

Evaluation of the interference of prebiotics in anaerobic bacteria

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Microbiota is essential for health maintenance, preventing the establishment of pathogens such as *Clostridioides difficile*. *Clostridium citroniae* and *Clostridium scindens* are bacterial components of the gut microbiota that may act as a barrier to infections caused by pathogenic bacteria. Fructooligosaccharides (FOS) and inulin (IN) are soluble fibers, widely used as prebiotics, that stimulate growth of beneficial intestinal bacteria. The present study aimed to evaluate the interference of prebiotics IN, FOS and the combination of both in growth and biofilm production of *C. citroniae*, *C. scindens* and *C. difficile* hypervirulent ribotypes 027 and 135. Bacterial suspensions were inoculated in microplates containing prebiotics at final concentrations of 0.5%, 1% and 2%, and the growth was measured by spectrophotometer (OD 620nm) for 24 hours. The results showed that combination of FOS and IN at 1% was the most effective concentration to stimulate bacteria growth. At this concentration, the growth of *C. scindens* and *C. citroniae* was equal to or greater than the control, while *C. difficile* strains had reduced growth in the same conditions. These results suggest that prebiotics administered together at a concentration of 1% are an alternative source to replenish bacteria from the microbiota after the use of antibiotics. Biofilm production assays showed that *C. difficile* strains tested were either strong (ribotype 027) or moderate (ribotype 135) biofilm producers. The same analysis demonstrated that *C. citroniae* and *C. scindens* were low biofilm producers. Further biofilm analysis showed that production of the microorganisms of the microbiota was increased in the presence of prebiotic compounds, while the biofilm production of the *C. difficile* was compared to the control. The results showed that the prebiotic also interferes with biofilm production. We will also analyze other aspects such as motility and sporulation in the presence of the prebiotics (FOS + IN). Quantitative PCR will be performed to evaluate alterations on the expression of genes of *C. difficile* related to these properties.

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