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# The absence of surface D-alanylation, localized on lipoteichoic acid, impacts the *Clostridioides difficile* way of life and antibiotic resistance



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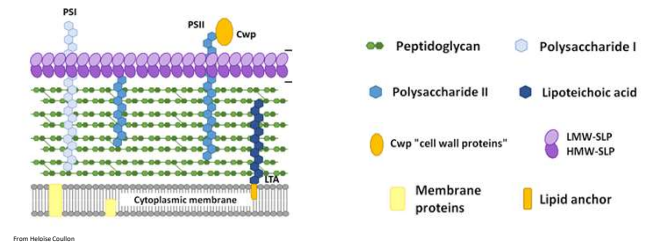
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## INTRODUCTION

*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). D-alanylation of surface polysaccharides reduces the affinity and efficacy of cationic antimicrobial compounds (CAMPs) on the bacterial surface. In *C. difficile*, the localization of D-alanylation is unknown and its implication in antibiotic resistance is not elucidated.



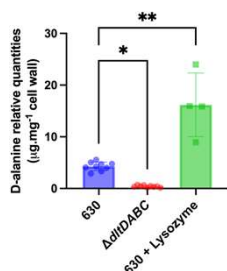
## AIMS OF THE STUDY

The aim of our study was to determine the site of D-alanylation in *C. difficile* and investigate its role in antibiotic susceptibility.

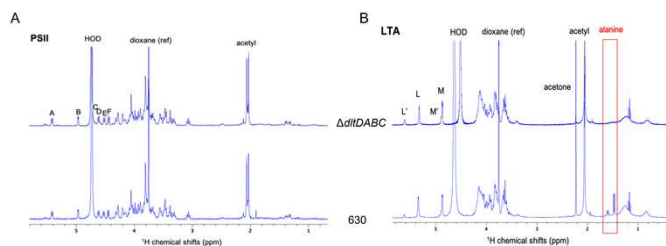
## RESULTS

### Location of the D-alanylation at the *C. difficile* surface

- D-alanylation is induced by the presence of lysozyme



- <sup>1</sup>H NMR analysis of LTA and PSII from the 630 and the 630  $\Delta dltDABC$  mutant strains



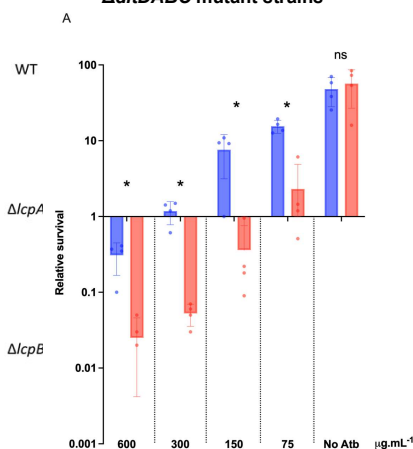
- D-alanylation in *C. difficile* is involved in sensitivity to antibiotics and CAMPs

Compounds	MIC ( $\mu\text{g.mL}^{-1}$ )	
	630	$\Delta dltDABC$
Bacitracin	550	75
Nisin	250	125
Teicoplanin	0.25	0.12
Vancomycin	2	1
Daptomycin	16	8
Amoxicillin	2	2
Imipenem	4	4
Cefotaxime	16	16

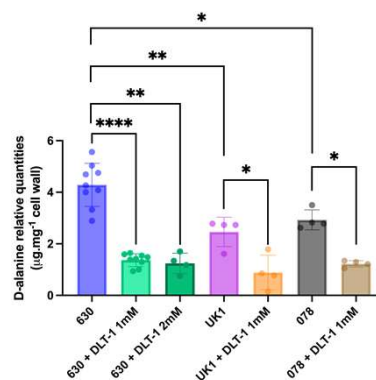
- D-alanylation was found in the LTA of the 630, but not in the 630  $\Delta dltDABC$  mutant strain
- D-alanylation is exclusively found in the LTA
- No modifications in susceptibility to  $\beta$ -lactams and cephalosporines
- Modification in susceptibility of antibiotics having a deep interaction or complete binding to the bacterial membrane

### The 630 $\Delta dltDABC$ mutant strain is more susceptible to bacitracin, DLT-1 inhibitor was then tested

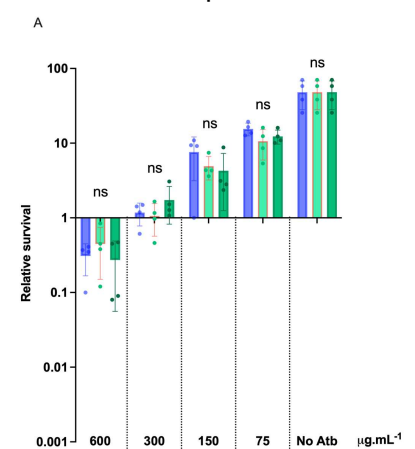
- Survival to bacitracin of the 630 and 630  $\Delta dltDABC$  mutant strains



- Test of DLT-1 inhibitor



- Survival of the 630 strain to bacitracin in the presence of DLT-1



- Significant decrease in survival to bacitracin of the  $\Delta dltDABC$  mutant in comparison with the 630 strain
- DLT-1 decreases the D-alanylation rate
- DLT-1 did not change the survival of the 630 strain against bacitracin

## CONCLUSION - PERSPECTIVES

In this study, we report the specific D-alanylation of *C. difficile* LTA. It is to note that the level of D-alanylation of both the UK1 and the 078 strains is reduced approximately 2-fold compared to that of the 630 strain, hinting at either a lowered expression of the *dltDABC* operon or lower quantities of LTA. We also confirmed the role of D-alanylation in the sensitivity to CAMPs and antibiotics such as vancomycin and bacitracin. All these antibiotics have a deep interaction or complete binding to the bacterial membrane, but these compounds differ from CAMPs in their structure and mechanisms of action. In addition, we did not observe modifications of susceptibility to the  $\beta$ -lactams families. The D-alanylation of LTA could create a steric hindrance at the surface reducing the sensitivity for antibiotics targeting or directly binding membrane. Our data suggest that the design of specific inhibitors for *C. difficile* represents an opportunity to impact *C. difficile* way of life and an additional tool for managing CDI.