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A strategy to challenge antibiotic resistance in *Streptococcus parauberis* using novel antimicrobial peptide "Octominin"

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Background: Antimicrobial resistance is one of the major concerns in world health, and Anti-Microbial Peptides (AMPs) have become an alternative solution to overcome resistant pathogens. Natural or synthetic AMPs consist of multiple modes of action against these pathogens, thus preventing the rapid development of resistance.
Objective: To synthesize the novel AMP, Octominin, based on defense protein of *Octopus minor*, and to test for its detailed mode of action against multi drug resistance *Streptococcus parauberis.*
Method: Octominin's Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by broth micro dilution, and agar diffusion methods, respectively. Field Emission Electron Microscopy (FE-SEM) was conducted to determine bacteria morphology changes after the Octominin treatment. Propidium iodide (PI) uptake assay, 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) staining and DNA binding ability were assessed for studying Octominin's additional mode of actions against *S. parauberis*. Moreover, transcriptional analysis was performed on selected genes related to homeostasis and antibiotic defense using cDNA which was synthesized from Octominin treated *S. parauberis.*
Results: Octominin had 92.5% purity with net charge of +5 and hydrophobic ratio of 43.0%. Octominin showed potent bactericidal activity with MIC and MBC of 50 and 100 µg/mL, respectively. FE-SEM analysis and PI uptake assay confirmed the bacterial surface morphology changes and membrane permeability alterations, respectively. H_2DCFDA staining confirmed the Octominin induced Reactive Oxygen Species (ROS) generation. We found, concentration dependent DNA binding activity of Octominin. Confirming each phenotypically identified Octominin actions in molecular level, genes related to membrane synthesis (*pgsA* and *cds1*) and DNA repairing (*recF*) were downregulated and genes related to DNA protection (*ahpF*) and ROS detoxification (*sodA*) were upregulated in transcriptional analysis.
Conclusion: Octominin showed potent antibacterial activity against *S. parauberis* with multiple mode of actions including, alterations in surface morphology and membrane permeability, DNA binding, ROS generation, and gene expression.