

Analyzing the phenotypic characteristics of environmental *Clostridioides difficile* Isolates

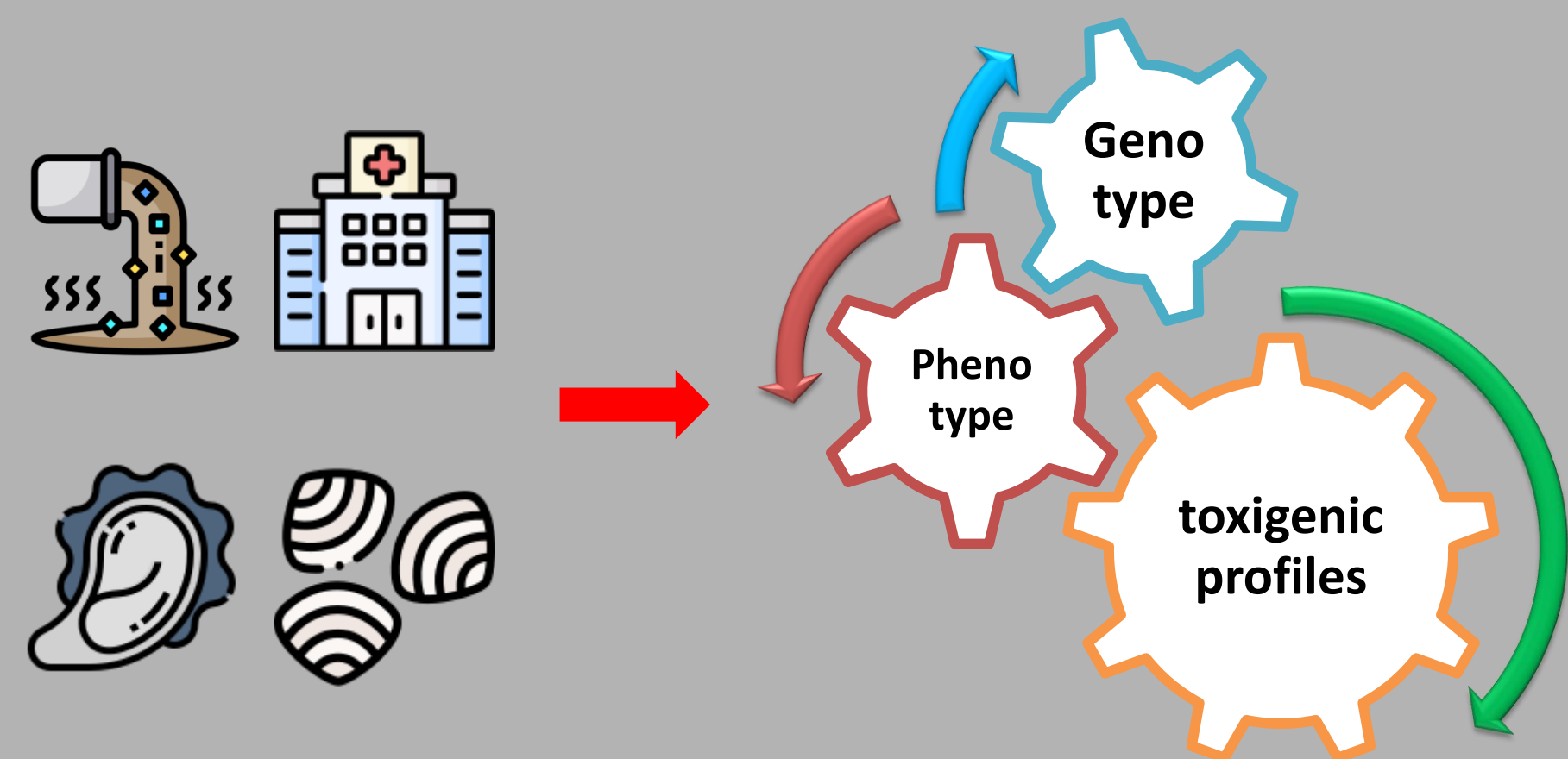


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INTRODUCTION

Clostridioides difficile (*C. difficile*) is an anaerobic, spore-forming bacterium. *C. difficile* is one of the most common causes of hospital-acquired antibiotic associated diarrhea and there are both toxigenic and non-toxicogenic *C. difficile* strains. Previously our lab isolated multiple *C. difficile* strains from several wastewater treatment plants in Taiwan. Ribotyping and antibiotic resistance profiling were performed for all 97 individual isolates. In total, 24 different ribotypes were represented.

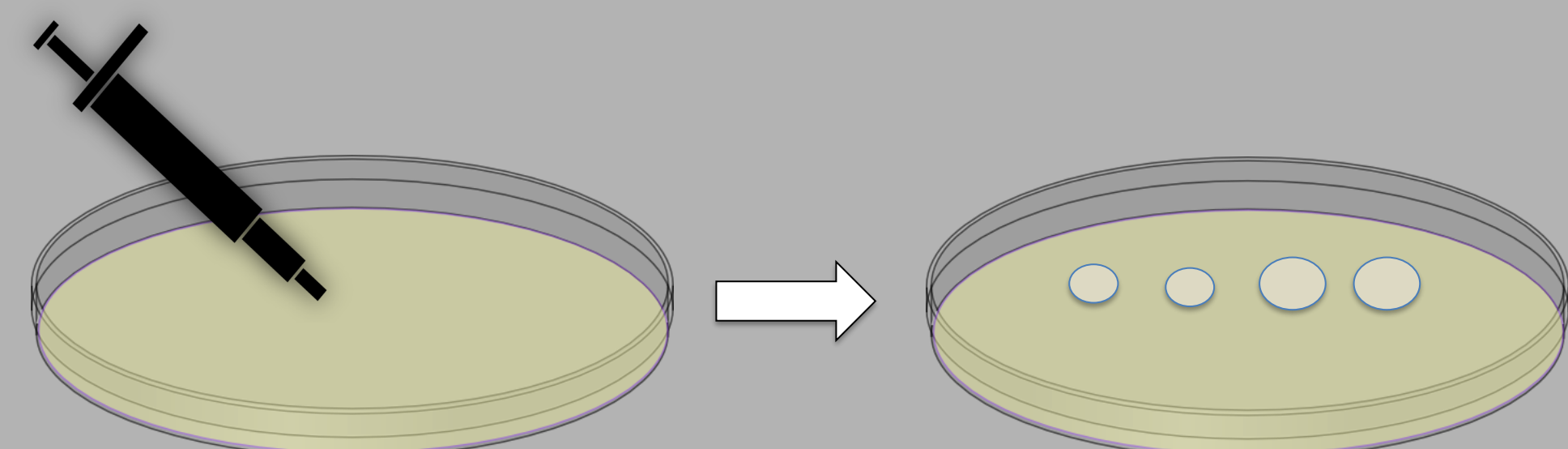
OBJECTIVES



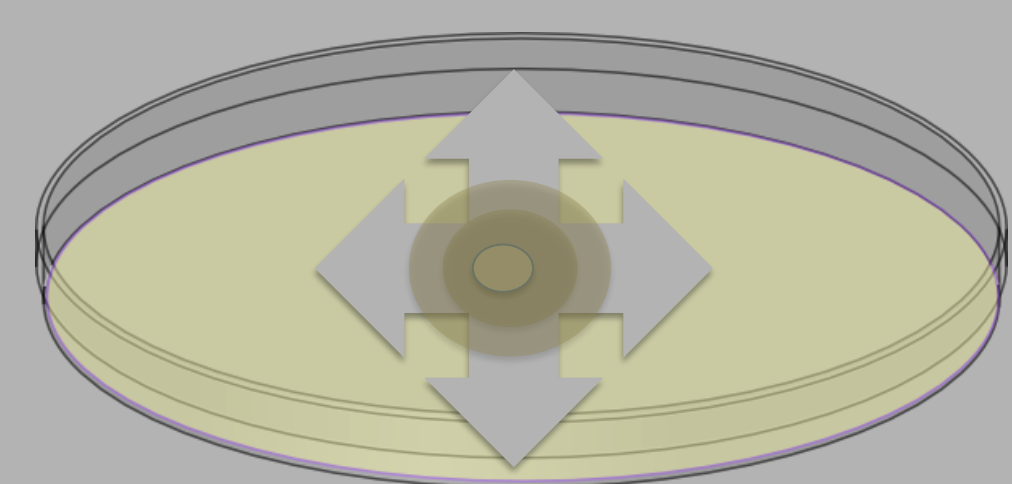
To investigate the phenotypic characteristics of environmental *C. difficile* isolates by examining colony morphology and gliding motility.

METHODS

- Colony morphology was examined by visually evaluating the morphology (i.e. size and shape) of a single colony for each isolate grown on BHIA.



- Gliding motility was quantified by recording the migration diameter for each isolate at 24-hour intervals over a period of 5 days. This experiment was done in triplicates. Statistical analysis was performed using GraphPad Prism 9.02 (161)



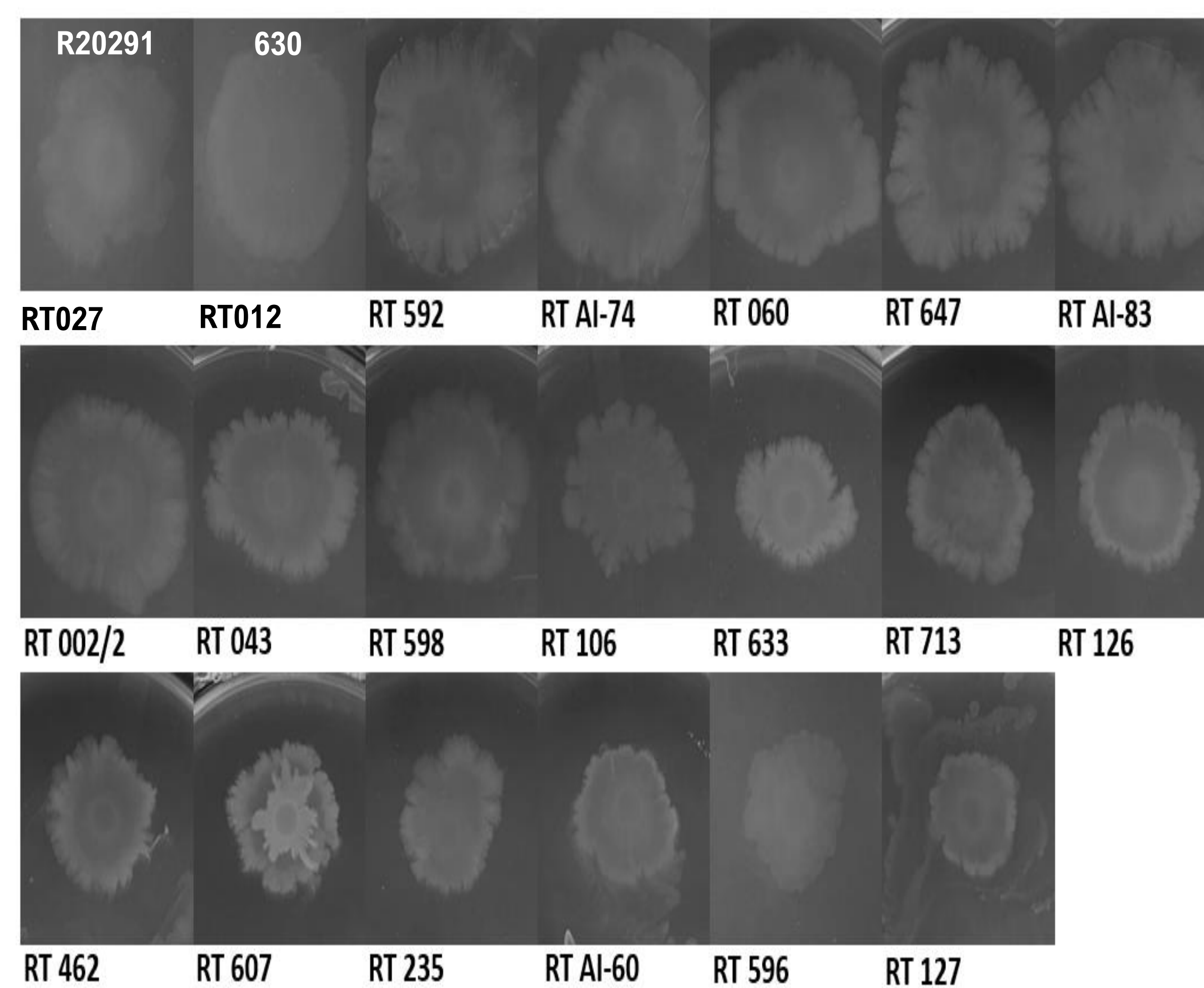
RESULTS

Table 1. *C. difficile* isolates used in this study

Ribotype					Toxin genotype				
	16S rRNA	Cd tpi	RT 078 lineage	tcdA	tcdB	cdtB	cdtA	tcdC	tcdA truncation
RT 002/02	(+)	(+)	(-)	(+)	(+)	(-)	(-)	WT	No
RT043	(+)	(+)	(-)	(+)	(+)	(-)	(-)	WT	No
RT060	(+)	(+)	(-)	(-)	(-)	(-)	(-)	WT	n.d.
RT106	(+)	(+)	(-)	(+)	(+)	(-)	(-)	WT	No
RT126	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ 39 bp	No
RT127	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ 39 bp	No
RT235	(+)	(+)	(-)	(+)	(+)	(-)	(-)	Δ 18 bp	No
RT462	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
RT592	(+)	(+)	(-)	(-)	(-)	(-)	(-)	WT	n.d.
RT596	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
RT598	(+)	(+)	(+)	(+)	(+)	(+)	(+)	Δ 39 bp	No
RT607	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
RT633	(+)	(+)	(-)	(+)	(+)	(-)	(-)	WT	No
RT647	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
RT713	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
AI-60	(+)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	n.d.
AI-74	(+)	(+)	(-)	(-)	(+)	(-)	(-)	WT	n.d.
AI-83	(+)	(+)	(-)	(-)	(+)	(+)	(+)	WT	n.d.

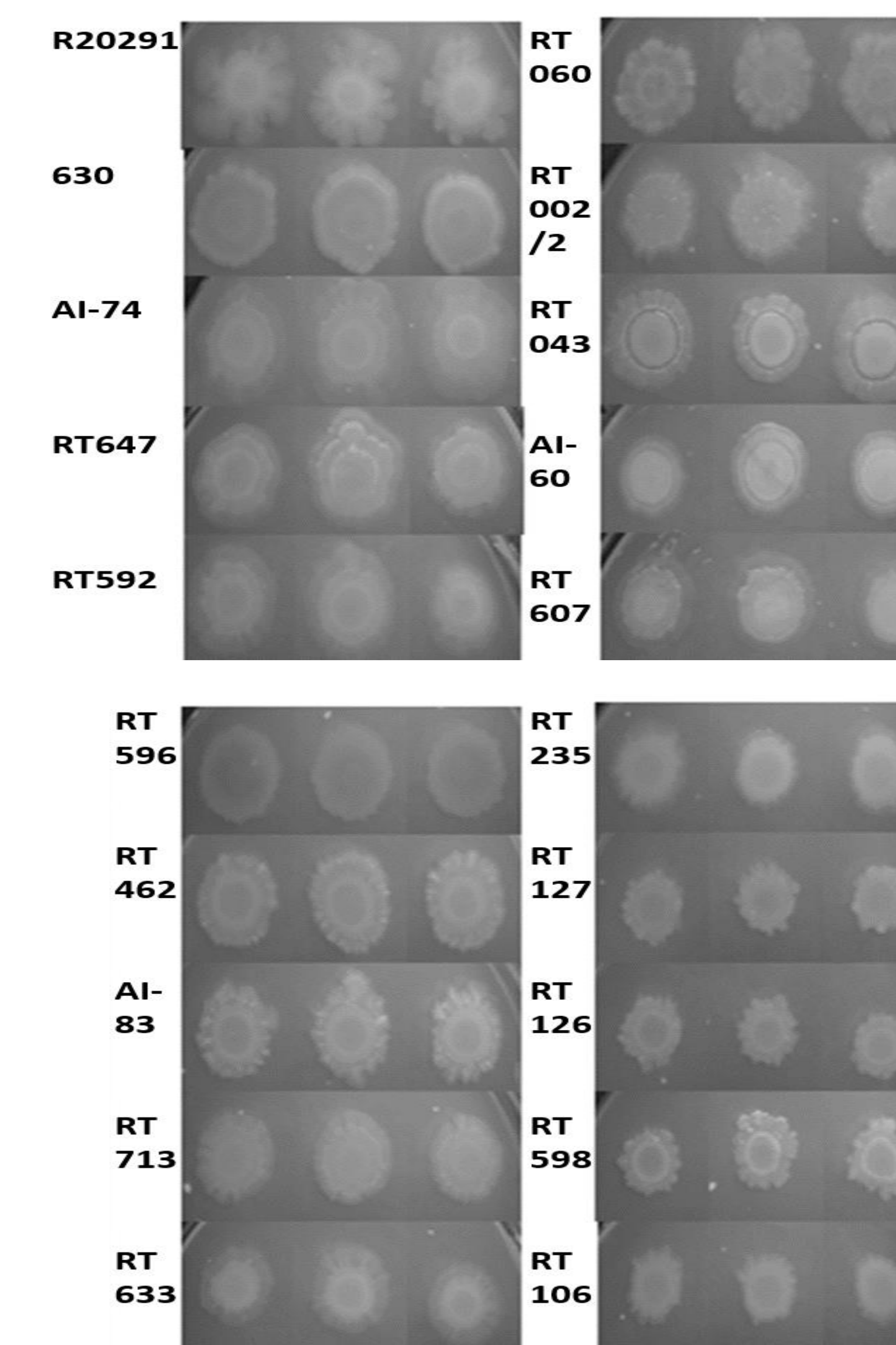
Ribotype and toxin genotype information of the isolates used in this study are listed here. This table was adapted from Ya-Ru Li's thesis.

Figure 1. Colony Morphology



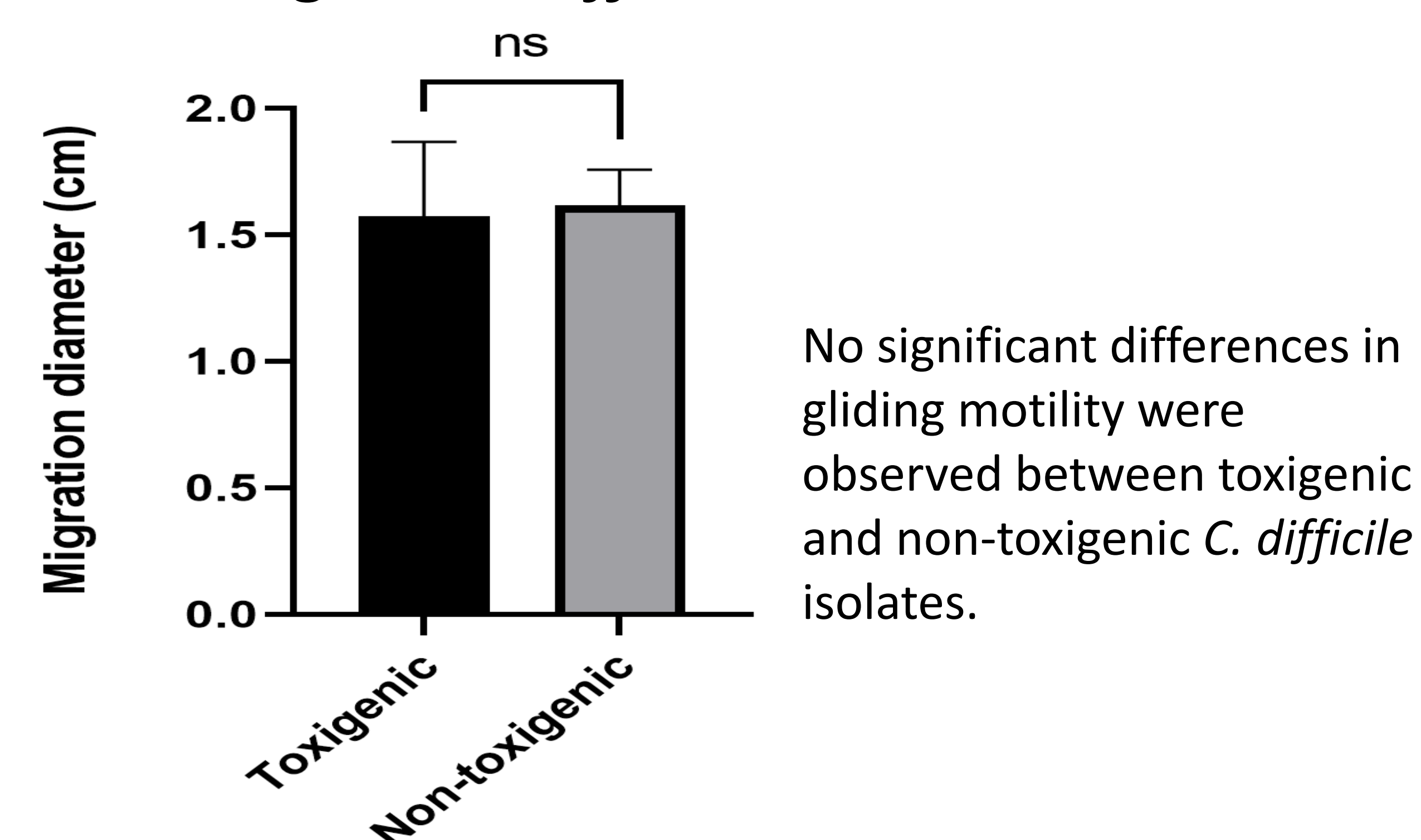
Colony morphology of environmental *C. difficile* isolates. Photographs of colonies were taken after 7 days of anaerobic incubation and are arranged based on visual similarities. Broad diversity among different ribotypes was observed. *C. difficile* strains R20291 and 630 were used as controls.

Figure 2a. Gliding Motility (Type IV pilus movement)



Gliding motility of environmental *C. difficile* isolates anaerobically grown on 2.1% BHIA with 1% glucose. Images are organized by visual similarities and migration diameter. *C. difficile* strains R20291 and 630 served as controls.

Figure 2b. Gliding Motility of toxigenic and non-toxicogenic *C. difficile* isolates



Summary and future work

- Established *C. difficile* isolate bank at OSU-CHS
- Wide diversity in colony morphology among isolates tested
- Gliding motility on agar surface measured
- C. difficile* displayed varying degree of gliding motility
- No significant differences between toxigenic vs non-toxicogenic strains in gliding motility
- We will next measure swimming motility (flagellar motility) and sporulation efficiency

REFERENCES

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