

CELL SIGNALING AND CANCER THERAPY & CELL METABOLISM AND CYTOPATHOLOGY

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Synthesis and characterization of novel antimicrobial peptides

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Statement of the Problem: The use of antibiotics as growth enhancers is a common practice in meat industry and extensive use of antibiotic in the meat industry causes an alarming increase of antibiotic resistance microbes across the world. Antibiotic resistance has been posing an increasingly serious concern to the public, health specialist, and animal food producers. Therefore, there is a need for an alternative group of drugs which do not induce bacterial resistance and have no effect on the food chain. Antimicrobial peptides particularly defensin and cathelicidin are prevalent throughout the nature as part of the intrinsic defenses of most organisms. In the present study tissue samples of reproductive tract were collected from apparently healthy indigenous cattle (*Bos indicus*) from local slaughter house to characterize the cathelicidin gene from the reproductive tract epithelial layer for in-silico designing and synthesis of novel antimicrobial peptides from the predicted peptide.

Methodology & Theoretical Orientation: RNA was extracted from the reproductive tract epithelial layer and RT-PCR of extracted RNA yielded a specific product of 530 bp for reproductive cathelicidin gene. The purified PCR product was cloned and positive clones were sequenced at Sci Genom, Hyderabad. The cDNA sequence was compared using homologous sequences retrieved by BLAST. The predicted peptide from complete ORF of reproductive tract cathelicidin gene was comprised of 144 amino acids. Active peptides of *Bos indicus* reproductive tract cathelicidin was from 131-144 and this domain had 3 arginines, 1 lysine, 3 proline and 4 tryptophan. Support Vector Machine (SVM) algorithm was used for designing and prediction of antimicrobial potency from the functional peptide exist in the C-terminal domain of 131-144. Two peptides designed, synthesized and found to be effective against both gram positive and negative bacteria. Minimum Inhibitory Concentration (MIC) of the antimicrobial peptides were determined on the fresh culture of *S. aureus*, *L. monocytogenes*, *E. coli* and *S. Typhimurium*. Cytotoxicity of the synthesized peptides was also recorded by erythrocyte lysis ability at different concentrations (4, 40 and 80 μ M) and was found to be less than 3% up to 40 μ M concentration.

Conclusion & Significance: From the present study, it can be concluded that the epithelial layer of the reproductive tract of *Bos indicus* synthesized a very potent antimicrobial peptide and this can be used as a blueprint for in-silico designing and synthesis of short novel Antimicrobial peptides.

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