

## Abstract

Diabetic foot infections (DFIs) are a major complication of diabetes mellitus and are commonly associated with *Staphylococcus aureus*, a pathogen capable of forming biofilms and exhibiting antibiotic resistance. The increasing prevalence of multidrug-resistant *S. aureus* highlights the need for alternative antimicrobial strategies. This study aimed to isolate and characterize a lytic bacteriophage targeting *S. aureus* InaCC B4 from wastewater sources in Jakarta and evaluate its antibacterial and anti-biofilm activities. Bacteriophages were isolated using enrichment culture, spot test screening, and plaque assay titration. Morphological characterization was performed using cryogenic electron microscopy (cryo-EM). Antibacterial activity was evaluated through in vitro killing curve assays at different multiplicities of infection (MOIs), while anti-biofilm activity was assessed using biofilm inhibition and eradication assays quantified by crystal violet staining. Phage stability against commonly used antiseptics was also investigated. A lytic bacteriophage was successfully isolated from Tanah Tinggi wastewater and produced clear plaques with an average diameter of  $1.23 \pm 0.12$  mm. Cryo-EM analysis revealed an icosahedral capsid and a contractile tail, consistent with the morphology of tailed bacteriophages within the class *Caudoviricetes*. The isolated phage effectively suppressed bacterial growth and demonstrated dose-dependent anti-biofilm activity, achieving up to 57% biofilm inhibition and 80% biofilm eradication at MOI 10. Stability testing showed that the phage remained viable in saline but was completely inactivated after 15 minutes of exposure to 70% ethanol, povidone iodine, and hydrogen peroxide. The results show the potential of the isolated bacteriophage as an alternative antimicrobial and anti-biofilm agent against *S. aureus* infections.

**Keywords:** bacteriophage, *Staphylococcus aureus*, diabetic foot infection, wastewater, phage isolation, MRSA, biofilm, phage therapy