CHARACTERIZING THE ACTIVITY OF ANTIMICROBIAL PEPTIDES AGAINST THE PATHOGENIC BACTERIUM CLOSTRIDIUM DIFFICILE IN AN ANAEROBIC ENVIRONMENT

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ABSTRACT

Clostridium difficile is an anaerobic Gram-positive pathogen with high treatment costs and mortality, and very high antibiotic tolerance. Antimicrobial host-defense peptides (HDPs) produced naturally by animal immune systems are promising candidates to develop novel therapies for bacterial infection because they cause oxidative stress that damages multiple targets in bacterial cells, so it is difficult for bacteria to evolve resistance to these attacks. Piscidin, fish-derived HDPs that can also form complexes with copper (Cu) to enhance their activities, are very active against multiple bacterial species in an aerobic environment. We examined their activity against *C. difficile* and other species in an anaerobic environment and found that the interaction of piscidins and copper is different in different oxygen environments. Piscidins are highly active against *C. difficile* and could be a good candidate for drug development.

Introduction

- Clostridium difficile is a Gram positive, obligate anaerobic, spore forming bacterium found in the animal large intestine
- C. difficile is highly antibiotic resistant and has high reoccurrence rate in an infected person
- The exposure to the spores can lead to infection

Antony M. B.: et al J Science reports. 03-21-2016

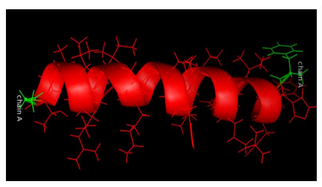
Host-defense (antimicrobial) peptides HDPs

- More than 2700 HDPs have been reported and they function through innate immune system
- HDPs have highly encompassing antimicrobial activities in mammals which help in the immunological defenses
- They employ physicochemical properties mechanistic approach
- Covalent modifications through oxidative stress against pathogens and piscidins is part of these family

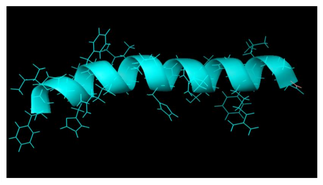
Fox JL (2013) Antimicrobial peptides stage a comeback. Nat Biotechnology 31, 379–382

Piscidins

- These are HDPs with broad spectrum antimicrobial effect and first discovered in vertebrate mast cells
- Two of the isoforms of these antimicrobial peptides extract from pieces are employed in this study, called Piscidin 1 & 3
- Piscidin 1&3 peptides are intrinsically disordered & helical structure in solution



PDB CODE: 2OJM Lee, S.A., Kim, Y.K., Kim, Y. 12-07 2007



PDB CODE: 2MCX. P3 Lee, S.A., Kim, Y.K., Kim, Y. 12-07-2007

Figure 1: PDB CODE: 2OJM and 2MCX. The α-Helix structure of piscidin 1and 3 found by solid-state NMR. Figure adapted from pymol protein structure software

M. Daben J. Libardo "Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persisters and biofilms". J. FEB 09-05-2017

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2MCX:A|PDBID|CHAIN|SEQUENCE FIHHIFRGIVHAGRSIGRFLTGX
2OJM:A|PDBID|CHAIN|SEQUENCE FFHHIFRGIVHVGKTIHRLVTG-
                             *:*********.*.:* *::**
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Figure 2: The amino acid residues conserved in p1 & p3. The three underlined N-terminal amino acid residues represent the ATCUN region for binding of Cu2+ and Ni2+ respectively

THE EXPERIMENTAL REPORTS

The imaging of Piscidin colocalization with a single *C. difficile* cell

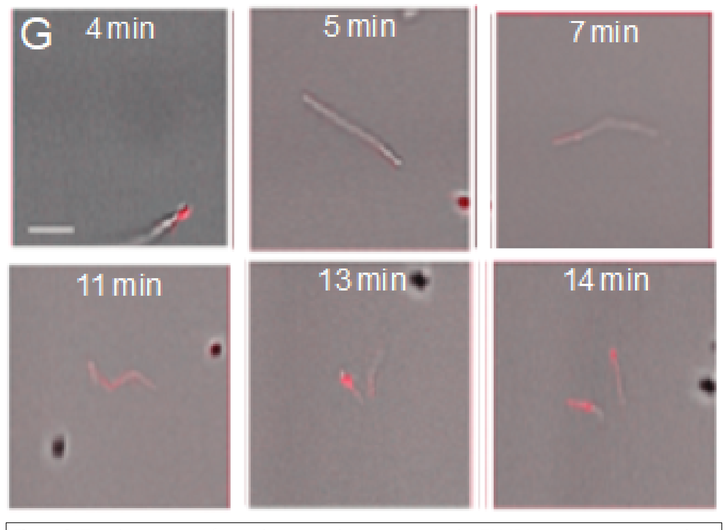


Figure 3: The time course of a cell incorporating labeled p1 with TAMRA at a total concentration of 1.5 µM p1 undergoing rupture (Dr. David Courson)

The Minimal Inhibitory Concentration Assay of p1, p3 & Cu on two strains of *C. difficile*

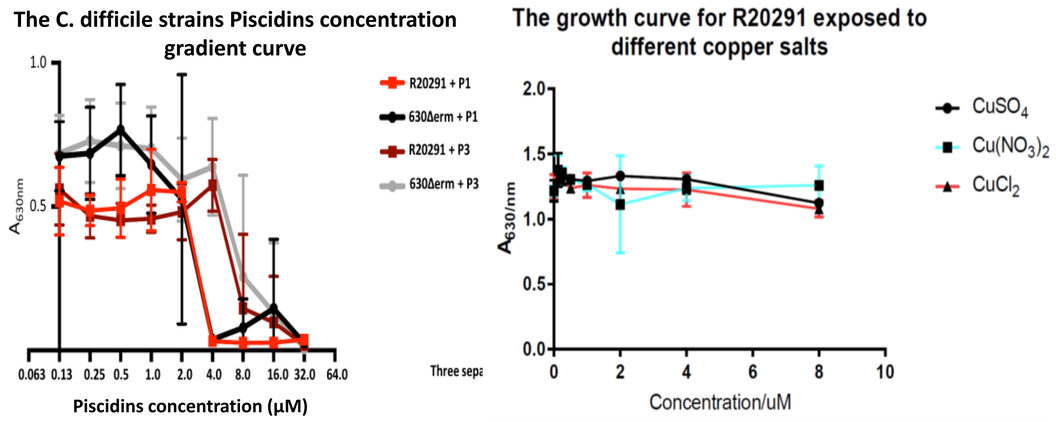
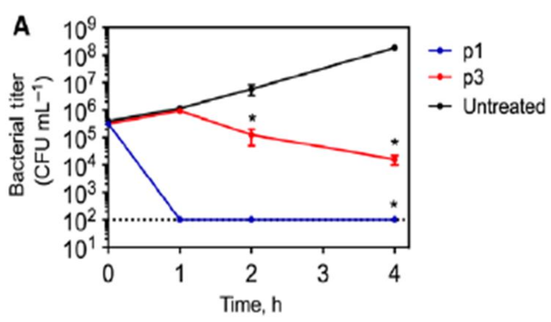


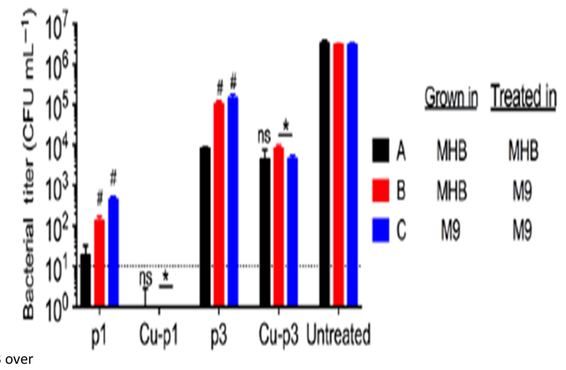
Figure 4: The MICs for p1 & p3 against both strains of C. difficile 630Δerm & R20291 and the copper concentration gradient for three copper(II) salts inhibitory effect against the virulent strain of *C. difficile*

The piscidin and its copper complexes time kill assay in aerobic and anaerobic conditions

E. Coli time kill assay in an aerobic condition against p1 & p3 alone

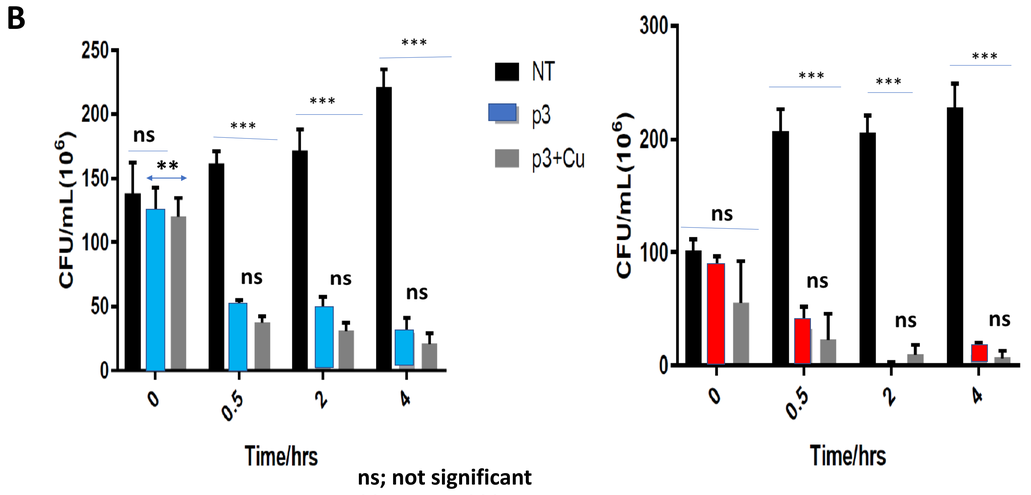


E. Coli time kill assay in an aerobic condition treated with piscidins and their copper complexes in different media



M. Daben J. Libardo "Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persisters and biofilms". J. FEB 09-05-2017

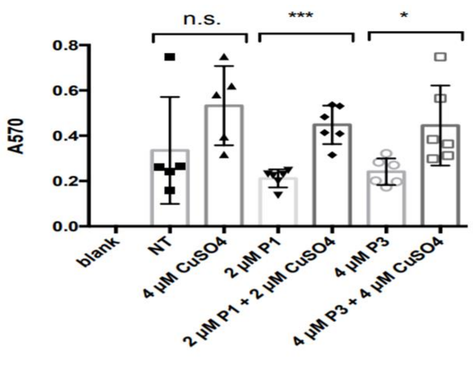
The time kill assay of *C. difficile* treated with psicidins and their copper complexes in an anaerobic conditions



ns; not significant
****p< 0.05, ***p< 0.001**
P1, p3 and Cu complexes are compared by two way ANOVA

Figure 5: The time kill assay between aerobic and an anaerobic bacteria treated with piscidins and their copper complexes. (A) The time kill assay for E.coli with p1 & p3 alone, along with the second for the time kill assay for E. coli in different media and the synergistic effect of the peptide with their complex. (B) time kill assay of the two peptides and their complexes not working synergetic in an anaerobic condition or addition of copper do not increase the bactericidal activity of the peptides

Biofilm inhibitory assay

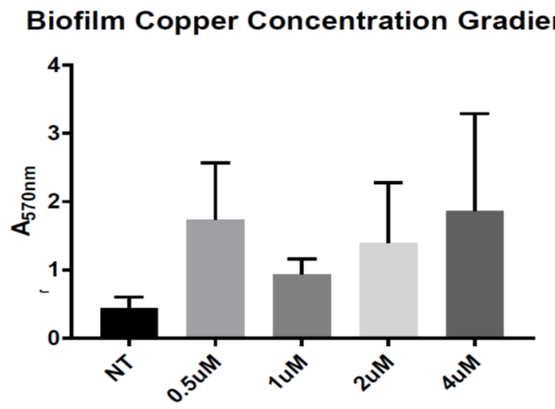


conditions +/- CuSO4 were compared by unpaired t test
n.s. not significant
* $p < 0.05$
*** $p < 0.001$

Figure 6: The pisdidins and their complexes biofilm inhibitory activity and copper alone. The showed biofilm formed in 24 well plates

Cu2+ appear to stimulate biofilm formation in *C. difficile*

A



B

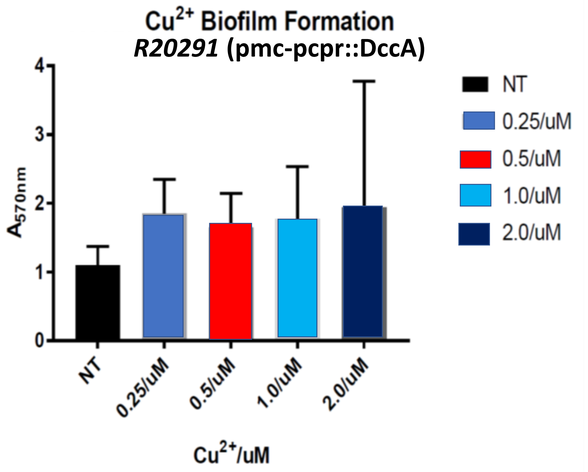


Figure 7: The graphical representation of Cu(II) biofilm inhibitory assay of two C. difficile strains (A) The Cu concentration gradient used to treat R20291 virulent strain. (B) The treatment *of C. diffiicile* strain RT527 with gradient copper concentrations

Conclusions

- Piscidins are active antimicrobial peptides against *Clostridium difficile* and p1 is better than p3 in bactericidal activity.
- Rate of p1 *Clostridium difficile* killing is fast than p3.
- There is no significant difference between the peptides complexes and peptides alone in time kill assay.
- Biofilm formation is effectively inhibited by peptides alone than their complexes

Future directions

- I would like to investigate oxidative stress response of *Clostridium difficile* in more transition metals.
- Drug design purpose in the future.
- Copper antimicrobial effect in the presence and absence of oxygen in relation to C. difficile and other bacteria biofilms.

Acknowledgements

- Dr. Erin B. Purcell, ODU, Norfolk VA.
- Dr. David Courson, ODU, Norfolk VA.
- Dr. Daben J. Libardo, UC, Storrs, CT.
- Dr. Myriam Cotten, W & M, VA.