RESEARCH PAPER

Targeting Phage-Mediated Gene Transfer: A Novel C3-Symmetric Polymer (PTriAce-1) Inhibits Temperate Phage Replication in *Staphylococcus aureus*

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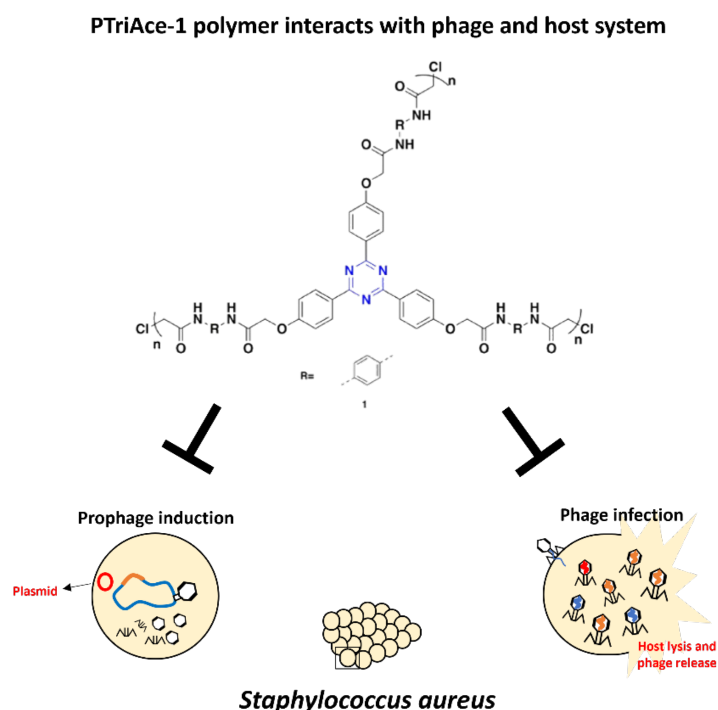
Abstract
Bacteriophages play a critical role in bacterial evolution by mediating horizontal gene transfer, including the dissemination of virulence determinants and antibiotic resistance genes. In *Staphylococcus aureus*, temperate bacteriophages contribute significantly to genomic plasticity through mechanisms such as transduction, lysogenic conversion, and mobilization of mobile genetic elements. Strategies capable of suppressing bacteriophage replication therefore represent a promising approach for limiting phage-mediated gene transfer and controlling the spread of antimicrobial resistance. In this study, the effect of the C3-symmetric triazine-based polymer (PTriAce-1) on bacteriophage replication was investigated. The impact of PTriAce-1 on phage infection dynamics was evaluated through phage propagation assays, mitomycin C–mediated prophage induction, plaque formation assays, and quantitative phage titration. Exposure to PTriAce-1 resulted in a huge reduction in the replication of prophage Φ11 compared with untreated controls. Plaque assays further demonstrate alterations in plaque morphology and reduced plaque size under polymer-treated conditions, indicating impaired phage infection dynamics. These findings suggest that PTriAce-1 interferes with key stages of the prophage life cycle and suppresses productive phage propagation. Because prophages are major drivers of horizontal gene transfer in bacterial populations, polymer-mediated inhibition of phage replication may reduce the dissemination of virulence factors and antibiotic resistance determinants. Together, these results highlight the potential of functional polymer materials as modulators of bacteriophage activity and suggest that targeting bacteriophage-mediated genetic exchange may represent a complementary strategy for combating antimicrobial resistance..

Graphic abstract:



Keywords: Temperate bacteriophage, Anti-prophage, Horizontal gene transfer, antimicrobial resistance transmission, Anti-diseases transmission, Polymer-mediated inhibition

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Introduction

Antimicrobial resistance (AMR) has emerged as one of the most urgent global health challenges of the twenty-first century. The rapid rise of multidrug-resistant bacterial pathogens threatens the effectiveness of existing antibiotics and complicates the treatment of both community- and hospital-acquired infections. Among clinically significant pathogens, *Staphylococcus aureus* remains a major cause of skin infections, pneumonia, sepsis, and device-associated infections worldwide. The adaptability of *S. aureus* is largely driven by its remarkable genomic plasticity, which allows rapid acquisition of resistance determinants and virulence factors through horizontal gene transfer (HGT) and mobile genetic elements (MGEs)[1], [2]. Understanding the molecular mechanisms that facilitate bacterial evolution and gene exchange is therefore essential for developing strategies to mitigate the spread of antimicrobial resistance. Bacteriophages play a central role in these evolutionary processes. Temperate phages integrated into bacterial chromosomes frequently act as vehicles for gene transfer and virulence evolution in *S. aureus*. Through mechanisms such as generalized and specialized transduction, lysogenic conversion, and mobilization of pathogenicity islands, phages facilitate the dissemination of antibiotic resistance genes and toxin-encoding determinants among bacterial populations[3], [4], [5], [6], [7]. Helper phages such as Φ80α have been shown to mobilize staphylococcal pathogenicity islands (SaPIs), which encode superantigens and other virulence factors that contribute significantly to disease severity[4], [8], [9], [10]. Similarly, temperate phage Φ11 represents an important model for studying lysogenic-lytic switching and phage-mediated gene transfer in *S. aureus*. Because prophage induction results in active phage replication and release of transducing particles, bacteriophages are increasingly recognized as critical drivers of bacterial pathogenicity and genome evolution. Despite the growing recognition of phage-mediated gene transfer in bacterial evolution, most antimicrobial strategies continue to focus primarily on targeting bacterial viability. While antibiotics effectively inhibit bacterial growth, they often exert strong selective pressure that accelerates the emergence of resistant strains. Moreover, antibiotic-induced stress responses can promote prophage induction and enhance horizontal gene transfer, paradoxically facilitating the spread of resistance genes within microbial communities [11], [12], [13]. These observations highlight an important limitation of traditional antimicrobial approaches: suppression of bacterial growth alone does not necessarily prevent dissemination of virulence factors and resistance determinants. Consequently, strategies that target bacteriophage activity or interfere with phage-mediated gene transfer have begun to attract increasing attention as complementary approaches to antimicrobial therapy[14], [15], [16]. In parallel with these developments, synthetic materials and nanostructured polymers have emerged as promising tools for modulating microbial systems. A wide range of polymeric materials has demonstrated antimicrobial properties through mechanisms including membrane disruption, interference with intracellular processes, and modulation of microbial signaling pathways[17], [18]. Among these materials, triazine-based polymers have attracted interest due to their structural stability, tunable functionality, and potential biological activity. Recently, a novel class of C3-symmetric triazine polymers containing aromatic acetamide linkers (PTriAce-1–4) was synthesized and shown to possess antimicrobial activity against several bacterial pathogens while also affecting the replication of *S. aureus* temperate bacteriophage 80α[15]. The unique structural architecture of these polymers provides multiple functional groups capable of interacting with biological molecules, suggesting that they may influence microbial processes beyond direct antibacterial activity. Building on previous observations that PTriAce-1 interferes with *S. aureus* Φ80α, the present study investigated whether this polymer exerts a similar inhibitory effect on the temperate bacteriophage Φ11. Because these phages represent important mediators of horizontal gene transfer in staphylococci, understanding how synthetic polymers influence their replication may provide new insights into strategies for limiting the spread of virulence determinants and antimicrobial resistance. By integrating polymer chemistry with bacteriophage biology, this work explores a novel conceptual framework in which antimicrobial materials are designed not only to inhibit bacterial growth but also to disrupt the genetic mechanisms that drive bacterial evolution.

Materials and Methods

2.1 Polymer Preparation

The C3-symmetric triazine-based polymer PTriAce-1 was obtained and synthesized following the procedure previously described for triazine-acetamide polymers[15]. Briefly, the triazine monomer 1,3,5-tris(4-hydroxyphenyl)-1,3,5-triazine was reacted with acetamide-based linker derivatives through nucleophilic substitution in dimethylformamide (DMF) using potassium carbonate as a base under nitrogen atmosphere. The reaction mixture was heated under reflux and then allowed to cool to room temperature. The resulting polymer was collected by filtration, washed with DMF and distilled water, and dried under vacuum at 80 °C.

2.2 Bacterial Strains and Culture Conditions

Staphylococcus aureus RN4220 was used as the primary bacterial host for bacteriophage propagation and infection assays. Bacterial cultures were grown in tryptic soy broth (TSB) at 37 °C with shaking at 120 rpm. For solid media,

tryptic soy agar (TSA) plates were used. Overnight cultures were prepared from single colonies and diluted 1:50 in fresh medium before experiments. Cultures were grown to early exponential phase ($OD_{540} \approx 0.15–0.20$) prior to phage infection or antimicrobial assays.

2.3 Phage Propagation and Induction

Temperate bacteriophages Φ11 was propagated using *S. aureus* RN4220[19]. Bacterial cultures in exponential phase were infected with phage lysates and incubated at 37 °C to allow phage replication. For prophage induction experiments, mitomycin C was added to lysogenic cultures at a final concentration of 2 $\mu g\ ml^{-1}$ to activate the bacterial SOS response and initiate the lytic cycle. Following incubation for approximately 4 h, cultures were centrifuged to remove cell debris and the supernatant containing phage particles was filtered through 0.22 µm membranes.

2.4 Phage Titration Assays

Phage titers were determined using a standard double-layer agar plaque assay[19]. Serial 10-fold dilutions of phage lysates were prepared in phage buffer. Aliquots of exponentially growing RN4220 cells were mixed with molten soft agar and overlaid onto TSA plates. Diluted phage samples were spotted onto the bacterial lawn and plates were incubated overnight at 37 °C. Plaques were counted and titers were calculated as plaque-forming units per milliliter (PFU ml^{-1}).

2.5 Statistical Analysis

Experiments were performed in at least three independent replicates. Data are presented as mean standard deviation (±SD). Statistical comparisons between groups were performed using Two-way ANOVA followed by appropriate post hoc tests. Differences were considered statistically. Graphs and statistical analyses were generated using Microsoft Excel and GraphPad Prism software.

3. Results

3.1 Evolutionary of the Φ11 regulatory system among staphylococcal prophages

Temperate bacteriophages play a central role in the evolutionary dynamics of *Staphylococcus aureus* by facilitating HGT and mediating the mobilization of virulence determinants, pathogenicity islands, and antibiotic resistance genes. The regulatory circuitry controlling lysogeny and induction in these phages is typically governed by CI-like repressors that maintain the prophage state by repressing lytic gene transcription until environmental stress signals trigger the lytic cycle. To better contextualize the biological system investigated here, we first examined the evolutionary relationship between the CI-like repressor encoded by bacteriophage Φ11 and homologous regulatory proteins present in other *S. aureus* prophages (Figure 1). Phylogenetic analysis revealed that the Φ11 repressor clusters most closely with the CI repressor of Φ80α, a well-characterized helper phage known to mobilize staphylococcal pathogenicity islands (SaPIs). The grouping of these two repressors into a distinct branch suggests that Φ11 and Φ80α share conserved regulatory mechanisms controlling prophage maintenance and lytic induction. Such similarity is biologically significant because CI repressors in λ-like phages act as master transcriptional regulators that determine the developmental fate of the phage. They bind operator sequences within the phage genome and repress transcription of genes required for lytic replication while simultaneously promoting expression of their own regulatory circuits that stabilize lysogeny[20], [21], [22]. The extended branch length observed for Φ11 indicates higher sequence divergence relative to other prophages included in the analysis, including Φ12, Φ29, Φ37, and Φ47. Nevertheless, the conserved clustering with Φ80α suggests that the regulatory architecture of Φ11 likely operates through mechanisms analogous to those of other lambda-like temperate phages. This observation has important implications for interpreting polymer-mediated inhibition of phage replication, as any material capable of interfering with CI-dependent regulation could potentially disrupt the delicate balance between lysogenic persistence and lytic replication. Prophages related to Φ80α are particularly relevant to *S. aureus* pathogenicity because they frequently serve as helper phages responsible for mobilizing SaPIs. These genomic islands encode virulence factors such as superantigens and toxins and are disseminated through phage-mediated transduction event [10], [23] Consequently, interventions capable of suppressing phage replication or induction could theoretically reduce the mobilization of such pathogenicity determinants. The phylogenetic analysis indicates that Φ11 belongs to a regulatory lineage closely related to Φ80α, providing a strong rationale for investigating whether materials capable of inhibiting Φ80α replication may also affect Φ11 infection dynamics

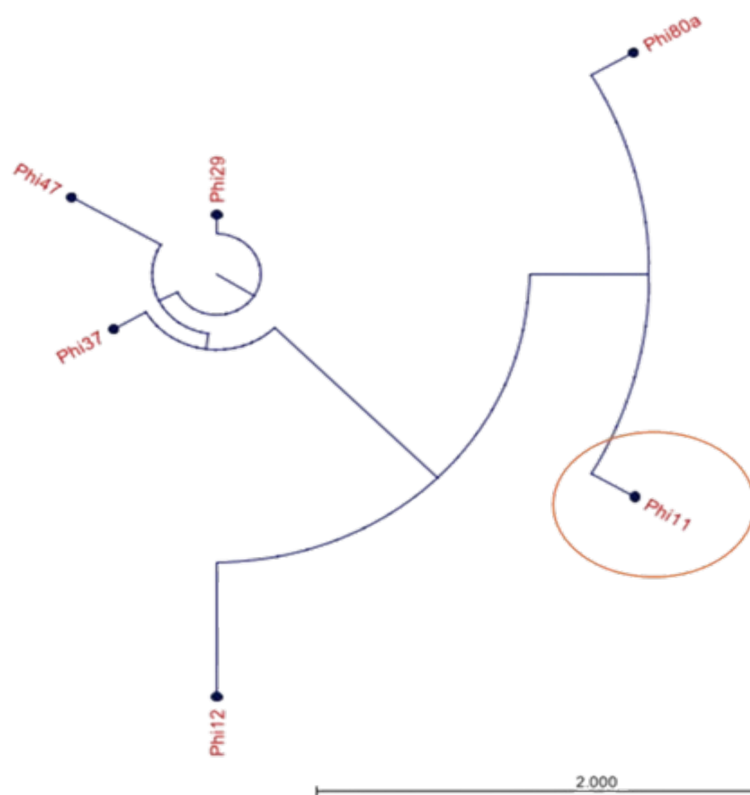

Figure 1. Phylogenetic tree of the phage 11 lambda-like repressor in *S. aureus* phages. Φ11 (arrow) groups closest to Φ80α and forms a distinct branch from Φ12, Φ29, Φ37, and Φ47. The longer Φ11 branch suggests higher sequence divergence (scale bar = 2.0 substitutions per site). The tree was inferred in CLC Genomics Workbench and visualized in iTOL.

3.2 Conserved domains support canonical λ-like control

To complement the evolutionary analysis, bioinformatic investigation of Φ11 regulatory proteins was performed to identify conserved functional domains associated with transcriptional regulation and replication initiation (Figure 2). Sequence alignment of Φ11 and Φ80α CI repressors revealed extensive conservation within the N-terminal helix–turn–helix (HTH) DNA-binding domain characteristic of Cro/CI family transcriptional regulators. The HTH motif represents one of the most common DNA-binding architectures in bacterial and phage regulatory proteins. In CI repressors, this domain allows the protein to bind specific operator sequences within the phage genome, thereby repressing transcription of early lytic genes and maintaining the prophage state. Structural studies of λ-like repressors have demonstrated that these proteins function as dimers that bind cooperatively to operator sites, creating a regulatory network capable of stabilizing lysogeny through autoregulatory feedback loops[24], [25]. Domain prediction analysis further identified conserved motifs associated with replication initiation proteins in Φ11. The N-terminal region contains a replisome organizer domain commonly associated with recruitment of host replication machinery, whereas the C-terminal region contains a DnaB/C-like domain involved in DNA binding and helicase interaction. These domains suggest that Φ11 replication proteins interact with host helicases and replication complexes to coordinate genome amplification during the lytic cycle. The presence of these conserved domains reinforces the conclusion that Φ11 replication follows canonical mechanisms characteristic of prophages. Consequently, disruption of regulatory pathways controlling transcriptional repression or replication complex assembly could effectively suppress phage propagation. Importantly, the structural features identified in this analysis provide potential molecular targets that could be affected by synthetic polymers capable of interacting with proteins or nucleic acids through electrostatic or hydrophobic interactions. Although the precise molecular target of PTriAce-1 remains to be determined, the conservation of regulatory domains identified here suggests that interference with DNA-protein interactions or replication complex stability could plausibly contribute to the observed inhibition of phage replication.

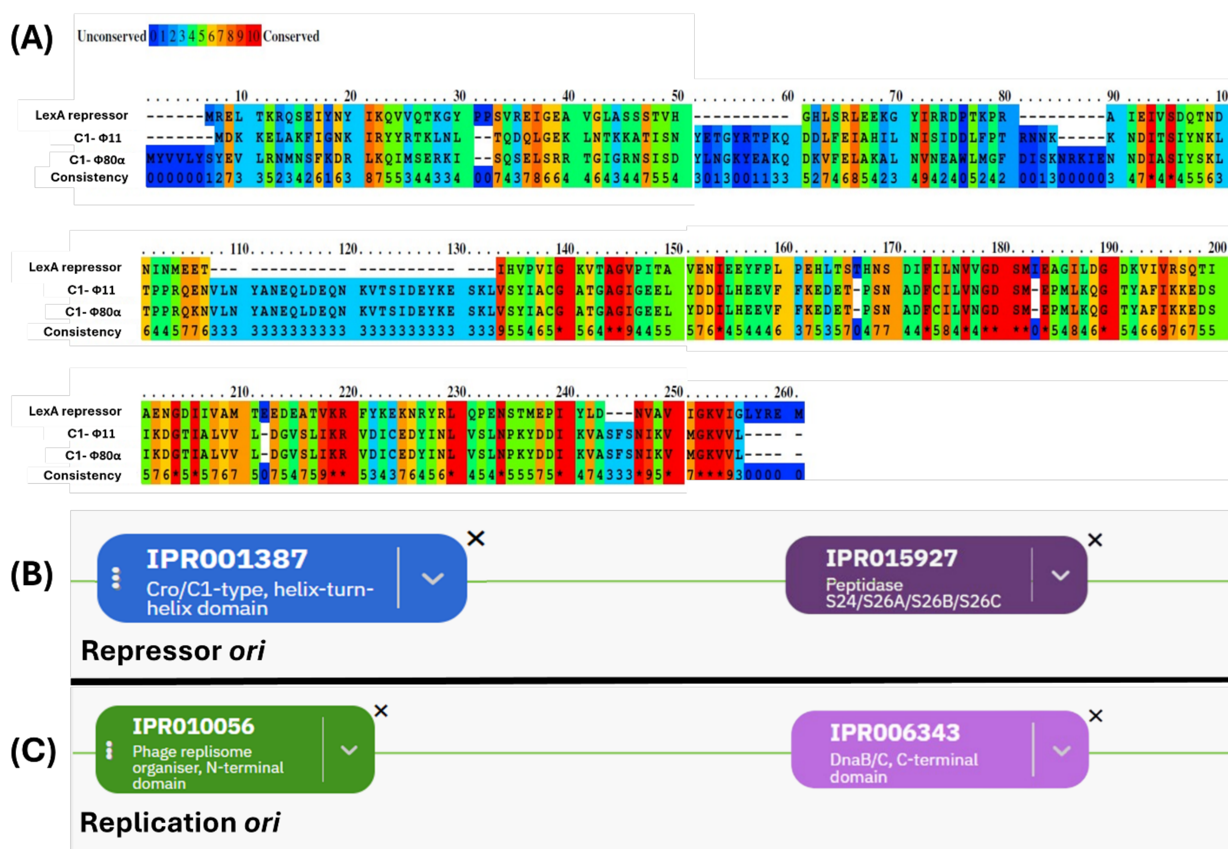

Figure 2. Bioinformatic analysis highlights regulatory domains potentially associated with PTriAce-1 polymer activity. (A) Multiple sequence alignment of Φ11 and Φ80α CI-like repressors revealing a shared domain organization and conserved motifs consistent with LexA/CI-type repressors. (B) Protein structure of the master regulator. The N-terminal region contains a lambda repressor-like helix–turn–helix (HTH) DNA-binding domain, consistent with CI/Cro-family transcriptional repressors involved in regulating the lysogenic/lytic switch. (C) Domain organization of the phage replication initiation protein. The N-terminal phage replisome organizer domain lies adjacent to a low-complexity region containing direct repeats that may function as the phage origin of replication, while the C-terminal DnaB/C-like domain is associated with DNA binding and DNA-dependent oligomerization during replication initiation. Analysis was done by InterPro.

3.3 PTriAce-1 strongly suppresses Φ11 lytic replication

Having established the regulatory context of Φ11, we next examined whether the C3-symmetric polymer (PTriAce-1) influences the replication dynamics of this phage during productive infection. Phage titration assays using the restriction-deficient *S. aureus* strain RN4220 demonstrated that polymer supplementation results in a dramatic reduction in phage titers compared with untreated controls (Figure 3A). While control cultures exhibited high titers consistent with efficient lytic replication, polymer-treated cultures produced markedly fewer phage particles, indicating strong inhibition of phage propagation. Visual inspection of infection cultures further supported these observations. Untreated samples displayed extensive bacterial lysis consistent with active phage replication, whereas cultures supplemented with PTriAce-1 exhibited reduced lysis after four hours of infection (Figure 3B). Together, these results indicate that the polymer effectively blocks the lytic cycle of Φ11 under the experimental conditions tested. Several mechanisms could explain this inhibitory effect. One possibility is that PTriAce-1 interferes with phage adsorption to bacterial cell surfaces, thereby preventing efficient infection. Adsorption of bacteriophages typically depends on recognition of specific cell surface receptors such as teichoic acids, membrane proteins, or polysaccharides [26]Polymers capable of interacting with bacterial cell surfaces could potentially mask these receptors or alter membrane properties, reducing the probability of successful phage attachment. Alternatively, PTriAce-1 may affect intracellular stages of the infection cycle. Following adsorption, phage DNA must be replicated using host replication machinery, and the resulting genomes are subsequently packaged into capsids before host cell lysis releases progeny virions. Disruption of any of these processes could lead to the observed reduction in phage titers. For example, polymers containing aromatic and charged functional groups may interact with nucleic acids or replication proteins, thereby interfering with DNA replication or transcriptional processes required for phage development. Another possible explanation involves modulation of host physiological conditions.

Because bacteriophage replication depends heavily on host metabolic resources, any polymer-induced stress affecting bacterial metabolism could indirectly suppress phage replication. Similar indirect inhibition mechanisms have been reported in studies where nanoparticles or polymeric materials alter bacterial physiology in ways that reduce susceptibility to phage infection[27], [28]. Although the precise molecular mechanism remains to be clarified, the strong reduction in phage titers observed in the presence of PTriAce-1 demonstrates that the polymer substantially alters the outcome of Φ11 infection.

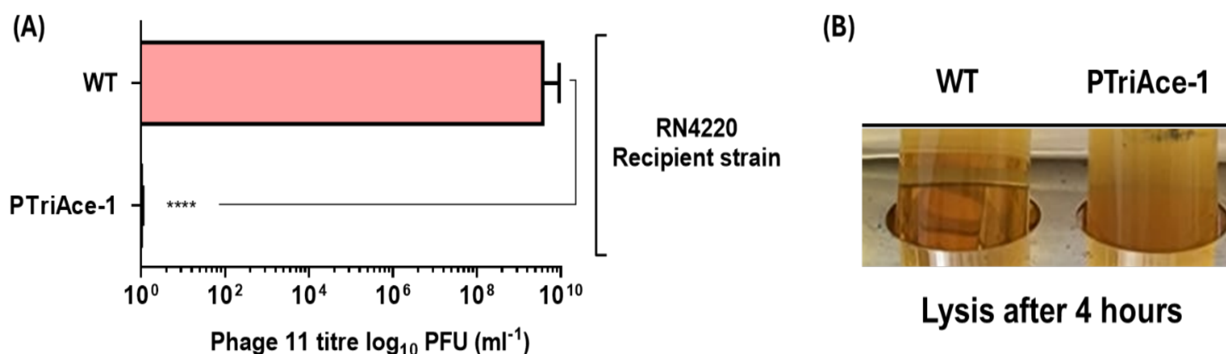

Figure 3. PTriAce-1 has blocked the lytic phage cycle completely. (A) RN4220 strain reached exponential phase. Then, fresh lysate of Φ11 was used to infect the different strains; (WT) RN4220 strain with no polymer added and other strain supplement with PTriAce-1 polymer. (B) Four hours following infection, phage lysates were examined. Three biological replicates' means are shown. Statistical analysis was performed on log_{10} transformed data using Two-Way ANOVA followed Sidak's multiple comparisons test. *p*-values are as follows: **** $p \leq 0.0001$.

3.4 PTriAce-1 polymer treatment reduces prophage induction

To determine whether PTriAce-1 also affects lysogenic induction, the activation of Φ11 was examined as prophage in RN4220 lysogenic derivatives following mitomycin C treatment (Figure 4). Mitomycin C induces DNA damage and activates the bacterial SOS response, which triggers cleavage of CI repressors and initiates the lytic cycle in many temperate bacteriophages [14], [19]. In untreated cultures, mitomycin C induction resulted in high phage titers consistent with efficient activation of the lytic cycle. However, cultures supplemented with PTriAce-1 displayed significantly reduced phage titers, suggesting that polymer treatment interferes with prophage induction or subsequent replication processes. Because mitomycin C directly activates the SOS response, the inhibitory effect of PTriAce-1 likely occurs downstream of CI cleavage. This polymer may interfere with replication initiation after induction, preventing amplification of phage genomes. Another possibility is that polymer interactions with host cellular components impair assembly of phage replication complexes. Suppression of prophage induction is biologically significant because prophage activation is a major mechanism driving horizontal gene transfer in bacterial populations. Prophages frequently mobilize virulence determinants and antibiotic resistance genes during lytic replication, thereby facilitating rapid genetic exchange among bacterial strains[3]. Consequently, materials capable of reducing prophage activation could limit dissemination of pathogenic traits within bacterial populations.

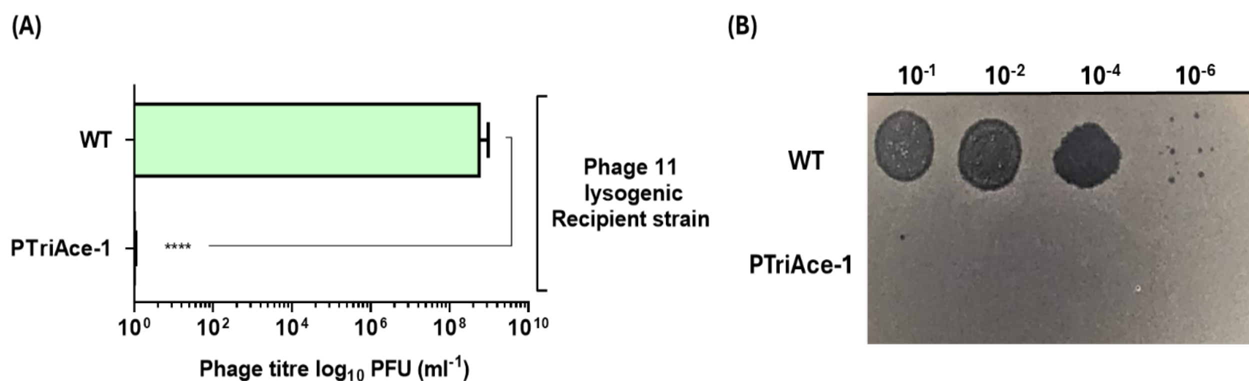

Figure 4: PTriAce-1 polymer has affected the phage lysogenic titre. (A) The indicated RN4220 derivatives lysogenic for Φ11 was grown to early exponential phase followed by mitomycin C induction of the lytic phage cycle and PTriAce-1 polymer was added in same time. **(B)** Plaque formation was assessed on a lawn of RN4220. Data shown are the means of three independent biological replicates and error bars represent the standard deviation from the mean. Statistical analysis was performed on log_{10} transformed data using Two-Way ANOVA followed Sidak's multiple comparisons test. *p*-values are as follows: **** $p \leq 0.0001$.

3.5 PTriAce-1 alters plaque morphology after infection

Further evidence for polymer-mediated modulation of phage infection dynamics was obtained through plaque assays comparing untreated and polymer-treated conditions (Figure 5). Plaques produced by wild-type Φ11 in untreated cultures displayed the characteristic morphology associated with efficient phage replication and spread through the bacterial lawn. In contrast, plaques formed in the presence of PTriAce-1 were noticeably smaller and less defined. Plaque morphology reflects several parameters of phage infection, including adsorption rate, replication speed, burst size, and diffusion through the bacterial lawn. Reduced plaque size typically indicates slower infection kinetics or decreased production of progeny virions[26], [29]. The altered plaque phenotype observed here therefore provides additional evidence that PTriAce-1 affects multiple stages of the phage infection cycle. Reduced replication efficiency, impaired virion assembly, or delayed host cell lysis could all contribute to the observed changes in plaque morphology.

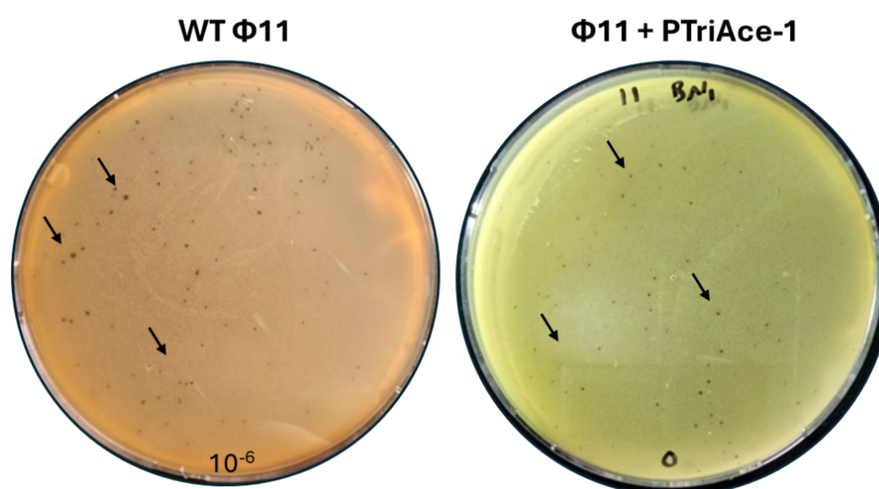

Figure 5. Polymer-dependent variation in phage plaque morphology showing differences in plaque size and phenotype.

4. Discussion

Temperate bacteriophages are major drivers of bacterial evolution and virulence in *Staphylococcus aureus*, where prophages frequently encode toxins and contribute to horizontal gene transfer of mobile genetic elements. Because the transition between lysogenic persistence and lytic replication is governed by CI-like repressors and associated regulatory networks, disruption of these pathways can strongly influence phage propagation and the spread of virulence determinants. In this study, the impact of the C3-symmetric triazine polymer (PTriAce-1) on the replication dynamics of the temperate bacteriophage Φ11 was investigated through phylogenetic analysis, protein domain characterization and functional infection assays. The findings indicate that polymer exposure substantially suppresses phage replication and alters infection dynamics in *S. aureus*. Phylogenetic analysis showed that the CI repressor encoded by Φ11 is closely related to the repressor of bacteriophage Φ80α, a helper phage involved in mobilization of staphylococcal pathogenicity islands. CI-like repressors maintain lysogeny by binding operator sequences and repressing transcription of lytic genes until environmental stress activates prophage induction[19], [30]. Conservation of the helix–turn–helix DNA-binding motif within the Φ11 repressor therefore suggests that the regulatory circuitry governing lysogenic maintenance in Φ11 resembles that of other λ-like phages[20], [21], [31]. Domain analysis further identified replication-associated motifs consistent with replisome recruitment and interaction with host helicases, indicating that genome amplification likely relies on host replication machinery. These structural features highlight potential regulatory and replication nodes that may be sensitive to perturbation by polymer exposure. Experimental infection assays demonstrated that PTriAce-1 markedly reduces Φ11 phage titers during lytic infection. Reduced bacterial lysis and diminished phage production were observed in polymer-treated cultures, indicating that the phage life cycle was disrupted. Although the precise mechanism remains to be determined, several possibilities may explain this inhibition. Polymer interactions with bacterial surfaces could modify accessibility of phage receptors, thereby reducing adsorption efficiency. Alternatively, interactions between polymer functional groups and nucleic acids or proteins could interfere with transcriptional regulation or genome replication within infected cells. However, the precise mechanism is still not clear yet. Suppression of phage propagation can be observed during mitomycin-induced prophage activation, suggesting that polymer activity more likely affects processes downstream of CI cleavage, such as replication or virion assembly. Plaque morphology analysis provided further evidence that infection dynamics were altered in the presence of PTriAce-1. Plaques formed under polymer exposure were smaller and less defined than those produced under untreated conditions, indicating reduced phage amplification or slower spread within the bacterial population. Plaque size is influenced by parameters including adsorption rate, latent period and burst size[32], [33], and the observed phenotype therefore supports the conclusion that polymer treatment interferes with productive infection. These findings may have broader implications for bacterial pathogenicity. Temperate bacteriophages are key mediators of horizontal gene transfer and contribute to the dissemination of virulence genes and antibiotic resistance determinants in *S. aureus* populations[1], [3]. Because mobilization of pathogenicity islands depends on active phage replication[5], [10], [34], [35], [36], suppression of phage propagation by PTriAce-1 could reduce the frequency of such genetic exchange events. Polymer-based modulation of phage activity therefore represents a potential strategy for limiting the spread of virulence traits without directly targeting bacterial viability. Further mechanistic studies will be required to determine whether polymer-phage interactions occur at the level of adsorption, replication or host regulatory pathways, and to assess the broader applicability of polymer-mediated phage inhibition as an antimicrobial approach.

5. Conclusion

This study demonstrates that the C3-symmetric triazine-based polymer (PTriAce-1) significantly influences the replication dynamics of temperate *Staphylococcus aureus* bacteriophages. Experimental analyses showed that exposure to PTriAce-1 markedly reduced the propagation of Φ11, as evidenced by decreased phage titers, reduced bacterial lysis, and alterations in plaque morphology. These observations indicate that the polymer interferes with key stages of the phage infection cycle, although the precise molecular mechanism underlying this inhibition remains to be clarified. Because temperate bacteriophages play a central role in horizontal gene transfer and mobilization of mobile genetic elements in *S. aureus*, suppression of phage replication may have important implications for limiting the dissemination of virulence factors and antibiotic resistance determinants. The findings therefore suggest that polymer-based materials such as PTriAce-1 could represent a novel strategy for modulating bacteriophage activity and reducing phage-mediated gene transfer in bacterial populations. More broadly, this work highlights the potential of functional polymers as tools for targeting microbial evolutionary processes rather than bacterial viability alone. Future studies should focus on elucidating the molecular interactions between PTriAce-1 and phage or host cellular components, as well as evaluating the impact of polymer exposure on phage adsorption, genome replication, and transduction frequencies. More investigations may provide valuable insights into new antimicrobial approaches aimed at limiting the spread of antimicrobial resistance through interference with bacteriophage-mediated genetic exchange.

Author: Mohammed A. Thabet: Conceptualization, Methodology, Investigation, Writing-original draft, Writing-review & editing.

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Declaration: The author declares no competing interests.

Data availability: All data was introduced in the text.

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