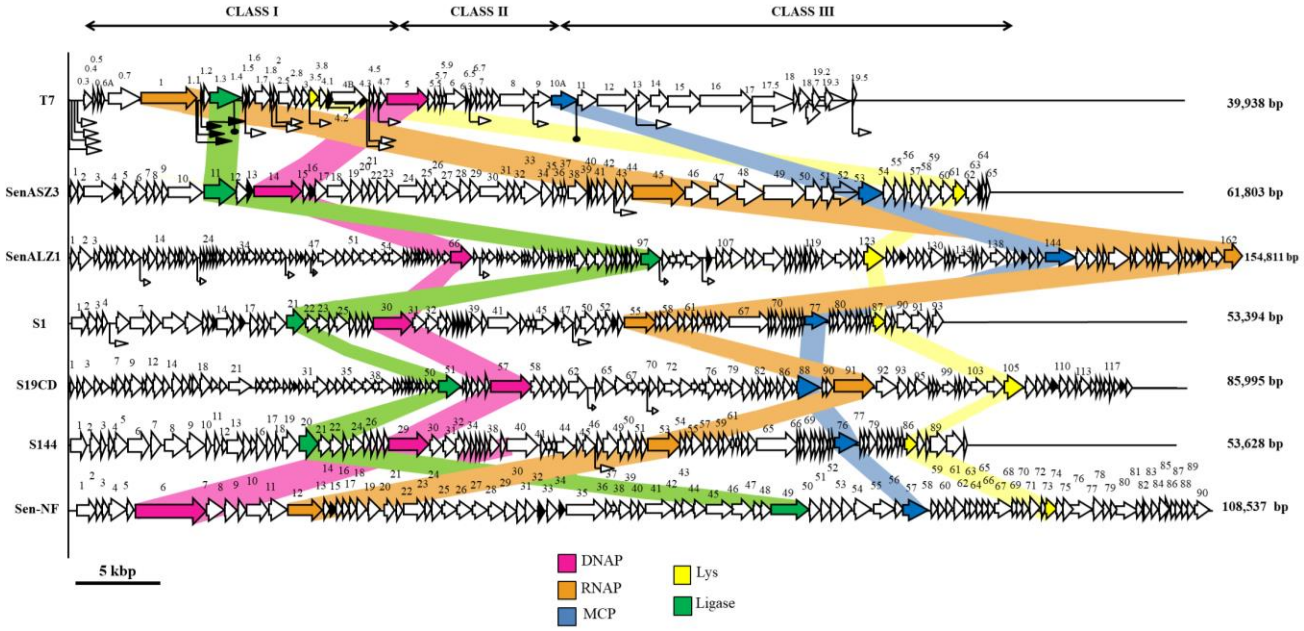

Figure 5. Comparative genomic analysis of seven different bacteriophages: T7, SeaKAS2, SeaALZ2, S1, S1DCP, S144, Sen-NF. Pink: DNA Polymerase (DNAP); Orange: RNA Polymerase (RNAP); Blue: Major Capsid Protein (MCP); Yellow: Lysin (Lys); Green: Ligase (Ligase)

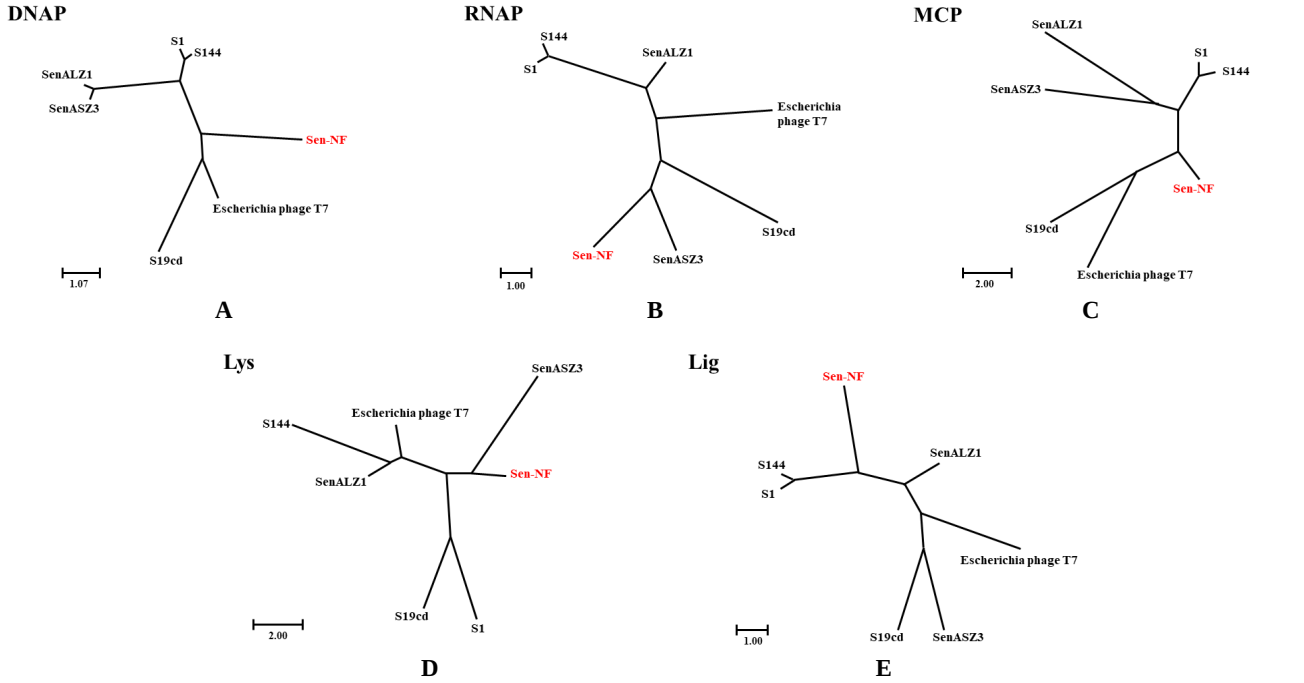

Figure 6. Phylogenetic tree with five important genes. A. RNAP; B. DNAP; C. ligase; D. MCP; E. lysozyme

Salmonella phage genomes MZ127825.1 and MT663719.1 are also in the same cluster, but slightly further away from the first two genomes. The genomes of Sen-NF, *Escherichia* phage T7, and MZ150758.1 *Salmonella* phage S19cd are on different branches, suggesting that they are more distant evolutionarily in relation to the main group. *Salmonella* phages tend to be more closely related to each other compared to *Escherichia* phages, which are on a more separate branch. Sen-NF (PV359490) appears as a separate branch, showing a significant evolutionary distance (4.953) from both *Salmonella* and *Escherichia* phages. Sen-NF has unique genetic characteristics compared to other phages in this tree. Sen-NF (PV359490) is genetically distinct from other *Salmonella* phages and *Escherichia* phage T7. It shares some evolutionary lineage with *Salmonella* phages, but it has undergone significant genetic divergence.

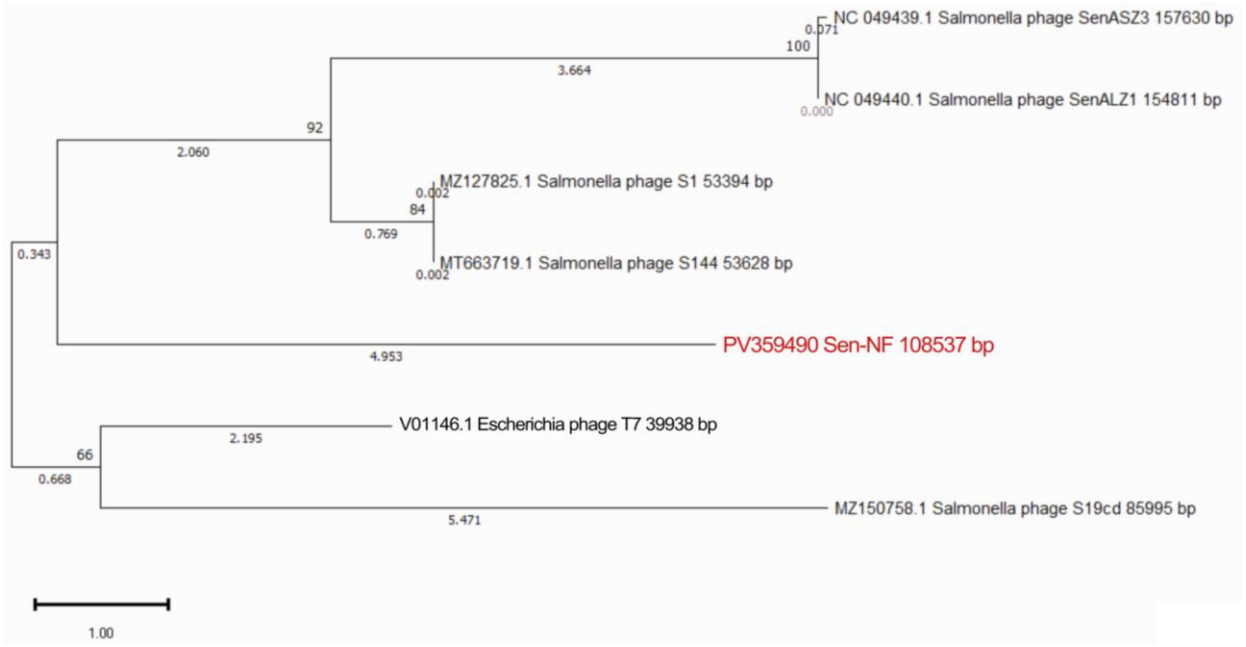

Figure 7. Phylogenetic tree of ϕSen-NF

Discussion

The plaque characteristics that were successfully purified have almost the same characteristics, namely the size and shape of the plaque is almost the same, which is ±1 mm in size. The plaque characteristics that were successfully purified have the characteristic of plaque, which is ±1mm in size. The size of the bacteriophage plaque obtained has the same plaque diameter size as the *Salmonella* phage SPYS_1 has a capsid 58.30±5.04 nm in diameter, while the tail is 133.15±8.65 nm in length and 11.37±2.63 nm in width (Song et al. 2024). The polyvalent phage vB_STM-2 within the family Myoviridae against *Salmonella typhimurium* serovars and non-*Salmonella* strains (Abdelhadi et al. 2021). Plaque size among bacteriophages can vary, with some forming plaque as small as ≤1mm in diameter. Some research indicates that bacteriophages belonging to the family Myoviridae tend to produce smaller plaques (Jurczak-Kurek et al. 2016; Narulita et al. 2018). *Salmonella enterica* became the primary host of bacteriophages isolated and purified from the same location because the isolated bacteriophages have receptors that can interact and infect the bacteria, allowing plaque formation. Receptors with different structures will not allow interaction, so there will be no plaque formed due to the absence of bacteriophage infection of bacterial cells (Shin et al. 2012; Esteves et al. 2023).

The plaque produced by the Myoviridae family is relatively small compared to the Siphoviridae and Podoviridae families, which can produce large plaques. This is because the Myoviridae family has a large capsid (head) size which results in a slower diffusion rate into bacterial cells compared to the Siphoviridae and Podoviridae families, which have smaller capsid (head) sizes so that the diffusion process in host cells is faster (Ramesh et al. 2019; Trofimova and Jaschke 2021). The capsid size of the Myroviridae family ranges from ~111 × 78 nm, larger than the Siphoviridae (~60 nm) and Podoviridae (~60 nm) families (Tamakoshi et al. 2011; Zinke et al. 2022).

Plaques that appeared were classified as lytic (virulent) bacteriophages because the lytic-type has a clear plaque while the lysogenic type is cloudy. Lytic-type bacteriophages replicate by injecting nucleic acids and taking over the metabolism of the host cell, which results in the lysis of the host cell, while lysogenic bacteriophages only integrate nucleic acids in the host cell so that the bacterial host cell can move (Gallet et al. 2012; Jurczak-Kurek et al. 2016). ϕSen-NF can only infect bacteriophages of *Salmonella enterica* ser. Typhi. Based on the level of plaque turbidity, the plaque results show a lytic-type bacteriophage because the plaque produced is very clear. A good bacteriophage is one with a lytic cycle, because it can cause complete lysis of bacterial cells and produce mature bacteriophages.

Conducted using a double-layer agar method to confirm lytic activity. The appearance of clear plaque that appears in lytic-type bacteriophage infection indicates that lysis has occurred in the host bacterial cells. Clear plaques (~1 mm diameter) indicated a strictly lytic lifecycle. In comparison, the cloudy plaque in lysogenic infection indicates that there is a symbiotic relationship between the host bacteria and the bacteriophage, characterized by the merging of the genetic material of both forming a prophage. In addition, cloudy plaque can also occur due to incomplete lysis due to a weak adsorption rate on host cells, resulting in immature bacteriophage particles in the lytic cycle process (Gallet et al. 2012; Jurczak-Kurek et al. 2016; Kawasaki et al. 2016). Spot tests on three *Salmonella* serovars (Enteriditis, Typhi, Typhimurium) revealed ϕSen-NF exclusively infects *S. enterica* ser. Typhi.

There are two types of bacteriophage nucleic acids, namely DNA and RNA. Both types of nucleic acids can be identified by extracting bacteriophage genomic DNA and DNAse, and RNAse treatments. Genomic DNA that is degraded by the DNAse enzyme indicates that the genomic DNA is classified as DNA, if the RNAse enzyme can be degraded then the bacteriophage nucleic acid is classified as RNA. Based on the results of the nucleic acid type test, it can be seen that the bacteriophage ϕSen-NF belongs to DNA nucleic acid (Figure 3). This is because the DNAse enzyme degrades the genomic DNA of the eight isolates but does not degrade by the RNAse enzyme. The DNAse enzyme itself is an enzyme that plays a role in the DNA degradation process (Singh et al. 2023).

The Sen-NF bacteriophage's genome is 108,537 bp with a G+C content of 46.8% (Figure 5), which is similar to that of the *Salmonella* bacteriophage (Sun et al. 2022). The genome sizes of *Salmonella enterica* phages ranged from 41 to 114 kb, while their GC contents were between 39.9 and 50.0% (Bhandare et al. 2024). *Salmonella* phage Season12 has a genome size of 59,059 bp and a GC content of 56.5% (Hendrix et al. 2015). Analysis of the bacteriophage genome revealed 96 Open Reading Frames (ORFs) (Table S1). The minimum and maximum lengths of the ORFs were 123 and 5,796 bp, respectively. According to the BLAST results, 49 ORFs had hypothetical functions, and the remaining genes encoded hypothetical proteins. This may be due to the diversity of bacteriophages and insufficient database information on phage functional genes. In addition, no tRNA genes were identified in the genome. Similar to most bacteriophage genomes, the ϕSen-NF genome is tightly packed: approximately 90% of the genome sequence encodes gene products.

Based on bioinformatics predictions, the ϕSEN-NF genome is functionally divided into four kinds, which include DNA replication/regulation, packaging, host cell lysis, and structural proteins. BLAST, HMMER, and InterProScan identified: Class I, early transcription regulators (e.g., RNA polymerase); Class II, DNA metabolism enzymes (e.g., DNA polymerase, ligase); and Class III, Structural proteins (e.g., major capsid protein). Synteny analysis with phages like ϕSE24 and ϕSw2 revealed conserved gene clusters (Figure 5). Five gene products are predicted to be involved in supporting bacteriophage infection and reproduction in bacterial cells, and the five gene products include DNA polymerase (ORF6, ORF7, ORF9), RNA polymerase (ORF18), and ligase (ORF55). The large subunit of terminase and the small subunit of terminase are predicted to be related to phage packaging, which is a functional protein required for phage assembly. Genes encoding phage structural modules include tail fiber protein (ORF46, ORF82, ORF83, ORF87, ORF89), head and tail connected protein (ORF36), tail protein (ORF47, ORF81) (Table S1).

Phylogenetic-related analysis of bacteriophages is based on several important genes, such as RNAP, DNAP, ligase, MCP, and lys. RNAP (RNA polymerase) shows evolutionary relatedness based on the gene encoding the RNA polymerase enzyme, while ϕSen-NF is along with SE24 and Sw2, showing significant genetic similarity. This cluster is separate from bacteriophages such as T3 and T7 as well as more distant groups such as LVR16A (Figure 6.A). DNAP (DNA polymerase) illustrates genetic relationships based on DNA polymerase genes. ϕSen-NF is again in the green cluster with SE24 and Sw2, indicating evolutionarily close relationships, and this cluster is significantly different from T3, T7, SenASZ3 and the LVR16A, S116 (Figure 6.B). Ligase gene analysis revealed a different pattern of evolutionary relationships. ϕSen-NF joined along with T3 and T7, suggesting that ligase gene function is more similar to bacteriophages in this cluster (Figure 6.C).

MCP (Major Capsid Protein) is the main capsid protein. The clusters again showed the association between ϕSen-NF, SE24, and Sw2, and there was a separation of T3, T7, SenASZ3 and LVR16A, S116, S133 clusters, which confirmed the differences in capsid structure between groups (Figure 6.D). Analysis was conducted on the lys gene (Lysin), which plays a role in bacterial lysis by bacteriophages. ϕSen-NF remained in the cluster, showing genetic proximity to SE24 and Sw2, reflecting variations in lysis mechanisms between bacteriophage groups (Figure 6.E). ϕSen-NF consistently falls within the SE24 and Sw2 in most phylogenetic analyses, indicating a close genetic relationship and similar evolutionary potential. Variations in relationships in certain genes, such as ligases (Figure 6.C), suggest differences in function or specific genetic adaptations. This analysis helps explain the evolutionary position of ϕSen-NF among other bacteriophages and provides insight into the underlying genetic diversity. Neighbor-joining trees placed ϕSen-NF closest to *Salmonella* phages NC_049439.1 and NC_049440.1, distinct from *E. coli* phage T7 (Figure 7).

In conclusion, bacteriophage ϕSen-NF can only infect *Salmonella enterica* ser. Typhi, which has a diameter of ±1mm with a clear plaque that shows lytic properties, and bacteriophage ϕSen-NF is a linear double-stranded DNA virus with a genome size of 108,537 bp and a G+C content of 46.8%. It encodes 96 potential Open Reading Frames (ORFs), which are classified into three functional classes based on genetic structure and replication mechanism. Comparative genomic analysis revealed similarities with the family Myoviridae, such as ϕSE24 and ϕSw2. Class I genes are involved in early transcription, Class II in DNA metabolism, and Class III in structural protein formation. Finally, phylogenetic reconstruction using whole genome sequences and key genes (RNAP, DNAP, ligase, MCP, and lysozyme) showed a close evolutionary relationship with phages such as ϕSE24 and ϕSw2.

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Table S1. List of Open Reading Frame (ORF) *Salmonella* Phage Sen-NF

Open reading frame	CDS position	Name of gene	E-value
1	1..1665	Putative DNA repair helicase	0.0
2	1666..2211	Hypothetical protein	9.05e-81
3	2212..2655	Hypothetical protein	3.03e-55
4	2656..3834	Hypothetical protein	0.0
5	3835..4140	Hypothetical protein	2.12e-14
6	4141..5415	Putative DNA polymerase I	0.0
7	5416..6690	Putative DNA polymerase I	0.0
8	6691..7452	Hypothetical protein	4.73e-32
9	7453..9324	Putative DNA polymerase I	0.0
10	9325..10329	Hypothetical protein	1.27e-45
11	10330..11040	Hypothetical protein	3.78e-13
12	11041..11661	Hypothetical protein	2.05e-65
13	11662..12834	Transposase IS605	1.48e-56
14	12835..13482	Oxygenase	6.19e-94
15	13483..14358	Asparagine synthase	3.85e-171
16	14359..16827	Putative reca protein	0.0
17	16828..17982	Putative single stranded Dna binding protein	3.37e-92
18	17983..19020	Putative rpod subfamily RNA polymerase Sigma-70 subunit	5.08e-164
19	19021..19452	Hypothetical protein	3.12e-10
20	19453..19929	Hypothetical protein	3.66e-12
21	19930..20133	Hypothetical protein	3.25e-18
22	20134..20787	Hypothetical protein	1.39e-87
23	20788..21156	Hypothetical protein	7.3e-12
24	21157..22170	Putative exonuclease	1.34e-141
25	22231..23406	Transposase IS605	2.4e-62
26	23407..24471	Putative DNA primase	1.4e-162
27	24472..25104	Hypothetical protein	5.82e-54
28	25105..27423	Putative LPXTG cell wall anchor	3.18e-17
29	27424..27987	Hypothetical protein	5.81e-55
30	27988..28551	Hypothetical protein	5.26e-77
31	28552..29655	Hypothetical protein	5.19e-135
32	29656..30696	Putative recombination Related endonuclease	1.08e-162
33	30697..31365	Hypothetical protein	6.97e-41
34	31366..32637	Hypothetical protein	3.36e-165
35	32638..33678	Hypothetical protein	2.03e-98
36	33679..34839	Putative head-to-tail joining protein	0.0
37	34840..35643	Hypothetical protein	1.14e-81
38	35644..36384	Hypothetical protein	2.35e-50
39	36385..36732	Putative HNH endonuclease	3.05e-41
40	36733..37515	Hypothetical protein	7.84e-75
41	37516..37986	Hypothetical protein	4.91e-19
42	37987..43782	Putative ATP-dependent DNA helicase	0.0
43	43999..44469	Hypothetical protein	2.84e-32
44	44470..46152	Putative tail sheath protein	0.0
45	46153..46770	Hypothetical protein	2.22e-90
46	46771..47391	Putative long tail fiber	1.47e-49
47	47392..51399	Putative major tail protein	0.0
48	51400..52179	Hypothetical protein	5.12e-110
49	52180..53139	Hypothetical protein	2.12e-123
50	53140..54237	Hypothetical protein	9.43e-135
51	54259..58083	Hypothetical protein	4.84e-117
52	58084..59964	Hypothetical protein	1.4e-112
53	59965..60831	Hypothetical protein	1.63e-35
54	60832..61650	Hypothetical protein	1.48e-36
55	61651..63591	Putative DNA ligase	0.0
56	63592..64485	Hypothetical protein	1.16e-78
57	64486..65580	Putative glycosyl transferase	1.56e-93
58	67183..68487	Hypothetical protein	6.51e-64
59	68488..70455	Putative atpase	7.54e-70
60	70456..71172	Putative structural protein	2.12e-124
61	71173..72261	Putative major capsid protein	0.0
62	72262..72753	Hypothetical protein	2.63e-16
63	72754..73449	Hypothetical protein	4.69e-51
64	73450..73794	Hypothetical protein	4.1e-48
65	73795..75483	Putative tail sheath protein	0.0
66	75484..75996	Putative tail tube protein	1.97e-89
67	75997..76686	Hypothetical protein	4.82e-58
68	76687..77205	Putative tail tube protein	3.08e-71
69	77206..77883	Hypothetical protein	1.71e-53
70	77884..78723	Hypothetical protein	1.75e-95
71	78724..81099	Putative vgrg protein	6.43e-70
72	81100..81855	Hypothetical protein	2.19e-80
73	81856..82149	Hypothetical protein	2.96e-27
74	82150..82350	Ssdna binding protein	1.96e-07
75	82351..84267	Hypothetical protein	0.0
76	84268..85098	Putative baseplate protein	1.31e-107
77	85099..85383	Putative baseplate spike protein	3.56e-15
78	85384..85791	Baseplate protein	6.94e-46
79	85792..87255	Putative baseplate wedge Subunit protein	9.59e-177
80	87256..91740	Hypothetical protein	0.0
81	94057..95487	Putative tail protein	7.44e-80
82	95488..96117	Putative tail fiber protein	1.64e-51
83	96118..96612	Putative tail fiber protein	3.59e-49
84	96613..98625	Hypothetical protein	0.0
85	98626..101481	Putative ILEI domain-Containing protein	6.29e-153
86	101482..101658	Hypothetical protein	3.64e-06
87	101659..102477	Putative tail fiber protein	1.4e-101
88	102478..102909	Hypothetical protein	2.27e-15
89	102910..103665	Putative long tail fiber	1.22e-54
90	103666..104247	Hypothetical protein	5.5e-06
91	104248..104514	Hypothetical protein	7.85e-11
92	104515..104919	Hypothetical protein	1.16e-14
93	104920..105666	Hypothetical protein	7.12e-97
94	105667..107127	Putative virion protein	8.94e-69
95	107128..107631	Hypothetical protein	1.17e-44
96	107632..108537	Hypothetical protein	2.51e-19