LysGN as a potential enzybiotics against Gram-Negative ESKAPE Pathogens

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Multidrug resistant (MDR) Gram negative bacterial infections call for development of alternative antibacterial strategy. The extended antibacterial approach of phage therapy involves the application of phage derived endolysin. Endolysins are peptidoglycan hydrolase, secreted during the late phase of viral replication for the purpose of disintegrating the host cell by hydrolyzing the various bonds present in the peptidoglycan. This study aimed at identification, cloning and purification of broad-spectrum *Salmonella* phage lysin (LysGN) and its characterization to determine lytic spectrum and shelf life followed by enhancing the lytic properties by engineering. Suitable candidate (LysGN) was selected by analyzing phage lysins, followed by synthetic cloning, expression, and purification. Lytic spectrum, thermostability and pH stability of LysGN were determined by RBB dye release assay. Bactericidal activity was determined as percentage reduction in CFU upon LysGN treatment with proper controls. The wild type LysGN was engineered to generate mutants with enhanced bactericidal activity along with its fusion with cell penetrating peptide. The selected candidate belongs to T4 type lysozyme family with an intrinsic potential of hydrolyzing the beta-1, 4 glycosidic bond between N-acetylmuramic acid and N-acetyl glucosamine in peptidoglycan. LysGN shows activity on wide spectrum of Gram Negative bacterial substrates. It remains active over a wide range of temperature and pH. On application of LysGN on different Gram negative bacteria (*Salmonella Typhimurium*, *Pseudomonas syringae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio Cholerae, E.coli, and Salmonella Newport)* significant reduction in CFU was observed in comparison to control plate. Also, LysGN engineered with cell penetrating peptide is able to cross the outer membrane barrier of Gram negative bacteria and have potential to be internalized by mammalian cell. It retains more than 90% bactericidal activity when stored at 4℃ for over a month. The screening of engineered lysGN with improved activity is under process. LysGN has effective intrinsic antibacterial activity against wide spectrum of Gram-negative bacteria over a range of temperature and pH. LysGN offers promising therapeutic perspective against MDR Gram-negative bacterial pathogens with further opportunities for in vivo studies and preclinical testing.

Keywords: Phage therapy, Endolysin, Multidrug resistant, Antibacterial