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Letter to the Editor

Effects of Lactobacillus rhamnosus and Lactobacillus acidophilus on bacterial vaginal pathogens

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Lucia Bertuccini,[1]* Rosario Russo,[2]* Francesca Iosi[1] and Fabiana Superti[1]

Abstract

The human vagina is colonized by a variety of microbes. *Lactobacilli* are the most common, mainly in healthy women; however, the microbiota composition can change rapidly, leading to infection or to a state in which potential pathogenic microorganisms co-exist with other commensals. In premenopausal women, urogenital infections, such as bacterial vaginosis and aerobic vaginitis, remain an important health problem. Treatment of these infections involves different kind of antibiotics; however, the recurrence rate remains high, and it must be also underlined that antibiotics are unable to spontaneously restore normal flora characterized by an abundant community of *Lactobacilli*. The main limitation is the inability to offer a long-term defensive barrier, thus facilitating relapses and recurrences.

We report here the antimicrobial activities of two commercially existing *Lactobacillus* strains, *Lactobacillus rhamnosus* HN001 and *Lactobacillus acidophilus* GLA-14 strains and their combination (Respecta® probiotic blend) against four different pathogens responsible for both bacterial vaginosis (*Gardenerella vaginalis* and *Atopobium vaginae*) and aerobic vaginitis (*Staphylococcus aureus* and *Escherichia coli*) by co-culturing assay. The probiotic combination, even if resulting in a different microbicidal activity against the different strains tested, demonstrated the efficacy of combined *Lactobacillus* strain treatment.

Keywords

aerobic vaginitis, antimicrobial, bacterial vaginosis, *Lactobacillus*

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Introduction

The human vagina is a complex environment colonized by a diverse community of microorganisms known as the vaginal microbiota; among these, *Lactobacillus* spp. represents the predominant microorganisms in the healthy vaginal ecosystem.[1,2] *Lactobacillus* species are able to colonize and to produce antimicrobial substances acting to prevent the growth of pathogenic microorganisms.[3] Alterations in the microbial composition of vaginal ecosystem are linked to several adverse health outcomes such as bacterial vaginosis (BV) and aerobic vaginitis (AV). BV is the most common vaginal infection worldwide, affecting women of all age groups, and is characterized by a vaginal pH of >

[1]Department of Technology and Health, National Institute of Health, Rome, Italy
[2]Giellepi S.p.A. Health Science, Lissone (MB), Italy

*Equal contributors.

Corresponding author:
Fabiana Superti, Ultrastructural Infectious Pathology Section, Department of Technology and Health, National Institute of Health, Viale Regina Elena 299, 00161 Rome, Italy.
Email: fabiana.superti@iss.it

4.5, absence of inflammation, and by an overgrowth of anaerobic bacteria with *Gardnerella vaginalis, Atopobium vaginae, Bacteroides* spp., *Mycoplasma hominis, Peptostreptococcus*, and *Prevotella* being typically prevalent.[4] AV is determined on the following criteria: enhanced yellow secretion; pH value $\geqslant$ 5; negative amino-odor test; increased number of leukocytes; absence of *Lactobacillus* spp.; and microbiologically isolated microorganisms: mainly *Escherichia coli, Staphylococcus aureus*, group *B streptococcus*, and *enterococci*.[5] Antibiotics are typically prescribed to treat BV whereas AV frequently requires combined local treatment with: antibiotic (infectious component); steroids (inflammatory component); and/or estrogens (atrophy component). Antimicrobial treatment is usually not fully effective due to antibiotic-resistant bacteria, or for the occurrence of re-infection. As antimicrobial therapy is often partially effective and antibiotics can also cause side effects,[6,7] researches on alternative or complementary approaches represents a medical priority. Even if there are different studies demonstrating a significant improvement in treating bacterial vaginal infections with probiotics versus traditional treatments,[6] results are often bacterial strain-specific suggesting that only certain probiotic bacteria seem to have effects against defined vaginal infections. In this study, we have analyzed the antimicrobial activity of two commercially existing probiotic strains, *L. rhamnosus* HN001 and *L. acidophilus* GLA-14, alone or in combination (Respecta® probiotic blend), against four different pathogens responsible for BV (*G. vaginalis* and *A. vaginae*) or AV (*S. aureus* and *E. coli*). Our results from mixed cultures with AV and BV pathogens strongly suggest that *L. acidophilus* GLA-14, alone or combined with *L. rhamnosus* HN001, can be used in probiotic products to prevent aerobic or anaerobic bacterial infections of the urogenital tract.

Materials and methods

Lactobacillus strains (*L. acidophilus* GLA-14®, *L. rhamnosus* HN001™) were stored in milk yeast extract (MYE) at −80°C. Before the experiments, each strain was transferred from the frozen stock culture to MRS (De Man Rugosa Sharpe) broth[8] incubated at 37°C under non-agitated aerobic conditions. *G. vaginalis* and *A. vaginae*, obtained from University of Göteborg (Sweden) were cultivated anaerobically using the GasPak anaerobic envelope system (Becton Dickinson, Erembodegem, Belgium) at 37°C on Trypticase Soy Agar (TSA) + 5% sheep blood (Becton Dickinson). UPEC *E. coli* CFT073 (O6:K2:H1, ATCC700928)[9] and *S. aureus* (ATCC29213) were cultured in Luria Bertani (LB) and Tryptone Soy (TSB) broths, respectively.

The capability of *L. acidophilus* GLA-14 and *L. rhamnosus* HN001 to interfere with the growth of the different pathogens was evaluated by a liquid co-culture assay in anaerobiosis or in aerobiosis, depending on the particular bacterial strain used.

The co-culture test was performed by incubating in Defined Medium Simulating Genital Tract Secretions (DMSGTS)[10] (capable of sustaining the growth of both probiotics and pathogens) different concentrations of the probiotic strains (10^7 and 10^8 cfu/mL), alone or in combination, with different concentrations (10^6 and 10^7 cfu/mL) of the target pathogen. Controls were carried out by inoculating DMSGTS with the different strains alone.

Incubation was carried out for different lengths of time (range, 6–48 h). To check whether the pathogens were inhibited or killed, 0.05 mL of co-culture suspensions were diluted and seeded on specific agar medium. After an incubation period at 37°C for 24–48 h, bacterial growth was evaluated. No growth was interpreted as microbicidal activity (100% inhibition).

Statistical analysis was performed by Student's t-test for unpaired data. Data were expressed as the mean and SD and P values of < 0.05 were considered significant.

Results

Results of co-culture assay have shown that the AV and BV pathogens were differently sensitive to the probiotics (Figure 1).

L. acidophilus GLA-14 was able to inhibit *S. aureus* growth after 6 h (Figure 1b) or 12 h (Figure 1a, b), whereas inhibition with *L. rhamnosus* HN001 was observed at 24 h (Figure 1d) and after 48 h (Figure 1c, d). The combination of both *Lactobacilli* (10^7 cfu/mL) with *S. aureus* inoculum (10^6 cfu/mL) caused complete inhibition of pathogen growth after 48 h (Figure 1e), whereas when the inoculum of *S. aureus* was higher (10^7 cfu/mL), complete inhibition of pathogen growth was observed after 24 h (Figure 1e). The combination of both *Lactobacilli* (10^8 cfu/mL) with *S. aureus* inoculum (10^6 cfu/mL) caused complete inhibition of pathogen growth since 6 h (Figure 1f), and when

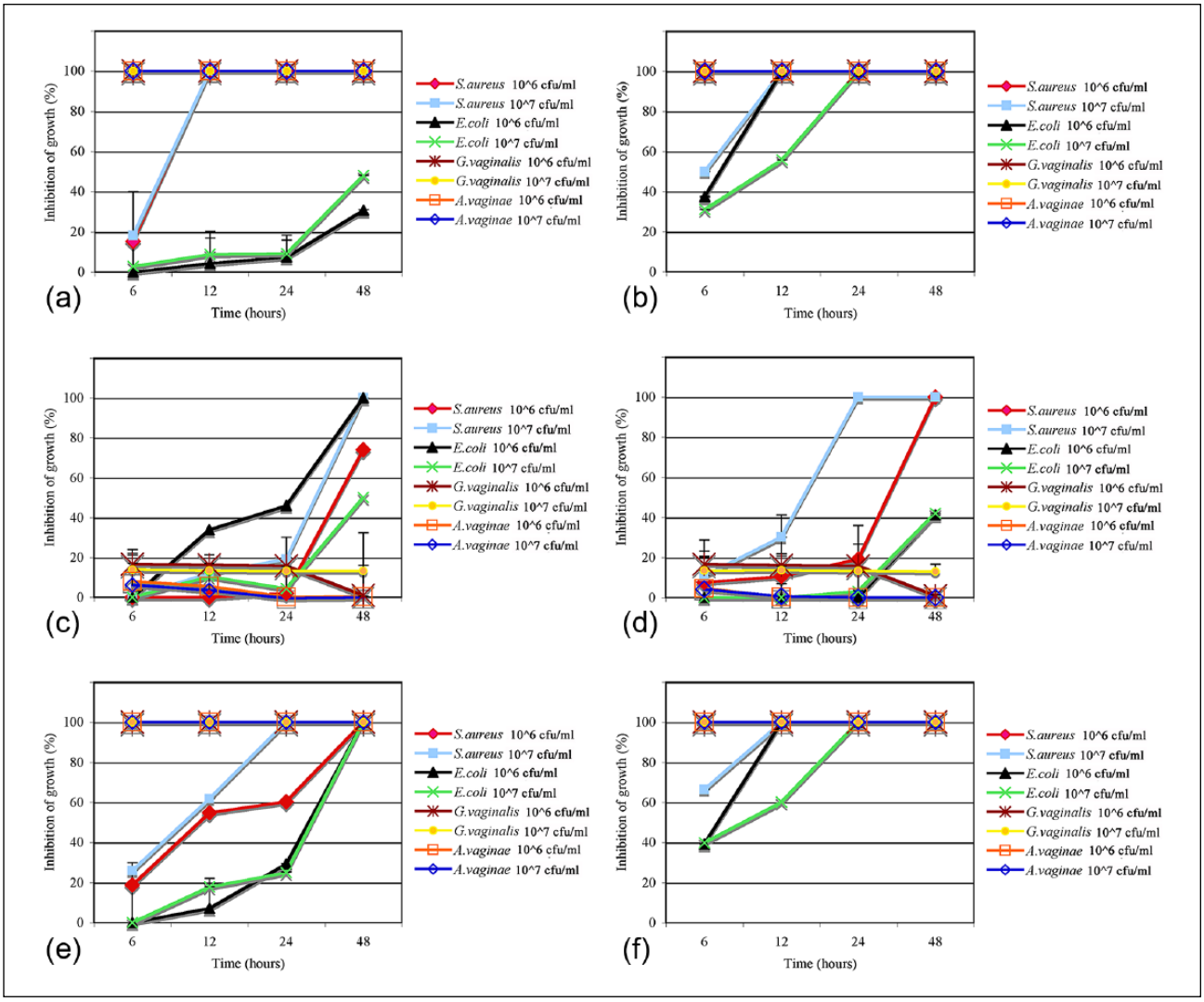

Figure 1. Effect of probiotic strains on AV and BV pathogens in co-culture assay. Percentage of growth inhibition was calculated as the recovered pathogen bacteria at the different time points after incubation with probiotics, alone (a–d) or in combination (e, f), compared with the control cultures (pathogens alone) taken as 100%. (a) *L. acidophilus* 10^7 cfu/mL; (b) *L. acidophilus* 10^8 cfu/mL; (c) *L. rhamnosus* 10^7 cfu/mL; (d) *L. rhamnosus* 10^8 cfu/mL; (e) *L. acidophilus* 10^7 cfu/mL and *L. rhamnosus* 10^7 cfu/mL; (f) *L. acidophilus* 10^8 cfu/mL and *L. rhamnosus* 10^8 cfu/mL.

the inoculum of *S. aureus* was 10^7 cfu/mL, complete inhibition of pathogen growth was observed after 12 h (Figure 1f). *L. acidophilus* GLA-14 was more active than *L. rhamnosus* HN001.

L. acidophilus GLA-14 and *L. rhamnosus* HN001were differently active against *E. coli* (Figure 1a–d). *L. rhamnosus* (10^7 cfu/mL) was more effective than *L. acidophilus* (10^7 cfu/mL) against *E. coli* at 10^6 cfu/mL (Figure 1a, c) and their combination was synergic against *E. coli* (10^7 cfu/mL), inducing a complete inhibition of growth after 48 h (Figure 1a, c, e). A probiotic combination of 10^8 cfu/mL and an *E. coli* inoculum of 10^6 or 10^7 cfu/mL resulted in a complete inhibition of pathogen growth after 12 h and 24 h, respectively (Figure 1f). This probiotic combination of 10^8 cfu/mL seems have some slight effects after 6 h incubation with both aerobic pathogens (inoculum of 10^7 cfu/mL) (Figure 1b, f).

L. acidophilus GLA-14 alone (10^7 cfu/mL or 10^8 cfu/mL) was able to inhibit both concentrations of *G. vaginalis* and *A. vaginae* after 6 h (Figure 1a, b), whereas *L. rhamnosus* HN001 had little inhibitory activity (Figure 1c, d). As expected, the combination of both *Lactobacilli* showed the same inhibition degree of *L. acidophilus* GLA-14 alone on both anaerobic pathogens.

Taken together, the results obtained showed that *L. acidophilus* GLA-14 and *L. rhamnosus* HN001 (Respecta® probiotic blend) were able to inhibit the growth of all tested pathogens at different incubation time, depending on the initial inoculum of pathogen and, for anaerobic strains, from *Lactobacillus* strain concentrations.

Discussion

The vaginal microbiota is a dynamic ecosystem that in healthy individuals is usually colonized by the *Lactobacillus* genus but it can rapidly lead to microbiota dysbiosis where a range of microorganisms (such as *G. vaginalis* and *A. vaginae* or *E. coli, S. aureus*, and group B *Streptococcus*) become predominant and cause polymicrobial BV or AV, respectively.[1,11] Incompetent diagnosis and antibiotic resistance, together with the elimination of some helpful bacteria[7], are the main causes of the unsatisfactory results of conventional antimicrobic treatments of BV and AV. Evidence of decreased levels of *Lactobacillus* species in BV and AV has given rise to the concept of their replacement to restore the natural vaginal flora by utilizing probiotic strains. Probiotics, according to the World Health Organization definition, are "live microorganisms which when administered in adequate amounts confer a health benefit on the host."[12] Even though the use of probiotics to colonize the vagina and prevent or treat infection has been considered for some time, only recently their efficacy has been proven, and, different from that observed for antibiotics, no adverse effects have been reported.[3] Here we studied the antimicrobial activity of two commercially probiotic strains, *L. rhamnosus* HN001 and *L. acidophilus* GLA-14, alone or in combination (Respecta® probiotic blend), against four different pathogens responsible for both BV (*G. vaginalis* and *A. vaginae*) and AV (*S. aureus* and *E. coli*). The tested probiotic bacteria showed that they possess inhibitory activity towards BV and, mainly, AV pathogenic bacteria, *L. acidophilus* GLA-14 having, in general, the highest antagonistic effect against anaerobic strains. Such an effect could be due to several mechanisms including the production of toxic compounds such as lactic acid, hydrogen peroxide, and bacteriocins that are enhanced in *L. acidophilus* rather than *L. rhamnosus*.[13]

Our results demonstrate that the *Lactobacilli* combination was synergic against *E. coli*, demonstrating that the association of two probiotic strains can be helpful to treat bacterial vaginal infections.

One promising lead towards the treatment of BV and AV is also the vagina colonization by *Lactobacilli* which forms a barrier against infection.[14] In fact, in a recent pilot study it was demonstrated that oral consumption by healthy volunteers of the combination of the same probiotic strains utilized in the present research (*L. acidophilus* GLA-14 and *L. rhamnosus* HN001, together with bovine lactoferrin: Respecta® complex) leads to *Lactobacillus* spp. vaginal colonization.[15]

In conclusion, commercial probiotics, such as the ones examined here, represent very promising tools to provide protection from BV and AV.

Declaration of conflicting interests

The authors declared the following conflicts of interest with respect to the research, authorship, and/or publication of this article: RR is an employee of Giellepi S.p.A.

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