Short communication

Selection of polyvalent bacteriophages infecting *Salmonella enterica* serovar Choleraesuis

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Background: Ideally, bacteriophages of pathogenic bacterial hosts should be polyvalent to be able to replicate in an alternative nonpathogenic bacterium. Thus, accidental infection by the original host can be avoided when bacteriophage lysates are used in biocontrol protocols.

Results: From 15 wastewater samples, collected at different sites in the V Region in Chile, we selected three bacteriophages (FC, FP, and FQ) capable of productively infecting *Salmonella enterica* serovar Choleraesuis. By transmission electron microscopy (TEM) observation, the bacteriophages were found to belong to the order Caudoviridae. Molecular analyses indicated that FC, FP, and FQ contained double-stranded DNA genomes, of sizes similar to bacteriophage P22, and distinct recognition sites for the restriction endonucleases HaeIII and HindIII. Assays of host range revealed that the bacteriophages were polyvalent and thus capable of infecting different strains of *Escherichia coli* and other serovars of *Salmonella*.

Conclusion: We have isolated new bacteriophages of the serovar Choleraesuis with various potential applications in relation to this pathogenic bacterium.

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1. Introduction

First described in the beginning of the last century, polyvalent bacteriophages are capable of productively infecting more than one host [1]. These bacteriophages were reported for members of Enterobacteriaceae [2]. The authors obtained isolates infecting *Escherichia coli*, Klebsiella pneumoniae, and Aerobacter aerogenes.

Among polyvalent bacteriophages, those active against *Salmonella enterica* are promising as they can be isolated and used as alternative or complementary biocontrol agents against this pathogen [3,4]. In this respect, bacteriophages that can replicate in *Salmonella* and nonpathogenic strains of *E. coli* are particularly useful. Bacteriophage production in *E. coli* would be safer than propagation in the original pathogenic host as these bacteriophage preparations may accidentally deliver the pathogen to the host’s cells. However, studies related to this topic are scarce. Notably, Bielke et al. [5] tested the lytic activity of wide-host-range bacteriophages on *Salmonella* strains for reducing *Salmonella* counts in poultry products [6].

More recently, three bacteriophages isolated as part of the European project Phaghevet-P were tested for polyvalency. Of these bacteriophages, phi PVP-SE exhibited a lytic effect on different *Salmonella* serovars and two nonpathogenic *E. coli* strains [7]. In addition, phiKP26, another polyvalent bacteriophage, was found to infect *Salmonella* and *E. coli* [8].

Recently, Leon et al. [9] found that the classic *E. coli* lysogenic bacteriophage P1 could naturally infect and proliferate in *S. enterica* serovar Choleraesuis, in contrast to other serovars of *Salmonella*, which are not naturally susceptible to P1. These results indicate that this bacterium shares certain features with the original host of P1. *E. coli*. Thus, we hypothesized that the selection of bacteriophages active against serovar Choleraesuis could yield isolates capable of infecting *E. coli*.

In this study, for the first time, we report the isolation of bacteriophages using *S. enterica* serovar Choleraesuis as a selective host. The three DNA bacteriophages obtained, belonging to the order Caudoviridae based on morphology, were found to be polyvalent and capable of infecting different strains of *E. coli* and other serotypes of *S. enterica*.

2. Materials and methods

2.1. Isolation and purification of lytic bacteriophages against serovar Choleraesuis VAL 201

2.1.1. Sampling

Wastewater samples were collected from several estuaries in the V Region of Chile: San Antonio, El Tabo, Concón, Higueirillas, 2 Norte, Quintero, Loma Larga I, Loma Larga II, Cartagena, Algarrobo, and Caleta.
Portales. They were collected in sterile glass bottles, transported to the laboratory in a cooler, and maintained at 4°C until processing the next day.

2.2.1. Transmission electron microscopy

2.2. Phage characterization

Shaken cultures were monitored based on the OD550 values until a growing cultures of VAL 201 with individual phage plaques. The phage DNA was precipitated with ethanol and stored in TE buffer at -20°C until use [11]. To analyze the genetic material of bacteriophage virions, the genomic material of the three phages. Of the 15 samples studied, 13 displayed phages active against VAL 201, which formed either clear or turbid plaques. From the phage producing a clear plaque (1 mm in diameter), we consistently found that of the 43.5-kbp genome of the temperate bacteriophage P22. Were found to have similar molecular mass, approximately equivalent to that of the 43.5-kbp genome of the temperate bacteriophage P22. These results are shown in Fig. 2.

3. Results

3.1. Selection of lytic bacteriophages

3.2. Bacteriophage morphology

3.3. Characterization of viral genomic material

First, we tested whether the genomic material of FC, FP, and FQ was DNA. Upon treating the phage genomic material with DNase I, the nucleic acids of all three phages were found to be sensitive to this nuclease, indicating DNA as the phage genomic material. Furthermore, upon agarose gel electrophoresis, the DNA genomes of FC, FP, and FQ were found to have similar molecular mass, approximately equivalent to that of the 43.5-kbp genome of the temperate bacteriophage P22. These results are shown in Fig. 2.

In addition, we tested the sensitivity of bacteriophage DNA genomes to the restriction endonucleases BamHI, EcoRI, HaeIII, and HindIII to determine the differences between FC, FP, and FQ. We found that EcoRI discriminated between the three phages and BamHI between FP and the other two phages. Restriction with HaeIII and HindIII led to the formation of multiple fragments in the three viral genomes. The restriction patterns, shown in Fig. 3, helped clearly differentiate the genomes of the three phages under study. Moreover, restriction with the tested endonucleases indicated double-stranded DNA as the genomic material of the three phages.
3.4. Host range of bacteriophages

The plaque assays of the FC, FQ, and FP phages were conducted using different strains of *E. coli* and *Salmonella*. These phages were found to be polyvalent, capable of infecting many of the strains tested in our experiments, via clear plaque formation. These results are shown in Table 1. The FP bacteriophage showed the widest host range. Furthermore, we observed that bacteriophage-insensitive mutants arose at a frequency of about $10^{-7}$.

3.5. In vitro VAL 201 decay caused by FC, FP, and FQ

To assess the infective activity of our phage isolates against the serovar *Choleraesuis*, we determined the corresponding bacterial decay curves. FC, FP, and FQ were added separately to exponentially growing cultures (37°C) of serovar *Choleraesuis* strain VAL 201 using a MOI of 1. The resulting decay was followed by a decrease in optical density. The results are shown in Fig. 4.

4. Discussion

In this study, we aimed to isolate and characterize polyvalent bacteriophages that can infect both serovar *Choleraesuis* and *E. coli*. We obtained the FC, FP, and FQ phages, which were found to contain double-stranded DNA genomes. This finding is consistent with the high prevalence of this type of phage in nature [15].

Furthermore, analyses involving transmission electron microscopy (TEM) micrographs and phage DNA restriction patterns allowed us to distinguish between the three phages more precisely. In fact, the FP bacteriophage could be clearly differentiated from FC and FQ, because of its long, flexible tail, whereas the latter shared similar morphology.

The electrophoretic analyses of bacteriophage DNA indicated that all three phages had genomes of approximately the same size.
However, the analysis with restriction endonucleases showed differences between FC and FQ, which otherwise appear highly similar. Again, consistent with morphological data, DNA analyses showed that FP was clearly distinct from FC and FQ.

Based on these analyses, we then examined the International Committee on Taxonomy of Viruses (ICTV) criteria for the taxonomic classification of viruses [16]. Based on these data, we suggest that FC, FP, and FQ be classified in the order Caudovirales, characterized by bacterial viruses with double-stranded DNA in non-enveloped capsids, icosahedral heads, and rigid/flexible helical tails.

FC, FQ, and FP can be further distinguished by their tail morphology. We suggest that FC and FQ be classified under Myoviridae and FP under Siphoviridae [16].

It is well known that phages are host specific in their lytic activity, which depends on the presence of particular bacterial cell receptors and other factors that control the ability of bacteriophages to multiply in their hosts [17]. However, others [5,18] have argued that not all phages are host specific; moreover, polyvalent phages that are capable of proliferating in different bacterial genera have also been found. The latter were found in this study, as FC, FP, and FQ were capable of productively infecting strains of *E. coli* and *S. enterica*. This suggests the presence of common recognition sites for the three phages, at least in *E. coli* C and serovar Choleraesuis. Furthermore, this is also consistent with the close phylogenetic link between these two bacteria [19]. In addition, due to their polyvalent nature, of FC, FP, and FQ can be propagated in the nonpathogenic host *E. coli* C, precluding potential risks involved in the use of bacteriophage preparations [7].

In summary, we have isolated and characterized new bacteriophages of the serovar Choleraesuis, which can be applied in various ways [20] in relation to this pathogenic bacterium.

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**References**


**Table 1**

<table>
<thead>
<tr>
<th>Host</th>
<th>FC</th>
<th>FQ</th>
<th>FP</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em> Sw</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>Escherichia coli</em> B</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td><em>Escherichia coli</em> C</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td><em>Escherichia coli</em> K12</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella Choleraesuis</em> (VAL 201)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td><em>Salmonella Enteritidis</em> 13076</td>
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<tr>
<td><em>Salmonella Enteritidis</em> PF4</td>
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<td><em>Salmonella Typhimurium</em></td>
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<td><em>Salmonella Agona</em></td>
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<td><em>Salmonella Typhi</em></td>
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<tr>
<td><em>Salmonella Pullorum</em></td>
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<td>-</td>
<td>+</td>
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<tr>
<td><em>Salmonella Paratyphi</em> B</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
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(+) lytic plaque; (-): no plaque.


