PHAGE: Therapy, Applications, and Research
Volume 4, Number 3, 2023

DOI: 10.1089/phage.2023.0014

Producing Tailocins from Phages Using Osmotic Shock and Benzalkonium Chloride

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Abstract

In the light of the worldwide antimicrobial resistance crisis, new substitutes to antibiotics are urgently needed. Tailocins or phage tail-like bacteriocin particles, produced by bacteria for environmental competition, are a potential antimicrobial alternative to antibiotic treatment. Yet, the availability of characterized Tailocins is limited. We explored the possibility to produce new Tailocins from phage particles, using osmotic shock or chemical treatment by the ammonium quaternary compound benzalkonium chloride on *Ackermannviridae* phage S117 and using *Straboviridae* phage T4 as control. We report that phage S117 was resistant to such treatment, while successful production of Tailocins by osmotic shock was achieved for phage T4. Finally, chemical treatment with benzalkonium chloride was inefficient on phage S117 but successfully inactivated phage T4 without production of Tailocins. Further studies are needed to implement such treatments of phages for producing Tailocins with killing activity.

Keywords: Tailocin, phage tail-like particle, Bacteriophage, head-less phage, antimicrobial, osmotic shock, quaternary compound

Introduction

With an estimated 1.27 million deaths in 2019 related to bacterial antimicrobial resistance worldwide,[1] the need for alternative to antibiotics treatment is more urgent than ever.[2] Bacteriocins are compounds produced by bacteria that inhibit the growth of neighboring bacterial species.[3] They are used by bacteria to fight for resources against other competitive bacterial strains and are therefore considered a potential alternative to antibiotics. Tailocins, or phage tail-like bacteriocins,[4,5] structurally and functionally resemble phage tails without a head, therefore, containing no genetic material. They kill upon contact with their targeted bacteria by puncturing their membranes leading to cell lysis.[4] Two types of Tailocins have been characterized, the R-type that possess a contractile tail, and the F-type that has a flexible but noncontractile tail.[6] While Tailocins have been isolated from both Gram-positive and Gram-negative bacterial species, the availability of this antimicrobial is still limited as only a few have been characterized so far.[5] Therefore, there is a need for development of new strategies to produce Tailocins. In view of how structurally similar phages and Tailocins are, the latter could be thought of as a headless phage. Thus, phage particles may be converted into Tailocins by rupturing their head/tail junctions. Osmotic shock has been previously shown to produce empty-headed (or ghost) phage particles as well as phage tails.[7,8] Additionally, chemical inactivation by ammonium quaternary compounds of phage infecting lactic acid bacteria has shown to be able to rupture the phage head/tail junction.[9]

Here, we have explored both methods to obtain Tailocins from the *Ackermannviridae* phage S117. *Ackermannviridae* phages show high potential as antimicrobials as they encode multiple tail spikes that can target different bacteria.[10] We have submitted S117 to osmotic shock and chemical treatment by benzalkonium chloride, a class of quaternary ammonium compounds,[11] using *Straboviridae* phage T4 as a control.[11]

Materials and Methods

Phage stocks and host strains

Phage T4[12] was prepared according to the method for phage amplification on solid media[13] using *Escherichia coli* stellar cells as host. Briefly, the bacterial host was grown until exponential phase and added together with the phage to

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melted Luria agar broth. After overnight incubation, 5 mL of Sodium Magnesium buffer (SM) phage buffer[13] was added on top of the agar plate showing phage plaques confluence and incubated for 24 h at 4°C under 80 rpm shacking. Phages were then filter-sterilized using 45 and 22 μm filters and stored at 4°C for further use. *Ackermannviridae* Phage S117[10] stock was prepared using the same method as for phage T4, using *Salmonella enterica* LT2C strain[14] as a propagation host.

Osmotic shock

One volume of phage stock was mixed for 60 min in 2 volumes of a 4M solution of concentrated salts (Sodium Chloride NaCl, Sodium Acetate NaAc, Cesium Chloride CsCl) to a final concentration of 3M, and then rapidly diluted into 1000 volumes of distilled water. Residual phage activity was assessed as described in the phage activity section.

Chemical inactivation

One tenth of phage stock was mixed for 30 min with benzalkonium chloride (Merck©) at final concentrations of 10%, 1%, 0.1%, or 0.01%. Residual phage activity was assessed as described in the phage activity section.

Phage activity

Phage activity was assessed using plaque assay method.[15] Briefly, phages exposed to the compounds as well as untreated phages were 10-fold serial diluted from 10^{-1} to 10^{-8} and subsequently 10 μL of each dilution was spotted on a lawn of the host bacterium of the phage plated on Luria Broth plates in 0.6% top agar. Plates were incubated at 37°C overnight and number of plaques at each dilution used to calculate the pfu/mL.

Tailocin killing assay

The conversion of phage into Tailocin particles was assessed by evaluating the number of surviving cells after mixing Tailocin with its bacterial host for 20 min and then plated serial dilutions on Luria Broth plates. The number of surviving colonies is expressed as cfu/mL after overnight incubation at 37°C, in comparison with an untreated control, allowed to calculate the killing efficiency.

Results

Osmolarity change inactivates phage T4 but is ineffective on Ackermannviridae *phage S117*

Osmotic shock was considered as a possible way of producing Tailocins from phage particles. To investigate this, we mixed phages T4 and S117 at 3.0×10^9 and 3.0×10^{11} pfu/mL, respectively, with solutions of either NaCl, NaAc, or CsCl to final concentrations of 3M. Subsequently, active phage particles were enumerated by performing plaque assays on their respective host. In the high salinity buffers, no reduction in the number of active phage particles was observed for any of the phages as compared to control in SM buffer, as expected. Following rapid dilution in 1000 volume of distilled water, the number of remaining active phages was determined by plaque assay on their respective hosts. While phage T4 showed 90% reduction in infection efficiency after osmotic shock from high to low salinity, phage S117 showed no reduction of activity after the osmotic shock in any of the salts tested (Table 1). The experiment was repeated, and the same results were obtained.

Table 1. Osmotic Shock Effect on T4 and S117 Phages Infectivity

	SM buffer	*NaCl*[a]	*Sodium acetate*[a]	*CsCl*[a]
Control				
T4[b]	3.0×10^9	3.0×10^9	3.0×10^9	3.0×10^9
S117[b]	3.0×10^{11}	3.0×10^{11}	3.0×10^{11}	3.0×10^{11}
After Osmotic shock				
T4[b]	3.0×10^8	2.9×10^8	3.1×10^8	3.0×10^8
S117[b]	3.0×10^{11}	2.8×10^{11}	3.2×10^{11}	3.1×10^{11}
Residual infectivity, %				
T4[b]	10	10	10	10
S117[b]	100	100	100	100

[a]Concentration of 3M.
[b]Expressed as pfu/mL.
SM, Sodium Magnesium buffer.

To evaluate if the osmotic shocked T4 phages were converted into Tailocins, we determined the remaining killing activity of the treated phage stock, considering that 10% of the phage particles were still infective. Inactivated phages T4 (by NaCl, NaAc, or CsCl) were mixed with 10^8 *E. coli* cells at multiplicity of infection (MOI) of 1 and incubated for 20 min at room temperature before plating allowing only one phage infection cycle. As T4 life cycle takes $\sim$30 min from adsorption to burst of the infected cells,[12] it can be assumed that the remaining 10% infective phage particles only contribute to up to 10% reduction of the surviving colonies. After overnight growth at 37°C, surviving colonies were counted, and the residual killing activity caused by T4-derived Tailocin was calculated. Osmotic-shocked phage T4 showed a 2/3 residual killing activity on *E. coli* (Table 2), suggesting that osmotic-shocked phages T4 particles conserved their killing properties.

Benzalkonium chloride effectively inactivates phage T4 and S117, yet do not produce Tailocins

Benzalkonium chloride was tested for its efficiency in inactivating phages T4 and S117, and possibly for

Table 2. Tailocin Killing Efficiency

	SM buffer (control)	*Tailocin T4 (NaCl)*	*Tailocin T4 (NaAc)*	*Tailocin T4 (CsCl)*
Surviving colonies[a]	1.5×10^8	4.0×10^7	5.0×10^7	3.5×10^7
Tailocin killing efficiency (%)[b]	0	73	67	77

[a]cfu/mL.
[b](Surviving colonies)/(initial amount of cells)$\times$100.

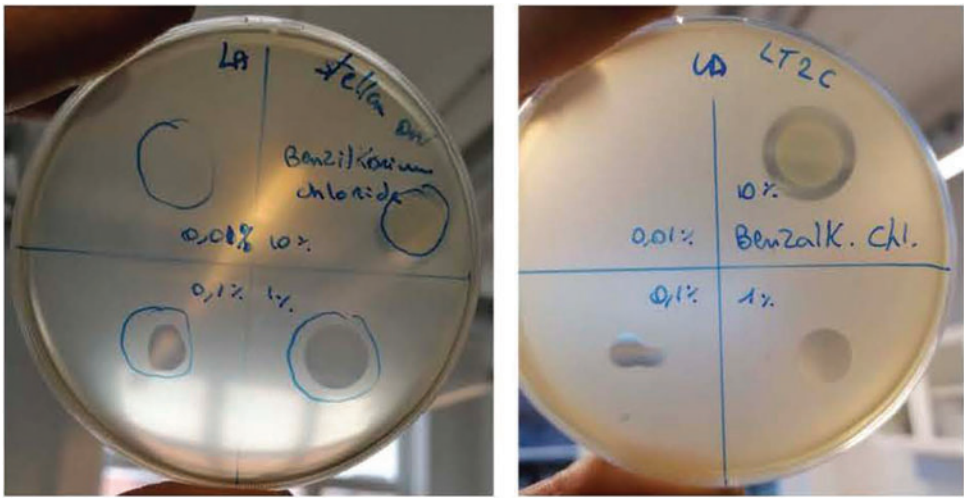

FIG. 1. Benzalkonium chloride effect on *Escherichia coli* stellar and *Salmonella* LT2C.

producing Tailocins. First, as negative control benzalkonium chloride was tested on a lawn of *E. coli* or *Salmonella* cells grown to exponential phase. Ten microliters of 10%, 1%, 0.1%, and 0.01% were tested for their direct effect on *E. coli* and *Salmonella*. At a concentration of 10%, 1% and 0.1% benzalkonium chloride produced clear lysis zone on both *E. coli* and *Salmonella* lawn, while no effect was observed at 0.01% (Fig. 1). Therefore, we used benzalkonium chloride at a final concentration of 0.01% in the following experiments.

Phages T4 and S117 at 3.0×10^{9} and 3.0×10^{11} pfu/mL, respectively, were mixed with benzalkonium chloride at a final concentration of 0.01%. The phages in presence of benzalkonium chloride were then incubated at room temperature for 20 min before testing for remaining phage activity by plaque assay. In presence of 0.01% benzalkonium chloride phage T4 showed a four-log activity reduction as compared to the SM buffer control (Table 3). On the contrary, phage S117 showed no activity reduction in presence of 0.01% benzalkonium chloride (Table 3). The experiment was repeated, and the same results were obtained.

The benzalkonium chloride T4 inactivated phages were then tested for remaining killing activity, which would indicate the conversion of phage into Tailocin particles. Inactivated phages with 0.01% benzalkonium chloride were mixed with 10^{8} *E. coli* cells at MOI of 1 and incubated for 20 min at room temperature before plating. After overnight growth at 37°C, surviving colonies were counted. Benzalkonium chloride-inactivated phage T4 showed no residual killing activity on *E. coli*, suggesting phage T4 were not converted into Tailocin particles.

Table 3. Benzalkonium Chloride Effect on Phages T4 and S117 Infectivity

	SM buffer	*Benzalkonium chloride*[a]
T4[b]	3.0×10^{9}	$3.1\ 10^{5}$
S117[b]	$3.0\ 10^{11}$	$2.8\ 10^{11}$

[a]At 0.01% finale concentration.
[b]Expressed as pfu/mL.

Discussion

The worldwide antimicrobial resistance crisis requires novel solutions replacing traditional antibiotics. While phage-tail like bacteriocin particles, called Tailocins, are produced by bacteria when competing for environmental niches, the number of characterized Tailocins are limited. Here, we proposed to rupture the head/tail junction of phages to produce Tailocins as an alternative to antibiotic treatment using osmotic shock or chemical exposure. For our studies, we chose *Straboviridae* phage T4 and *Ackermannviridae* phage S117 that both carry nonflexible contractile tails. It was expected that if produced, Tailocin particles from these phages would resemble those of R2 pyocins[6] and kill in a similar fashion their bacterial host.

We have confirmed that phage T4 is sensitive to osmotic shock, as it was previously demonstrated several decades ago in great details.[7,16,17] Indeed, phage T4 was used as control in our experiments. Our results confirm that dilution of phage T4 from a high concentrated to a low concentration salt suspension inactivated 90% of T4 phage particles infectivity. The particles produced were further tested for remaining killing activity, and they proved to still be able to kill their targeted host, as expected from previous published results,[7] suggesting the formation of Tailocin or ''ghost'' particles. Different salts were tested (NaCl, NaAc, CsCl), and all provided similar results. Considering that ghost T4 particles were observed only after treatment with NaCl,[7] but full Tailocin particles production has been documented after CsCl treatment,[18] we may speculate that both type of particles conserved their killing activity. On the contrary, when we exposed *Ackermannviridae* phage S117 to osmotic shock, no loss of infectivity was reported in any of the solution tested. Therefore, we can conclude that as for phages T1, T3, T5, and T7,[19] S117 is insensitive to osmotic pressure changes, indicating the phage selected may influence the production of Tailocin, as previously described.[19,20]

While we have confirmed the infectivity loss and maintenance of killing activity by phage T4 particles submitted to osmotic shock, we have not confirmed the presence of Tailocin particles. To do so, a reconcentration step of the inactivated particles,[21] followed by imaging the particles by transmission electron microscopy should be performed. This was unfortunately not possible during this work.

We investigated the possibility to produce Tailocin particles using chemicals. The ammonium quaternary compound benzalkonium chloride is part of a chemical family used to effectively disable phage particles in the dairy industry.[9] We effectively inactivated phage T4 using benzalkonium chloride at 0.01%, yet T4-inactivated particles did not transformed into Tailocin as no residual killing was observed. Phage S117 was also tested, yet was resistant to the treatment at the concentration used. Other chemical compounds could be foreseen to produce Tailocins by rupturing the head/tail phage junction, such as zero-valent iron nanoparticles,[22] or the food component indigo Carmine.[23] Components that are already in use in the food industry would be preferable as their innocuity for the consumer has been tested.

Also, one can wonder if the particles produced killed their targets by puncturing as described previously for Tailocins produced by *Pseudomonas* spp.,[24] or if the lysis-from-without phenomenon could be responsible. The authors refer here to lysis-from-without as described in the review of Abedon,[25] where a culture of host cells is exposed to sufficient number of phages to obtain a ''saturation'' in adsorption. Considering that the residual killing effect of T4 Tailocins were determined at a theoretical MOI of 1, this phenomenon can be dismissed from being responsible for the killing effect described here.

From these experiments, we conclude that it is possible to produce noninfective phage particles that remain biologically active in killing their targeted host by osmotic shock. Yet, the structural architecture of the head/tail junction may influence the sensibility of phages to lose their heads as we see different response to the exposure to osmotic shock. While we expect that Tailocin particles are produced from this treatment, further studies are needed to select phage suitable for the production, also considering host range and target bacterium. Thus, to develop the use of phages for producing Tailocins with killing activity, further studies are needed. Yet, taking advantage of the large diversity of phages found in nature, to produce novel antimicrobial Tailocins without genetic engineering, is attractive for providing novel antimicrobial solutions for the future.

Authors' Contributions

C.W.: Conceptualization, methodology, validation, formal analysis, investigation, writing–original draft, writing–review and editing. L.B.: Conceptualization, project administration, supervision, writing–review and editing, funding acquisition.

Author Disclosure Statement

No potential conflict of interest was reported by the authors.

Funding Information

This work was supported by the Danish Council for Independent Research (Grant No. 9041-00159B).

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