Type I toxin-antitoxin systems contribute to mobile genetic elements maintenance in *Clostridioides difficile*

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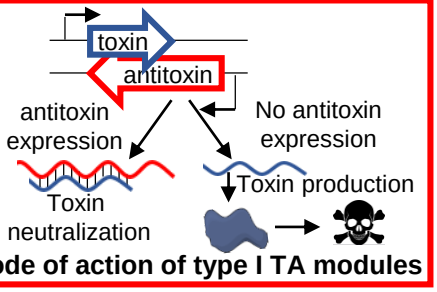
INTRODUCTION

- Toxin-antitoxin (TA) systems are widespread in bacteria: composed of a stable toxin and an unstable antitoxin.
- Toxin synthesis impacts growth and cell viability.
- Antitoxin expression neutralizes the toxin action or production.
- In type I TA, synthesis of the toxin protein is prevented by the transcription of an antitoxin RNA during normal growth.

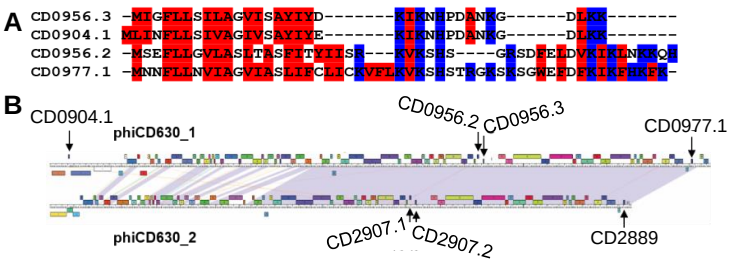
Here, we report the characterization of 7 type I TA systems present within phiCD630-1 and phiCD630-2 prophage regions of *C. difficile* strain 630



Mode of action of type I TA modules

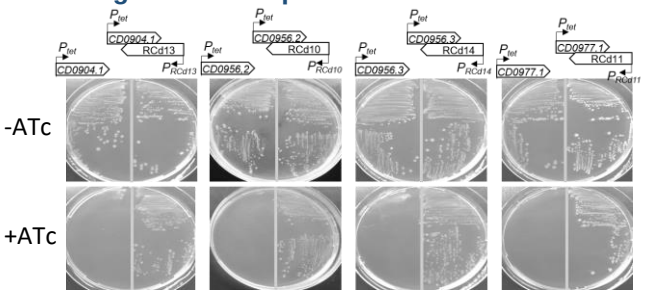
RESULTS

Identification of toxin genes within prophages of *C. difficile* 630



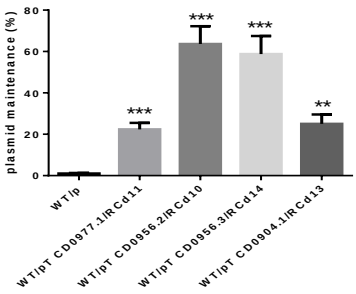
A. Protein alignment of newly identified toxins. The hydrophobic and positively charged amino acids are indicated in red and blue, respectively. **B**. Maps and alignment of the phiCD630-1 and phiCD630-2 genomes.

toxin genes within phiCD630-1 are functionnal



Growth of *C. difficile* 630Δ*erm* strains harbouring represented plasmids on agar plates with Tm and with (+ATc) or without 10 ng/ml of ATc (-ATc) after 24 hrs of incubation at 37°C.

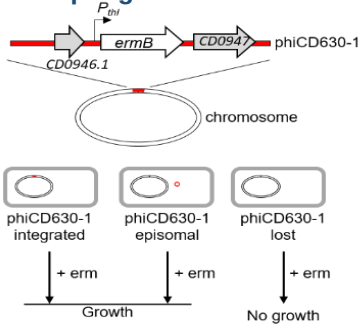
TA modules confer plasmid stabilization



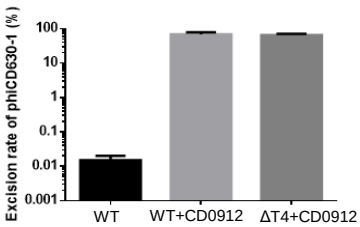
The stability of control vector (p) and derived vectors expressing the different TA modules under control of their own promoters in *C. difficile* 630Δ*erm* was determined after seven passages in TY broth without selection pressure.

A method to monitor phage loss *in-vivo*

A cassette containing an erythromycin resistance gene (*ermB*) under control of the strong *thl* promoter of *C. acetobutylicum* was introduced into an innocuous location of phiCD630-1. Cells that lost the prophage were selectively killed when plated on erythromycin-containing plates

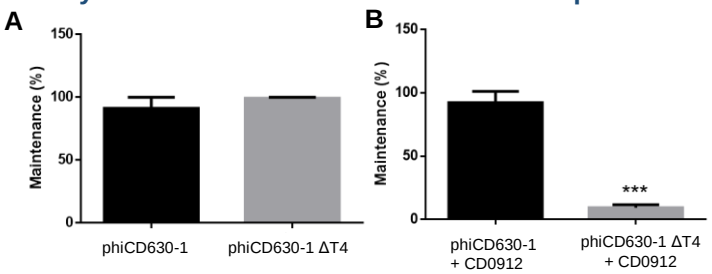


The excisionase CD0912 induces phiCD630-1 excision



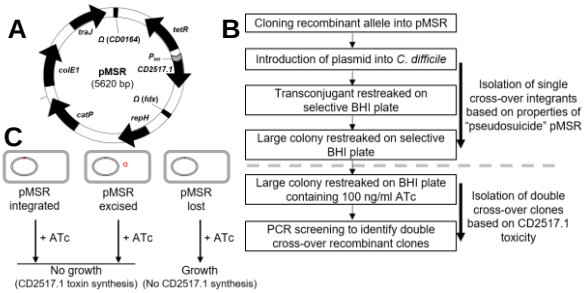
The frequency of prophage excision was estimated by quantitative PCR. Excision rate of phiCD630-1 was higher in *C. difficile* expressing CD0912 when compared to WT and was not impacted by the deletion of the 4 toxin genes (ΔT4).

TA systems are involved in maintenance of phiCD630-1



A. Maintenance of prophages phiCD630-1::*erm* and phiCD630-1-ΔT4::*erm* was determined after four passages in TY broth by plating serial dilutions on agar plates supplemented or not with Erm. **(B)** Maintenance of prophages in strains expressing CD0912 after 24 h of growth in TY was quantified by plating serial dilutions on agar plates supplemented or not with Erm.

TA as a counterselection marker for chromosomal manipulation



A. Features of the pMSR vector for allele exchange (ACE) in *C. difficile*. The toxin gene *CD2517.1* is under the control of the ATc inducible promoter Schematic overview of the ACE protocol (**B**) and of the counterselection method used to isolate double cross-over clones (**C**). Isolated single cross-over integrants were restreaked on ATc-containing agar plates to induce synthesis of toxin CD2517.1. Cells that retained pMSR (either integrated or excised) produced CD2517.1 and were selectively killed.

CONCLUSIONS

- novel functional type I TA identified in *C. difficile* 630 prophages
- Identification of phage excisionase gene of phiCD630-1 prophage
- TA modules contribute to prophage maintenance and stability upon excision.
- Toxins can be used as counter-selection markers for chromosomal manipulation in *C. difficile*
- new vector generated for highly efficient ACE in *C. difficile*: more than 50 mutants constructed so far, including multiple deletions and insertions in various *C. difficile* strains.

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