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Analysing human microbiota in health and disease

Inhibitory activity of vaginal lactobacilli towards *Candida* spp.

A. Marangoni[1], C.E. Parolin[2], C. Foschi[1], L. Laghi[3], R. Cevenini[1], B. Vitali[2]

[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, Bologna, Italy*

Objectives. Lactobacilli are the dominant bacteria of healthy vaginal microbiota and their principal function is to maintain an environment that restricts the growth of pathogenic and opportunistic microorganisms, as fungi belonging to the genus *Candida*. Lactobacilli form a critical line of defence against potential pathogens by lowering the environmental pH through lactic acid production and producing antimicrobial compounds, or through competitive exclusion. Anyway, the mechanisms underlying antifungal activity against *Candida* spp. are still not fully understood.

In this study, the potential activity against *Candida* spp. of different strains of vaginal lactobacilli was analysed, focusing on hydrogen peroxide generation, lactic acid production and antimicrobial supernatant fluids activity.

Methods. Seventeen strains of lactobacilli were isolated from vaginal swabs collected from pre-menopausal healthy women. They were taxonomically identified by sequencing the 16S ribosomal RNA gene.

Hydrogen peroxide generation was tested in a semi-quantitative assay on de Man, Rogosa, Sharpe (MRS) agar plates containing tetramethylbenzidine and horseradish peroxidase in anaerobic conditions. Isolates were scored as low, medium and high producing strains.

Lactic acid production was measured in cell free supernatants of *Lactobacillus* cultures by ^{1}H-nuclear magnetic resonance (NMR) analysis.

Lactobacillus culture supernatants were tested for their fungistatic or fungicidal activity against 9 *Candida* strains isolated from vaginal swabs submitted to the Microbiology Laboratory of Sant'Orsola-Malpighi University Hospital of Bologna for routine diagnostic procedures, belonging to C. *albicans, C. tropicalis, C. krusei, C. glabrata, C. parapsilosis,* and C. *lusitaniae* species. The in vitro activity of free-cell supernatants was determined by broth microdilution assay in accordance with EUCAST guidelines. To determine if *Lactobacillus* strains supernatants had a killing effect, samples from wells exhibiting less than 50% of growth were taken and spotted onto SD agar plates. Fungicidal activity was defined as a ≥3 $\log_{10}$ reduction from the starting inoculum.

Results. The *Lactobacillus* isolates were taxonomically identified as follows: 8 strains of *L. crispatus* (BC1-BC8), 6 strains of *L. gasseri* (BC9-BC14), and 3 strains of *L. vaginalis* (BC15-BC17).

All *Lactobacillus* strains exhibited a good generation of hydrogen peroxide, while the production of lactic acid, even if recorded for all the strains tested, showed concentrations ranging from 4.8 to 50.9 mM.

When the anti-fungal activity of *Lactobacillus* was assessed, *L. crispatus* supernatants were the most effective, especially versus *C. albicans* and *C. lusitaniae*. None of the *Lactobacillus* strains was able to interfere with *C. krusei* and *C. parapsilosis*. Detailed results of fungistatic or fungicidal activity are shown in figure 1.

Conclusion. A major potential application of this study concerns the identification of active *Lactobacillus* strains that could be administered as probiotics for prophylaxis and/or adjuvant therapy of vulvovaginal candidiasis. Further studies are ongoing to elucidate the mechanisms by which lactobacilli exert their protective functions against *Candida*.