Impact of *Saccharomyces boulardii* CNCM I-745 on the *in vitro* biofilm of *Clostridium difficile*

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Clostridium difficile is the leading cause of healthcare-associated diarrhea. Clinical signs range from mild diarrhea to pseudomembranous colitis. Recurrence, which occurs in more than 20% of patients after a first episode of *C. difficile* infection (CDI), can be due either to reinfection with a different strain or to a relapse caused by the initial strain. It is generally recognized that relapses are due to the persistence of *C. difficile* in the form of spores, but bacterial persistence within a biofilm could also be considered and *C. difficile* is known to be able to produce biofilm *in vitro*. *Saccharomyces boulardii* CNCM I-745 is a probiotic yeast that can be used, in association with vancomycin, for the treatment of recurrent CDI. This study evaluated the impact of *S. boulardii* on the *in vitro* biofilm-forming ability of *C. difficile*.

MATERIAL AND METHODS

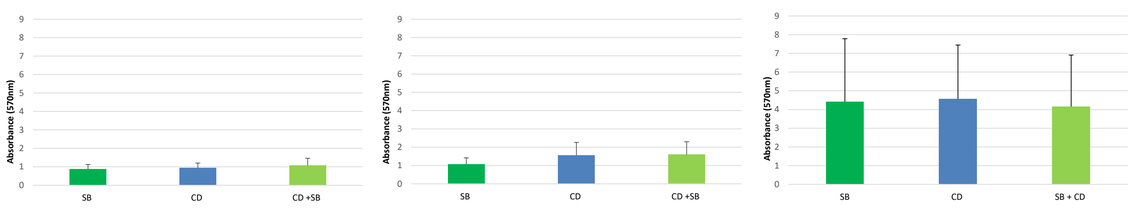
Biofilm assays were performed in 24-well polystyrene plates in Brain Heart Infusion broth supplemented with 1.8% D-glucose, 0.1% L-cysteine and 0.5% yeast extract (Difco). Overnight suspensions of *C. difficile* strain R20291 (CD) and *S. boulardii* CNCM I-745 (SB) were added to each well after dilution at different ratios (1:1, 1:100). After different times of incubation (24 h, 48 h and 72 h) under anaerobic conditions at 37° C, the biofilm biomass was quantified by crystal violet staining and microorganism enumeration and the architecture of the biofilm (24 h post incubation) was investigated by confocal laser scanning microscopy (LSM 510 microscope, Carl Zeiss Inc) post-live/dead Syto9 staining.

RESULTS

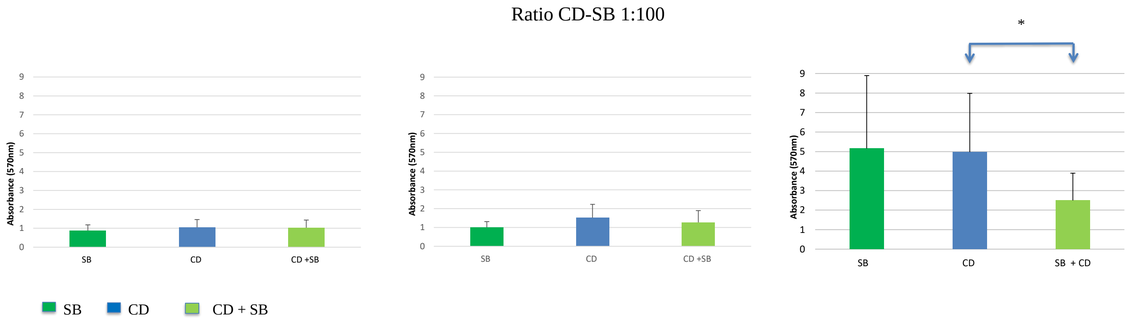
Crystal violet staining

24 h post-incubation | **48 h post-incubation** | **72 h post-incubation**

Ratio CD-SB 1:1



Ratio CD-SB 1:100

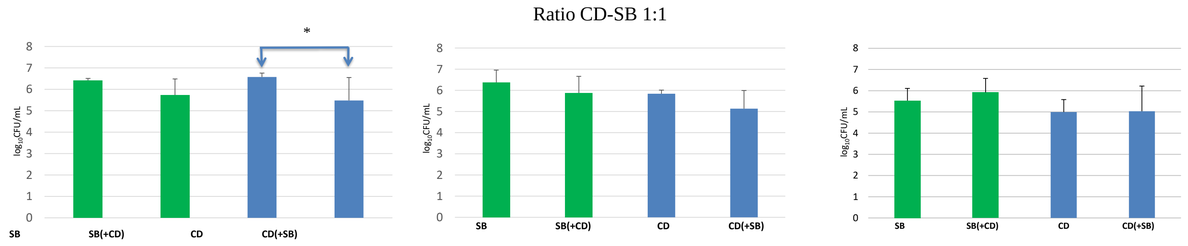


The biomass of *C. difficile* single and dual species biofilm was similar in all the conditions tested, except for the 72h-old dual species biofilm (ratio CD-SB 1:100), in which the biomass was significantly weaker than that of single species biofilm.

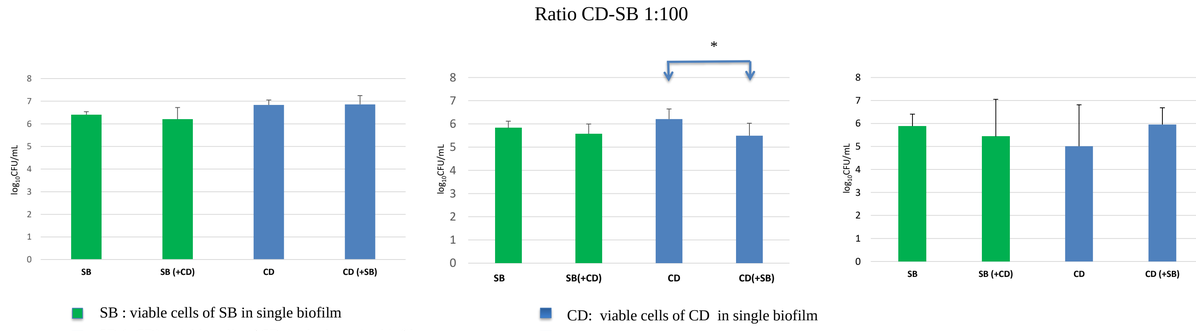
Cell counts of biofilm

24 h post-incubation | **48 h post-incubation** | **72 h post-incubation**

Ratio CD-SB 1:1



Ratio CD-SB 1:100



C. difficile viability was decreased in the dual biofilm (24h-old biofilm, ratio 1:1 and 48h-old biofilm, ratio 1:100). For other conditions, no significant differences of viable cells in single biofilm of *C. difficile* and dual species biofilm were observed. In addition, no differences were observed for a shorter incubation time (6 h) (data not shown).

Biofilm architecture (24 h post incubation)

C. difficile biofilm | *S. boulardii* biofilm | *C. difficile and S. boulardii* biofilm

Ratio CD-SB 1:1

These images represent a 3D projection of 24h-biofilm structures

As already observed[1], monospecies *C. difficile* biofilm formed in 24-well plates showed homogeneous architecture. In contrast, dual species biofilm was nonuniform and heterogeneous. The appearance of large holes evokes a weakening of *C. difficile* biofilm. This observation is consistent with the decrease in *C. difficile* viability in the same conditions.

CONCLUSION

Our results suggest that under certain conditions, the presence of *S. boulardii* influences biofilm formation by *C. difficile*. Indeed, the architecture of *C. difficile* biofilm is modified in presence of *S. boulardii* (presence of holes). Further studies are on-going to better understand the impact of *S. boulardii* on the formation of *C. difficile* biofilm.

[1]Pantaléon, V. et al. 2015. *PloS One*, *10*(4), e0124971

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