Abstract

Pseudomonas aeruginosa is a critical priority pathogen responsible for chronic infections throughout the body, and is commonly found in the wounds of diabetic patients. Bacteriophages represent a promising alternative antimicrobial strategy against such pathogens. This study isolated and characterized a lytic bacteriophage from river water samples capable of infecting *P. aeruginosa* InaCC B3, and evaluated its antibacterial and anti-biofilm potential. Plaque morphology analysis revealed a distinctive three-zone bullseye pattern consisting of a central clear lysis zone, a lytic plaque zone, and an outer halo zone, suggesting strict lytic activity and putative extracellular polysaccharide depolymerase activity. *In vitro* killing curve assays demonstrated significant bacterial growth suppression at multiplicity of infection (MOI) 10 at 8 hours ($p < 0.05$), while lower MOI levels (1, 0.1, 0.01, 0.001) did not achieve statistical significance. Stability testing against 70% ethanol, 10% povidone-iodine, and 3% hydrogen peroxide resulted in complete phage inactivation across all agents tested, with full viability retained in the saline control. Biofilm inhibition assays demonstrated a consistent MOI-dependent reduction trend, though without statistical significance. Biofilm eradication assays produced a statistically significant reduction in pre-formed biofilm biomass at MOI 10 ($p < 0.05$). Host range analysis revealed lytic activity against 10 of 15 tested *P. aeruginosa* strains, including multiple CRPA clinical isolates. These findings establish the isolated phage as a biologically active, moderately broad-spectrum lytic candidate warranting further genomic, structural, and in vivo characterization for therapeutic development against *P. aeruginosa* infections.

Keywords: Bacteriophage, *Pseudomonas aeruginosa*, phage therapy, biofilm eradication