Department of Biochemistry and Microbiology

Antimicrobial activity of a recombinant *Clostridium butyricum* bacteriocin (CBMB) against *Clostridioides difficile* and other gram positive and gram negative bacteria

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INTRODUCTION

Clostridioides difficile is the leading cause of antibiotic-associated diarrhea worldwide and infects approximately 200,000 people annually in the United States. *C. difficile* is a gram positive, anaerobic, spore forming bacterium that takes advantage of the disruption of the gut microflora during antibiotic treatment. *C. difficile* spores germinates in the small intestine and rapidly proliferate in the colon leading to infections that can lead to sepsis and death if left untreated. Vancomycin and metronidazole are the antibiotics of choice for *C. difficile* infections (CDIs). However, relapsing cases are often common while the risk of developing antibiotic resistance is high. Therefore, it is critical to develop alternative treatment strategies against *C. difficile*. Previously, our lab screened multiple non-pathogenic commensals for their ability to inhibit *C. difficile* in vitro. Currently commercialized as a probiotic, ***Clostridium butyricum* Mayairi bacteriocin (CBMB) was shown to have the highest inhibitory effect on multiple strains of *C. difficile* in vitro**. Currently we have successfully cloned and purified recombinant CBMB. Results from our laboratory show an equal to greater effectiveness of the bacteriocin compared to the current antibiotics. In this study, our goal is to assess the activity of the bacteriocin on several strains of *C. difficile* as well as other similar bacterium. The goal of the research would be to find a novel treatment for CDIs that would avoid reoccurring cases and antibiotic resistance risks.

OBJECTIVES

- To determine the inhibitory activity of CBMB on multiple *C. difficile* isolates collected from Taiwan
- To determine the inhibitory activity of CBMB on bacteria other than *C. difficile*

METHODS

- Determining the growth kinetic of *C. difficile* isolates collected from wastewater treatment plants and seafood
- Overexpression and purification of recombinant CBMB using affinity chromatography
- Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of CBMB using microbroth dilution assay and agar subculturing, respectively.
- Determine MIC and MBC of CBMB on bacteria other than *C. difficile*

RESULTS

Fig. 1 *C. difficile* sampling sites in Taiwan

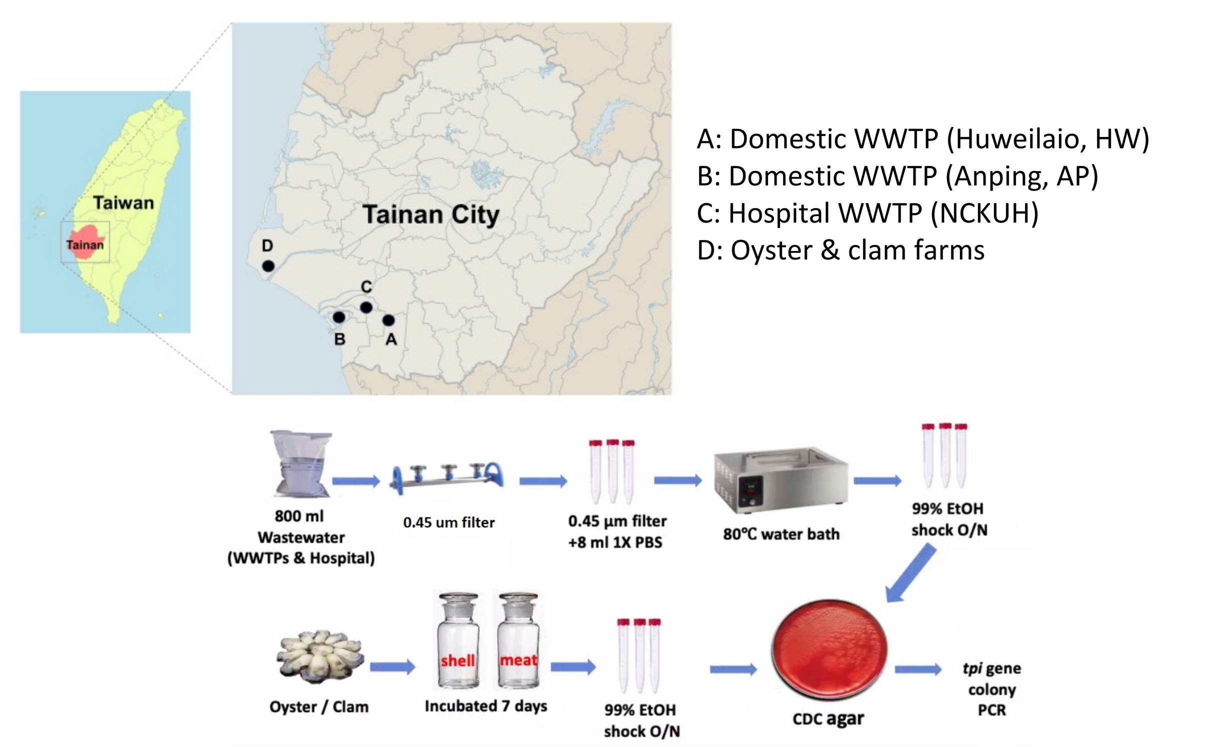

Table 1. Environmental *C. difficile* isolates

Ribotype	16S rRNA	Cd tpi	RT078-lineage	tcdA	tcdB	cdtB	cdtA	tcdC	tcdA truncation	No. of isolates	Total
			Toxin genotype								
RT002/2	(+)	(+)	–	(+)	(+)	–	–	WT	No	3	97
RT010	(+)	(+)	–	–	–	–	–	–	n.d.	2	
RT012	(+)	(+)	–	(+)	(+)	–	–	WT	No	1	
RT020	(+)	(+)	–	(+)	(+)	–	–	WT	No	1	
RT027	(+)	(+)	–	(+)	(+)	(+)	(+)	Δ18 bp	No	2	
RT043	(+)	(+)	–	(+)	(+)	–	–	WT	No	1	
RT060	(+)	(+)	–	–	–	–	–	WT	n.d.	1	
RT106	(+)	(+)	–	(+)	(+)	–	–	WT	No	3	
RT126	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ39 bp	No	3	
RT127	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ39 bp	No	55	
RT235	(+)	(+)	–	(+)	(+)	–	–	Δ18 bp	No	1	
RT462	(+)	(+)	–	–	–	–	–	–	n.d.	6	
RT590	(+)	(+)	–	–	–	–	–	–	n.d.	1	
RT592	(+)	(+)	–	–	–	–	–	WT	n.d.	1	
RT596	(+)	(+)	–	–	–	–	–	–	n.d.	1	
RT598	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ39 bp	No	1	
RT607	(+)	(+)	–	–	–	–	–	–	n.d.	2	
RT633	(+)	(+)	–	(+)	(+)	–	–	WT	No	1	
RT638	(+)	(+)	–	–	–	–	–	WT	n.d.	1	
RT647	(+)	(+)	–	–	–	–	–	–	n.d.	1	
RT713	(+)	(+)	–	–	–	–	–	–	n.d.	4	
AI-60	(+)	(+)	–	–	–	–	–	–	n.d.	3	
AI-74	(+)	(+)	–	–	(+)	–	–	WT	n.d.	1	
AI-83	(+)	(+)	–	–	(+)	(+)	(+)	WT	n.d.	1	

Fig. 4 Representative MIC and MBC for *C. difficile*

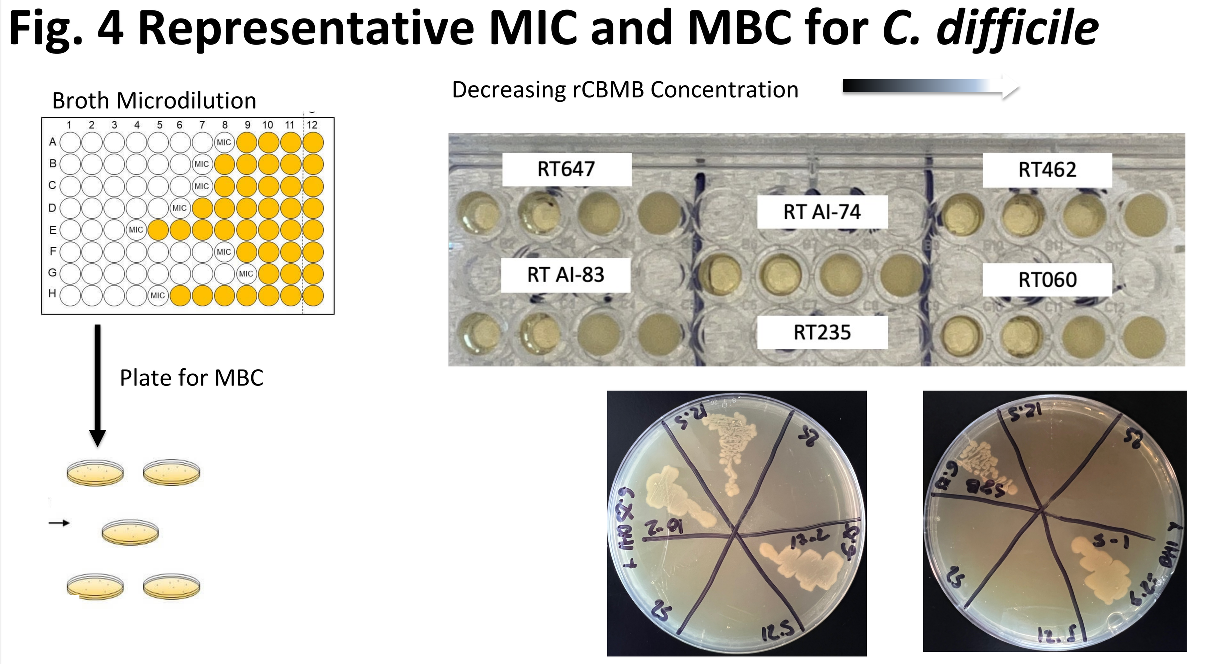

Fig. 2 rCBMB Purification

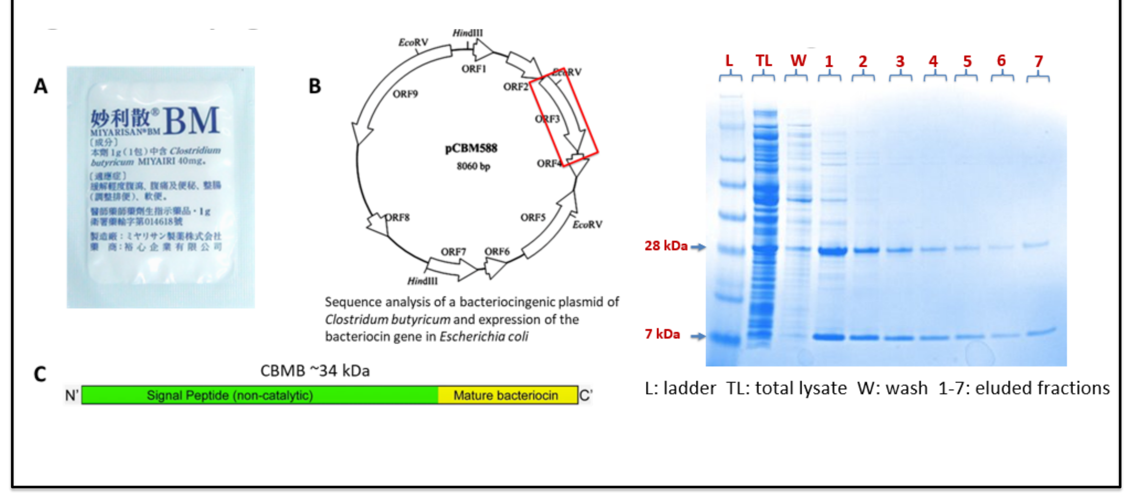

Fig. 3 *C. difficile* isolates growth curve

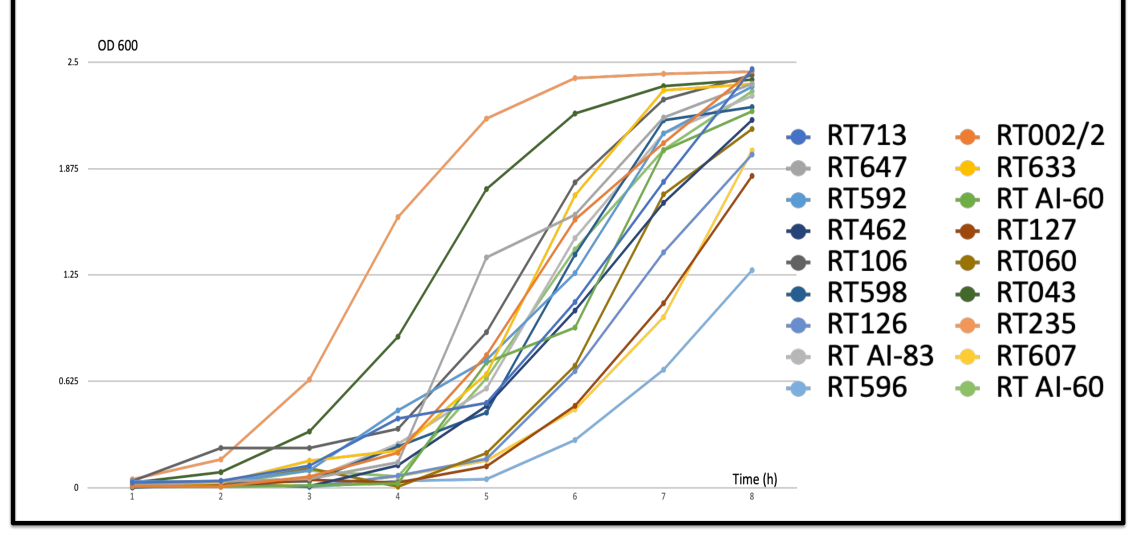

Table 2. MIC/MBC for *C. difficile*

Ribotype	MIC (cbmb ug/mL)	MBC (cbmb ug/mL)
RT002/2	12.5	12.5
RT043	12.5	12.5
RT060	12.5	12.5
RT106	12.5	25
RT126	12.5	25
RT127	12.5	12.5
RT235	12.5	12.5
RT462	12.5	12.5
RT 592	12.5	25
RT 596	12.5	25
RT 598	12.5	25
RT 607	12.5	12.5
RT633	12.5	25
RT647	12.5	12.5
RT713	12.5	25
AI-60	12.5	25
AI-74	12.5	25
AI-83	12.5	12.5

Table 3. Other bacteria tested

	Gram Positive	Gram Negative
Strict Aerobe	*Bacillus subtilis*	
Facultative	*Serratia marcescens*	*Serratia marcescens*
Strict Anaerobe	*Fusobacterium nucleatum*	*Clostridium difficile*

Fig. 5 Representative MIC and MBC for other bacteria

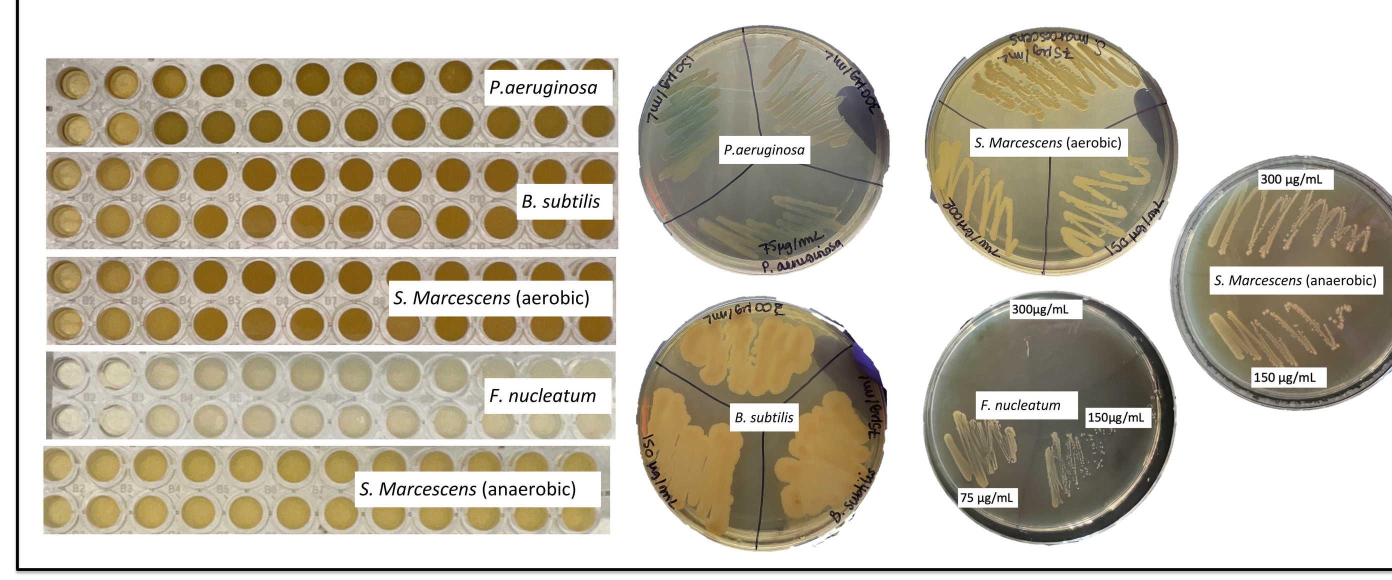

Table 4. MIC/MBC for other bacteria

Bacteria	MIC (μg/mL)	MBC (μg/mL)
P. aeruginosa	150	>300
B. subtilis	300	>300
S. marcescens (aerobic)	300	>300
S. marcescens (anaerobic)	150	300
F. nucleatum	>300	>300

SUMMARY & FUTURE WORK

- CBMB does have inhibitory effect on multiple *C. difficile* strains and isolates
- Some isolates are more resistant than other to the bacteriocin
- CBMB does have inhibitory effect on bacteria other than *C. difficile* but at high concentration
- Future work would include testing of the bacteriocin on *C. difficile* sporulation
- Testing of shortened CBMB against *C. difficile* and other bacteria

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