New functional aspects of *Clostridium difficile* CRISPR-Cas system

Maikova Anna[1,2,3,4], Boudry Pierre[2], Shiriaeva Anna[4], Vasileva Aleksandra[5], Boutserin Anaïs[3], Medvedeva Sofia[4], Semenova Ekaterina[6], Severinov Konstantin[4,5,6], Soutourina Olga[3,7]

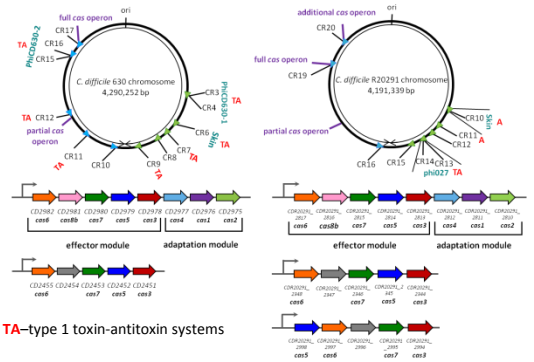
[1]Peter the Great St. Petersburg Polytechnic University, Saint Petersburg, Russia;
[2]Laboratoire Pathogenèse des Bactéries Anaérobies, CNRS-2001, Institut Pasteur, Université de Paris, Paris, France;
[3]Université Paris-Saclay, CEA, CNRS, Institute for Integrative Biology of the Cell (I2BC), Gif-sur-Yvette, France;
[4]Center of Life Sciences, Skolkovo Institute of Science and Technology, Moscow, Russia;
[5]Institute of Gene Biology, Russian Academy of Sciences, Centre for Precision Genome Editing and Genetic Technologies for Biomedicine, Institute of Gene Biology, Russian Academy of Sciences, Moscow, Russia;
[6]Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, USA;
[7]Institut Universitaire de France (IUF).

Email of presenting author: ann-maikova@yandex.ru

Abstract

Nosocomial diarrhoea caused by *Clostridium difficile* has become a key public health issue associated with antibiotic therapy in industrialized countries. Many aspects of *C. difficile* pathogenesis remain poorly understood. During its infection cycle, *C. difficile* interacts with bacteriophages and other mobile genetic elements, possibly by relying on its CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) system of adaptive immunity. Previous studies of our groups revealed the presence of an active and original CRISPR-Cas system in *C. difficile,* which contains an unusually large set of CRISPR arrays, some of which are localized in prophage regions, two or three I-B type *cas* operons and the toxin-antitoxin type I systems, linked to several arrays. However, the role CRISPR-Cas plays in the physiology and infectious cycle of this pathogen remains obscure. In the present work we determine the general PAM consensus using PAM libraries experiments and confirm the functionality of all PAM variants through plasmid conjugation efficiency assays in *C. difficile*. Additionally, we demonstrate the defensive function of all 12 CRISPR arrays from laboratory 630 strain. Finally, we show an adaptive function of *C. difficile* CRISPR-Cas system for the laboratory strain and confirm PAM consensus through naïve adaptation experiments. Altogether, these data highlight the original features of active *C. difficile* CRISPR system that might be important for *C. difficile* survival during its infection cycle.

Chromosomal organization of *C. difficile* CRISPR-Cas loci

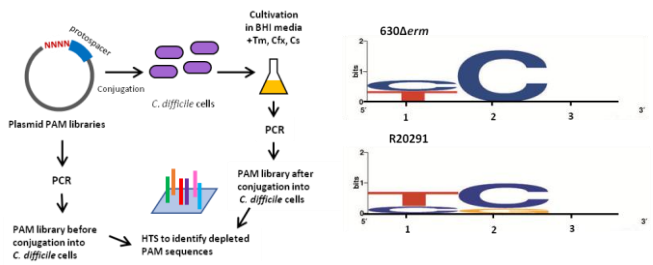


TA–type 1 toxin-antitoxin systems

Boudry *et al*. mBio. 2015; Maikova *et al*. NAR. 2018

PAM-sequences determination in *C. difficile* 630 and R20291

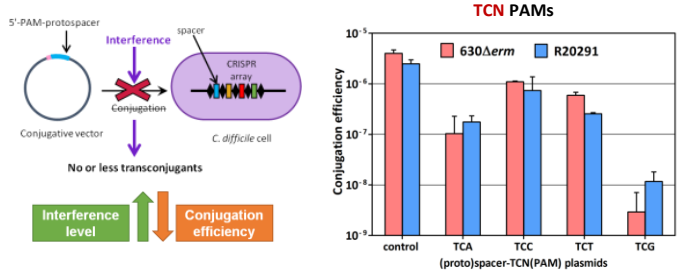
Plasmid depletion assay with a plasmid library for PAM screening in *C. difficile*



CCN/TCN PAM consensus in both strains

Maikova *et al*. in preparation

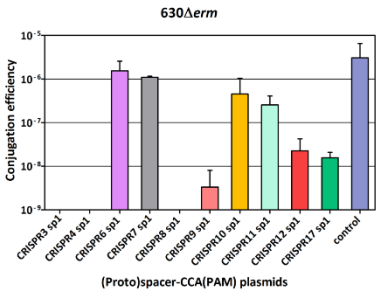
Plasmid interference assays to confirm PAMs



- CCN PAMs are fully functional in both 630 and R20291 strains
- TCN are less active in both 630 and R20291 strains

Maikova *et al*. in preparation

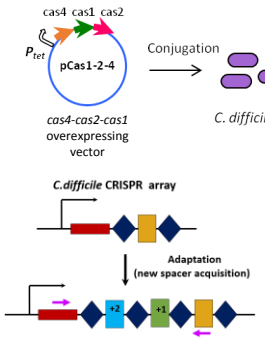
Functionality of CRISPR-Cas system for plasmid interference in *C. difficile* 630

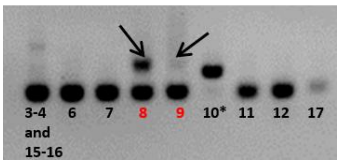


- **Almost all** CRISPR arrays in 630 strain **are functional**
- **Defense levels** of different arrays **generally** correspond **to their expression rates**

Maikova *et al*. in preparation

Naïve CRISPR adaptation assay in 630 strain





C. difficile 630 arrays

- CRISPR-Cas system in 630 strain is **functional for adaptation**
- New spacer acquisition was detected in **CR8 and CR9 arrays**
- **Most spacers** were acquired from the **pCas1-2-4 plasmid**
- **CCN PAMs** are confirmed by adaptation assays

Maikova *et al*. in preparation

Conclusions

- Enlarged **PAM (CCN/TCN)*** sequences were identified for *C. difficile* 630 and R20291 strains
  * **TCN** PAMs are less effective for interference
- **Active interference** of almost all **12 CRISPR** arrays in **630 strain**
- **New spacer acquisition** was shown for 2 CRISPR-arrays in **630 strain** and **CCN PAM** functionality was **confirmed for adaptation.**
- The **naïve adaptation** is **not as active as interference** in *C. difficile*

Basis for mechanistic and physiological analyses of CRISPR-Cas-mediated interactions of *C. difficile* with its genetic parasites