

Evaluation of the lactic acid bacteria efficacy in *Blastocystis* ST3 eradication and the relevance of the intestinal microorganisms in protozoan proliferation

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BACKGROUND. *Blastocystis* subtype 3 is an intestinal protist present in humans throughout the world. Its pathogenic potential remains still controversial. Researchers from many countries suggest that *Blastocystis* may cause gastrointestinal as well as skin disorders. It has been suggested that probiotic bacteria inhibit the multiplication of gut protozoans, while others are beneficial for their development. The aim of the study was to evaluate the efficacy of the lactic acid bacteria *Lactobacillus rhamnosus*, *Lactococcus lactis* and *Enterococcus faecium* in *Blastocystis* ST3 eradication and the relevance of the intestinal microorganisms *Escherichia coli*, *Candida albicans* and *Candida glabrata* in protozoan proliferation.

MATERIALS AND METHODS. *Blastocystis* xenic and axenic culture was co-incubated with the above-mentioned microorganisms and their cell free supernatants at three different concentrations *in vitro*. The pH of all solutions was measured. The number of *Blastocystis* cells each day of laboratory incubation used for the experiment was determined by counting in a Neubauer chamber. The Jones' medium with *Blastocystis* was used as a control sample. The number of viable morphological forms of *Blastocystis* in treatment and controls were compared using t-test (GraphPad Prism 7.04), as well as Pearson Chi-square and two-way ANOVA tests were used whenever appropriate. To compare the influence of the dilutions according to the time of co-incubation, three-way ANOVA (Tukey's test) was used. A p value of <0.05 was considered as statistically significant.

RESULTS. Both experiments, with xenic and axenic cultures, showed *Blastocystis* inhibition by *L. rhamnosus* and *L. lactis* and their supernatants from the 2nd day of co-incubation. Furthermore, co-incubation with both *E. faecium* and *E. coli* showed a beneficial influence on *Blastocystis* during the first 2 days. Only after 3 days did the above-mentioned bacteria start to inhibit *Blastocystis* growth in both cultures. The supernatant containing the metabolites of *E. coli* was effective to a lesser degree. Compared to the control samples, co-incubation with both *C. albicans* and *C. glabrata* showed a faster decrease in *Blastocystis* proliferation, but this was not statistically significant.

CONCLUSIONS. Our study has shown the potential of using *L. rhamnosus* and *L. lactis*, as well as *E. faecium* as probiotics against *Blastocystis* colonization. The fact that these probiotic bacterial strains are able to disrupt the cell cycle of *Blastocystis* shows a promising future in the use of probiotics for prophylactic treatment of blastocystosis, or as an additional treatment regimen in combination with standard drugs. The obtained results did not show what is the mechanism of *Blastocystis* inhibition by lactic acid bacteria. This issue requires further research.