The anchoring of the polysaccharide II is essential for Clostridioides difficile survival

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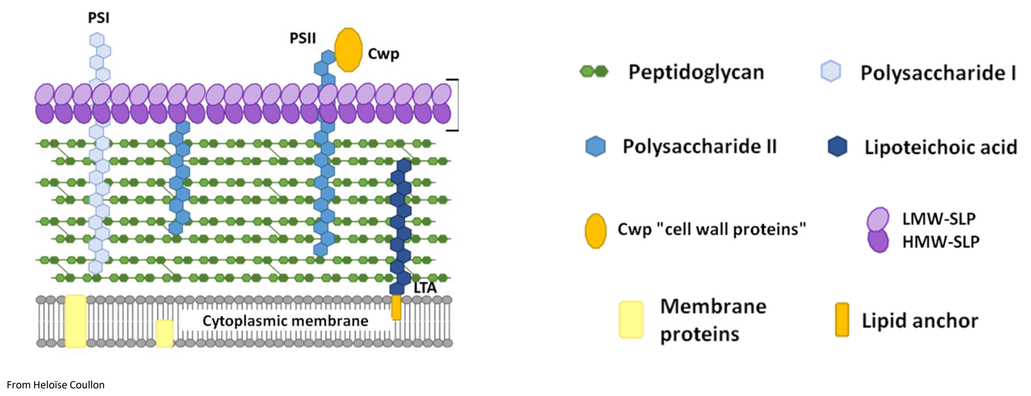
INTRODUCTION

C. difficile is an anaerobic, motile and spore-forming bacterium, responsible for 15 to 25% of post-antibiotic diarrhea and 95% of pseudomembranous colitis. While its toxins are described to be the major virulence factors in *C. difficile* infections, there is an increasing interest in the role of non-toxin factors in pathogenesis and virulence. In many other pathogens, cell wall glycopolymers influence the virulence. In *C. difficile*, three major carbohydrates are described : the polysaccharide I (PSI), the polysaccharide II (PSII) and the lipoteichoic acid (LTA). In the synthesis model suggested before[1], the polysaccharide II is synthetized in the cytoplasm, then exported to the surface by a flippase and finally anchored into the peptidoglycan by two proteins of the LytR family : LcpA and LcpB. The two *lcp* genes are supposed to be non-redundant and were previously disrupted by insertional inactivation[1].



From Heloïse Coullon

AIMS OF THE STUDY

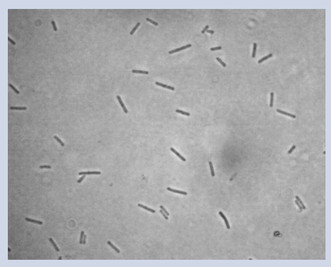
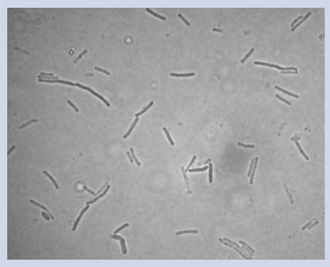
The aims of the study were first to investigate the **role of the polysaccharide II and its correct anchoring** for growth, surface set-up and survival of *C. difficile* and also to develop an innovative technique to **study essential genes** in *C. difficile.*

RESULTS

CONDITIONAL-LETHAL MUTANT TECHNIQUE TO STUDY ESSENTIAL GENES

▪ **Single and double mutation of the *lcp* genes**

➢ ***ΔlcpA*** *and* ***ΔlcpB*** : constructed by allele-coupled exchange technique

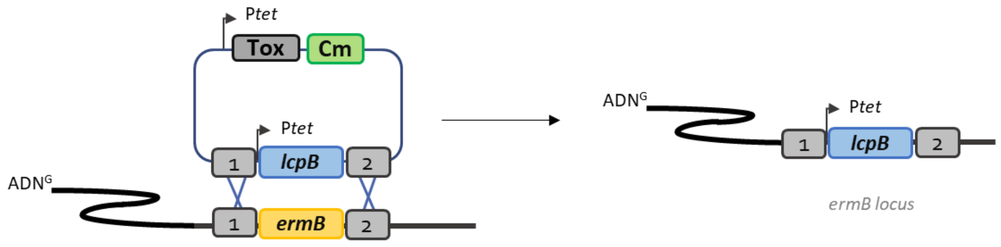
	ΔlcpA	***ΔlcpB***
Microscopic aspect		
Nb mutant / clones screened	21 / 25	3 / 187

➢ ***ΔlcpA ΔlcpB*** : unsuccessful to obtain double mutant
Only WT revertants found in PCR over 457 clones screened

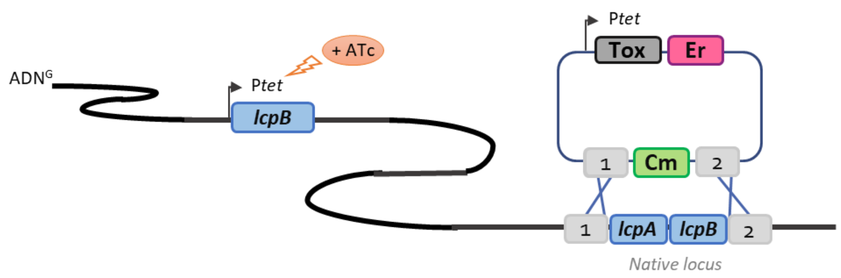
→ It suggests that LcpA and LcpB have redundant functions and the activity of at least one Lcp seems to be essential for survival

▪ **Obtention of a conditional-lethal mutant**

Step 1 : insert the supplementary copy into the *ermB* region of the chromosome by ACE and verify by PCR



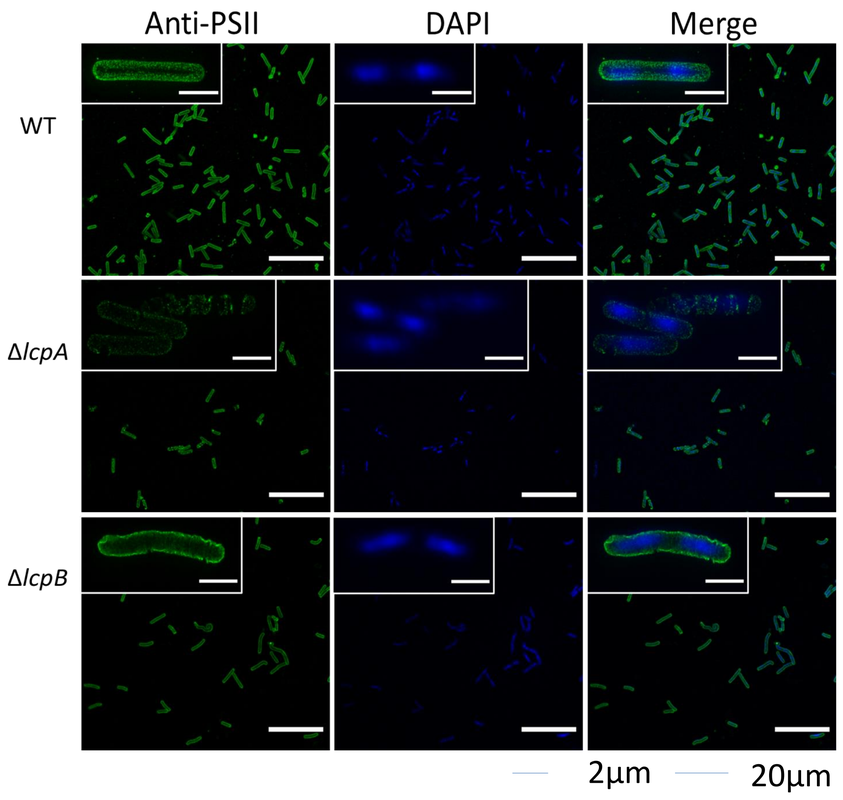
Step 2 : induce the additional copy with ATc and delete the gene in the native locus



→ Modulation of the gene expression to allow survival and observe phenotype
→ Double *lcp* mutant is not able to grow without ATc → LcpA and LcpB are redundant
→ PSII anchoring is essential

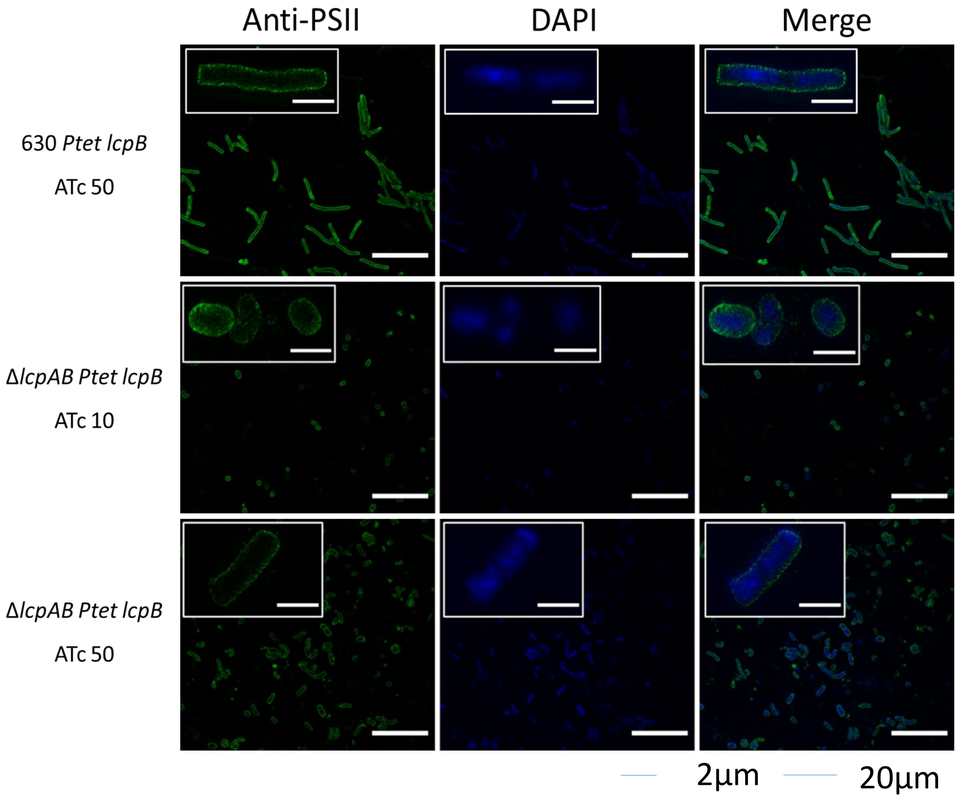
MUTANTS ANALYSIS BY IMMUNOFLUORESCENCE MICROSCOPY AND WESTERN BLOT

▪ **Single mutants PSII layer**



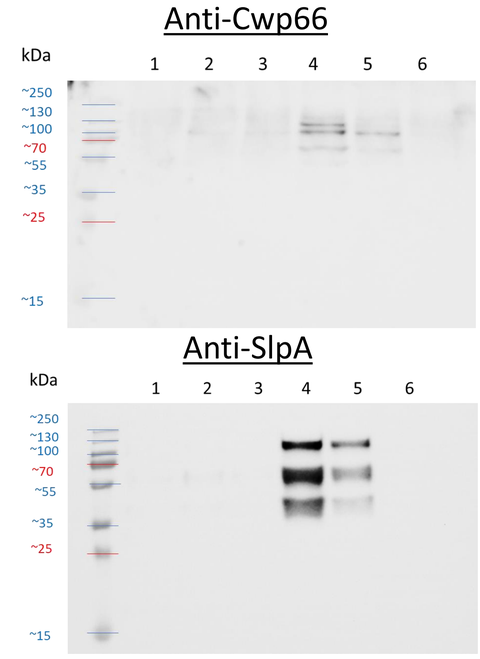
Δ*lcpA* mutant strain has a holed layer of PSII and Δ*lcpB* mutant strain has a smooth layer and deformed cells

▪ **Double conditional-lethal mutant PSII layer**



Low induction of *lcpB* gene leads to ellipsoid cells, unable to elongate correctly → PSII anchoring is important for a correct bacterial elongation

▪ **Protein analysis of the supernatant by WB**



1 : JMV1
2 : 630 pTet *lcpB*
3 : Δ*lcpAB* Ptet *lcpB* + p*lcpA* (ATc 0)
4 : Δ*lcpAB* Ptet *lcpB* (ATc 10)
5 : Δ*lcpAB* Ptet *lcpB* (ATc 50)
6 : Δ*lcpAB* Ptet *lcpB* + p*lcpA* (ATc 50)

SlpA and Cwp66 proteins are found in the culture supernatant of the mutant

CONCLUSION - PERSPECTIVES

Thanks to the construction of the single *lcp* mutants by allelic exchange, we confirmed the phenotypes previously observed[1]. The impossibility to isolate a double *lcp* mutant clone tends to confirm the essentiality of the PSII anchoring into the peptidoglycan. This was confirmed using to the conditional-lethal mutant of both *lcp*, which is completely unable to grow without induction of the supplementary copy of *lcpB* added in the chromosome. When *lcpB* expression is very low, we observe ellipsoid cells, unable to elongate. Our results show the critical role of polysaccharide II anchoring in growth, elongation, and correct surface set-up of *C. difficile*. Our technique provides new opportunities to study essential genes in *C. difficile*.

[1] Chu M *et al*, Plos Pathogens, 2016