

Bioactive influence of Cell-Free Supernatant from *Lactobacillus plantarum* strains towards probiotic Bifidobacteria: a preliminary study on growth and survival kinetic parameters.

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INTRODUCTION & AIM

The study of the positive influence of probiotic strains on human health has become increasingly evident and there are numerous scientific studies that highlight how the interaction between different probiotic strains provides an even more beneficial action.

Lactobacilli and *Bifidobacterium* spp. are among the most studied probiotic and/or functional genera and are prominent members of the normal flora of the gastrointestinal tract throughout the host life.

Today's scientific research in the field of probiotics, prebiotics and paraprobiotics focuses its attention on the study of the mechanisms that underlie the interaction between these two genera of microorganisms and between the multiple strains belonging to the different species.

This paper shows the first results of a preliminary study on the bioactive action of cell-free supernatants obtained by potential probiotic *Lactiplantibacillus plantarum* strains towards probiotic strains of Bifidobacteria.

MATERIALS AND METHODS

Microorganisms

Lactiplantibacillus plantarum c3, *Lactiplantibacillus plantarum* c4 and *Lactiplantibacillus plantarum* c15 were examined throughout this study for the bioactivity of their cell-free filtrate towards bifidobacteria.

All strains were isolated from Italian table olives "*Bella di Cerignola*" (Campaniello et al., 2005), and stocked at -80 °C in appropriate media.

The bifidobacteria strains studied under the influence of *L. plantarum* cell-free filtrates were the following: *Bifidobacterium animalis* DSM 10140, *Bifidobacterium subtilis* DSM 20096 and *Bifidobacterium breve* DSM 20213, purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany), and stocked at -80 °C in appropriate media, as recommended by the producer (www.dsmz.de).

Purification of Biological Cell-Free Factor from *L. plantarum* strains

Lactiplantibacillus plantarum strains were grown in MRS broth (Oxoid, Milan, Italy) (MRS), at 30 °C for 48 h. The cells were harvested from the broth by centrifugation at 5000 rpm at 5 °C, for 15 min and then washed twice with sterile saline solution (NaCl 9 g/l). The supernatant was filtered through a 0.45 µm Millipore filter (Whatman, Dassel, Germany) and the filtrate represented the cell-free hydrosoluble fraction (CFF) (Kaneko et al., 1994).

Assay of the cell-free factor

Frozen cultures of *B. animalis*, *B. subtilis*, *B. breve*, were thawed and pre-cultured in MRS broth (Oxoid, Milan, Italy) added with cysteine (0,05 w/v) (cMRS) (Sigma-Aldrich, Milan, Italy). Serial dilutions of cell suspensions were carried out to get the desired inoculum level (10² cfu ml⁻¹).

The assays were performed in 250-ml Erlenmeyers, containing 100 ml of cMRS broth. The cell-free filtrate from lactobacilli, was added as a medium ingredient (1% v/v).

The samples were inoculated with bifidobacteria initial inocula (10² cfu/ml) and for each assay pure cultures of the microbial targets, without cell-free filtrate, were used as controls. The samples were incubated at 37 °C in an anaerobic condition and the viable count of bifidobacteria was periodically evaluated on cMRS agar, incubated at 37 °C.

Assay of the cell-free factor

The experimental data obtained were modeled using the Gompertz equation modified by Zwietering et al. (1990) in order to monitor the effectiveness of the bioactive effect of the supernatant on the kinetic parameters of the microbial growth curve. Specifically, the bioactive action was evaluated both in the growth and death phases to also evaluate how the bioactive action acted on the survival of the target strains.

RESULTS & DISCUSSION

A stimulation/inhibition effect, during bifidobacteria growth and death phases, respectively, were recorded.

In our experimental conditions, a potential "prebiotic" activity and/or an inhibition exerted by the cellular extracts of *L. plantarum* strains on the growth and death of bifidobacteria was observed, furthermore a strain-dependence on the stimulating strains and a species-dependence on the stimulated bacteria was recorded.

Figure 1 (a, b). Evolution of *Bifidobacterium animalis* in growth and death phase with and without cell-free supernatants (CFS) obtained by *L. plantarum* strains (Lc). The curves represent the best fit of the model to the experimental data. (○ *B. animalis* □ CFS Lc3 ◇ CFS Lc4 △ CFS Lc15)

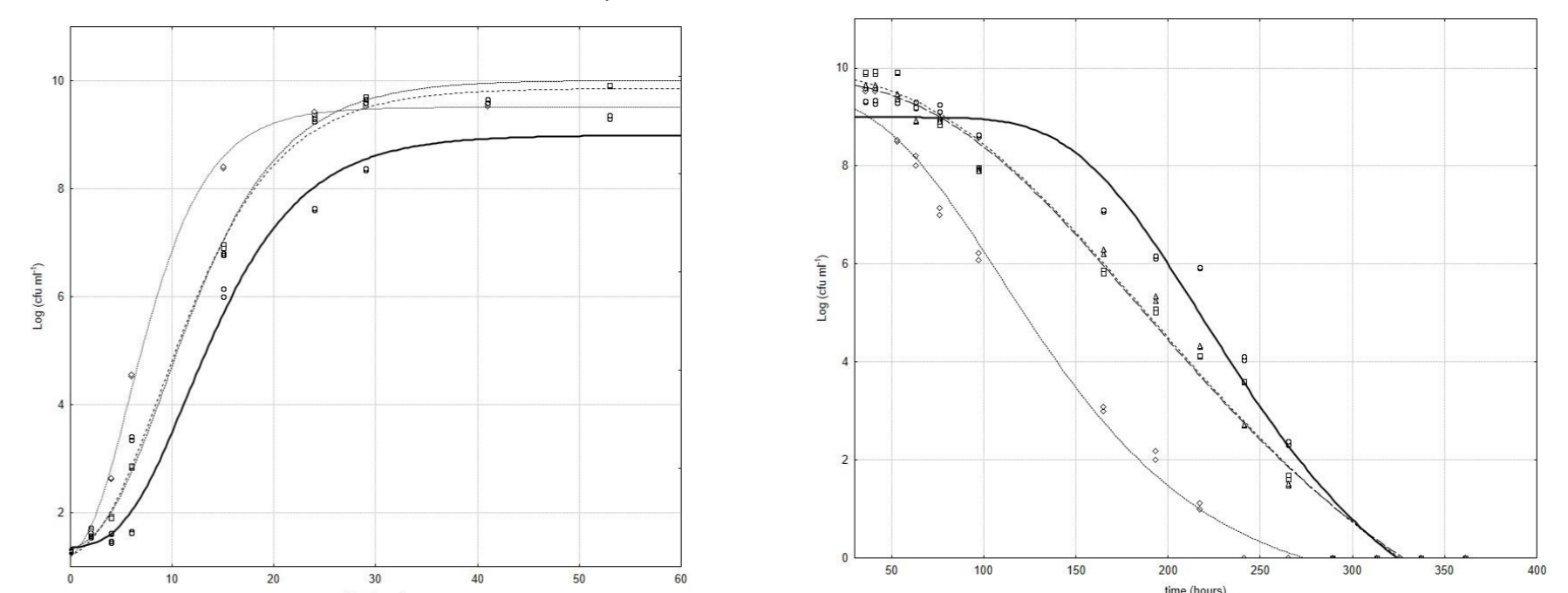


Figure 2 (a, b). Evolution of *Bifidobacterium subtilis* in growth and death phase with and without cell-free supernatants (CFS) obtained by *L. plantarum* strains (Lc). The curves represent the best fit of the model to the experimental data. (○ *B. subtilis* □ CFS Lc3 ◇ CFS Lc4 △ CFS Lc15)

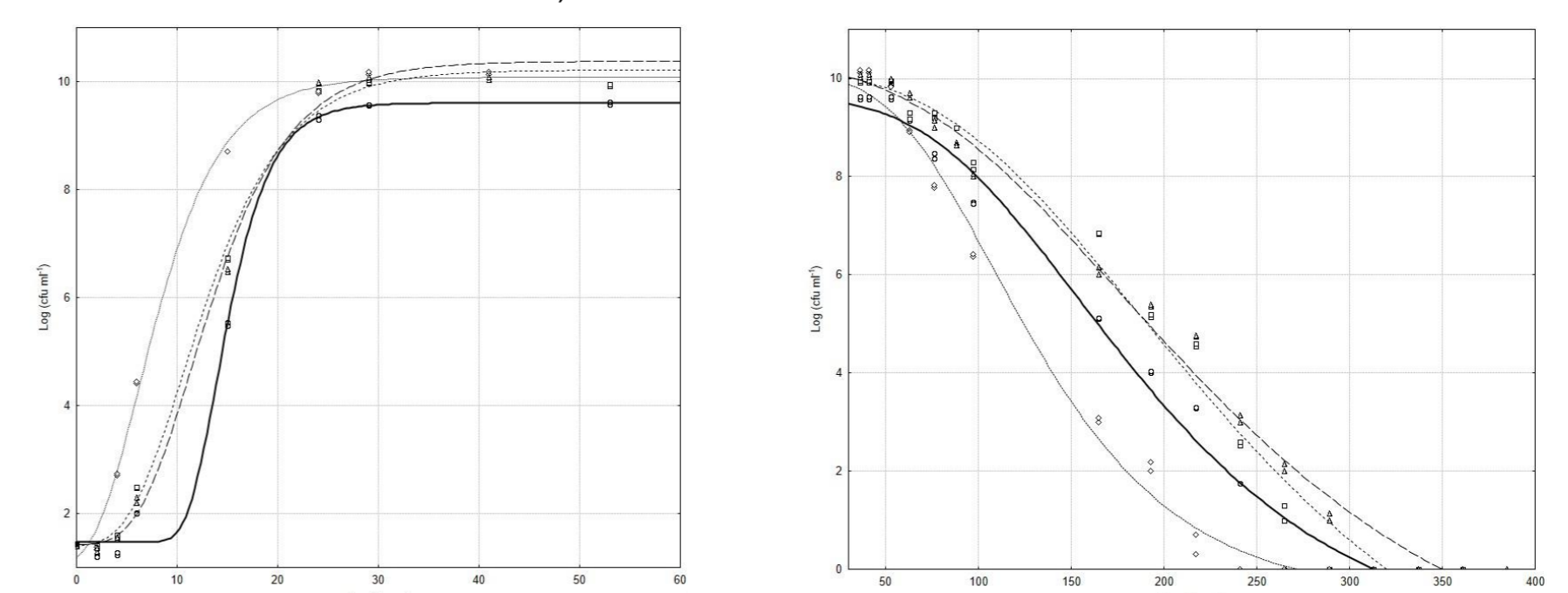
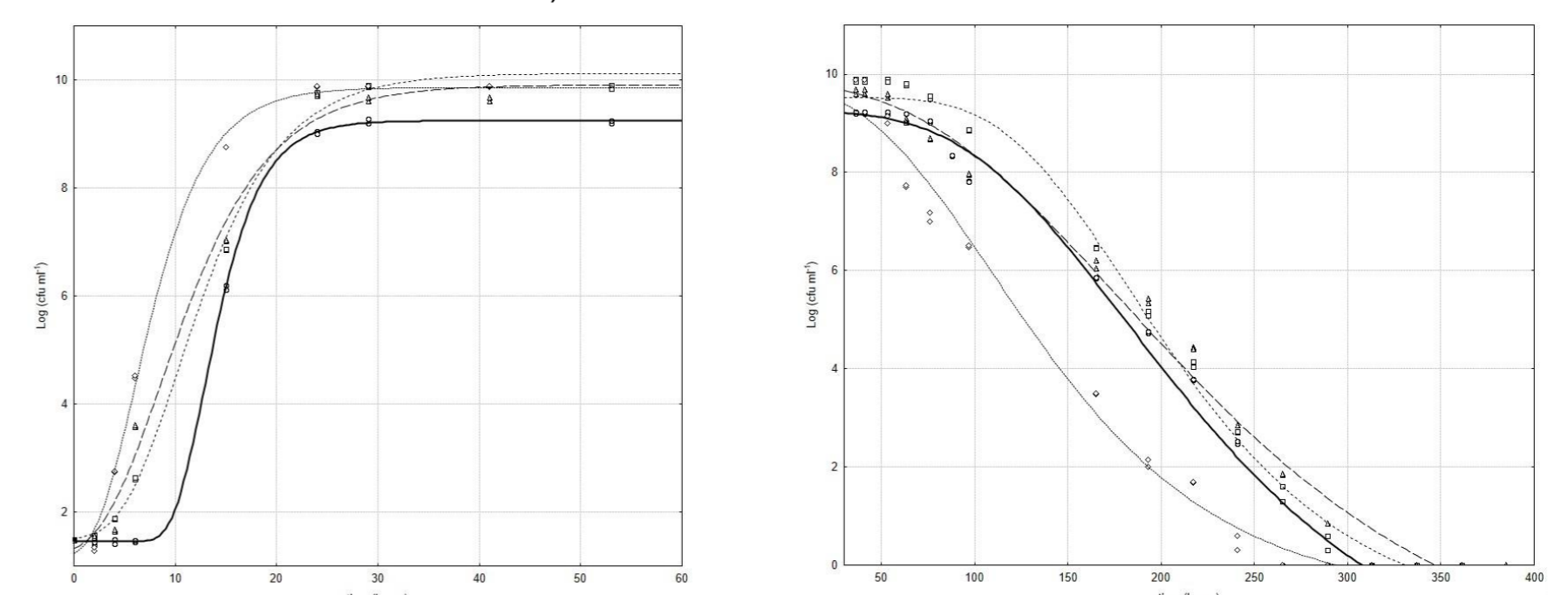


Figure 3 (a, b). Evolution of *Bifidobacterium breve* in growth and death phase with and without cell-free supernatants (CFS) obtained by *L. plantarum* strains (Lc). The curves represent the best fit of the model to the experimental data. (○ *B. breve* □ CFS Lc3 ◇ CFS Lc4 △ CFS Lc15)



CONCLUSION

This preliminary study could be considered as the first report about the study of the bioactivity by cell-free supernatants from probiotic *L. plantarum* strains that exert a potential "prebiotic" activity on Bifidobacteria.

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