Clostridioides difficile virulence genes expression in an insert plate model

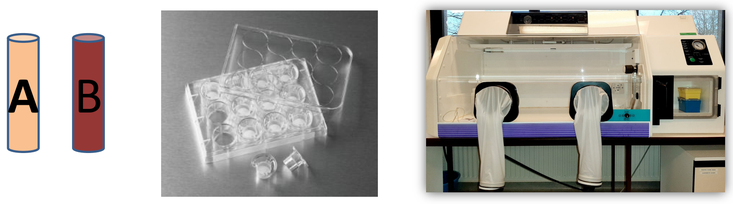
E. Martinez[1], L. Doumont [1], B. Taminiau [1], G. Daube[1]

1. *Département des Sciences des denrées alimentaires, FARAH, Faculty of veterinary Medicine, University of Liege, Belgium*

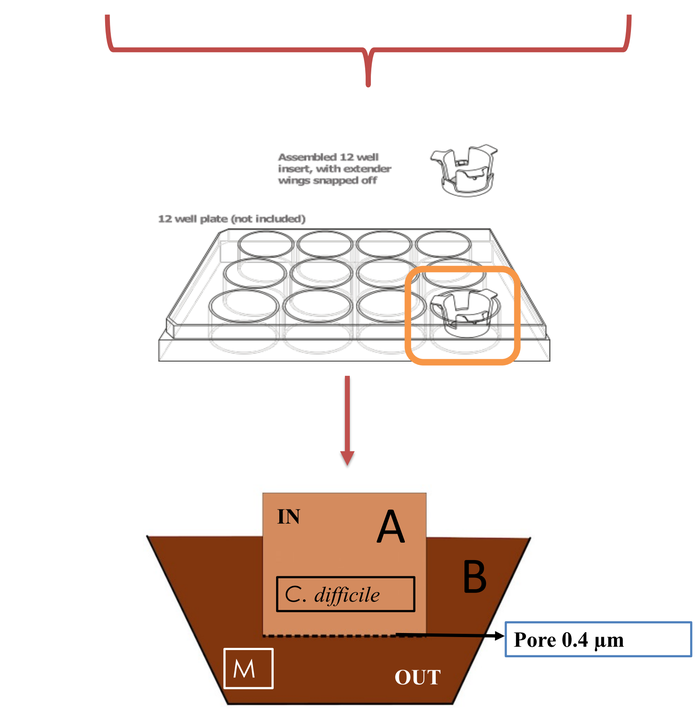
Introduction

C. difficile is a strictly anaerobic Gram + bacteria. The intestinal carriage of *C. difficile* can be asymptomatic, but, in cases of disease, it is associated with different clinical signs ranging from mild diarrhea to pseudomembranous colitis or even death. Transmission of *C. difficile* infection (CDI) occurs by the fecal-oral route. The objective of this study was to identify the pattern of the expression of the virulence genes in an insert plate model.

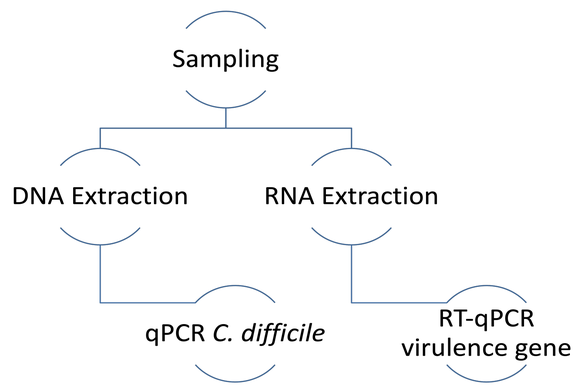
Materiel and Methods



A: Nutritive matrix + bile salts + *C. difficile* spores
B: Nutritive matrix + bile salts with or without human feces



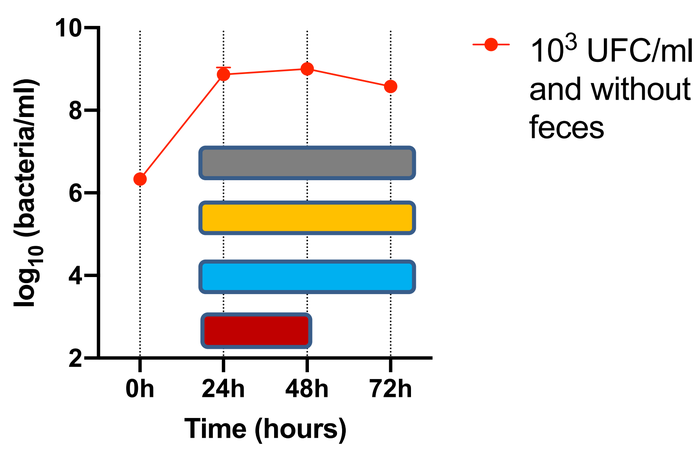
M1→ M4: human feces suspension



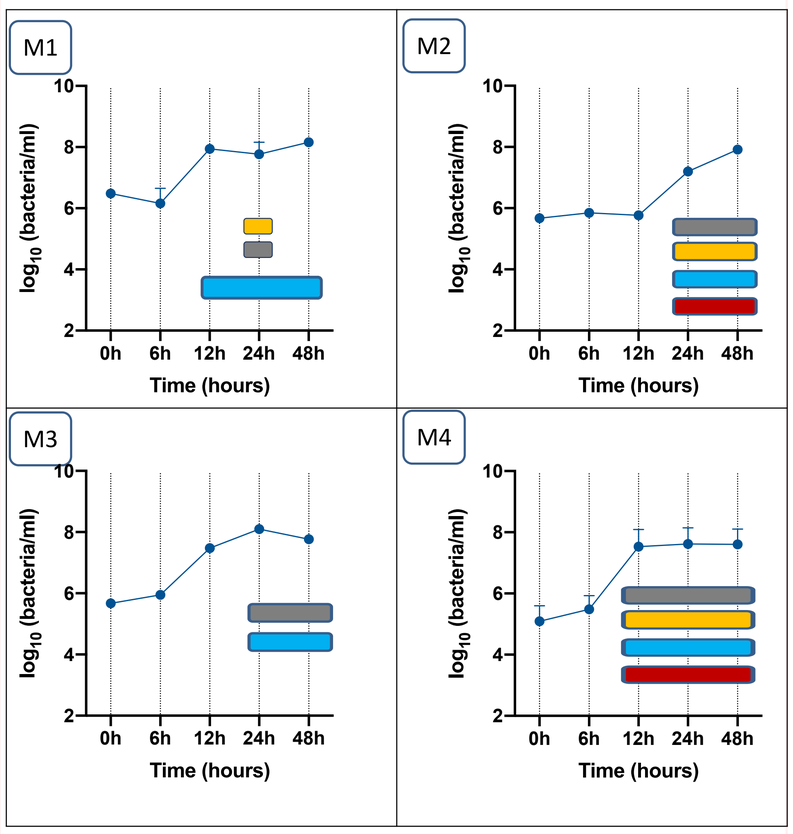
tcdA and ***tcdB*** are toxins genes;
gluD is a housekeeping gene of *C. difficile;*
mldA is a gene involved in cell division.

Results

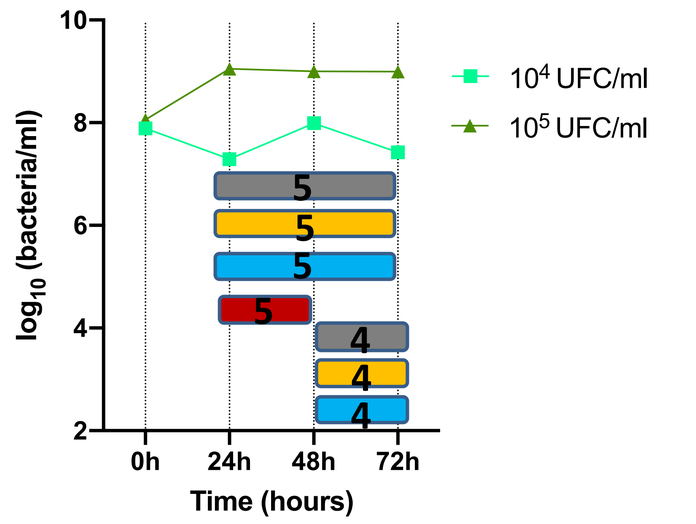
(1) *C. difficile* growth and virulence gene expression without microbiota (10^3 UFC/ml) (control)



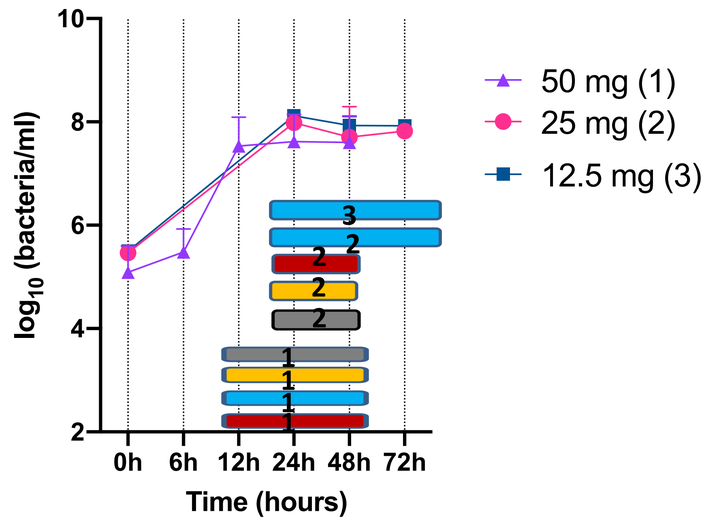
(2) *C. difficile* growth and virulence gene expression with four different microbiota (M1, M2, M3, M4) (10^3 UFC/ml *C. difficile* spores)



(3) *C. difficile* growth and virulence gene expression with microbiota (M4) and different spores concentrations (10^4 and 10^5 UFC/ml *C. difficile* spores)



(4) *C. difficile* growth and virulence gene expression with different microbiota concentrations (M4) (10^3 UFC/ml *C. difficile* spores)



Legend of figures:

tcdA gene
tcdB gene
gluD gene
mldA gene
Timing of virulence gene expression (RNA)
C. difficile growth curve (DNA)

Conclusions

This model allows the study of *C. difficile* growth and gene expression. The insert maximizes the recovery of *C. difficile* genetic content when co-cultured with microbiota. Using this model, changes in gene expression patterns have been observed when the same *C. difficile* strain grows in interaction with different feces microbiota (M2 vs M4 vs Control). M4 shows the most similar expression pattern to the control. Ongoing *C. difficile* transcriptomic and microbiota metatranscriptomic analyses will allow us to complete the gene expression patterns in this model and will help us to better understand the interactions between this pathogenic bacteria and the human gut microbiota.