Identification and Characterization of the Phage φK08-48 Depolymerase Targeting Biofilm-Forming Strains of *Klebsiella pneumoniae*

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INTRODUCTION

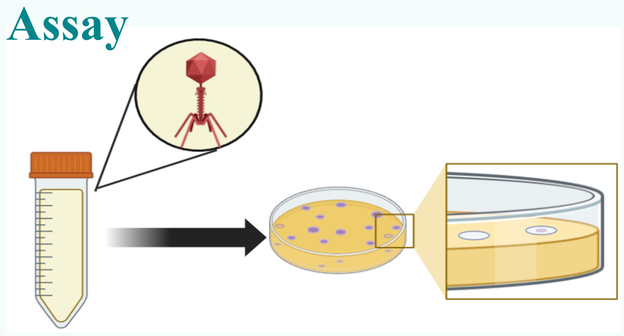
Klebsiella pneumoniae is an opportunistic Gram-negative pathogen that has become one of the leading causes of healthcare-associated infections (HAIs) worldwide[1]. This bacterium primarily affects immunocompromised individuals, including hospitalized patients, neonates, and the elderly, leading to severe conditions such as pneumonia, urinary tract infections, and sepsis[1]. A major factor contributing to its pathogenicity is its remarkable ability to form biofilms, providing protection against environmental stress and antimicrobial treatments, severely limiting therapeutic options[2]. This growing resistance crisis underscores the urgent need for alternative or complementary strategies to conventional antibiotics[2]. In this context, bacteriophages (phages) and their associated enzymes, particularly depolymerases, have gained significant attention as potential therapeutic tools[3]. Depolymerases are phage-encoded enzymes capable of degrading the polysaccharide components of the bacterial capsule and the extracellular matrix of biofilms[3]. By breaking down these protective barriers, depolymerases facilitate phage adsorption, enhance antibiotic penetration, and improve immune clearance of the pathogen[3]. Therefore, understanding and characterizing phage depolymerases provide a promising avenue for developing novel approaches to combat biofilm-associated and antibiotic-resistant *K. pneumoniae* infections[3].

OBJECTIVES

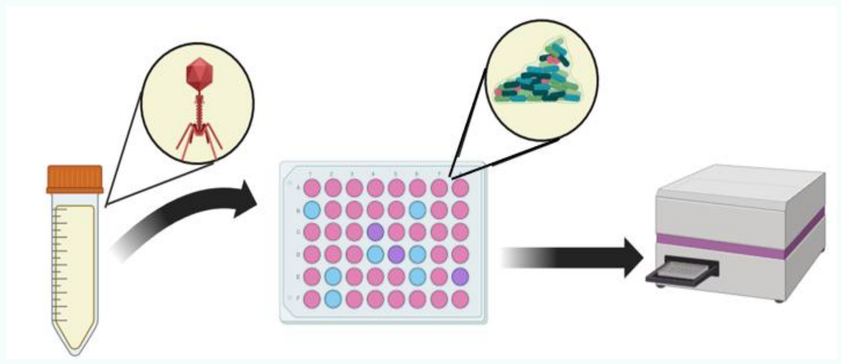
1. To identify depolymerase-producing phages
2. To evaluate the effect of phage φK08-48 on biofilm formation and degradation.
3. To perform a genomic analysis to identify a depolymerase gene.

METHODS

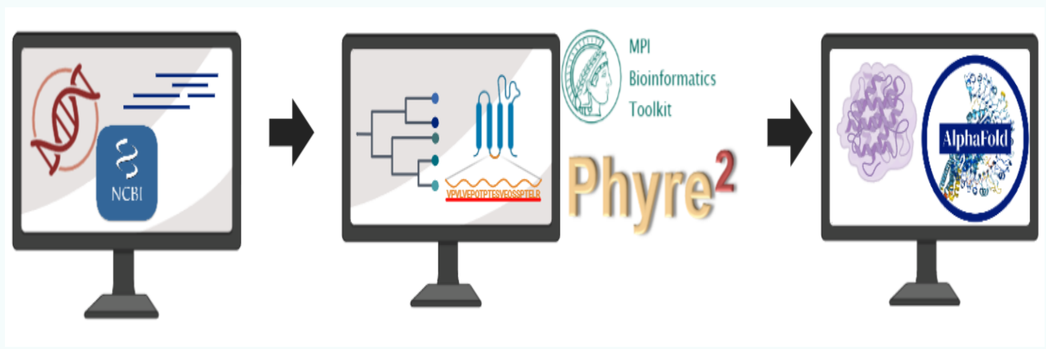
1.-Plaque Morphology Analysis by Soft Agar Overlay Assay



2.-Biofilm Modulation by Phage φK08-48



3.-Bioinformatic Characterization of the φK08-48 Depolymerase



RESULTS

1.- Depolymerase-producing phages

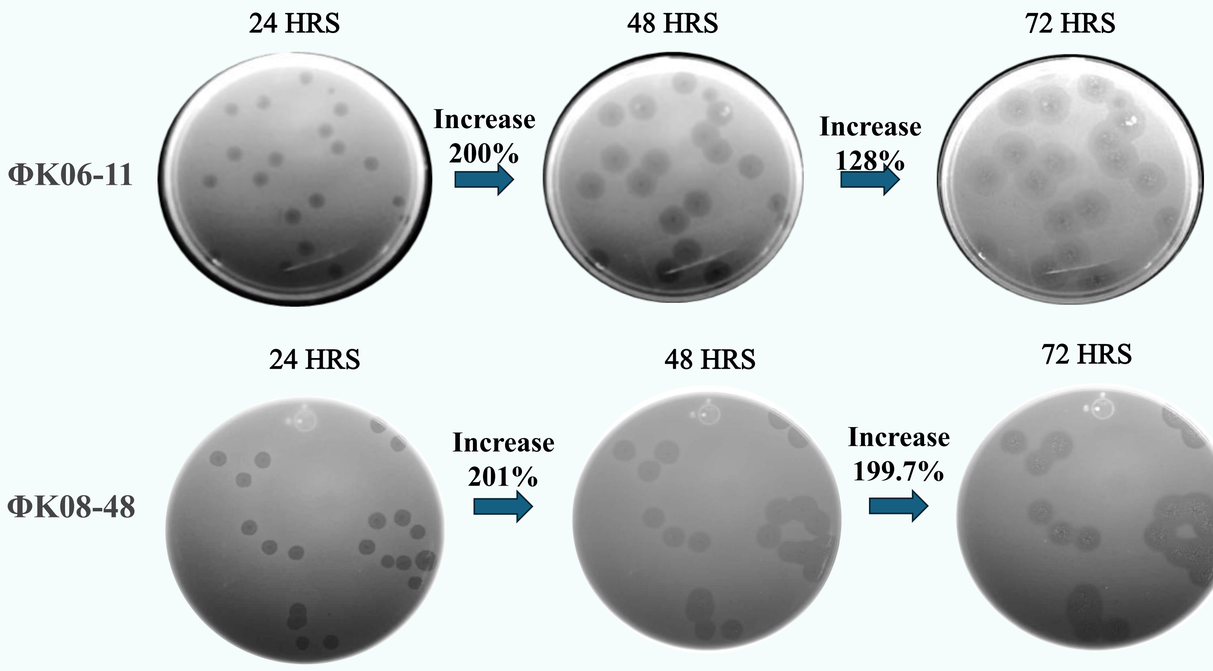


Figure 1. Plaque morphology. The phages show double halos. A clear halo, indicative of the phage's predominant lytic replication. A turbid halo that increases over time, indicates the effect of the depolymerase

2. - Effect of phage φK08-48 on biofilms

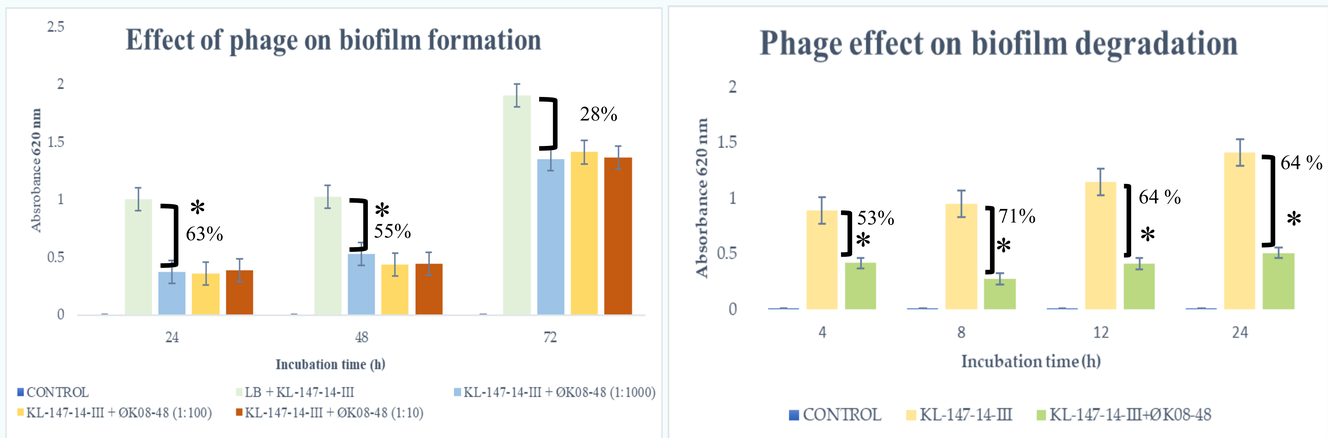


Figure 2. Effect of Phage φK08-48 on *K. pneumoniae* Biofilm. The data are presented as the mean ± standard deviation (SD) of three independent replicates. Asterisks (*} denote statistical significance ($p < 0.05$) as determined by a one-way ANOVA test

3.1.- Functional Annotation of φK08-48 Predicted ORF

DNA replication, repair and modification	Regulation of gene expression	Assembly, morphogenesis and structure of the capsule and tail	Lysis	Genome packaging	Hypothetical proteins
13 ORF's	3 ORF's	20 ORF's	3 ORF's	2 ORF's	34 ORF's

Figure 3. Functional annotation of predicted ORFs in the genome of phage φK08-48. The complete genome of phage φK08-48 (48,421 bp) was analyzed using bioinformatic tools. Open reading frames (ORFs) were identified, predicted, and classified based on their putative functions.

3.2.- Identification and 3D Modeling of a Novel Depolymerase

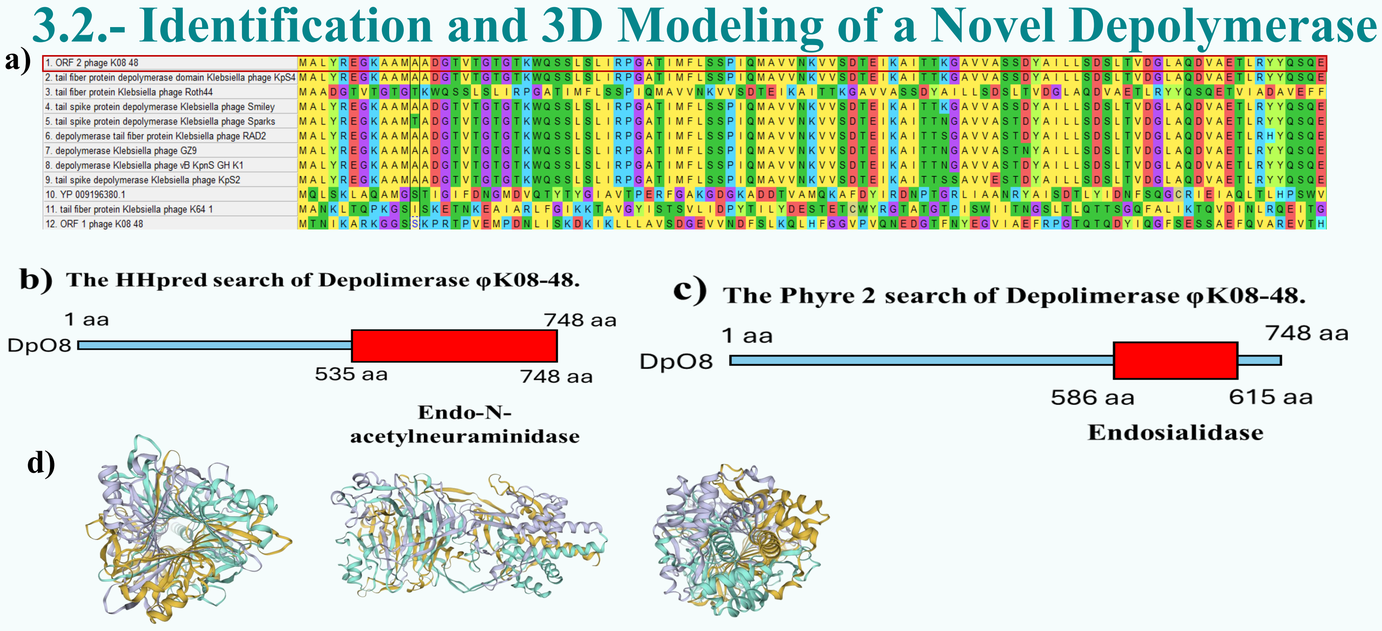


Figure 4. Bioinformatic identification and modeling of a putative depolymerase from phage φK08-48. a)Sequence alignment. b)-c)Conserved domain prediction (HHpred, Phyre2). d) Structural modeling (AlphaFold)

CONCLUSION

The phage φK08-48 exhibited plaques with a clear center surrounded by a progressively expanding turbid halo, a characteristic feature suggesting depolymerase activity. Functional assays suggested that this phage effectively inhibits biofilm formation and promotes *K. pneumoniae* biofilm degradation. Bioinformatic analysis of its genome identified a putative depolymerase, based on sequence homology, conserved domain architecture, and 3D structural modeling. Together, these findings support the presence of an active depolymerase in φK08-48, highlighting its potential as a biocontrol tool against biofilm-associated *K. pneumoniae* infections.

BIBLIOGRAPHY

1. Guilhen, C., Miquel, S., Charbonnel, N. *et al.* Propiedades de colonización e inmunomodulación de células dispersas en biopelículas *de Klebsiella pneumoniae* . *npj Biofilms Microbiomes* **5** , 25 (2019).
2. Topka-Bielecka, G., et al., (2021). Bacteriophage-Derived Depolymerases against Bacterial Biofilm. Antibiotics
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