BACTEROIDES STRAINS INHIBITS ADHERENCE OF *CLOSTRIDIOIDES DIFFICILE* TO HUMAN COLON CELLS AND BIOFILM FORMATION *IN VITRO*

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Background

Probiotics are defined as microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotics act by inhibition of growth or expression of bacterial virulence factors, preventing the host from colonization by pathogenic bacteria, improving the gastrointestinal barrier, and modulation of mucosal or/and systemic immune response. There is some evidence that *Bacteroides fragilis* possess probiotic properties and can prevent *C. difficile* infection (CDI) in the mouse model and directly inhibit adhesion of *Clostridioides difficile* to HT-29 cells. This study aimed to investigate the effects of non-toxigenic *B. fragilis* (NTBF) and *Bacteroides thetaiotaomicron* on *C. difficile* adhesion to various human epithelial cell lines and biofilm formation *in vitro*.

Material and methods

Three reference strains were used in this study: *B. thetaiotaomicron* ATCC29741, non-toxigenic *B. fragilis* (NTBF) IPL323, and *C. difficile* 630. The effect of *Bacteroides* strains on *C. difficile* adhesion was assessed by co-culture with three different human epithelial cells *in vitro* (HT-29, mucus-secreting HT-29 MTX, and CCD 841 CoN cells). The Influence of *Bacteroides* on biofilm formation by *C. difficile* was assessed by co-culture those strains on 96 microplates and crystal violet staining, also confocal laser scanning microscopy (CLSM) method was adapted to visualize biofilm formation.

Results

Two tested *Bacteroides* strains significantly inhibited the adhesion of *C. difficile* 630 to the cells of three examined cell lines ($p<0.05$)(Fig.1). Co-incubation of *B. thetaiotaomicron* and NTBF with *C. difficile* resulted in significantly higher biofilm amounts than *C. difficile* and *Bacteroides* strains in monocultures ($p=0.002$)(Fig. 2). In monoculture average CFU of *C. difficile* was 14, co-incubation with *B. fragilis* and *B. thetaiotaomicron* reduced *C. difficile* colonies numbers within mixed biofilm to 1,33 ($p=0.0001$) and 3,33 respectively ($p=0.001$)(Fig. 3). Images from CLSM showed that co-culture *C. difficile* with *Bacteroides* stains resulted in increased biofilm formation compared to monocultures (Fig. 4).

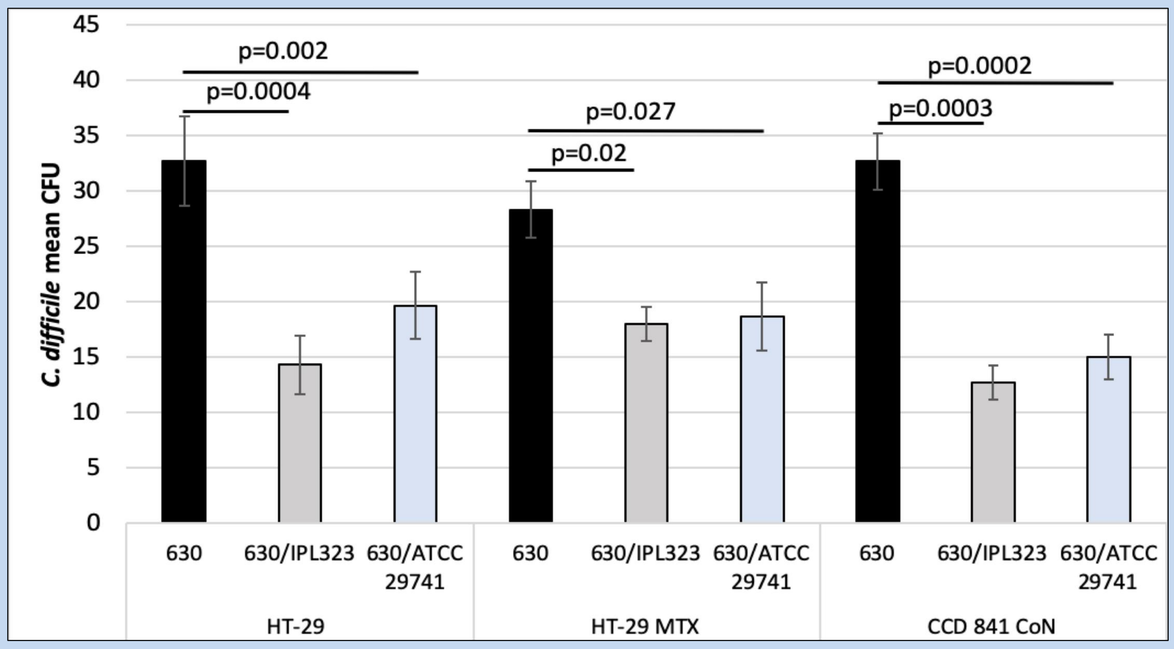

Figure 1. The effect of *B. fragilis* IPL323 and *B. thetaiotaomicron* ATCC 29741 on adhesion of *C. difficile* 630 to the three different cell lines. Data are shown as means ± standard deviation.

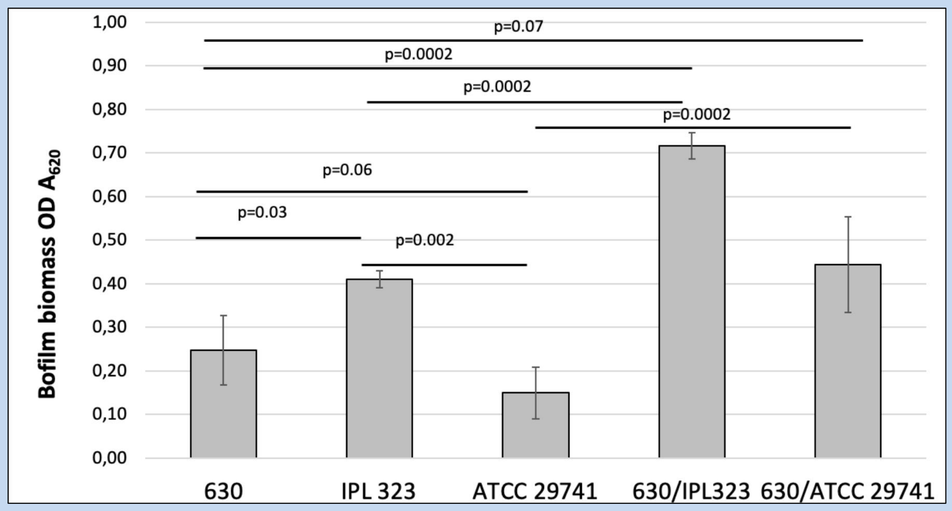

Figure 2. The effect of *B. fragilis* IPL323 and *B. thetaiotaomicron* ATCC 29741 on biofilm formation by *C. difficile* 630. Data are shown as means ± standard deviation. Biofilm biomass stained with CV

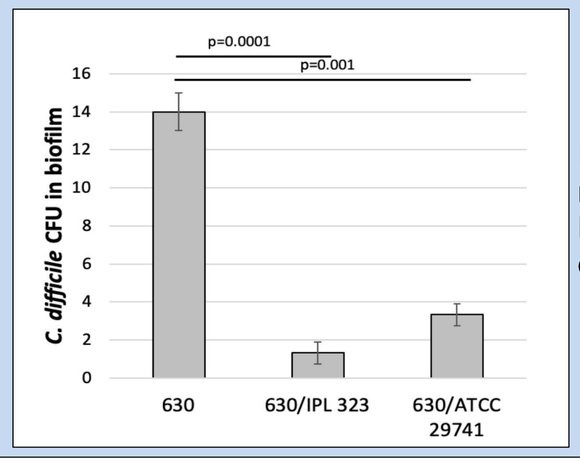

Figure 3. *C. difficile* counts within monocultured and co-cultured biofilms. Data are shown as means ± standard deviation.

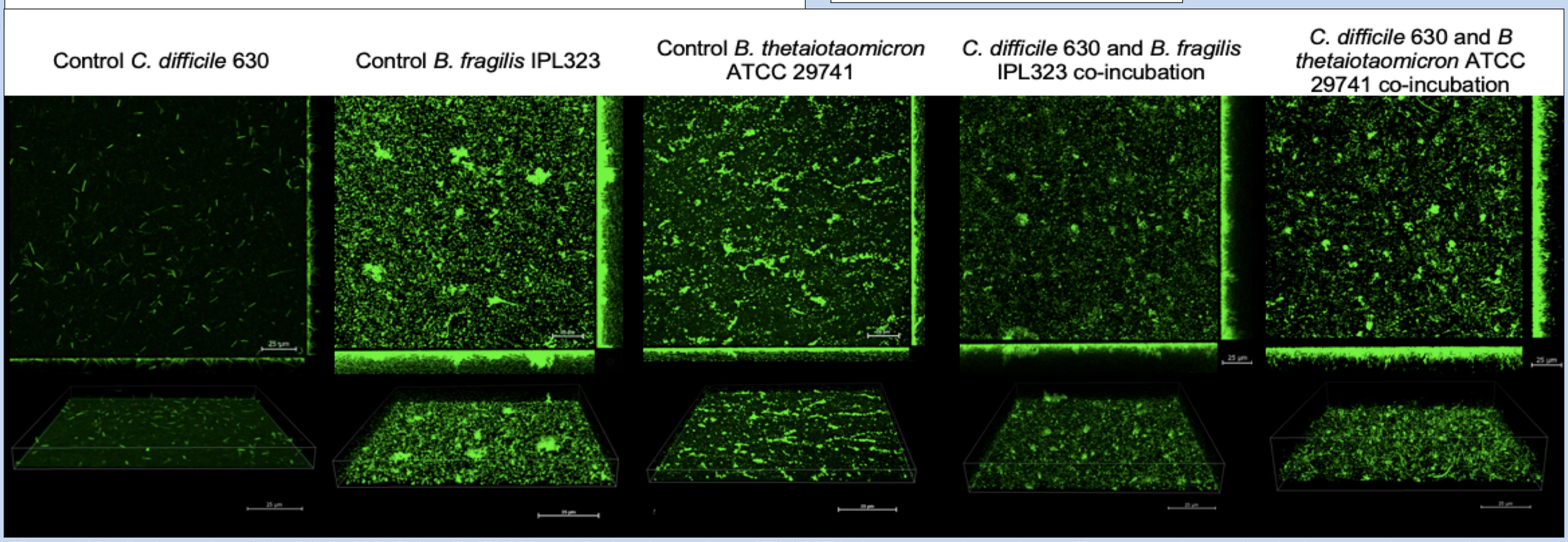

Figure 4. Biofilm formation of *C. difficile* 630, *B. fragilis* IPL323 and *B. thetaiotaomicron* ATCC29741 in mono and co-cultures. Representative confocal microscopy images of horizontal (xy) and vertical (xz) projections of *C. difficile* biofilm structures. Slices views with maximum intensity projection.

Conclusions

Non-toxigenic *B. fragilis* and *B. thetaiotaomicron* had an observable impact on decreasing *in vitro* adhesion and biofilm formation by *C. difficile*. In this light, those strains seem to be a proper candidate for probiotic microorganisms which may help in treatment or prophylaxis CDI. However, further examinations in this field are planned.

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