

Lactobacillus acidophilus, Strain NAS (H₂O₂ Positive), in Reduction of Recurrent Candidal Vulvovaginitis

Julius Metts, MD*

Thomas R. Famula, PhD†

Natasha Trenev‡

Roger A. Clemens, DrPH§

*University of California, Cowell Student Health Center, Davis, California

†University of California, Department of Animal Science, Davis, California

‡Natren Inc, Westlake Village, California

§University of Southern California, School of Pharmacy, Los Angeles, California

KEYWORDS: recurrent vulvovaginal candidiasis; *Lactobacillus acidophilus*, NAS strain; *Candida albicans*

ABSTRACT

The incidence of recurrent vulvovaginal candidiasis was compared among female university students using *Lactobacillus acidophilus*, NAS strain (H₂O₂ positive), vaginally (group 1), *L acidophilus*, NAS strain (H₂O₂ positive), vaginally in combination with oral probiotic capsules (group 2), and placebo controls. The selected students had recent vaginitis and a total of 4 or more vulvovaginal infections in the past 12 months. Out of 27 women participating for an average 3.3 months in a randomized, double-blind, placebo-controlled trial, 9 women were in group 1, 8 women were in group 2, and the remaining 10 women were in the control group. The total number of infections in group 1 differed from the control group ($P = 0.005$), as did the number of infections in group 2 ($P = 0.011$). The incidence of infection between the two treatment groups did not differ ($P = 0.81$).

These important findings demonstrate that vaginal inserts of *L acidophilus*, NAS strain (H₂O₂ positive), with or without oral probiotic capsules, may significantly reduce the incidence of candidal vulvovaginitis in women with recurrent infections.

INTRODUCTION

An estimated 10 million women present with vaginitis each year. The introduction of specific strains of lactobacilli may reduce the incidence and duration of vaginitis. Vulvovaginal candidiasis is a common condition occurring in an estimated 75% of all women during their lifetime. *Candida albicans* cause 90% of these infections. There are indications that 5% of women with vulvovaginal candidiasis may develop recurrent vulvovaginal candidiasis (RVVC), which is defined as four or more episodes of vulvovaginal candidiasis in the previous year.¹

Lactobacilli are the predominant flora in the vagina.² Lactobacilli produce lactic acid, which maintains vaginal acidity (normal pH, 3.8–4.2), and some strains pro-

duce H₂O₂. These peroxide-producing strains, along with their antimicrobial agents, may suppress bacterial growth of potential pathogens including *C albicans* and genital microorganisms, which cause urogenital tract infections.^{3,4}

Some women with RVVC present with obvious exogenous factors such as diabetes, typically use antibiotics, or are immunodeficient.⁵ In most cases, there is no obvious explanation for recurrences in women with RVVC. Systemic prophylaxis has been recommended when the infection in RVVC is caused by *C albicans*.⁶ An initial 14-day course of oral imidazole therapy to induce clinical remission followed by a 6-month maintenance regimen has been suggested.¹ The therapies have a significant medical cost and may potentially cause side effects such as jaundice,⁷ hepatotoxicity,⁶ nausea, headaches, and diarrhea.

A cross-over trial by Hilton et al.⁵ demonstrated that daily consumption of yogurt containing high colony counts (>10⁸ cfu/mL) of H₂O₂-producing *Lactobacillus acidophilus* significantly reduced the incidence of candidal infections in women with RVVC. Another preliminary study by Hilton et al.⁸ showed that *Lactobacillus rhamnosus*, GG strain, vaginal suppositories self-administered twice daily for 7 days reduced the symptoms associated with candidal vaginitis. Conversely, a cohort study done by Hawes et al.⁹ found that H₂O₂-producing vaginal lactobacilli did not protect against vulvovaginal candidiasis. No *L acidophilus* supplementation was implemented in that study.

This study examines the efficiency of *L acidophilus* NAS strain (Gy-Na•tren[□]) vaginal suppositories alone or in combination with oral probiotic strains (*L aci - dophilus* NAS strain; *Bifidobacterium bifidum* Malyoth strain, and *Lactobacillus bulgaricus* LB-51 strain [Healthy Trinity[□]]) to reduce the incidence of candidal vulvovaginitis in women with RVVC relative to placebo controls.

METHODS AND MATERIALS

Participants

Thirty-four undergraduate and graduate female students (19 to 40 years of age) with RVVC were recruited from a university student health center. Eligible women had 4 or more candidal vulvovaginal infections in the past 12 months, with at least 2 documented by a medical provider. Participants with confirmed immunocompromised condition, pregnancy, or the presence of other vaginal pathogens such as bacterial vaginosis, gonorrhea, or trichomonas were excluded from the study. Women receiving antibiotic therapy were unable to enter the study until 1 month after they completed therapy. The participants were also observed free of symptoms of vulvovaginitis for 1 month.

All patients provided written informed consent and the study was approved by the institutional review board. At the beginning of the study, each woman completed a questionnaire that provided information on gynecologic, dietary, medical, and sexual history. A history of the number of episodes of vaginitis was obtained, including the number of episodes diagnosed by a medical provider. The women were instructed to avoid eating yogurt or other *L acidophilus*-containing products during the study period. In addition, the participants were advised not to alter their sexual behavior. Financial incentives were not offered, nor were there any charges for the study evaluations, laboratory analysis, or study products.

On admission to the study, each woman was given a pelvic examination. Two cervical swabs were obtained and directly inoculated onto Thayer Martin media and tested for chlamydia. Two other swabs were taken from the posterior fornix and vaginal walls, inoculated onto blood agar, and examined with potassium hydroxide (KOH) digestion and wet mount microscopy.

Selected women were randomized using a random number generator into 1 of

2 treatment groups or a control group. Participants and health care providers (investigators/assessors) were blinded and unaware of allocation. At the participating pharmacy, women collected the probiotics/control in identical opaque paper bags identified by the randomized number. The paper bags contained capsules and brown bottles identical in appearance.

The study included 2 treatment groups in addition to a control. Treatment group 1 used the preparations *L acidophilus*, NAS strain (H₂O₂ positive; [2x10⁹ cfu/capsule] from Gy-Na•tren[□]), vaginal suppositories with placebo (~300 mg cellulose [Solka-Floc[™]]) oral capsules. Treatment group 2 used the preparations *L acidophilus*, NAS strain (H₂O₂ positive; [2x10⁹ cfu/capsule]), vaginal suppositories with probiotic oral capsules (*L acidophilus*, NAS strain (H₂O₂ positive) [5x10⁹ cfu/capsule]; *B bifidum*, Malyoth strain [20x10⁹ cfu/capsule] and *L bulgaricus*, LB-51 strain [5x10⁹ cfu] from Healthy Trinity[□]). The control group received placebo preparations vaginally and orally.

All preparations were kept refrigerated (4°C) as specified by the probiotic manufacturer. Oral capsules were self-administered daily and vaginal suppositories were self-administered 3 times weekly according to the instructions provided. The women were followed for up to 6 months and seen by a staff physician on a monthly basis and additionally when symptoms of vaginitis were present.

Data Collection and Analysis

Candidal infections, presumed candidal infections, and asymptomatic candidal infections were assessed in each study participant. Candidal vulvovaginal infections were assessed by symptoms of vulvovaginal/pruritus or irritation with the presence of budding yeast or pseudohyphae on KOH digestion or a positive culture for *Candida* species. Presumed candidal infections were assessed as symptoms of vulvovaginal pruritus or irritation, which resolved with over-the-counter anti-candidal vaginal

medication. Asymptomatic colonization with *C albicans* was determined on monthly examinations during the study period. Self-reported data relating to symptoms of vaginitis, sexual activity, menses, and changes in diet or medications were recorded by the medical staff during scheduled monthly visits. All subjects were encouraged to report symptoms and schedule an appointment as soon as symptoms developed. Vaginal samples were collected at each visit. These samples were subjected to pH measurement, KOH digestion with microscopy, wet mount microscopy, and whiff test. Candidal vulvovaginal infections were treated with topical terconazole (Terazol[®] 3) 0.8% vaginal cream for 3 days. No systemic antifungals were used to avoid interference with the study. No test of cure was done after treatment.

Data were analyzed on the total number of infections, a value computed as the sum of documented infections plus presumed infections in each study group. Differences between treatments were evaluated through the analysis of covariance with the number of months in the study as covariate. The significance tests on single degree of freedom contrasts were performed in the General Linear Models procedure of SAS (Versions 6, 12; 1996).

RESULTS

During 36 months (May 1998 to May 2001), 1,090 women reported to the student health center with incidence of vaginitis. Thirty-four of the 43 women who met the inclusion criteria were entered into the study. None of these women was infected with *Gardnerella* species, *Neisseria gonorrhoea*, *Trichomonas* species, or chlamydia at the time of entry. Seven women did not return for any follow-up visits. Thus, 27 women participated in total, for an average 3.3 months and 9 women completed the 6-month program (Table 1).

Five women in the control group discontinued follow ups due to frequent candidal vulvovaginitis. One woman dropped

Table 1. Study Group Distribution and Participation.

Study Group	Number of Participants	Average Age (yrs; range)	Total participation (months)	Participants returned for follow-up visits (0.5 – 6 months)	Participants followed up \leq 3 months	Participants completed entire 6 months (%)	Number discontinued
Control	10	25; 21-34	25.8	10	3	2 (20)	5
Treatment 1 NAS + placebo	9	27; 19-31	34.5	9	5	4 (44)	0
Treatment 2 NAS + probiotic	8	29; 21-40	30.0	8	4	3 (38)	1
Total = 27							

out due to vaginal discharge that she thought was associated with the vaginal suppository. A third woman did not return for follow up. In group 2, 1 woman discontinued due to a brown vaginal discharge the morning after using the insert. Three other women in this group failed to return for follow up. There was 1 episode of bacterial vaginosis in each of the treatment groups. Bacterial vaginosis did not occur in women in the control group.

The number and rate of documented infections in each group throughout the study period is shown in Table 2 and in Figure 1, respectively. A test of normality based on the residuals from the analysis of covariance (ANCOVA), called the Shapiro–Wilks statistics, reveals that the residuals for the total number of infections or number of documented infections plus presumed infections are normally distributed. Table 3 summarizes significance tests of various treatment-control comparisons for the number of infections. There is a significant difference ($P = 0.013$) between the rate of total infections of the combined two treatment groups and the control group when combined documented and presumed infections are considered. The rate of documented infections between the combined treatment groups and the placebo control differs significantly ($P = 0.001$).

The average number of months spent on the trial by a patient is 3.3. Using the values in Table 1, the calculated means

(SD) of documented infections for control, group 1, and group 2 patients were 1.55 (0.25), 0.27 (0.25), and 0.60 (0.27), respectively.

Figure 2 illustrates that incidence of infections within the first 3 months of follow-up did not differ among the 3 study groups, whereas the average number of infections within the second 3-month period was significantly reduced ($P = 0.032$) between the combined treatment groups and the control.

DISCUSSION

The results indicate that *L acidophilus* strain NAS (H_2O_2 positive) vaginal inserts administered three times weekly with or without oral probiotic capsules may significantly decrease candidal infections in an at-risk population of women with RVVC.

RVVC is a common problem encountered in primary care and frequently ignored by the medical community.¹ Numerous oral and topical therapies are available; however, adverse side effects and expense may limit their use.^{6,7}

The application of probiotic preparations of appropriate strains of *L acidophilus*, such as NAS strain, may prove to be an inexpensive and efficacious approach for treatment of women with RVVC.

Vaginal suppositories may be a more reliable, direct form of establishing H_2O_2 positive strains of lactobacillus population in

Table 2. Summary of Regression Parameters for Number of Documented Infections, Asymptomatic Yeast Infections, and Total Infections plus Presumed Infections During 6-Month Study Period

Study Group	Number of Infections	Slope	Intercept	Correlation Coefficient
Control	10	0.714	- 0.843	0.857
Treatment group 1 Lactobacillus NAS + placebo	2	- 0.101	0.611	0.512
Treatment group 2 Lactobacillus NAS + probiotic	5	0.067	0.373	0.197

Study Group	Number of Confirmed and Presumed Infections	Slope	Intercept	Correlation Coefficient
Control	15	0.610	-0.013	0.733
Treatment group 1 Lactobacillus NAS + placebo	5	-0.091	0.903	0.299
Treatment group 2 Lactobacillus NAS + probiotic	6	0.104	0.358	0.323

the urogenital region than the oral route.

It may be noted that the study did not include the general population of premenopausal women who suffer from RVVC, and the results should be cautiously interpreted due to the small sample size and the demographic nature of the study participants. Of those women who completed the study, some graduated, some did not reenroll for the next quarter, or they withdrew because of continued vaginal symptoms. Those who had vaginal symptoms such as discharge or itching were in the control group receiving a placebo.

Reid et al.¹⁰ reported on a 6-month study with 41 premenopausal women, mean age 23 (\pm 4.4 years). The women entered the study through a university outpatient

clinic and 31 completed the treatment. All women received 3-day antimicrobial therapy followed by vaginal suppositories to determine recurrence of UTI. Failure to complete the program related to university exams, travel problems, illness, relocation, and clearance of vaginal symptoms. Shalev et al.¹¹ studied the incidence of recurrent bacterial vaginosis and candidal vaginitis in women who ingested either a pasteurized yogurt or a yogurt containing live *L acidophilus*. Twenty-eight of the 46 participants completed the first 4 months of the study.

Hilton et al.⁵ indicated that consumption of yogurt containing at least 10^8 cfu/mL of H_2O_2 -producing *L acidophilus* to be effective in reducing the incidence of RVVC. It is important to note that most

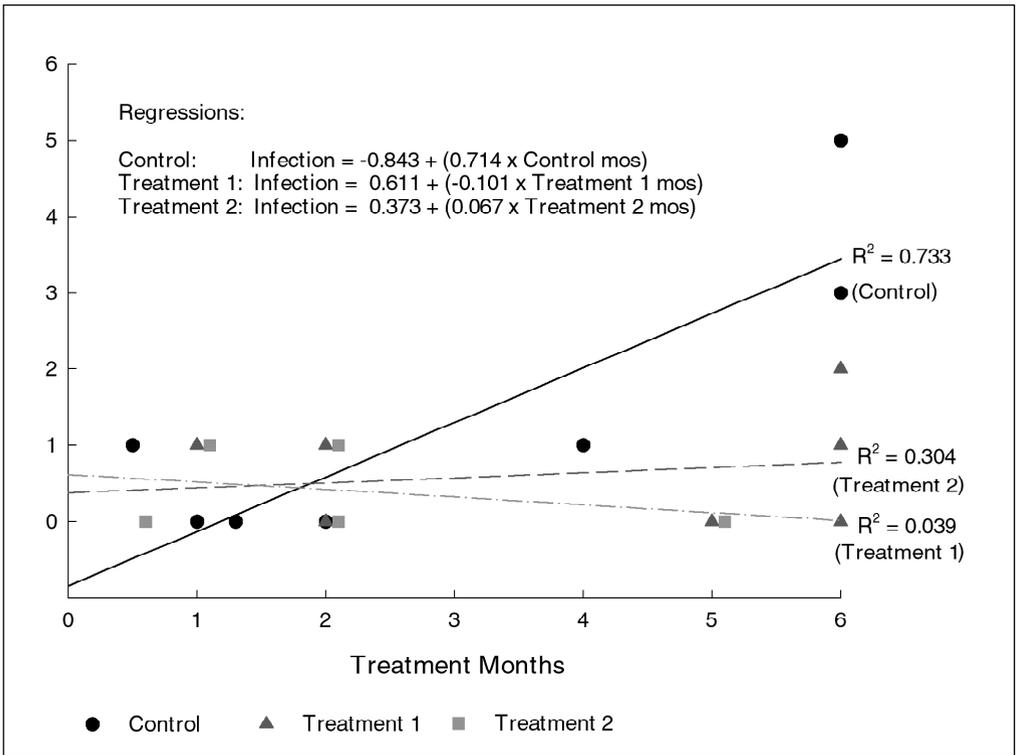


Figure 1. Total Candidal Infections by Month.

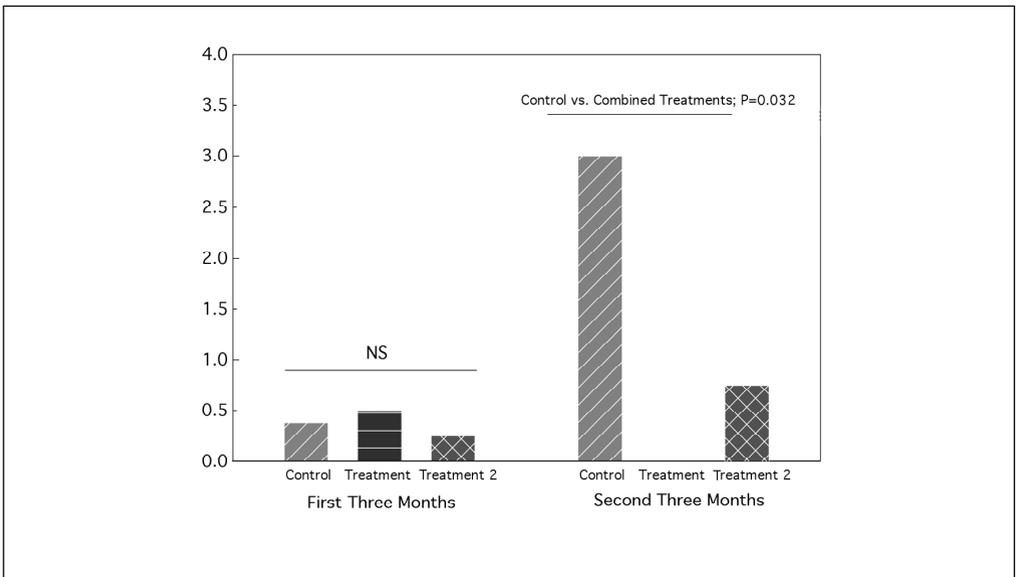


Figure 2. Average 3-Month Incidence of Infections by Study

Table 3. Significance Values for the ANOVA of Number of Infections, Asymptomatic Yeast, and Total of Infections plus Presumed Infections Regression Parameters During 6-Month Study Period

Number of Documented Infections	<i>P</i> -Value
Treatment differences (Intercepts)	0.062
Month effect different from zero	0.004
Month effect differences by treatment	0.001
Asymptomatic Yeast	
Treatment differences (Intercepts)	0.646
Month effect different from zero	0.790
Month effect differences by treatment	0.975
Documented Infections + Presumed (Total)	
Treatment differences (Intercepts)	0.460
Month effect different from zero	0.024
Month effect differences by treatment	0.013

commercial yogurts contain only unknown strains of *L bulgaricus* and *Streptococcus thermophilus*. Unlike the FDA, which does not specify culture concentration, the National Yogurt Association (NYA) requires at least 10^8 cfu/mL, but does not require members to guarantee potency through a printed expiration date. Neither the FDA nor the NYA require identification of microbial strains. None of the current regulatory statutes mandate that commercially available yogurts contain *L acidophilus* that produce H_2O_2 or, for that matter, even contain *L acidophilus* at all.

In another study, Hilton et al.⁸ reported that *L rhamnosus*, GG strain, vaginal suppositories self-administered twice-daily for 7 days reduced the symptoms associated with candidal vaginitis. The current study, which administered a lower dose of *L acidophilus*, NAS strain (H_2O_2 positive), has produced more effective results in preventing RVVC over a longer period of time.

A review by Famularo et al.¹² indicated that H_2O_2 -positive lactobacilli may not always yield a protective role for bacterial vaginitis, yet an alternate mechanism via arginine deaminase may decrease

polyamine levels in the vaginal microenvironment, thus improving clinical symptoms. Another study by Velraeds et al.¹³ reported that lactobacilli isolates produced biosurfactants that reduced the adherence of pathogenic microbes. A later study by Boris et al.¹⁴ suggested lactobacilli may be competitive with pathogenic microbes such as *C. albicans* not only because of self-aggregation and adhesion to epithelial cells, but also through coaggregation and receptor binder interference.

For lactobacilli to be effective as a prophylactic, production of H_2O_2 was considered a necessary characteristic. Antonio et al.² reported that vaginal colonization by H_2O_2 -producing lactobacilli was advantageous for maintenance of normal microflora and the prevention of sexually transmitted diseases.

While the cohort study by Hawes et al.⁹ found that H_2O_2 -producing vaginal lactobacilli do not protect against vulvovaginal candidiasis, Hillier et al.⁴ found that pregnant women vaginally colonized by H_2O_2 -positive lactobacilli were less likely to have genital colonization with pathogens and conditions such as symptomatic candidiasis.

Similarly, in one study, Reid reported that selected strains of probiotics may not only protect women from urogenital tract infection,¹⁵ and also found, in a separate study, that it may also assist in the normalization of urogenital microflora and thus reduce the risk of urinary tract infection (UTI) and bacterial vaginitis.¹⁶

CONCLUSION

The study, conducted among college women aged 19 to 40 years with RVVC provides additional clinical evidence that H₂O₂ positive strain of lactobacillus (*L acidophilus* NAS) may significantly decrease candidal infections. Even though the sample size is small and the demographic nature of the study participants unique, the results indicate the suppository and/or oral administration of these strains to premenopausal women who suffer from RVVC is beneficial. An evaluation of the study statistics on documented infections plus presumed infections reveals that group 1 differs from the control group ($P = 0.005$), as does group 2 from the controls ($P = 0.011$), but the two treatments groups do not differ significantly ($P = 0.81$). Relative to intention-to-treat, the number needed to treat (NNT) is $1 / (\text{proportion affected in controls} - \text{proportion affected on treatment})$. The NNT on Treatment group 1 (NAS) is 2.08, and NNT on group 2 (NAS and probiotic) is 13.33. The number needed to harm was not calculated since there were no adverse events. Self-report events such as an occasional brown discharge following the use of the vaginal insert was not considered clinically relevant by the attending staff. The study provides evidence on the safe use of selected strains of probiotics for a 6-month period to reduce the incidence of candidal infections.

It was not determined whether using the vaginal inserts less frequently would be equally effective or if using these or other selected strains of *L acidophilus* for shorter periods of time (2 to 4 weeks) would yield a prolonged or prophylactic effect.

ACKNOWLEDGMENTS

This study was supported by a grant from the American Academy of Family Physicians Foundation.

Probiotic strains were provided by Natren Inc, Westlake Village, CA.

Subir Ghosh, Professor of Statistics, University of California, Riverside, provided valuable comments on statistical analysis and interpretation.

REFERENCES

1. Nyirjesy P. Chronic vulvovaginal candidiasis. *Am Fam Physician*. 2001;63(4):697-702.
2. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. *J Infect Dis*. 1999;180(6):1950-1956.
3. Naidu AS, Clemens RA. Probiotics. In: Naidu AS, ed. *Natural food antimicrobial systems*. New York, NY: CRC Press; 2000:431-462.
4. Hillier SL, Krohn MA, Klebanoff SJ, Eschenbach DA. The relationship of hydrogen peroxide-producing lactobacilli to vaginosis and genital microflora in pregnant women. *Obstet Gynecol*. 1992;79(3):369-373.
5. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. *Ann Intern Med*. 1992;116(5):53-57.
6. Sobel JD. Recurrent vulvovaginal candidiasis. A prospective study of the efficacy of maintenance of ketoconazole therapy. *N Engl J Med*. 1986;315(23):1455-1458.
7. Franklin IM, Elias E, Hirsch C. Fluconazole-induced jaundice. *Lancet*. 1990;336(8714):565.
8. Hilton E, Rindos P, Isenberg HD. Lactobacillus GG vaginal suppositories and vaginitis. *J Clin Microbiol*. 1995;33(5):1433.
9. Hawes SE, Hillier SL, Benedetti J, et al. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. *J Infect Dis*. 1996;174(5):1058-63.
10. Reid G, Bruce AW, Taylor M. Influence of three-day antimicrobial therapy and lactobacillus vaginal suppositories on recurrence of urinary tract infections. *Clin Ther*. 1992;14(1):11-6.
11. Shalev E, Battino S, Weiner E, Colodner R, Keness Y. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. *Arch Fam Med*. 1996;5(10):593-6.

12. Famularo G, Pieluigi M, Coccia R, Mastroiacovo P, De Simone C. Microecology, bacterial vaginosis and probiotics: perspectives for bacteriotherapy. *Med Hypotheses*. 2001;56(4):421-30.
13. Velraeds MM, van der Mei HC, Reid G, Busscher HJ. Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. *Appl Environ Microbiol*. 1996; 62(6):1958-63.
14. Boris S, Suárez JE, Vázquez F, Barbés C. Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infect Immun*. 1998; 66(5):1985--9.
15. Reid G. Probiotic agents to protect the urogenital tract against infection. *Am J Clin Nutr*. 2001; 73(2 Suppl):437S--443S.
16. Reid G. Probiotics for urogenital health. *Nutr Clin Care*. 2002; 5(1):3--8.