Effects of a chionodracine-derived antimicrobial peptide against bacteria virulence factors

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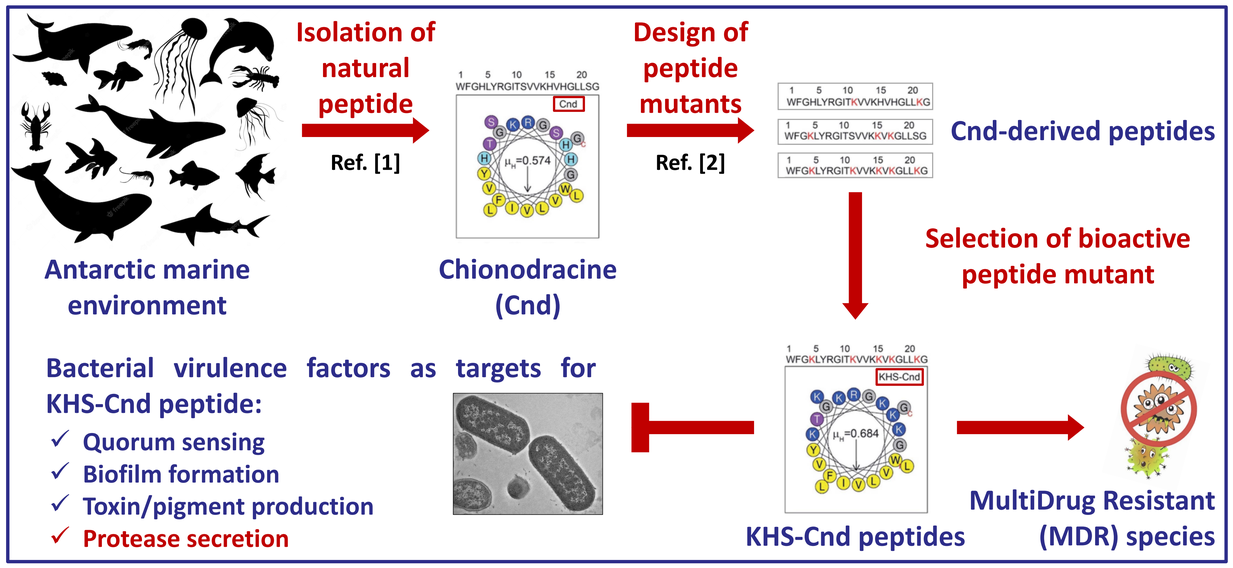
Antarctic fishes, living in an extreme environment and normally exposed to pathogens, are a promising source for antimicrobial peptides (AMPs), fundamental for the innate immune responses of these vertebrates. These natural peptides are emerging as next-generation therapeutics due to their action against bacteria, viruses, yeasts and protozoa. As they show a broad spectrum of activity against multidrug resistant (MDR) bacteria, strong efforts are in progress to bring AMPs into clinical use, in order to counteract the increasing resistance to classical antibiotics. Beyond intrinsic/acquired resistance, MDR species also use virulence factors (like biofilm formation and protease secretion) to infect hosts. Hence, there is a need for innovative approaches targeting these virulence factors especially in the case of bacteria involved in chronic pathogenesis.

In our research, we used a mutant peptide, named KHS-Cnd, that was obtained from the scaffold of the chionodracine (Cnd), a natural peptide identified in the icefish *Chionodraco hamatus*.

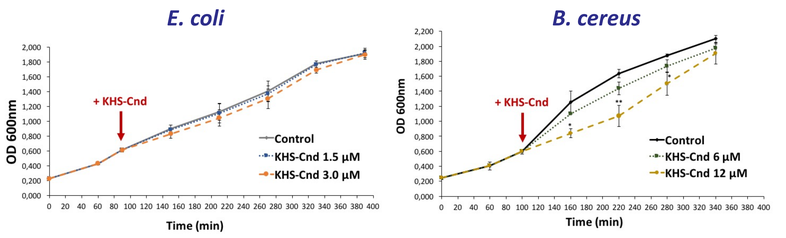


Among virulence factors, we investigated the effect of KHS-Cnd on protease production of two model Gram-negative/positive bacteria, *Escherichia coli* and *Bacillus cereus*.

The peptide was tested both at minimum inhibitory concentrations (MICs) and 2x MICs previously determined for the two bacterial strains. A significant reduction in protease activity was observed for both bacteria at the tested concentrations within 1-3 h from the treatment.

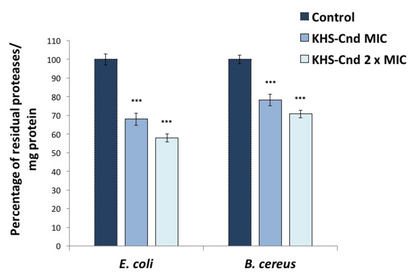
Moreover, we determined that KHS-Cnd has low cytotoxicity on human primary cells and no hemolytic activity on mammalian erythrocytes at concentrations displaying anti-virulence activity, thus confirming the interesting potential of the peptide as a new drug.

Effect of KHS-Cnd on protease production of Gram-negative *Escherichia coli* and Gram-positive *Bacillus cereus*

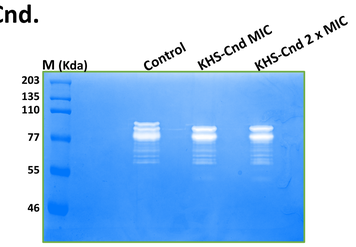


Growth curves of bacteria treated with MICs and 2 x MICs of KHS-Cnd. KHS-Cnd displayed MIC values of 1.5 µM and 6.0 µM for *E. coli* and *B. cereus*, respectively.
*** $p < 0.05$, ** $p < 0.01$.**

Assay of proteolytic activitiy in bacterial sovranatants after 1 h (for *E. coli*) and 2 h (for *B. cereus*) treatment with respective MIC and 2 x MIC of KHS-Cnd.
Data are expressed as the percentage of residual proteolytic activity compared with untreated control bacteria. *** $p < 0.0001$.



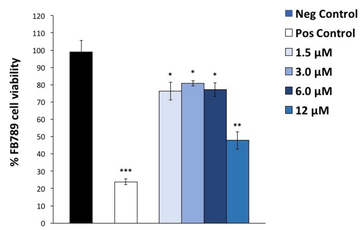
Gelatin-zymography analysis of protease secreted by *B. cereus* after 2 h treatment with KHS-Cnd.



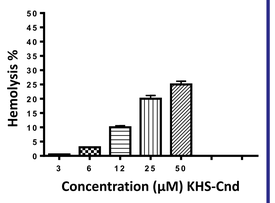
Cytotoxic and hemolytic activities of KHS-Cnd

Cell viability was assessed by MTT assay on normal human FB789 fibroblasts after 24 h of treatment at the four concentrations tested for anti-virulence activity.

Data are expressed as the percentage of viable cells in presence of peptide compared with negative control cells. Positive control cells are represented by cells treated with 2% NaN_3. * $p < 0.001$, ** $p < 0.0001$, *** $p < 0.00001$.



Hemolytic activity was tested against human erythrocytes treated for 2 h with KHS-Cnd up to 50 µM.



Data are expressed as the percentage of hemolysis in presence of peptide compared with positive control (cells treated with 10% Triton X-100 thus representing 100% of hemolysis).

Conclusion and perspective

This study highlights the potential of KHS-Cnd as an anti-virulence agent able to mitigate protease secretion, a key virulence factor produced by antibiotic-resistant pathogens. For a possible application, anti-virulence activity of KHS-Cnd displayed at MICs and 2 x MICs for both considered bacteria; but a toxicity of about 50% was observed in human cells only at one of the highest tested peptide concentrations. Despite this, no hemolytic activity was observed.

References

1. Buonocore F *et al*. **2012** *Fish & Shellfish Immunol* 33, 1183-1191.
2. Olivieri C *et al*. **2018** *RSC Adv* 8, 41331-41346.