Application of Bacteriophage Derived Endolysins to Combat Economic Loss Associated with Antimicrobial Resistant Pathogens Responsible for Bovine Respiratory Disease

Sidra Moqaddes[1], Jaeen Hyun[1], Tahreem Asrar[1], Kim Stanford[2], Brenda Ralston[3], Tim McAllister[4], Dongyan Niu[1]

[1]Faculty of Veterinary Medicine, University of Calgary, Alberta, Canada
[2]Biological Science, University of Lethbridge, Lethbridge, Alberta, Canada
[3]Parkway Research Ltd., Rocky View County, Alberta, Canada
[4]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada
*E-mail: sidra.moqaddes@ucalgary.ca

ABSTRACT

Keywords: Bovine respiratory diseases, Bacteriophage endolysins, Mannheimia haemolytica, Multidrug resistance.

INTRODUCTION

Bovine respiratory disease (BRD) is associated with primary viral and secondary bacterial infections is a leading cause of mortalities in cattle posing a great economic impact on Canadian beef and dairy industry. Among commonbacterial BRD pathogens (*Mannheimia haemolytica, Histophilus somni, Pasteurella multocida) M. haemolytica*is of prime importance. It lives in upper respiratory tract of cattle as commensal organism but immunosuppression due to primary viral infection or environmental stress conditions trigger the penetration of bacterium in lungs where it replicates and damage tissue lining. Early age (two weeks old calf) vaccination is recommended but infection still persists despite vaccination and extensive use of antimicrobial agents. The reason for vaccine failure is existence of multiple bacterial strains which sometimes render vaccination ineffective. On the other hand, continual development of antimicrobial resistance made the therapeutic measures using antibiotics more challenging. The second concern about use of antibiotics is the presence of their residues in milk and meat if withdrawal period is not considered. An alternative treatment strategy is therefore required to combat antimicrobial resistance and cure the diseased animals from opportunistic bacterial pathogens.

OBJECTIVES

Endolysins are the viral proteins naturally produced by bacteriophages at the end of their lytic cycle to hydrolyze bacterial hostby degrading peptidoglycan layer present in their cell wall. Application of these endolysins can be a promising solution towards control of bacterial infection. Current study aimed to produce phage endolysins and evaluate their antimicrobial activity against multidrug resistant*M. haemolytica*.

METHODOLOGY

Previously, the lysogenic bacteriophages induced from *M. haemolytica*showed antimicrobial activity against it. In this study, four endolysins from lysogenic phages were successfully extracted as two original phage proteins (185(3-2), 587AP2(3-5)) and two peptide (polycationic nanopeptide) fused endolysins (PCNP-185(P-2), PCNP-587AP2(P-5)). Muralytic assay was performed to estimate peptidoglycan degradation ability of all

four endolysins. To check the antimicrobial activity, bacterial stains were incubated with endolysins followed by spreading on blood agar to count viable bacteria after treatment.

RESULTS AND FUTURE DIRECTIONS

Muralytic assay revealed that enzymatic activity of unmodified endolysins 185(3-2) and 587AP2(3-5) were19560 unit/ug and 19770 units/ug which was greater than the modified PCNP-185(P-2) and PCNP-587AP2(P-5) endolysins with 2280 units/ug and 6930ug/ml, respectively. These results indicated that unmodified endolysins have greater relative activity than the modified ones. Antibacterial activity of endolysins against *M. haemolytic* strain 535 was evaluated using unmodified 185(3-2) and modified PCNP-185(P-2) endolysins which demonstrated a 1.41 log and 1.98 log reduction in bacterial growth, respectively. Combination of endolysins with 0.5mM EDTA produced zero colonies indicating synergistic effect. In future, the study is directed towards engineering of these endolysins to enhance their efficacy. *In vitro* model will be used to optimize the dose of engineered endolysins followed by *In vivo* challenge study in suitable animal model.

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