PP 110

A novel lytic Klebsiella phage KPP-1 for the therapeutic discovery

Nikapitiya C[1], Lee J[2], De Zoysa M[1*]

[1]College of Veterinary Medicine, Chungnam National University, Rep. of Korea, [2]Fish Vaccine Research Center, Rep of Korea.

Background: Bacteriophage (phage) therapy is current spotlight against Multi-Drug Resistance (MDR) pathogenic bacteria, due to many advantages over conventional antibiotics. *Klebsiella pneumoniae* is MDR, Gram negative, opportunistic pathogen, causing infections to lungs, urinary tract, gastrointestinal tract of humans, and also present in terrestrial and aquatic animals. *K. pneumoniae* belongs to the ESKAPE (ESKAPE; an acronym consisting the six scientific names of highly virulent and antibiotic resistant bacterial pathogens) priority group, spreads in hospitals and community, thus, continuous development of new therapies against *K. pneumoniae* is at high priority.

Objective: To newly isolate and characterize lytic Klebsiella phage KPP-1 and its genome, and evaluate antibacterial activity of recombinant KPP-1 endolysin (*r.KEnd*) against *K. pneumoniae*.

Method: KPP-1 was isolated from a natural water stream, and characterized by morphology (Electron Scanning Microscopy; TEM), growth and MDR *K. pneumoniae* inhibitory activity. Using zebrafish model, phage efficacy was performed. Additionally, KPP-1 complete genome sequence was annotated, and investigated *r.KEnd* antibacterial activity and mode of action.

Results: TEM results revealed KPP-1 belongs to family *Myoviridae.* KPP-1 (0.1 multiplicity of infection; MOI) latent period and burst size were 20 minutes and 88 plaque forming units (PFU) per infected cell, respectively. Phage was stable at broad temperature (4-50 ^{o}C), pH (3-11), and salinity (0.1-3%), and showed potent lytic spectrum *in vitro* against *K. pneumonia.* KPP-1-treated zebrafish (*K. pneumoniae* + KPP-1) exhibited, 67% cumulative survival, while *K. pneumoniae*-challenged fish had 21%. KPP-1 genome was 143,379 bp consisting 256 coding sequences, including host recognition, lysozyme activity, and putative endolysin. *r.KEnd* had higher antibiotic activity against *K. pneumoniae* than control group. Increased dead cells and reactive oxygen species indicate, *r.KEnd* induced clear membrane damage and high oxidative stress to bacteria cells, respectively.

Conclusion: KPP-1 showed high host specificity toward *K. pneumoniae,* postulating damage to the normal microbial community is minimal. Induced cell membrane penetration and elevated oxidative stress could be reasons for *r.KEnd* antibacterial effect. As a drug candidate, synthetic peptide could be designed (incorporating active residues) to improve the efficacy.

Acknowledgement: This research was supported by the National Research Foundation (NRF) grant funded by the government of Korea (MSIT) (2017010990) and a part of the project titled 'Fish Vaccine Research Center' funded by the Ministry of Oceans and Fisheries, Korea.