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Antimicrobial activity of "Octopromycin"; A novel peptide derived from the proline-rich protein of *Octopus minor* against *Acinetobacter baumannii*

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Background: The emergence of carbapenem-resistant *Acinetobacter baumannii* raises fears of a range of nosocomial infections and poses the greatest health threats. There is an urgent need to find alternative therapies, including broad-spectrum Antibacterial Peptides (AMPs).

Objective: To design, characterize, and study the antibacterial activity and potential mechanism of a novel synthetic peptide; Octopromycin derived from a proline-rich protein 5 of *Octopus minor*.

Method: The peptide, Octopromycin was synthesized by a solid-phase peptide synthesis technique. The Minimum Inhibitory Concentration (MIC) and time-kill kinetics (broth microdilution), Minimum Bactericidal Concentration (MBC) (agar plating method), and cell viability (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; MTT) of Octopromycin against *A. baumannii* were determined using respective assays. The potential antibacterial mechanisms of Octopromycin were detected by cell morphology changes (Field Emission Scanning Electron Microscopy; FE-SEM), membrane permeability (Propidium iodide; PI and Fluorescein diacetate; FDA uptake), and production of Reactive Oxygen Species (ROS) (2',7'-dichlorodihydrofluorescein diacetate; H_2DCFD assay) in *A. baumannii*.

Results: Octopromycin consists of 38 amino acids. It has a positive charge (+5) with high hydrophobic residue ratio (36%), and predicted to have an alpha helix secondary structure. MIC and MBC were 50 and 200 μg/mL, respectively. MBC/MIC ratio was 4.0, which indicated the efficient bactericidal activity. Time-kill kinetics and bacterial viability assays confirmed the concentration-dependent antibacterial activity of Octopromycin. FE-SEM revealed that Octopromycin caused ultrastructural cell wall deformities to *A. baumannii*, and showed a low propensity to induce resistance. Besides, Octopromycin penetrating to *A. baumannii* cells, it further demonstrated loss of cell membrane integrity that caused cell death at both MIC and MBC. Moreover, Octopromycin treatment increased the production of *A. baumannii* intracellular ROS and decreased cell viability in a concentration-dependent manner.

Conclusion: Collectively, it is apparent that the antibacterial peptide Octopromycin may achieve rapid control of *A. baumannii*, by multi-target interactions, and presents a desirable therapeutic option for the prevention and control of infections.

Acknowledgement: This work was supported by the Research Program of National Marine Biodiversity of Korea (MABIK2021M00600), funded by the Ministry of Oceans and Fisheries, and the National Research Foundation of Korea (NRF) grants, funded by the Korean government (MSIT) (2019R1A2C1087028 and 2018R1A2B6007841).