An Antimicrobial Protein with Broad-Spectrum Activity Against Drug-Resistant Bacteria: The Phage-Derived Holin

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Abstract

Objective: This study aims to investigate the lytic activity against host bacteria, fundamental biological characteristics, and antimicrobial activity against other clinical strains of the holin protein HolKpV318, which is derived from *Klebsiella pneumoniae* phage vB_Kp_XP4. *Methods*: Bioinformatics analysis was performed on the gene and amino acid sequence of HolKpV318. The recombinant plasmid pET-28a(+)-HolKpV318 was constructed using homologous recombination and transformed into *Escherichia coli* BL21 for induced expression. The minimum inhibitory concentration (MIC), antimicrobial and anti-biofilm activities, morphological effects on the host bacteria, and tolerance to temperature and pH stability of the protein were evaluated. Additionally, the antimicrobial spectrum against non-host bacteria was determined using the spot assay. *Results*: The HolKpV318 gene is 252 bp in length and encodes a protein consisting of 83 amino acid residues, which belongs to the phage Holin family, with a predicted molecular weight of approximately 9 kDa. HolKpV318 exhibited concentration- and time-dependent antimicrobial effects, with a minimum inhibitory concentration (MIC) of approximately 4 μg/mL. When combined with HolKpV318, the MIC of sulfamethazine decreased to 1/256 of its value when used alone. The optimal anti-biofilm effect of HolKpV318 was observed at a concentration of 12 μg/mL. Treatment with this protein significantly increased the permeability of the host bacterial cell membrane, ultimately leading to cell lysis. HolKpV318 exhibited the highest antimicrobial activity at 50°C and effectively inhibited host bacterial growth within a pH range of 3–10. Furthermore, HolKpV318 demonstrated lytic activity against a variety of clinical pathogenic bacteria, including *Klebsiella pneumoniae, Klebsiella pasteurii*, *Klebsiella grimontii*, *Enterobacter hormaechei*, and *Raoultella planticola*. Notably, it exhibited robust lytic capability against *Klebsiella pneumoniae* strains carrying carbapenemase genes (such as KPC-2, NDM-1, and NDM-5) and those of different ST and K types, including hypervirulent strains. *Conclusion*: The holin HolKpV318 derived from phage vB_Kp_XP4 exhibits potent broad-spectrum antibacterial activity, effectively lyses various drug-resistant strains, inhibits biofilm formation, and demonstrates favorable stability under varying pH and temperature conditions. These characteristics highlight its promising potential for clinical translation and its viability for the development of novel antimicrobial agents.

Keywords

Holin, *Klebsiella Pneumonia*, Endolysin, Antibacterial Activity, Biofilm