Anti-bacterial activity of 17 strains of lactobacilli against elementary bodies of *Chlamydia trachomatis*

Antonella Marangoni[1], Paola Nardini[1], Carola Parolin[2], Rogers Alberto Nahui Palomino[2], Beatrice Vitali[2], Luca Laghi[3], Claudio Foschi[1], Roberto Cevenini[1]

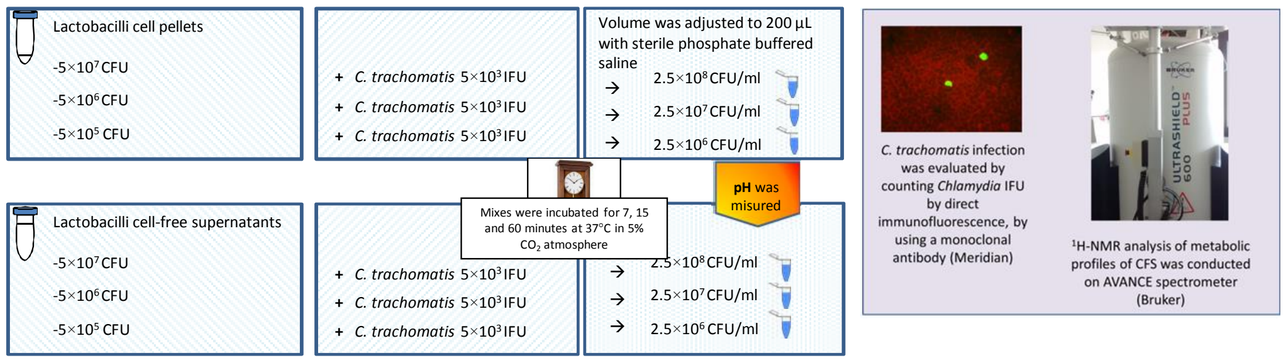
[1]Microbiology, DIMES, University of Bologna, Bologna, Italy; [2]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; [3]Centre of Foodomics, Department of Agro-Food Science and Technology, University of Bologna, Cesena, Italy

INTRODUCTION AND PURPOSE

Lactobacillus species dominate the vaginal microbiota of healthy reproductive-age women and protect the genitourinary tract from the attack of several infectious agents. *Chlamydia trachomatis*, a leading cause of sexually transmitted diseases worldwide, can induce severe sequelae, i.e. pelvic inflammatory disease, infertility and ectopic pregnancy. In the present study we investigated the interference of *Lactobacillus crispatus, L. gasseri* and *L. vaginalis,* known to be dominant species in the vaginal microbiome, with the infection process of *C. trachomatis*.

METHODS

Seventeen *Lactobacillus* strains, isolated from vaginal swabs of healthy pre-menopausal women and cultured in MRS broth were used: in particular, 8 strains of *L. crispatus* (BC1-8), 6 of *L. gasseri* (BC9-14) and 3 of *L. vaginalis* (BC15-17). After turbidimetric determination of cell concentration culture were centrifuged to separate cell pellets (CP) from cell-free supernatants (CFS). All CP and CFS corresponding to 2.5×10^8, 2.5×10^7 and 2.5×10^5 CFU were mixed with 5×10^3 IFU of EBs of CT serotype D. pH were measured in the final volume; EBs diluted in PBS were used as controls. Tubes were incubated for 7, 15 and 60 minutes and then centrifuged. Supernatants were used to infect confluent Hela cells in single tubes containing sterile coverslips. After 48h of incubation at 37°C we evaluated CT infectivity by immunofluorescence. IFUs were counted in 30 randomly chosen 200× microscopic fields and compared with controls. Statistical analyses were performed using GraphPad Prism software, applying Wilcoxon test. ^{1}H-NMR analysis of metabolic profiles of CFS was conducted on AVANCE spectrometer (Bruker); signals were assigned comparing their chemical shifts and multiplicity with Chenomx software data bank (Chenomx, ver8.02). Statistical analysis of ^{1}H-NMR data was performed using R computational software, applying Wilcoxon test.



C. trachomatis infection was evaluated by counting *Chlamydia* IFU by direct immunofluorescence, by using a monoclonal antibody (Meridian)

^{1}H-NMR analysis of metabolic profiles of CFS was conducted on AVANCE spectrometer (Bruker)

RESULTS

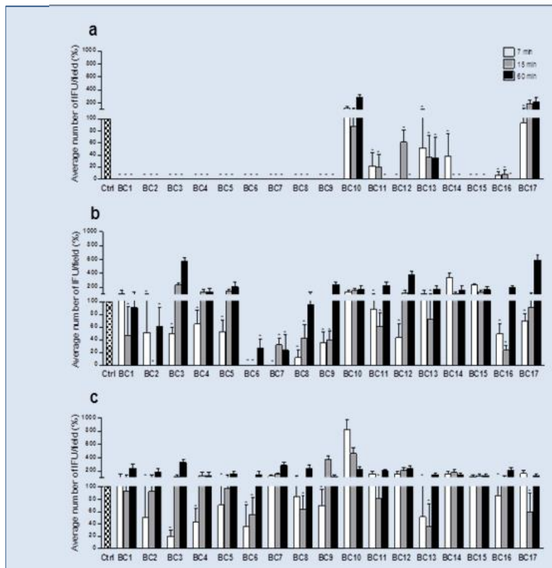


Figure 1. Effect of lactobacilli supernatants on *C. trachomatis* infectivity

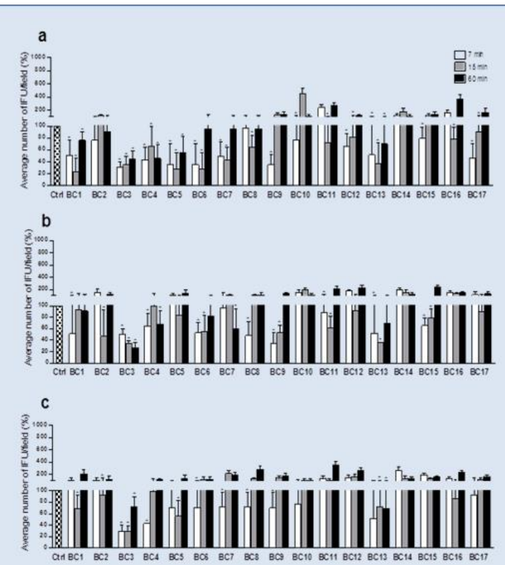


Figure 2. Effect of lactobacilli cell pellets on *C. trachomatis* infectivity

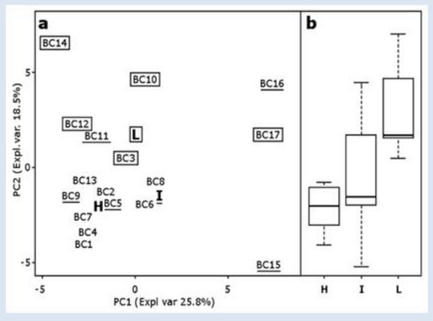


Figure 3. Correlation between metabolome of lactobacilli and killing activity towards *C. trachomatis*

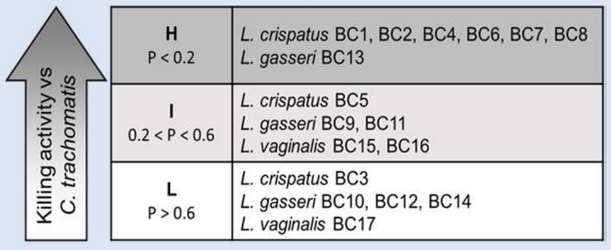


Figure 4. Ranking of lactobacilli in relation to anti-*Chlamydia* activity

CONCLUSIONS

Our results demonstrate the ability of different *Lactobacillus* strains of vaginal origin to inactivate *C. trachomatis* EBs through the production of extracellular metabolites in an acidic environment.

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