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ORALLY ADMINISTERED MULTISPECIES PROBIOTIC FORMULATIONS TO PREVENT URO-GENITAL INFECTIONS: A RANDOMIZED PLACEBO-CONTROLLED PILOT STUDY

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ABSTRACT

Purpose The aim of this study was to evaluate in the vagina of 60 pre-menopausal women the detection of orally administered multispecies probiotic formulations showing anti-microbial properties in test in vitro.Methods A randomized, double-blind, three-arm parallel pilot study was carried out on 60 pre-menopausal women.Subjects were randomly divided in three groups (F_1, F_2, F_3). Each group received a daily oral administration of probiotic mixtures (for 14 days and at the day 21, 7 days after the wash-out) containing: Lactobacillus acidophilus and Lactobacillus reuteri (F_1), or Lactobacillus plantarum,Lactobacillus rhamnosus and Bifidobacterium animalis subsp. lactis (F_2), or placebo (F_3), respectively. Vaginal swabs were collected at four experimental times,at t0 and at t7, t14 and t21 days, and analyzed by qPCR. At the same time, the anti-microbial activity of the probiotic formulations was verified by assays in vitro against microorganisms as Escherichia coli and Candida albicans.Results L. acidophilus and L. reuteri as well as L. plantarum,L. rhamnosus and B. lactis were significantly increased on 7 days in the groups administered with F_1 and F_2, respectively, compared to group F_3. A similar significant trend was observed on 21 days, 7 days after the wash-out. F_1 and F_2 showed coherent anti-microbial properties.Conclusion Both probiotic formulations F_1 and F_2,chosen because of their anti-microbial activity against pathogens responsible for vaginal dysbiosis and infections,led to vaginal detection and enhancement of the amount of species of formulates when orally administered. This work provides the basis for further clinical investigations of the F_1 and F_2 capacity to prevent or treat uro-genital infections.

INTRODUCTION

Probiotics are, according to the FAO and WHO's definition,"live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1].Several health benefits of probiotics are known: their administration is beneficial in preventing and curing different types of diarrhea [2]; they are used in the treatment of inflammatory intestinal diseases [3] and it has been demonstrated that they help preventing from allergies [4].Usually, the gut is the target organ for probiotic formulations,but recently other organs and tissues have been related to probiotics, such as skin, hair [5], the oral cavity [6] and the vagina [7], by showing their positive effects for human health. In the specific case of vagina, the microbial species play an important role in the maintenance of health and prevention from infections throughout several mechanisms: occupation of specific adhesion sites of the urovaginal epithelium, maintenance of a low pH and production of anti-microbial metabolites such as acids, bacteriocins,hydrogen peroxides and anti-adhesive polysaccharides [8]. In physiological conditions, the vagina hosts mainly Lactobacillus spp. which concurs to a healthy microbiota, hence preventing from colonization of pathogenic bacteria and fungi [9, 10]. On the contrary, the depletion of vaginal lactobacilli facilitates the overgrowth of diverse species such as Gardnerella vaginalis, Atopobium vaginae, Candida albicans, Escherichia coli,responsible for vaginal dysbiosis and uro-genital infections [9–12]. Different probiotics were reported [13–15] to restore the normal vaginal homeostasis by colonization of lactobacilli, when administered topically. Recent works [16, 17] also suggest that oral administration of lactobacilli and bifidobacteria would colonize

both the intestinal and vaginal mucosal surfaces. This opens a new prospect for probiotic therapy: the challenge is to develop probiotics formulation to treat the uro-genital infections and to reduce the recurrences of disease in time,thanks to the capacity to control the pathogenicity of other microbes and to restore the normal ecological balance in vagina. However, these studies did not describe the efficacy of oral administration in term of presence and persistence of probiotic at the vaginal mucosa.

In this context, the aim of the present work is to evaluate in the vagina of 60 pre-menopausal healthy women the detection of orally administered multispecies probiotic formulations showing anti-microbial properties in test in vitro. A formulation F_1 containing L. acidophilus PBS066 and L. reuteri PBS072 and the other F_2 composed by L. plantarum PBS067, L. rhamnosus PBS070 and B. animalis subsp. lactis PBS075 were compared with the placebo (F_3). Data on these two mixtures supported the anti-microbial activity exerted by the above-mentioned single strains by assays in vitro using cell-free supernatants against microorganisms as E. coli and C. albicans.

The pilot study developed in this paper represents an example to investigate how oral consumption of probiotics formulations can lead to increased levels of the consumed species in the vagina of the women recruited in the study that showed anti-microbial activity against microorganisms potentially involved in uro-genital infections.

RESULTS

Anti-microbial activity of the probiotic formulates

The anti-microbial activity of formulates F_1 and F_2 was tested against C. albicans and E. coli, as an example of microorganisms responsible for uro-genital infections, by growth inhibition with non-neutralized cell-free supernatants and by overlay assay on living cells. Both probiotics mixtures showed a strong inhibition rate against E. coli mediated by microbial culture supernatants during 24 h, while no influence was detected vs C. albicans. The inhibition degree of each probiotic formulation compared to the corresponding single strain is reported in Fig. 1.The curve of activity of formulates is in line with the expected profile from single strain performance. The inhibition capacity of the mixtures F_1 and F_2 was further investigated by direct contact of their cultures against the same microorganisms. The growth inhibition halos around the multiple colonies were coherent with the anti-microbial inhibition profiles detected for the single strains. F_1 showed a moderate activity against both C. albicans and E. coli according to a better performance of L. acidophilus contrary to L. reuteri, while a stronger inhibition capacity vs both pathogens.

Evaluation of vaginal probiotic amount in the subjects of the study

The selected primers were used to estimate the presence and persistence of each probiotic species in vagina from swab samples of the enrolled subjects by qPCR. PCR analyses were performed on DNA extracted from vaginal swabs at time t0, t7, t14 and t21 days.

The qPCR analysis demonstrated that the speciesspecific sequences associated with the probiotics of the formulations were detected only in vaginal DNA from subjects treated with the formulations F_1 and F_2 and not with the formulation F_3. Figure 3 shows the abundance of the different probiotic species for each treatment group at the different experimental times. The subjects treated with F_1 showed an increase in the level of both L. acidophilus and L. reuteri compared with

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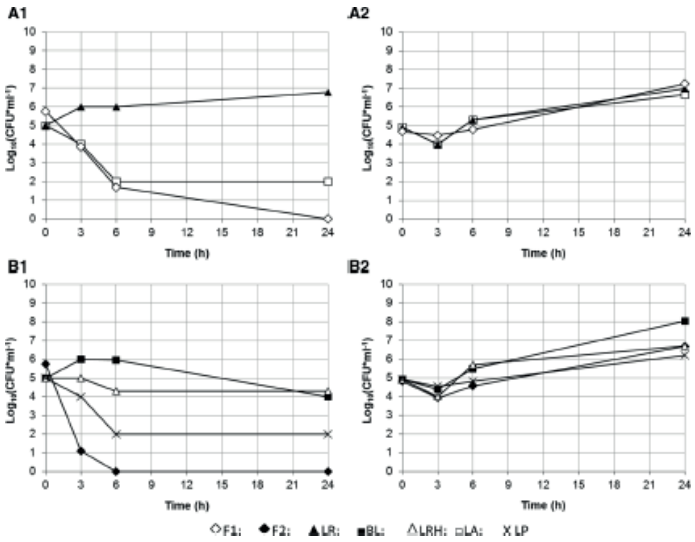

Fig. 1 Effect of non-neutralized culture supernatants of L. aci-dophilus (square), L. plantarum (cross), L. rhamnosus (triangle), B. animalis subsp. lactis (filled square), L. reuteri (filled triangle), mixture F_1 (diamond), mixture F_2 (filled diamond) on the growth

F_3. This has been observed at all the times compared with the t0, including t21, 7 days after the follow-up from the last probiotics administration. The same trend was observed for L. rhamnosus, L. plantarum and B. animalis subsp. lactis in women treated with F_2 formulation. Dif-ferently, the amount of all five strains remained constant throughout the study in the vaginal DNA of women treated with the placebo F_3.The increase of microbial cell number and the perma-nence of probiotic species at higher levels after the end of the oral administration (t21) indicate that these species are actually more abundant in the vaginal microbiota. This increase was observed with statistical significance (p value\0.05) since 7 days after the beginning of treatment for L. reuteri and L. acidophilus of the F_1 group and for L. rhamnosus and B. animalis subsp. lactis of the F_2 group. Instead, the increase of L. plantarum resulted statistically significant (p value \0.05) since 14 days from the begin-ning of the treatment. The permanence of bacteria was observed for all the species studied until the t21, 7 days from the last probiotics intake. In fact, the differences of E. coli and C. albicans: A1 mixture F_1 vs E. coli; A2 mixture F_1 vs C. albicans; B1 mixture F_2 vs E. coli; B2 mixture F_2 vs C. albicans t14 and t21 from t7 are not statistically significant (p value [0.05) for all probiotics, with the exception of L. plantarum.For F_3 formulation there were not so variations in the amount of selected probiotics for the entire 21-day period.

DISCUSSION

The microbial species that inhabit the vaginal tract play an important role in the maintenance of health and prevention of infections. In particular, the presence of high numbers of lactic acid bacteria in the vagina is often equated with an health status [26]. It appears evident that the balance between a healthy and diseased state involves an equilib-rium which can depends on different factors, such as hor-mone levels, douching, sexual practices, as well as bacterial interactions, and host defenses [27, 28]. A way to increase the level of vaginal lactobacilli is through the use of probiotics; two ways are commonly applied: direct application through vagitories or indirect application by oral consumption of probiotics. Several probiotics have been found to both increase the overall level of vaginal lactobacilli and to aid in the treatment of bacterial vagi-nosis [26]. Moreover, the capacity of probiotics to exert beneficial effects in human health is recognized to be strain-specific and a multi-species formulation could take the advantage of combining a greater spectrum of activities.In this context, the present work allowed us to assess in the vagina of 60 pre-menopausal healthy women of a pilot study, the detection of orally administered multispecies probiotic formulations showing anti-microbial properties in test in vitro. First, we tested the antagonistic capacity against E. coli and C. albicans of the formulation F_1 and F_2 through cell supernatants and through the overlay contact between probiotic formulations and pathogens. Both formulations showed a strong anti-microbial activity against E. coli in both the conditions, as expected by the average trend of the single strains included in the formulations, while inhibition against C. albicans occurred only in the overlay assay, likewise the tested single strains. This is probably due to the reliable inhibitory activity of lower pH upon the E. coli growth, while the same condi-tions are not effective against yeasts. Moreover, it is reported that bacteriocins produced by probiotics are active only at certain pH

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ranges but can be neutralized at different pHs; this could be the reason for not having observed any inhibitory effect on the pathogens growth by using the neutralized cell supernatants [18].

Probiotics can reach the target of vagina when orally consumed, as an alternative way to a direct local admin-istration; so they can locally exert their effect by compe-tition–displacement of pathogens, reducing the infection relapses and related symptoms [26, 29]. Although previous studies [30–32] had already suggested this possibility, it is not so expected and the mechanism is still unclear. The current pilot study was aimed to investigate the vaginal detection after oral consumption of mixtures of probiotics would lead to increased levels of the consumed multispecies in the vagina of the enrolled subjects.Our analysis showed an increased levels of all probiotics species (p value\0.05) detected in the vaginal swabs of women consuming the formulates F_1 and F_2 in comparison to women of the placebo group. The only exception was for L. plantarum, which was higher starting from 7 to 21 days of administration. This might suggest that: (1) L.plantarum is not as efficient as other probiotics, in colonizing vaginal mucosa from the intestinal region, (2) L.plantarum is already present at high levels in vaginal mucosa of healthy women and its abundance is not easily perturbed by the oral administration of probiotics. For this reason, L. plantarum might need a longer treatment or may require a higher concentration in administered capsules. The abundance of probiotic strains in the vaginal DNA was assessed for both formulations until the last day of the experiment (t21 days), 7 days after the last intake (p value \0.05). This indicates that probiotics can actually colonize the vaginal microbiota in a short time. It would be particularly interesting to demonstrate if these can persist also after the menses. Indeed, the set-up of this study was purposely planned to match menstrual cycle of volunteers,with the first swab collected after menses and the last 21 days later, before the next menses. This was done to prevent from having blood traces in the vaginal swabs, so possibly altering the results.The DNA markers used allowed to monitor the assessment and persistence of each probiotic species in the vaginal microbiota of healthy pre-menopausal women during the treatment and after the follow-up period. Our findings suggested that the five selected DNA markers can detect the increasing level and persistence of the studied bacteria into the vagina by using qPCR methods. As lactobacilli and bifidobacteria have a fundamental role on the vaginal well-being, and more specifically an anti-microbial activity, we would highlighted that the antimicrobial effect detected against several pathogen microorganisms suggests that the five selected strains could similarly exert the antagonistic activity in vivo.

CONCLUSIONS

In conclusion, this study reports that the oral intake of two probiotic multi-species mixtures leads to an evident colonization of vagina of 60 volunteers of probiotic bacteria showing in vitro anti-microbial activity against pathogens involved in uro-genital infections. The adopted molecular tool represents a valid instrument to be used in future clinical trials to correlate the eventual clinical outcomes with the effective colonization in the treatment or prevention of vaginal dismicrobism and uro-genital infections.

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