

Can probiotic bacteria be used to return the normal vaginal flora?

I. Introduction

Bacteria vaginosis is one of the most common vaginal infections found in women of child-bearing age^{1,3}. Many women complain of a vaginal discharge and often have reoccurring symptoms with months of their initial diagnosis. *Gardnerella vaginalis* (Figure 1) may be responsible for causing BV and the classification of this bacterium may give some insight on its ability to dominate the vaginal system. Jennifer Patterson et al characterize BV by a loss of these protective lactobacilli, an increase in vaginal pH to > 4.5 , and the proliferation of a variety of anaerobic species.³ Lamont, RF et al suggest that BV correlates with the acquisition of HIV, and the changes in the vaginal microbiota may increase the susceptibility to other STIs, such as Herpes Simplex Virus 2, Gonorrhoea, *Trichomonas*

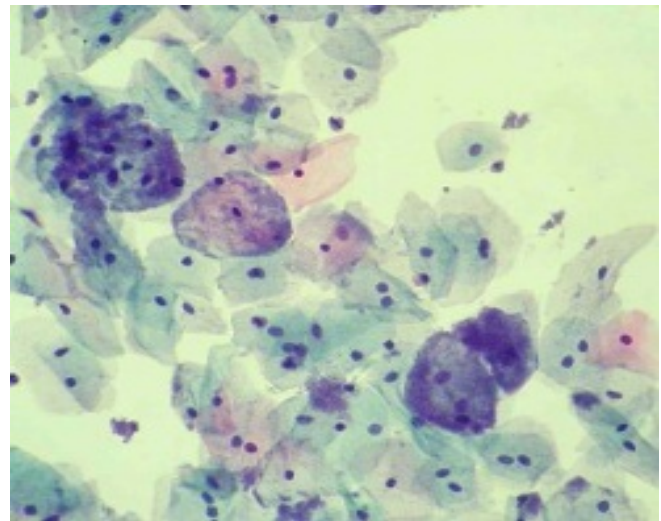


Figure 1: Gardnerella vaginalis CDC.gov

vaginalis, and *Chlamydia trachomatis*.¹ There is sufficient evidence that states women's health is affected by the composition and function of the microbial ecosystem⁴. Even though *G. vaginalis* has been primarily responsible for causing BV, there still may be a possibility that BV is polymicrobial condition caused by the collective effects of an altered microbial flora.³

Subjective evidence of heterogeneous mycobacteria that is correlated with *G. vaginalis* has initiated a quest to determine what microorganisms are involved in maintaining the vaginal ecosystem.⁶ A traditional process of Gram-staining vaginal secretions limited the ability to identify the true classification of the species that is responsible for vaginal health. Initially, the pH level of the vagina and the anti-pathogenic control was related to *Lactobacillus* bacterium,

primarily *Lactobacillus acidophilus*.⁵ Iara M. Linhares et al suggest that vaginal secretions was analyzed strictly on the basis of microscopic observation traditional Gram stain smears. Therefore, the vaginal infection was only seen responsible for a single organism.^{5,7} Tae Kyung Kim et al explain that recent studies have demonstrated that *Lactobacillus* is not the predominant bacterial genus within the vaginal tracts of healthy women.⁶ May A. D. Antonio et al used DNA homology test to evaluate 27 healthy women to confirm that *Lactobacillus gasseri*, *Lactobacillus jensenii*, and *Lactobacillus crispatus*, are the predominant vaginal *Lactobacillus* species colonizing asymptomatic women.⁵ These strains of *Lactobacillus* may play a critical role of protection that helps to maintain a highly acidic environment.

The combination of bacillus species may provide an acidic environment of the vagina is recognized as an important defense mechanism that is used to prevent colonization from pathogenic bacteria.⁸ This characterization allows them to maintain a highly acidic environment that is intolerable to most bacteria.⁹ In respect, these bacteria have become classified as Lactic Acid Bacteria mainly because they naturally convert sugars into lactic acid as an end-product of fermentation. Yueh-Ting Tsai explains that lactobacilli (LAB) are effective at enhancing immunity by setting up a line of defense against pathogen related infections.¹⁰ U.

Forsum et al explain that the primary ecological fundamentals of life (niche) of lactic acid bacteria are the mucous membranes of humans and animals and the vagina.¹¹ LAB may be used as probiotic therapy by providing a

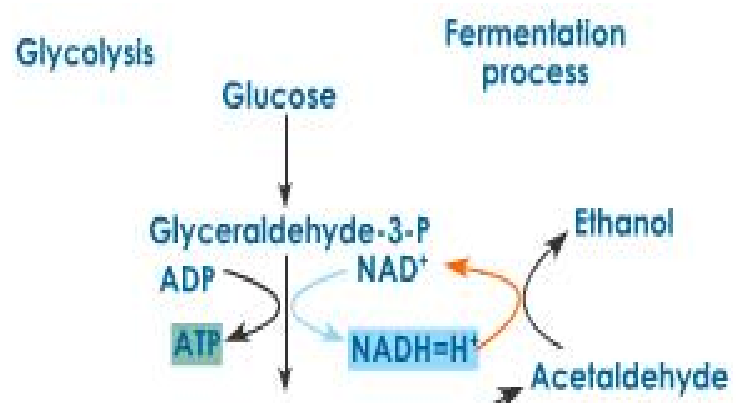


Figure 2: Tutorvista.com¹²

microbial disinfectant that can be used to destroy pathogenic bacteria. The use of certain LAB strains may be used to provide the host with mucosal immunity, which is the prevalent factor in BV-related cases symptomatic women.

Probiotic therapy may have the ability to naturally defend the vaginal ecosystem. The colonization of these species has been found in healthy women, and the summarizations of characteristic behavior identify their level of importance for maintaining a healthy vaginal flora. The purpose of the experiment described in this proposal is to test whether this is the case.

II. Experiment

The aim of this experiment is to determine if some LAB strains have the probiotic capabilities to might destroy Gardnerella and to determine the concentration of the various LAB strains that may be needed in order to reestablish a healthy vaginal flora. New characteristics for determining BV-related cases can use the Nugent system to help identify those who may be colonized with the pathogenic bacteria. Scientific advancements is DNA profiling kits allow the use of primers to get genomic sequences that can be used for DNA profiling. Subspecies can be classified by conducting a Quantataive PCR assay.

II.A. Introduction of Clinical Study, Nugent Score System, and DNA Profiling

A clinical trial will be used to initiate a study of women that are of child-bearing age as previously described and each person is required to provide two vaginal samples at every visit as previously descibed.¹³ One sample will be used for a Gram-stain. A Gram-positive stain will be used to stain the bacteria so they can be visualized by a microscope and the vaginal slides will be used to evaluate the vaginal secretions according to the Nugent scoring system.¹⁴ The second sample will be used to provide a sample of DNA.¹³ The DNA sample is mixed in a blender along with water and a salt solution. The solution is mixed for about 15 minutes, and the liquid is

filtered to gain the necessary type of DNA. Enzymes were added according to the DNA kit used in the experiment. Alcohol and buffer solution were added for the supernatant was profiled.¹³ Six strains were cultured on Columbian agar (supplemented 5% Defibrinated Horse Blood) and incubated in an anaerobic chamber for 24 hours at 35°C. The supernatant that was made was mixed with 400 µL of deionized water. DNA concentration was calculated using the NanoDrop (ND-1000).

II.B Quantative PCR

For this experiment, 6 species were used for the Quantative PCR test.¹³ Real-time polymerase chain reaction is A reaction may be performed using primers unique to each region to be amplified and tagged with different fluorescent dyes. Absolute quantification gives the exact number of target DNA molecules by comparison with DNA standards.¹⁵ The six primers that used are described in Figure 3. The primers were synthesized by Eurogentec, Seraing, Belgium.¹³ 25µL of PCR mixture (QuantiTect SYBR Green PCR) was added to all primers, except *Lactobacillus vaginalis*. The reactions were applified with the machine and programs as described¹³ Each sample was run twice and statistical data was gathered with each successful run.

Figure 3: Primers for Quantitative PCR

PCR	Reference	Primers	Target gene	Cycling conditions	Concentration
<i>L. species</i>	Zariffard MR [28]	F-LBF: 5'- ATGGAAGAACACCAGTGGCG-3' R- LBR: 5'- CAGCACTGAGAGGCGGAAAC-3'	16 S r RNA	15 min 95 °C, (15 sec 95 °C, 45 sec 50 °C, 45 sec 72 °C) x37	150 nM
<i>L. crispatus</i>	Byun R [29]	LcrisF: 5'- AGCGAGCGGAACTAACAGATTTAC-3' LcrisR : 5'- AGCTGATCATGCGATCTGCTT-3'	16 S r RNA	15 min, 95 °C, (15 sec 95 °C, 60 sec 60 °C, 20 sec 72 °C) x40	100 nM

Figure 3: Primers for Quantitative PCR

PCR	Reference	Primers	Target gene	Cycling conditions	Concentration
<i>L. gasseri</i>	Tamrakar R [30]	LgassF: 5'- AGCGAGCTTGCCTAGATGAATTTG-3' LgassR: 5'- TCTTTTAAACTCTAGACATGCGTC-3'	16 S r RNA	15 min 95 °C, (15 sec 95 °C, 60 sec 57 °C, 60 sec 65 °C) x40	200 nM
<i>L. iners</i>	De Backer E [31]	InersFw: 5'- GTCTGCCTTGAAGATCGG-3' InersRev: 5'- ACAGTTGATAGGCATCATC-3'	16 S r RNA	15 min 95 °C, (15 sec 95 °C, 55 sec 60 °C, 60 sec 65 °C) x35	200 nM
<i>L. jensenii</i>	Tamrakar R [30]	LjensF: 5'- AAGTCGAGCGAGCTTGCCTATAGA-3' LjensR: 5'- CTTCTTTCATGCGAAAAGTAGC-3'	16 S r RNA	15 min 95 °C, (15 sec 95 °C, 55 sec 60 °C, 60 sec 72 °C) x40	300 nM
<i>L. vaginalis</i>	In-house designed primers	LV16s_23s_F: 5'- GCCTAACCATTTGGAGGG-3' LV16s_23s_R3: 5'- CGATGTGTAGGTTTCCG-3'	16 S- 23 S r RNA	15 min 95 °C, (15 sec 95 °C, 30 sec 56 °C, 30 sec 72 °C)x37	200 nM
<i>G. vaginalis</i>	Zariffard MR [28]	F-GV1: 5'- TTACTGGTGTATCACTGTAAGG-3' R-GV3: 5'- CCGTCACAGGCTGAACAGT-3'	16 S r RNA	15 min 95 °C, (45 sec 95 °C, 45 sec 55 °C, 45 sec 72 °C) x50	260 nM
<i>A. vaginae</i>	De Backer E [31]	ATOVAGRT3Fw: 5'- GGTGAAGCAGTGGAAACACT-3' ATOVAGRT3Rev: 5'- ATTCGCTTCTGCTCGCGCA-3'	16 S r RNA	15 min 95 °C, (20 sec 95 °C, 45 sec 60 °C, 45 sec 72 °C) x45	300 nM

The data collected will represent the baseline characteristics using range and proportion of the total volumes for DNA and total volumes of the entire sample. We can profile the species based on the baseline data collected from women who participated in the clinical trial. The Quantative results obtains will express data related to copies/5 μ L

III. Discussion

If all goes well, the baseline ratios from healthy women will be used to determine the evolution of the corresponding subspecies. According to K. Makarova et al, phylogenetic analyses can be used to compare related gene content between a set of species by studying the evolution process that occurs through the horizontal gene transfer. The log base analysis can be graphed with copies/ μ L to help determine the originating species.

The main problem that might occur in the experiment is primarily defined in the clinical study. There may be a possibility that were may lose participants based on participation and possible conception during the clinical study. Full corporation and honesty is required to gather accurate results. .

I feel that this study should confirm that there are other predominate species that control the vaginal system, very similar to this experiment. If these species are found predominant, it would be highly beneficial to gather the DNA sequence for further insight on gene function. Some species have been identified with having a stable genome structure that provides the organism integrity and basic immunities that promote survival. The fact that the species are naturally found in the vaginal environment greets us with a good starting point, but further taxonomy classifications could lead us to determine what makes this *G. vaginalis* virtually unstoppable. In the end, Quantative PCR may be a value tool to accurately determine the

presence of probiotic bacteria and by confirming the presence of the bacteria; vaginal probiotics may become a basis for pathogenic infections.

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