

Cathelicidin Reduces Internalization of *Staphylococcus aureus* in Bovine Mammary Epithelial Cells

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Background: *Staphylococcus aureus* is a main cause of bovine mastitis. Mastitis caused by *S. aureus* is especially problematic, as there is an increase of strains with resistance to conventional antibiotics. In search of antibiotic alternatives for mastitis, this project focuses on the function of cathelicidin peptides innately secreted by the mammary epithelium in the pathogenesis of *S. aureus* mastitis and udder host-microbial defenses. Cathelicidins have demonstrated antimicrobial and anti-inflammatory effects in skin and intestine. Moreover, it has been reported that *S. aureus* induces cathelicidin in bovine mammary epithelial cells. However, why cathelicidin is important for udder defenses in bovine mastitis remains largely unknown.

Objectives: We aimed to determine the time response of *S. aureus* on internalization into bovine epithelial cells and to determine the effect of cathelicidins on *S. aureus* internalization.

Material and Methods: Bovine mammary epithelial cells (MAC-T) were grown until confluence (24 well-plates; initial inoculum 1.5×10^5 cells/well), washed with phosphate buffer solution (PBS) and exposed to *S. aureus* for 1, 2 and 4 h (3×10^6 CFU/mL, equivalent to a multiplicity of infection (MOI) of 30:1). To count bacteria that invaded the mammary epithelium, media was removed, MAC-T cells washed with PBS (3X) and replaced with DMEM plus gentamicin (200 μ g/mL) that kills extracellular bacteria. One hour later, the media was removed and MAC-T cells washed with PBS and treated with Triton-X 1% to lysis the cells and release internalized bacteria. The lysates were diluted 10X and plated on LB agar to count forming colonies of *S. aureus*. To verify the effect cathelicidin in the bacteria internalization, MAC-T cells were pre-treated with synthetic cathelicidin peptides for 4 h (1, 5 and 10 μ g/ μ L). Statistical differences in bacteria internalization among treatments were analyzed by t-student tests (GraphPad Prism).

Results and Conclusions: This study showed *S. aureus* invaded the mammary epithelium after 2h or 4 h. Importantly, pre-treatment with increasing doses of cathelicidins decreased the amount of *S. aureus* internalized inside the bovine mammary epithelium. Therefore, cathelicidins promote mammary epithelial defenses to prevent the invasion by pathogenic *S. aureus*. Thus, cathelicidins may be able to enhance natural resistance in the udder against mastitis.