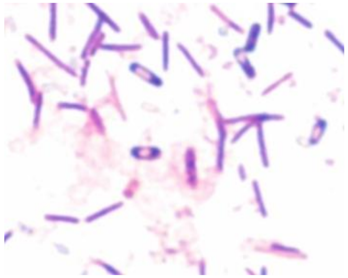
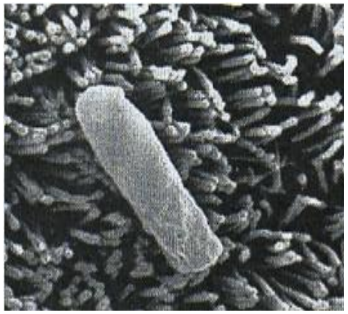
5th ICDS – May 2015 – Bled, Slovenia

Clostridium difficile virulence factors





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Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

The ClosTron: A universal gene knock-ou

John T. Heap, Oliver J. Pennington [1], Stephen T. Ca

OPEN ACCESS Freely available online

Expanding the Repertoire of Gen
Manipulation of the *Clostridium*
Exchange Using *pyrE* Alleles

Yen Kuan Ng[1,9], Muhammad Ehsaan[1,9], Sheryl Philip[1], Mark M
Stephen T. Cartman[1], Nigel P. Minton[1]*

Published online 18 January 2012 *Nucleic Acids Research, 2012, Vol. 40, No. 8* ***e59***
doi:10.1093/nar/gkr1321

Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker

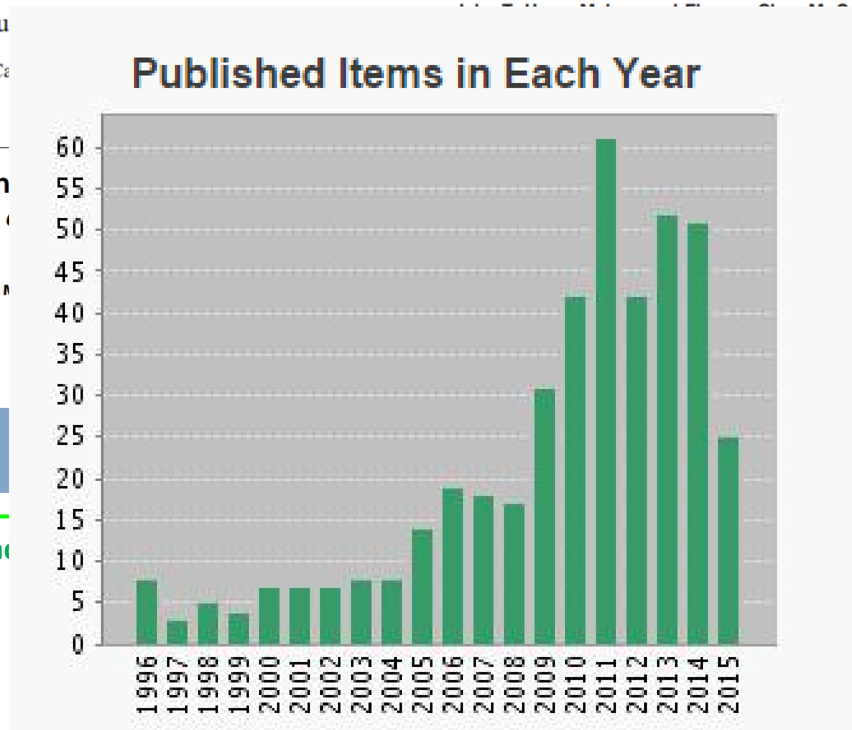
oksley, Yen-Kuan Ng,
Minton*

doi:10.1111/j.1365-2958.2006.05315.x
First published online 1 August 2006

hromosomal

De

1893	1935	1977	2011	2015

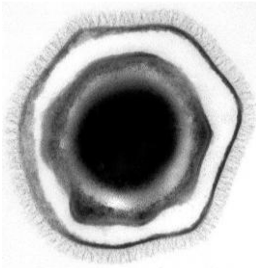


Un

lopment of genetic tools

lopment of animal models

Pathogenic model of *C. difficile* infection (CDI)



Contamination

Modification of intestinal microbiota
Disruption of the barrier effect

Antibiotics

Spore germination

Gut colonization

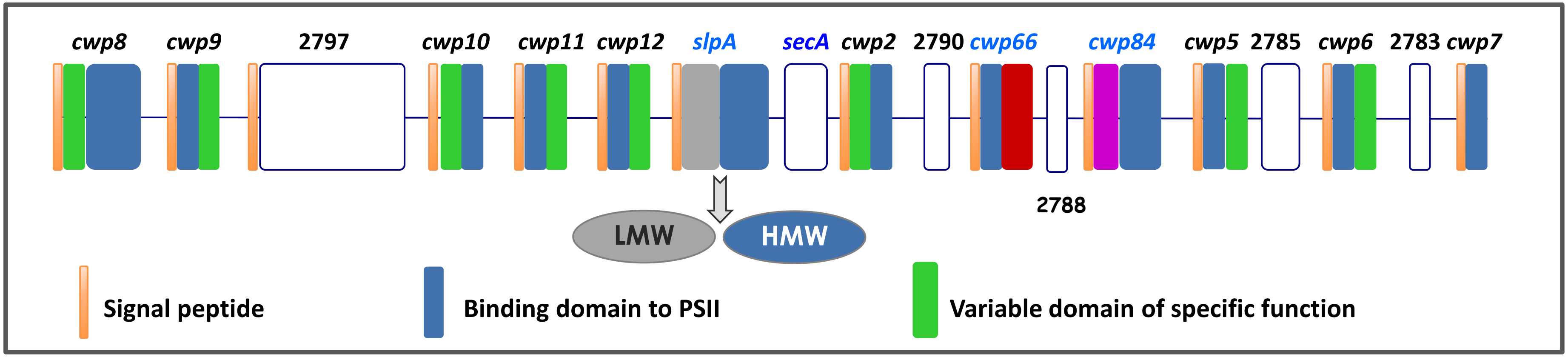
Toxin production

Production of virulence factors:
- adhesins
- proteolytic enzymes
- resistance factors to host defenses
- toxins

Clinical manifestations: Diarrhea, PMC
Frequent relapses

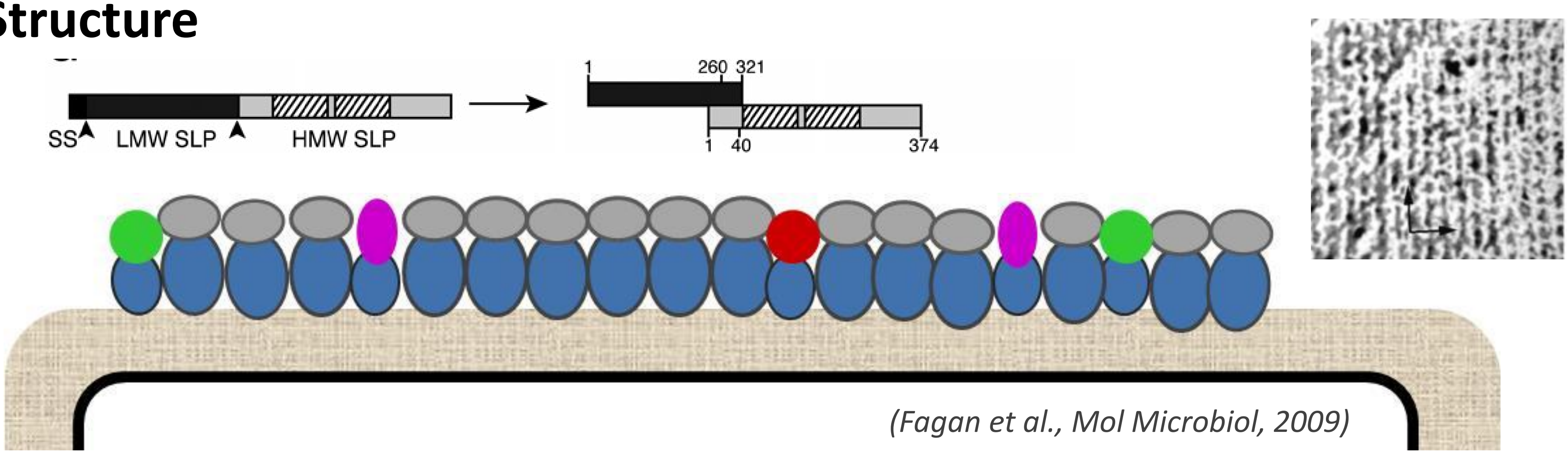
S-layer proteins

Genetic organization



(Karjalainen et al., Infect Immun, 2001; Fagan et al., J Med Microbiol, 2011; Willing et al., Mol Microbiol, 2015)

Structure



(Fagan et al., Mol Microbiol, 2009)

Functions

- **Role in adherence to the host**

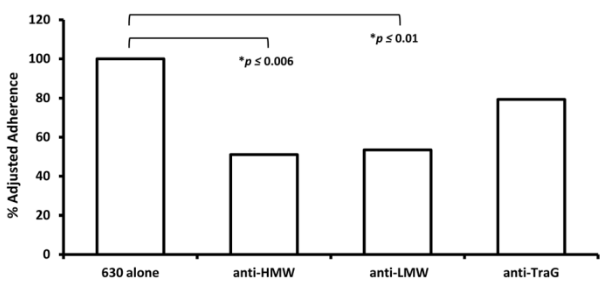
Adherence of native SLPs and rSlpA to human gastrointestinal tissues

(Calabi, Infect Immun, 2002)

Adhesion to colon: **Control protein** **Native SLPs** **Purified recombinant SlpA**

Important contributor to *C. difficile* adherence to Caco-2 cells

(Merrigan et al., Plos One, 2013)



Role as MSCRAMM: adherence of HMW to ECM components

No study of mutant

- ***slpA* essential for in vitro growth of the R20291 strain** *(Dembek et al., mBio, 2015)*

Fibronectin binding protein Fbp68/FbpA

- **Surface localization**
- **Structure** *(Lin et al., J Biol Chem, 2011)*

- **Function:**

  Direct binding to fibronectin *(Hennequin et al., Microbiology, 2003)*

  Study of mutants (630 background):

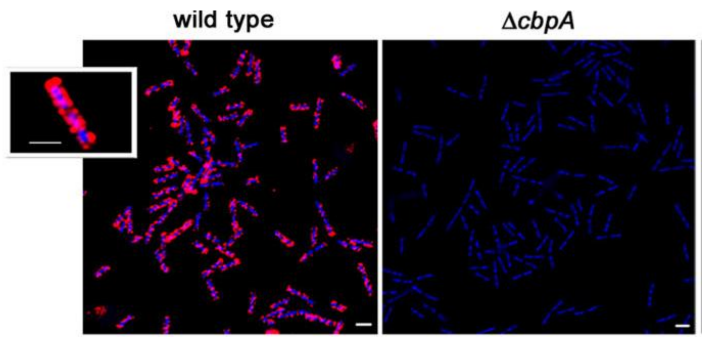
Study	In vitro adherence rates / WT	Colonization rate in mouse model / WT
Lin et al., J Biol Chem, 2011	**No inhibition / WT (Caco-2 cells)**	**ND**
Barketi-Klai et al., J Med Microbiol, 2011	**Small increase / WT (Caco-2 & HT29-MTX cells)**	**Decrease fitness of colonization & decrease adhesion to caeca of mutant**

- **Gene of the core genome** *(Janvisliri et al., J Bacteriol, 2009)*

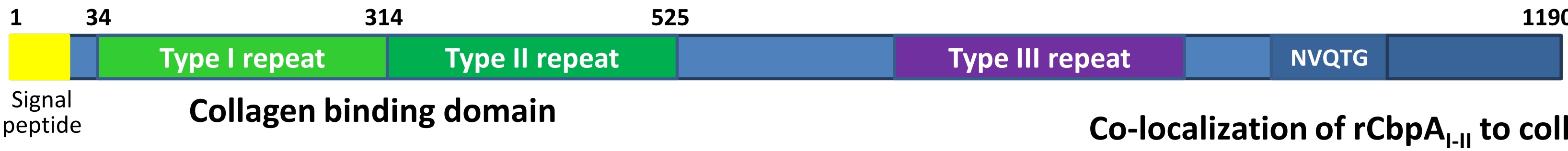
Collagen binding protein CbpA

Tulli et al., Cell Microbiol, 2013

- **Surface localization**



- **Structure**



- **Functions**

  Adherence to type I and V collagen

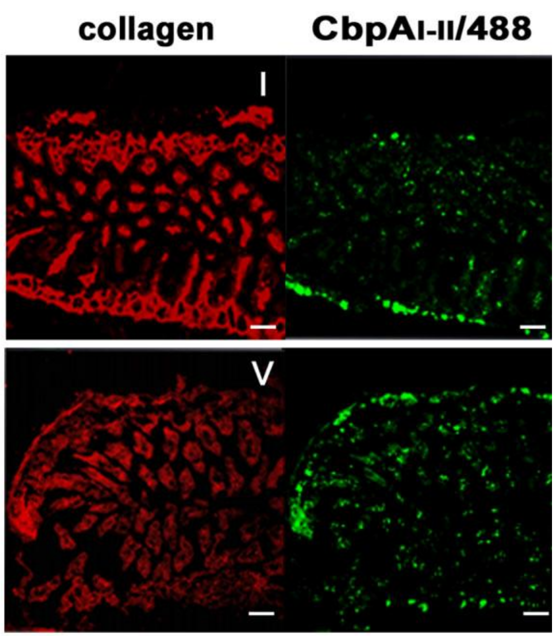
  Enhance bacterial adherence to collagen when expressed in *L. lactis*

  No difference in adherence of a mutant/ WT

- **No decrease of colonization fitness in a competitive dixenic mouse model**

  (Janoir et al., Infect Immun, 2013)

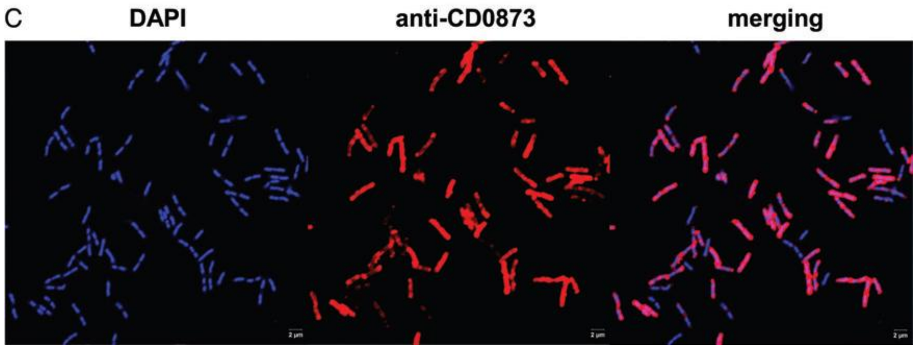
Co-localization of $rCbpA_{I\text{-}II}$ to collagen fibers in mouse intestine sections



Lipoprotein CD630_08730

(Kovacs-Simon, J Infect Dis, 2014)

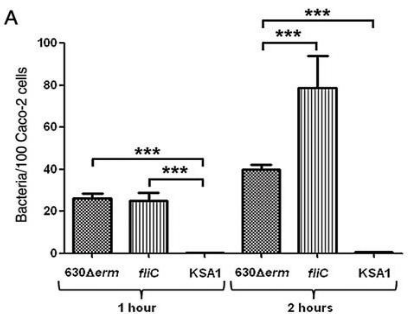
- **Surface localization**



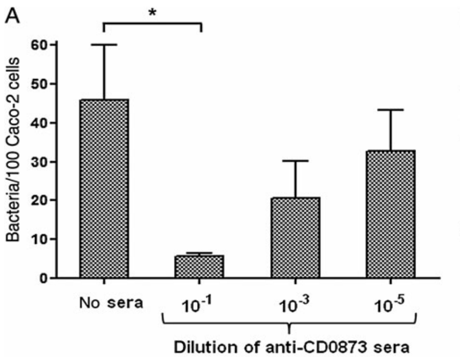
- **Structure**

  Lipoprotein part of a ABC transporter

- **Function:** mediates adherence to Caco-2 cells



WT and mutant strains



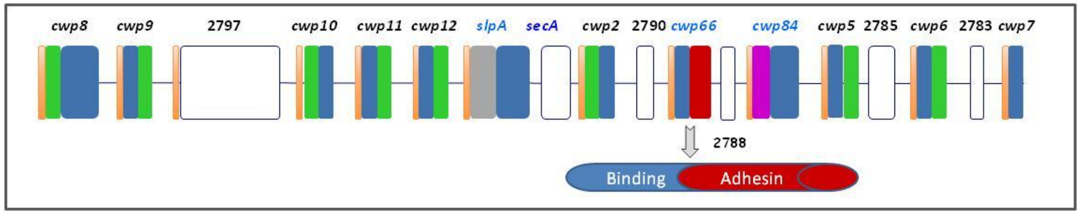
WT strain pre-incubated with anti-CD0873

- **No study in animal model**

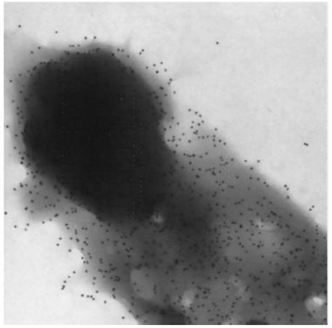
Other adhesins

Cell wall protein Cwp66 *(Waligora et al., Infect Immun, 2001)*

- **Surface localization**



Labelling of *C. difficile* with anti-Cwp66$_{\text{C-ter}}$ Ab

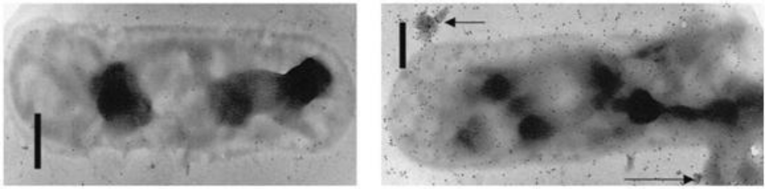


- **Heat shock induced**
- **Adherence on Vero cells partially inhibited by anti-Cwp66 antibodies and pre-incubation with purified recombinant proteins**

Heat-shock protein GroEL *(Hennequin et al., Microbiology, 2001; Péchiné et al., Plos One, 2014)*

- **Surface localization**

Labelling of *C. difficile* with anti-GroEL Ab

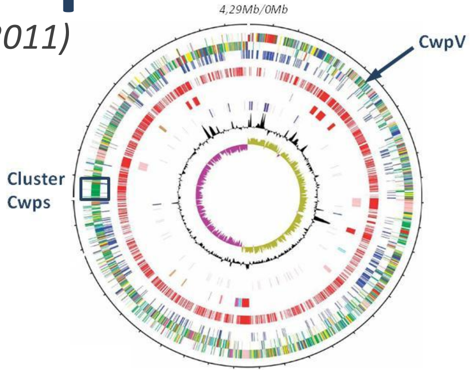


Incubation at 37°C Heat-shocked 48°C

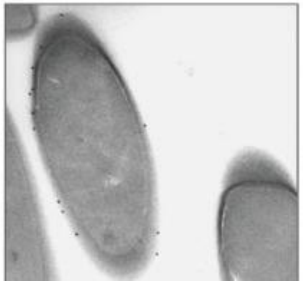
- **Adherence on Vero cells partially inhibited by anti-GroEL antibodies**
- **Reduced colonization of murine gut after GroEL immunization**

Surface-associated protein CwpV

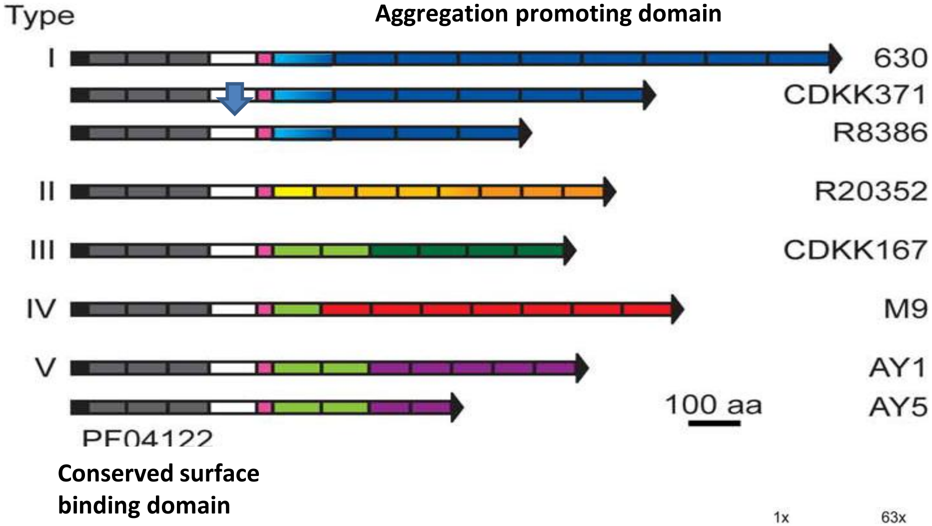
(Emerson et al., Mol Microbiol, 2009; Reynolds et al. PlosPathog, 2011)



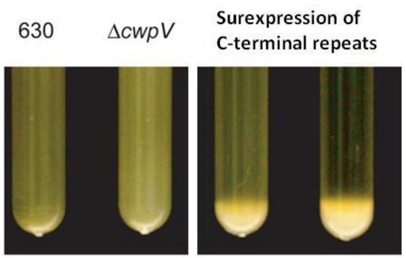
- **Surface localization**

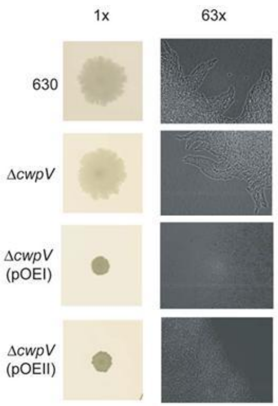


- **Structure**

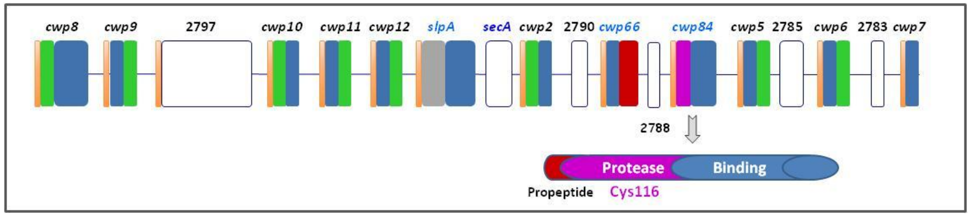


- **Function:** aggregation-promoting factor



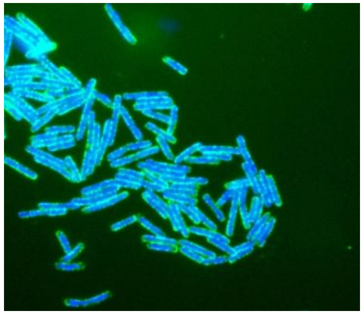


Cysteine protease Cwp84



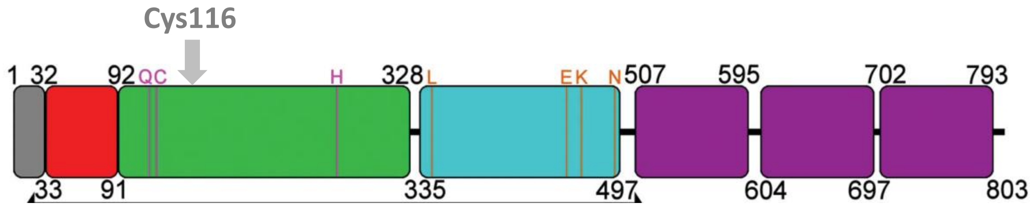
- **Surface localization**

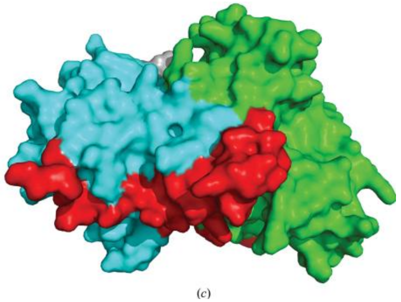
  (Janoir et al., J Bacteriol, 2007; Chapeton-Montes et al., J. Bacteriol, 2011)



- **Structure**

  (Bradshaw et al., Acta Cryst, 2014)

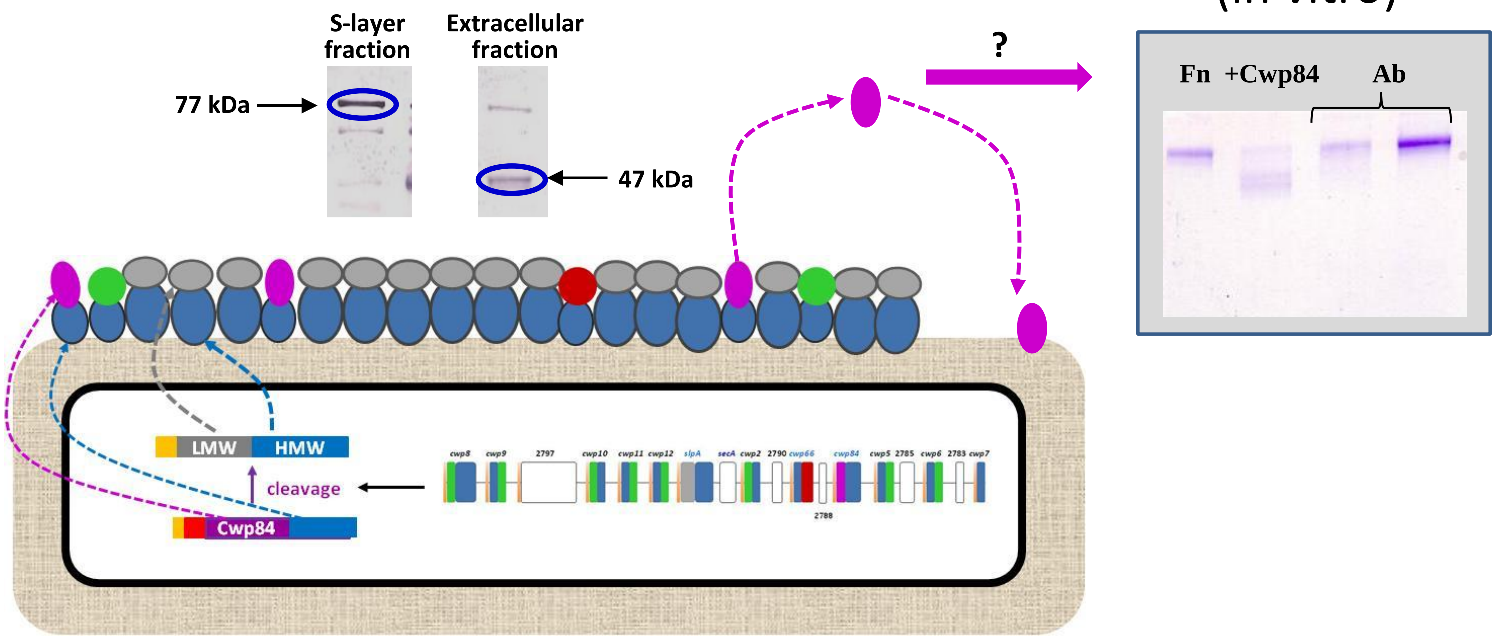




- **Function**

  (Janoir et al., J Bacteriol, 2007; Kirby et al., J Biol Chem, 2009; Dang et al., ACS Chem Biol, 2010; de la Riva et al., J Bacteriol, 2011; Chapeton-Montes et al., J. Bacteriol, 2011)

SlpA processing **Degradation of ECM proteins (in vitro)**



- **Mutant as virulent as the parental strain in the hamster model**

  (Kirby et al., J Biol Chem, 2009)

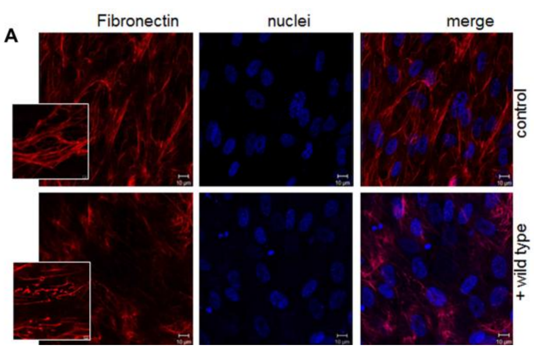
Metalloprotease CD2830-Zmp1

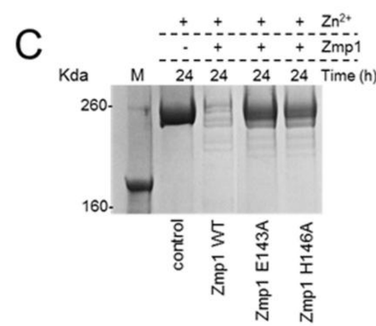
(Cafardi et al., PlosOne, 2013; Hendsberger et al., Mol Cell Prot, 2014)

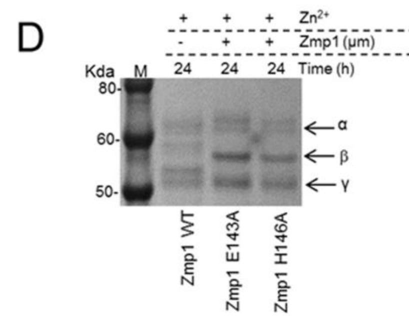
- **Secreted Zn metalloprotease**
- **Structure**

- **Functions**

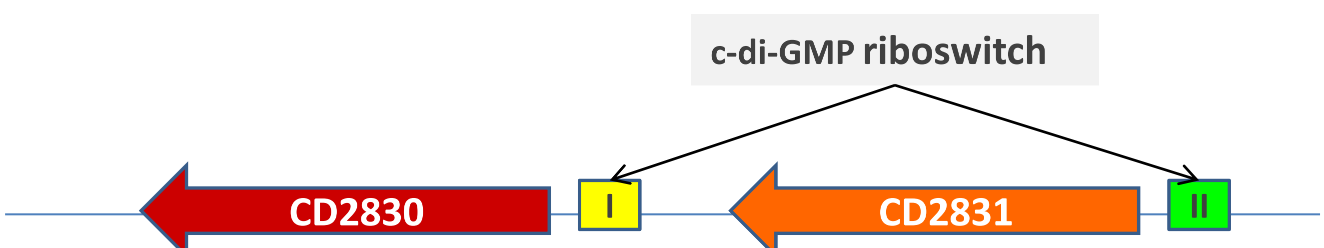
Cleavage in vitro of host proteins (HSP90β, IgA2, fibrinogen, fibronectin)



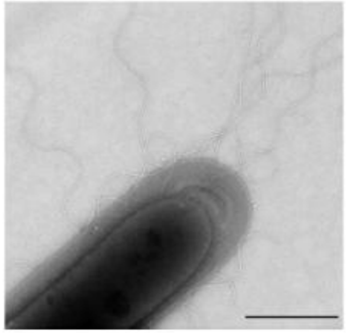




Cleavage of bacterial surface protein (putative adhesin CD2831)



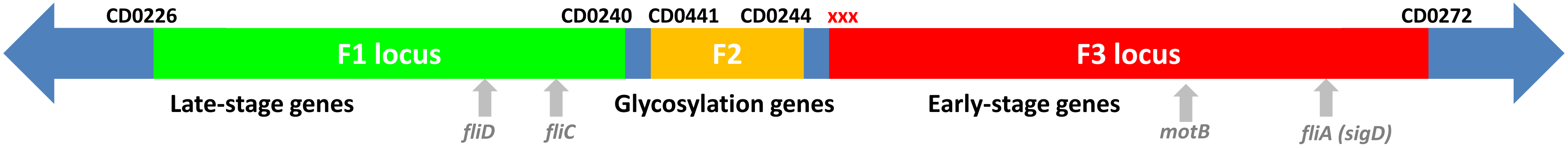
Flagella



Genetic organization

(Sebaihia et al., Nature, 2006; Stabler et al., J Bacteriol, 2006; Stabler et al., Genom Biol, 2009)

630 C. difficile chromosome



/630	F1	F2	F3
027	Divergence	Divergence	+
078	+	Divergence	-
A-B+	+	+	+

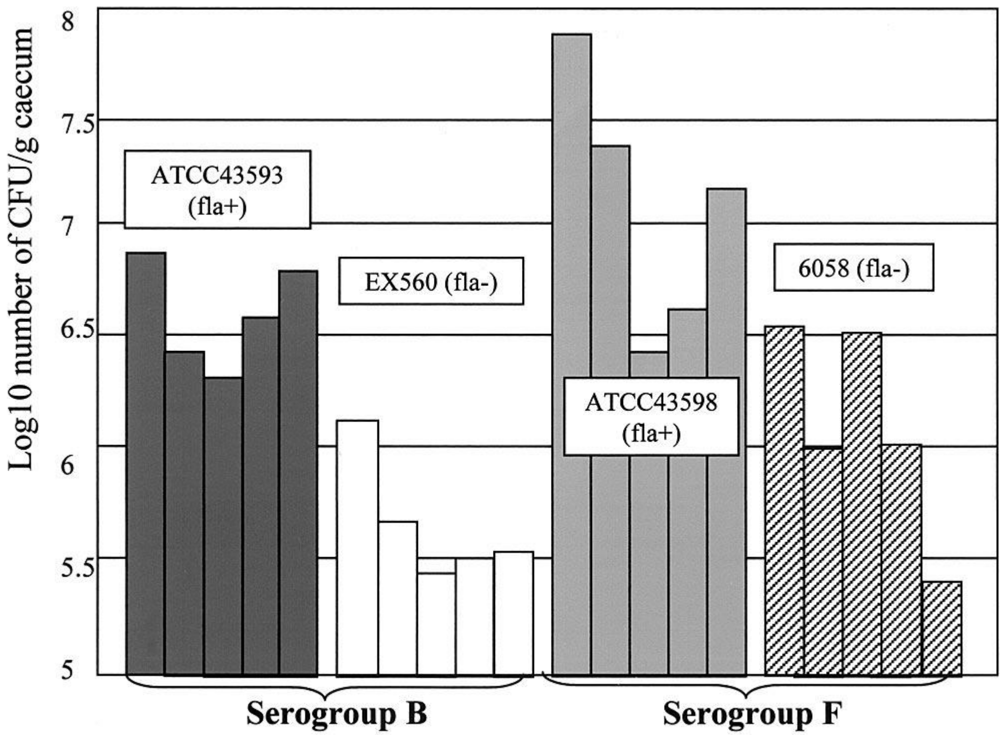
Clinical non flagellated strains

Functions

- **In colonization**

  Role in adherence to murine **mucus** for FliC and FliD *(Tasteyre et al., Infect Immun, 2001)*

  Comparison between non flagellated and flagellated strains:



Functions

- **In colonization**

Study of mutants generated with the Clostron method:

Study	Strain	Mutated genes	Adherence to Caco-2 cells	In vivo	
				Animal model	**Colonization rate relative to WT**
Dingle et al., Infect Immun, 2011	**630Δ*erm***	***fliC***	↑*	Hamster	=
		fliD	↑		=
Faulds-Pain et al., Mol Microbiol, 2014	**630Δ*erm***	***fliC***		Conventional mouse (individual experiment)	=
Baban et al., PlosOne, 2013	**630Δ*erm***	***fliC***	↑	Dixenic mouse (competitive experiment)	Subtle decrease fitness
	R20291	***fliC***	↓**		Decrease fitness
		fliD	↓**		

* significant

10 fold decrease of CD0873

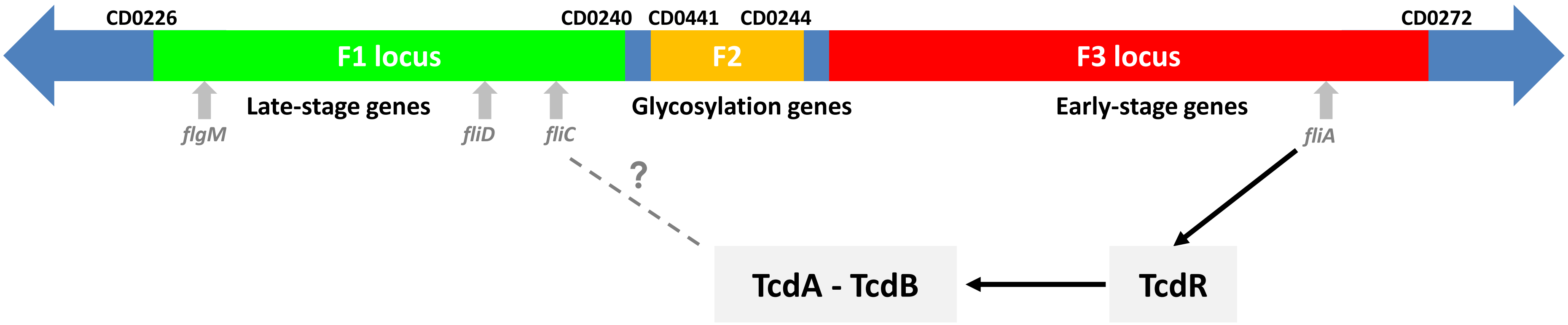
(Barketi-Klai at al., Plos One, 2013)

Functions

- **Flagella and virulence**

Study	Strain	Mutated genes	In vivo		Toxin production in vitro / WT
			Animal model	Virulence/WT	
Dingle et al., Infect Immun, 2011	630Δerm	*fliC*	Hamster	↑	
		fliD		↑*	
Aubry et al., Infect Immun, 2013	630Δerm	*fliC*			
Baban et al., PlosOne, 2013	R20291	*motB*	*C. difficile* mono-associated mouse	=	
		fliC		↑**	

- **Flagella and toxins: the 630 example**



(El Meouche et al., Plos One, 2014)

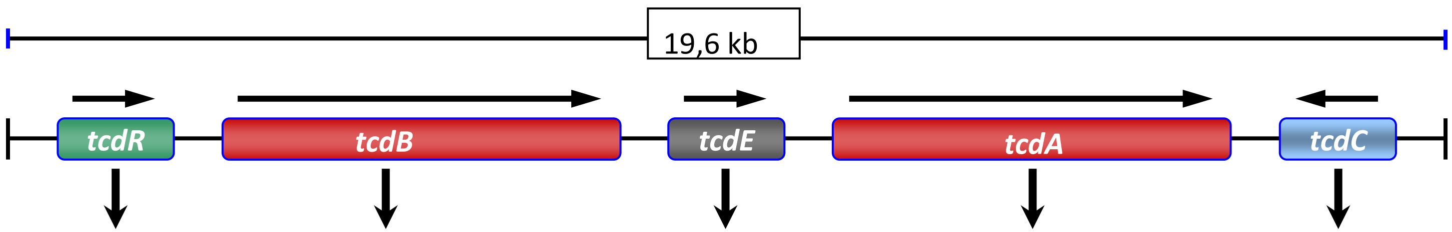
- **Pro-inflammatory role of flagella: TLR5 signaling**

(Yoshino et al., Life Sci, 2013; our unpublished data, see posters P61 & P62)

Toxins: major virulence factors

TcdA and TcdB

- **Genetic organization**



TcdR
Positive regulator
σ factor

TcdB
Toxin B

TcdE
Extracellular release of toxins

TcdA
Toxin A

TcdC
Negative regulator ?

(Mani & Dupuy, PNAS, 2001)

(Govind & Dupuy, 2012)

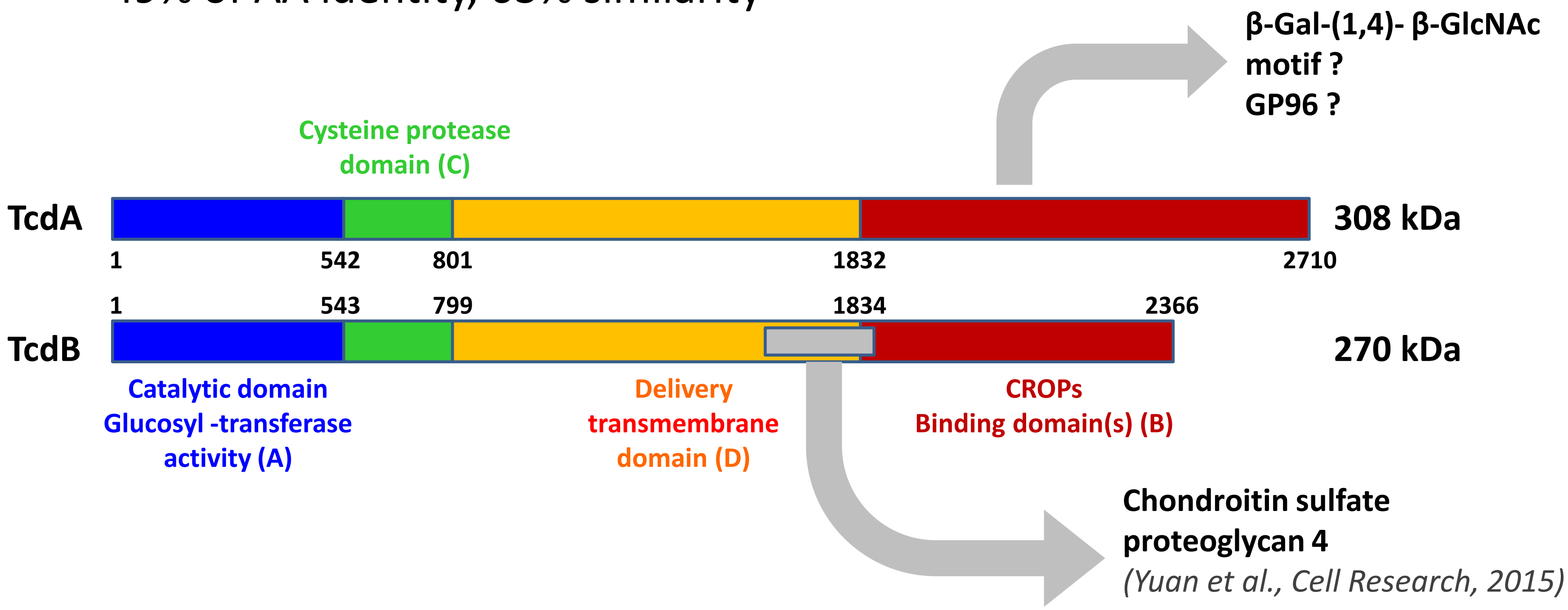
(Matamuros et al., Mol Microbiol, 2012)

PaLoc could be horizontally transfered *(Brouwer at al. Naure Com, 2014)*

TcdA and TcdB

- **Structure**

49% of AA identity, 63% similarity



TcdB_{027} more cytotoxic on several cell lines than $\text{TcdB}_{\text{Historical}}$, due to variations within the C-terminal binding domain

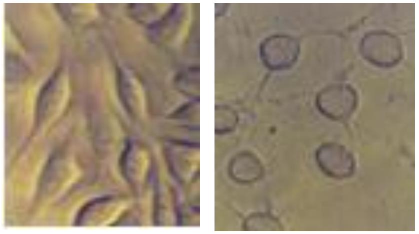
(Stabler et al., Genome Biol, 2009; Lanis et al., Plos Path, 2010)

TcdA and TcdB

- **Function: large clostridial toxins glucosylating of Rho family GTPases**
  (*reviewed by Pruitt & Lacy, Front Cell Infect Microbiol, 2012, and Shen, J Innate Immun, 2012)*

  Effects on cells:

  - Cytopathic effect: cell rounding, disruption of the tight junctions
  - Cytotoxic effect: cell death, via apoptosis and necrosis



  TcdB: cytotoxic;TcdA: enterotoxic in animal models

  Pro-inflammatory effects

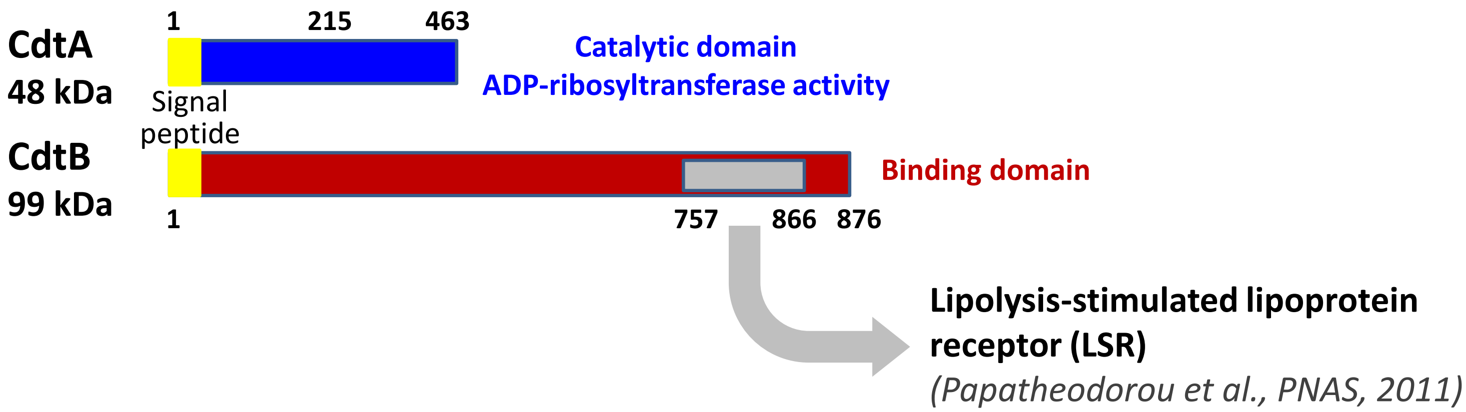
- **Regulation**

  By environmental conditions, via regulators of bacterial metabolism *(reviewed by Bouillaud et al., Res Microbiol., 2014)*

  By global regulators , such as Spo0A *(Mackin et al., Plos One, 2013; Pettit et al., BMC Genom, 2014)*

Binary toxin CdtAB

- **Structure**



- **Distribution of binary toxin among *C. difficile* strains**

  About 20% in sporadic cases

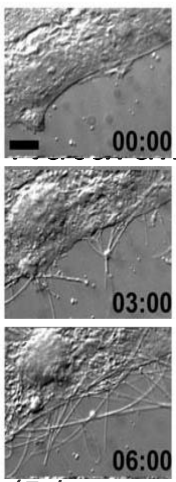
  Presence in epidemic PCR-ribotypes 027, 078

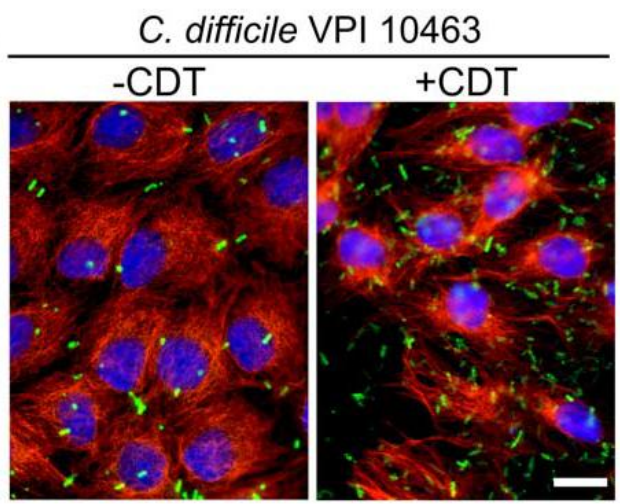
- **Function**

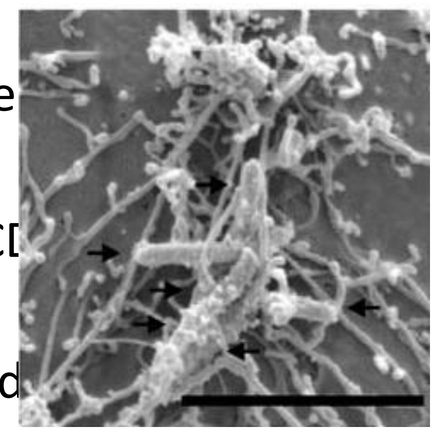
ADP ribosylation of actin: inhibition of actin polymerization and depolymerization of actin filaments
(Popoff et al., Infect Immun, 1988; Perelle et al., Infect Immun, 1997)

Cell rounding

Formation of protrusions promoting bacterial adherence and mouse caecum colonisation *(Schwan et al., Plos Pathog, 2009)*







Caco-2 cells

Relative importance of each toxin in CDI

Mutant analyzes

- Mutants generated in 630 derivative strains
- No difference in hamster colonisation rates

Study	Genetic background	Method	Genes		Hamster model	
			tcdA	*tcdB*	Protocol	Virulence
Lyras et al., Nature, 2009	JIR8094	Recombination	+	+	Antibiotic: clindamycin D-5 Challenge: 10^3 spores	++
			+	-		-
			-	+		++
Kuehne et al., Nature, 2010	630Δerm	Clostron	+	+	Antibiotic: clindamycin D-5 Challenge: 10^2 spores	++
			+	-		+
			-	+		++
			-	-		-

- **Mutant generated by the Clostron method in the R20291 strain**
- **Hamster model: Clindamycin, 7 days before challenge with 10^4 spores; no difference in colonisation rates**

Study	Genetic background	Genes			Virulence in hamster model
		tcdA	*tcdB*	*cdtA*	
Kuehne et al., JID, 2014	R20291	+	+	+	++
		-	-	-	-
		-	+	-	++
		+	-	-	+
		+	-	+	++
		-	+	+	++

Thanks to:

EA4043 UBaPS

Unité Bactéries Pathogènes et Santé

and

All of you