

The SigmaD factor is a positive regulator of flagella and toxins A and B in *Clostridium difficile*

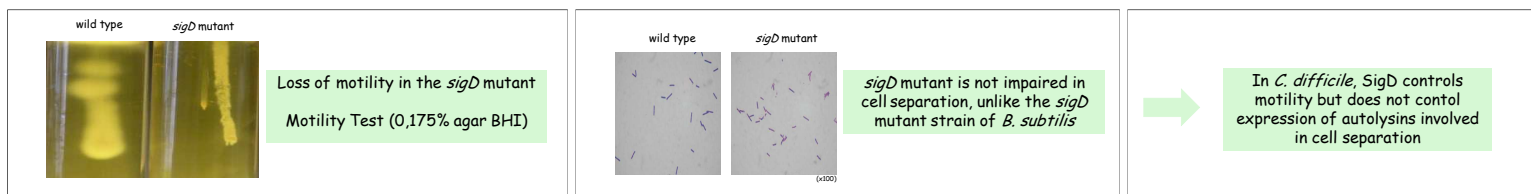
Imane El Meouche¹, Johann Peltier¹, Marc Monot², Martine Pestel-Caron¹, Bruno Dupuy², and Jean-Louis Pons^{1, 3}

¹Laboratoire G.R.A.M. (EA 2656 IFR 23 IHURBM), Université de Rouen, ²Laboratoire Pathogénèse des Bactéries Anaérobies, Institut Pasteur, Paris, ³Laboratoire Ecosystème intestinal, Probiotiques, Antibiotiques (EA 4065, IFR IMTCE), Université Paris Descartes, Paris, France

Introduction

The mechanisms of regulation of virulence genes is of major concern for *C. difficile*, since the spectrum of intestinal disease is highly variable. The intestinal colonization by *C. difficile* depends on the expression of bacterial adherence and colonization proteins such as the flagellar components *FliC* and *FliD*. Beyond intestinal colonization, the major virulence factors are toxins A and B, whose encoding genes *tcdA* and *tcdB* are located in a 19.6 Kb pathogenicity locus, with three accessory genes encoding the positive regulator *TcdR*, the negative regulator *TcdC* and the holin-like protein *TcdE*. We found in the *C. difficile* 630 genome a gene encoding a putative SigD factor homologous to SigD of *Bacillus subtilis* which controls flagellar synthesis, autolysis and sporulation in this species. The aim of the present work was to investigate the regulatory properties of SigD in the control of genes implicated in motility, autolysis and toxin production in *C. difficile*.

Impact of *sigD* inactivation in *C. difficile* 630E (Clostron®)

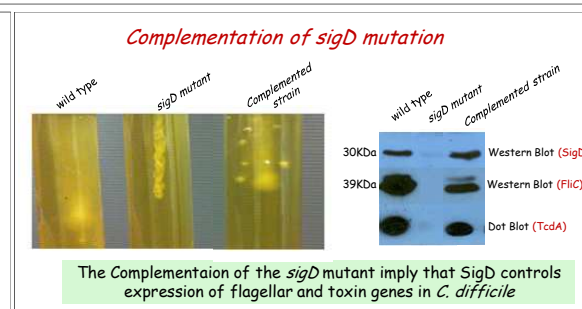
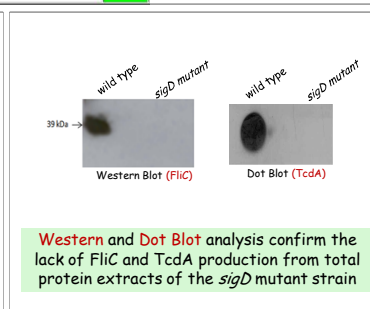
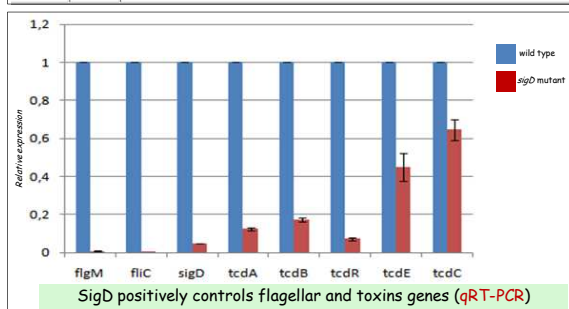


Comparative transcriptomic analysis of gene expression profiles of the 630E strain and the *sigD* mutant (Microarrays)

Gene ID	Gene Name	Description	6H SigD 6H WT	Gene ID	Gene Name	Description	6H SigD 6H WT	?
CD0626		Putative lytic transglycosylase	0.06	CD0652	tcdR	Alternative RNA polymerase sigma factors	0.54	±
CD0627		Conserved hypothetical protein	0.06	CD0660	tcdB	Toxin B	1.00	±
CD0628	fliV	Flagellar motor switch protein FliV	0.06	CD0661	tcdE	Holin-like pore-forming protein	1.00	±
CD0629	fliH	Negative regulator of flagellin synthesis (Anti-sigma-D factor)	0.06	CD0663	tcdA	Toxin A	0.24	±
CD0630		Putative flagellar biosynthesis protein	0.06	CD0664	tcdC	Negative regulator of toxin gene expression	1.00	±
CD0631	fliG	Flagellar hook-associated protein FliG (or HAP1)	0.06	CD0641		Phosphoserine phosphatase	0.04	±
CD0632	fliL	Flagellar hook-associated protein FliL (or HAP3)	0.06	CD0642		Conserved hypothetical protein	0.04	±
CD0633	fliW	Flagellar assembly factor FliW	0.06	CD0643		Conserved hypothetical protein	0.04	±
CD0634	csrA	Carbon storage regulator homolog CsrA	0.06	CD0644		Putative CDP-glycerol:Poly(glycerophosphate) glycerophosphotransferase	0.04	±
CD0635	fliS	Flagellar protein FliS	0.06					
CD0636	fliS2	Flagellar protein FliS2	0.06					
CD0637	fliD	Flagellar hook-associated protein 2 FliD (or HAP2)	0.06					
CD0638		Conserved hypothetical protein	0.06					
CD0639	fliC	Flagellin C	0.06					
CD0640		Flagellin C	0.06					
CD0666	fla	RNA polymerase sigma-28factor for flagellar operon	0.13					
CD0667		Putative flagellar protein	0.14					
CD0668	fliG1	Flagellar hook-basal body complex protein FliG1	0.14					
CD0669	fliG	Flagellar basal body rod protein FliG	0.14					
CD0670	fliM	Flagellar motor switch protein FliM	0.14					
CD0671	fliN	Flagellar motor switch phosphatase FliN (CheY-P phosphatase FliN) (Flagellar motor switch FliN)	0.13					
CD0672		Conserved hypothetical protein	0.16					

One hundred-three genes are differentially expressed (at least two fold) between the wild-type and the *sigD* mutant

Among them, numerous genes are involved in various functions such as motility (*fliC*, *fliD*, *fliG*,...), metabolism, virulence (*tcdA*, *tcdR*), or protein regulation



Conclusions and Perspectives

In *C. difficile*, SigD appears as a major regulator of motility, but not of autolysis, unlike in *B. subtilis*

SigD positively controls three genes of the PaLoc, *tcdA*, *tcdB* and *tcdR*

Further studies will help to elucidate the fine mechanism of toxins regulation by SigD