Department of Biochemistry and Microbiology

Characterization of a bacteriocin that targets *Clostridioides difficile*

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Abstract

Clostridioides difficile is a bacterium of concern for anyone undergoing antibiotic treatment. *C. difficile* can resist most antibiotics and is currently only treated with Metronidazole and Vancomycin. Both antibiotics are non-specific *to C. difficile* and have the side effect of killing the normal microbiome of the gut. This microbiome helps to keep the body resistant to *C. difficile* infections. The lack of specific treatment options perpetuates the problem of infection and can lead to relapses of disease. *Clostridium butyricum*, a non-pathogenic probiotic, has been shown to produce a highly specific antimicrobial product called a bacteriocin that targets *C. difficile*. Previously, the gene encoding for the *C. butyricum* bacteriocin (CBMB) was cloned into *E. coli* and purified as recombinant protein. The recombinant CBMB was shown to exhibit potent activities multiple strains of *C. difficile*. In my project, I continue the characterization of CBMB by performing disk agar diffusion assays, growth curve analysis showing the antimicrobial effect of CBMB on *C. difficile*. In silico analysis using the new AI system Alphafold 2 was performed to predict the 3-dimension structure of CBMB. Based on this analysis, we also designed peptide fragments derived from the different regions of CBMB to determine the site of catalysis. Furthermore, we are also working on determining the minimal residues required for CBMB to still retain antimicrobial activity. The ultimate goal of my project is to generate potentially novel alternative treatment of *C. difficile* infections.

METHODS

Protein Prep:

- Transformed BL21 *E. coli* with plasmid containing CBMB (pET21b-CBMB)
- Induced and collected *E. coli* for protein purification
- Sonication and prepping for protein purification
- Column purification using nickel resin to collect CBMB using His-Tag
- Further purification of mature CBMB using FPLC

Growth Curve Analysis:

- 15 test tubes with 25mL of BHIS broth were assigned to either a control or 1 of 4 possible treatments
- Each test tube got appropriate amount of antibiotic or CBMB (6 and 3 µg/mL Vancomycin or 12 and 6 µg/mL CBMB)
- Test tubes were then inoculated with a 1:100 dilution of *C. difficile* R20291 from an overnight culture
- OD was measured and recorded every hour for 8 hours

Modified Disk Diffusion Assay:

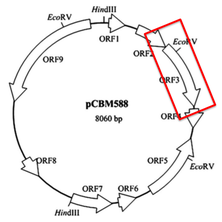
- *C. difficile* R20291 and 630 were grown in an overnight culture and inoculated into fresh BHIS broth at a 1:100 dilution
- This was allowed to grow until OD ~1
- The *C. difficile* was then diluted to an OD of 0.08 with fresh BHIS broth
- 100 µL was then pipetted onto 1 of 3 BHIS agar plates, 3 plates per strain, and spread to make a lawn using a sterile Q-tip
- Paper disks were impregnated with 100, 50, or 25 µg/mL Vancomycin or CBMB. This was done 3 times allowing the disk to dry between each impregnating
- Triplicates were prepared for each concentration and placed on plates.

Results

Previous Results

Sequence analysis of a bacteriocinogenic plasmid of *Clostridium butyricum* and expression of the bacteriocin gene in *Escherichia coli*

Anaerobe, 2010 Jun;16(3):253-7



Bacterial species	MIC (µg/ml)	Bacterial species	MIC (µg/ml)
Clostridium perfringens	6.25	*Proteus mirabili*	>25
Clostridium tetani	12.5	*Enterobacter aerogenes*	>25
Clostridium butyricum MIYAIRI 588	6.25	*Klebsiella pneumoniae*	>25
Staphylococcus aureus	>25	*Serratia marcescens*	>25
Methicillin-resistant *Staphylococcus aureus*	>25	*Salmonella spp*	>25
Methicillin-sensitive *Staphylococcus aureus*	>25	*Pseudomonas aeruginosa*	>25
Staphylococcus epidermidis	>25	*Shigella sonnei*	>25
Bacillus subtilis	>25	*Shigella flexneri*	>25
group A streptococci	>25		

Bacterial species		MIC (µg/ml)	MBC (µg/ml)
Clostridium difficile	Strain R20291	1.56	3.13
	Strain DPS630	3.13	6.25
	Strain TNHP 1	1.56	3.13
	Strain TNHP 3	3.13	6.25
	Strain TNHP 6	1.56	3.13
	Strain TNHP 79	3.13	6.25
	Strain TNHP 82	1.56	3.13
	Strain TNHP 403	3.13	6.25
	Strain TNHP 20	3.13	6.25
	Strain TNHP 59	3.13	6.25
	Strain TNHP 207	1.56	3.13

(Chang et al, Unpublished)

Figure 1. Recombinant bacteriocin purification

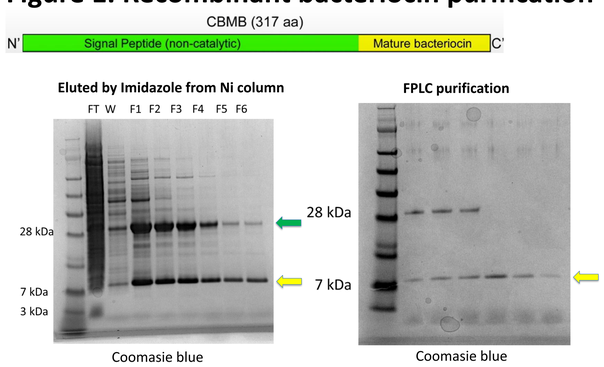
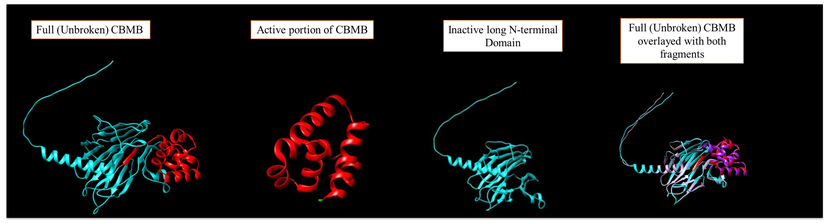
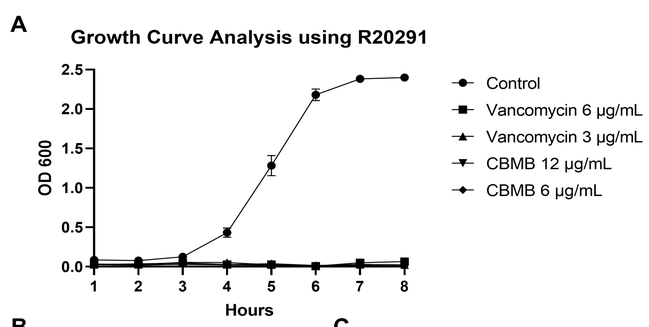


Figure 2. Alphafold structure prediction



CBMB PSAWQITKCAGSIAWALGSGIFAGAKLLKIKKYIKALGGVKEAAALLLGATTWAEKMEAGGSALVNLAAEISGVKDIKENCFS
---HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH--HHHHHHHHH----HHHHHHHHHHHHHHHHHHH--HHHHH---

Figure 3. Inhibition of *C. difficile* growth by purified bacteriocin

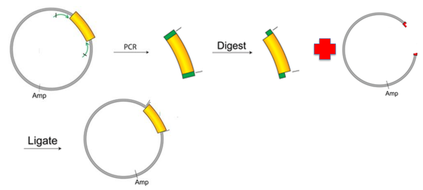


Summary

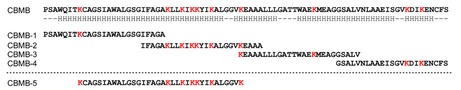
- Reestablish protocols for the recombinant purification of CBMB
- CBMB has bactericidal activity at predicted both concentrations of 6 and 12 µg/mL
- Preliminary disc diffusion assay suggest CBMB not diffused in membrane
- In-silico prediction of mature bacteriocin structure

Future Directions

- Optimizing Disk Diffusion Assay
- Cloning of mature bacteriocin for recombinant protein purification



- Testing the antimicrobial activity of peptides derived from CBMB



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REFERENCES

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- Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. 2021;596(7873):583-589. doi:10.1038/s41586-021-03819-2