Abstract

Background

Probiotic microorganisms are receiving increasing interest for use in the prevention, treatment, or dietary management of certain diseases, including antibiotic-associated diarrhea (AAD). *Clostridium difficile* is the most common cause of AAD and the resulting *C. difficile*–mediated infection (CDI), is potentially deadly. *C. difficile*–associated diarrhea (CDAD) is manifested by severe inflammation and colitis, mostly due to the release of two exotoxins by *C. difficile* causing destruction of epithelial cells in the intestine. The aim of this study was to determine the effect of probiotic bacteria *Lactobacillus delbrueckii* ssp. *bulgaricus* B-30892 (LDB B-30892) on *C. difficile*-mediated cytotoxicity using Caco-2 cells as a model.

Methods

Experiments were carried out to test if the cytotoxicity induced by *C. difficile*-conditioned-medium on Caco-2 cells can be altered by cell-free supernatant (CFS) from LDB B-30892 in different dilutions (1:2 to 1:2048). In a similar experimental setup, comparative evaluations of other probiotic strains were made by contrasting the results from these strains with the results from LDB B-30892, specifically the ability to affect *C. difficile*–induced cytotoxicity on Caco-2 monolayers. Adhesion assays followed by quantitative analysis by Giemsa staining were conducted to test if the CFSs from LDB B-30892 and other probiotic test strains have the capability to alter the adhesion of *C. difficile* to the Caco-2 monolayer. Experiments were also performed to evaluate if LDB B-30892 or its released components have any bactericidal effect on *C. difficile*.

Results and discussion

Co-culturing of LDB B-30892 with *C. difficile* inhibited the *C. difficile*-mediated cytotoxicity on Caco-2 cells. When CFS from LDB B-30892-*C. difficile* co-culture was administered (up to a dilution of 1:16) on Caco-2 monolayer, there were no signs of cytotoxicity. When CFS from separately grown LDB B-30892 was mixed with the cell-free toxin preparation (CFT) of separately cultured *C. difficile*, the LDB B-30892 CFS was inhibitory to *C. difficile* CFT-mediated cytotoxicity at a ratio of 1:8 (LDB B-30892 CFS:*C. difficile* CFT). We failed to find any similar inhibition of *C. difficile*-mediated cytotoxicity when other probiotic organisms were tested in parallel to LDB B-30892. Our data of cytotoxicity experiments suggest that LDB B-30892 releases one or more bioactive component(s) into the CFS, which neutralizes the cytotoxicity induced by *C. difficile*, probably by inactivating its toxin(s). Our data also indicate that CFS from LDB B-30892 reduced the adhesion of *C. difficile* by 81%, which is significantly (*P* < 0.01) higher than all other probiotic organisms tested in this study.

Conclusion

This study reveals the very first findings that *Lactobacillus delbrueckii* ssp. *bulgaricus* B-30892 (LDB B-30892) can eliminate *C. difficile*-mediated cytotoxicity, using Caco-2 cells as a model. The study also demonstrates that LDB B-30892 can reduce the colonization of *C. difficile* cells in colorectal cells. More study is warranted to elucidate the specific mechanism of action of such reduction of cytotoxicity and colonization.