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Clostridioides difficile Spore Production in Response to Antibiotic and Immune Stress

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ABSTRACT

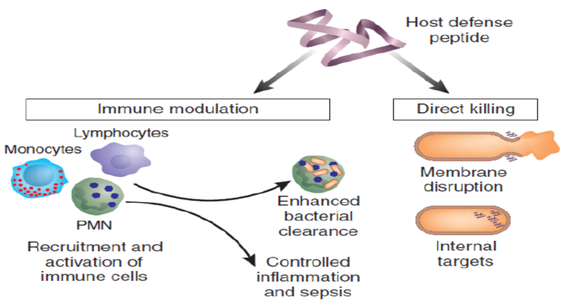
***Clostridioides (Clostridium) difficile*, an anaerobic, spore-forming Gram-positive pathogenic bacterium, is a major cause of hospital-acquired infections and can persist as surface-attached biofilms for protection from antibiotic and immune stress. *C. difficile* can form biofilms as a single species or with other anaerobic intestinal bacteria. The environmental signals that cause individual cells to secrete toxins, form biofilms, or develop into spores that can spread the infection to new patients are unknown. In these studies, we investigate bacterial responses to different stress. Antimicrobial host-defense peptides (HDPs) produced 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. We investigate antibiotic treatments, metal ions and sugars, and antimicrobial peptide treatments to determine how. *C. difficile* reacts to multiple environmental stresses like those from antibiotic treatment or the human immune system. In our investigation of *C. difficile* and HDPs in an anaerobic environment, we found that the interaction of piscidin and copper is different in different oxygen environments. Antibiotics and oxidative stresses from other sources cause the cells to form spores and/or biofilms to protect themselves, but piscidin kill vegetative *C. difficile* cells without triggering these protective responses. Piscidins are highly active against *C. difficile* and could be a good candidate for drug development.**

Introduction

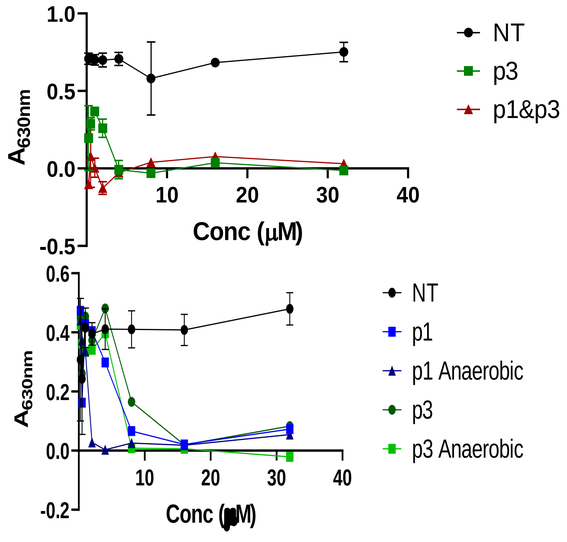
***Clostridioides difficile (C. difficile)* are Gram-positive, anaerobic, spore, and biofilm-producing bacteria (1)**

- **Highly resilient to multiple classes of common antibiotics (2)**
- **Several outbreaks caused by *C. difficile* infection (CDI) have been reported worldwide over the last decade**
- **Infection spread through spores (3)**
- **Hospital death rate and cost are more than 25,000 death and $6 billion annually in the US alone (1)**
- **Antibiotic treatment is the major risk factor for *C. difficile* infection (CDI) (2)**
- **Recurring episodes affect 15–35% of patients and present a particular risk for the elderly (1,2)**

HDPs killing roles in the innate immune system



Piscidin act synergistically and most effective in an equilibrated environment



Sub-inhibitory concentrations of piscidins synergize with antibiotics

Antibiotic (μg/mL)	MIC: no peptide	+ 0.5X p1 (2μM)	+ 0.5X p1Cu (2μM)	+ 0.5X p3 (4μM)	+ 0.5X p3Cu (4μM)	+ 0.5X p1&p3
Fid	≥0.50	0.10	0.05	0.20	0.05	0.02
Met	≥4.00	1.00	2.00	2.00	0.50	0.25
Van	≥4.00	2.00	1.00	0.50	0.25	0.50
Cipro	>128	n/a	n/a	n/a	n/a	n/a

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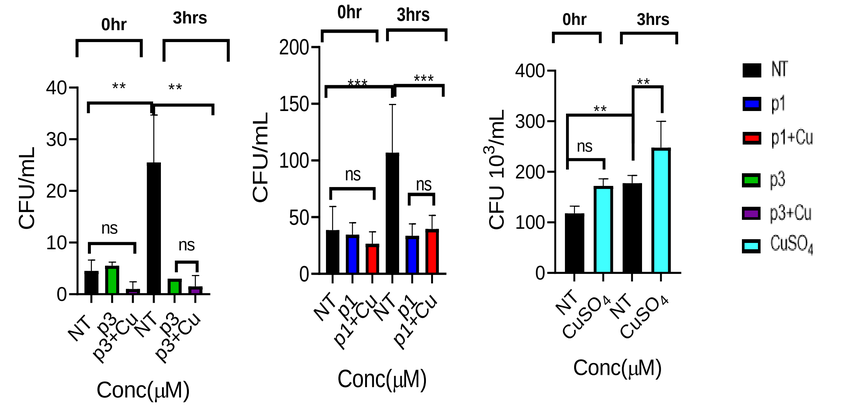
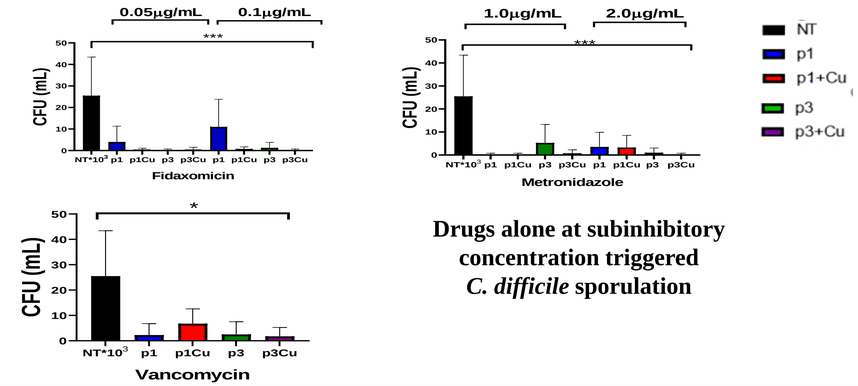
Copper induces sporulation but piscidin and piscidin-copper complexes do not



Piscidins + antibiotics reduce sporulation more than either treatment alone



Drugs alone at subinhibitory concentration triggered *C. difficile* sporulation

Conclusions

- **Piscidins, copper complexes alone and in synergy with themselves or antibiotics significantly reduce *C. difficile* proliferation**
- **Stress from the peptides and their copper complexes do not trigger sporulation in *C. difficile***
- **Biofilm formation may be stimulated by piscidin in sub-leather dosage.**

Future Directions

- **Elucidation of the mechanism of action of the two peptides in synergy with antibiotics**
- ***In-vivo a*pplications and drug design**

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