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P-178 - CONTROL OF PORPHYROMONAS GINGIVALIS USING A BACTERIAL-DERIVED PEPTIDOGLYCAN HYDROLASE

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Background

The human periodontium health is commonly compromised by inflammatory conditions, which are designated by periodontal diseases. *Porphyromonas gingivalis* is considered a keystone periodontal pathogen, capable of breaking the homeostatic relationship with the host and initiating an inflammatory disease. It is thought that the dysbiotic changes caused by this pathogen could be reversed by its specific removal, being an excellent target candidate for therapy. Due to limitations of the current therapies that often leads to the recurrence of the disease and the increasing bacterial resistance to antimicrobials, there is a need for novel approaches to control periodontal disease. Peptidoglycan hydrolases (PGHs) are lytic enzymes that cleave bonds in the bacterial peptidoglycan (PG). In contrast to the well-known bacteriophage-encoded PGHs, such as endolysins, the exogenous use of bacterial-derived PGHs to control pathogenic bacteria remains poorly explored. In this work we explored the use of a *P. gingivalis*-encoded PGH against itself.

Method

An *in silico* analysis was carried out on the genome of *P. gingivalis* 2561 (GenBank AP009380.1) for the identification of putative PGHs. A probable N-acetylmuramoyl-L-alanine amidase, denominated by PgPALys, was cloned in the pET-28a expression vector and its expression in *E. coli* BL21 (DE3) and subsequent purification was optimized. The antibacterial activity of the recombinant protein (alone or in combination with the outer membrane permeabilizer EDTA) was assessed against *P. gingivalis* 2561 and *P. gingivalis* HG66 after incubation at 37 °C in the anaerobic chamber for up to 24 hours.

Results & Conclusions

Antibacterial assays demonstrated that PgPALys is active against *P. gingivalis*, reducing 1.08 ± 0.38 and 1.45 ± 0.37 logarithm units of *P. gingivalis* 2561 and *P. gingivalis* HG66 cells after 24 hours of incubation, respectively. The well-known chelator EDTA was used in combination with PgPALys to increase the permeability of *P. gingivalis* outer membrane (OM) and thus enhance the antibacterial activity. Overall, the antibacterial activity of PgPALys/EDTA improved with the time, resulting in an inactivation of 2.03 ± 0.58 and 2.53 ± 0.71 logarithm units of 2561 and HG66 cells after 24 hours of contact.
This work shows the potential of a *P. gingivalis*-encoded enzyme for the control of the aforementioned periodontal pathogen, providing new insights into the use of a bacterial-derived PGH for the control of bacterial diseases.

References & Acknowledgments