Glycogen availability and pH variation influence the growth of vaginal Lactobacillus species and Gardnerella vaginalis

Stephany Navarro¹, Habib Abla², Betsaida Delgado³, Jane A. Colmer-Hamood¹,⁴, Gary Ventolinį⁵, Abdul N. Hamood¹

¹Dept. of Immunology & Molecular Microbiology, TTUHSC, Lubbock; ²School of Medicine, TTUHSC, Lubbock; ³Honors College, TTU, Lubbock; ⁴Dept. of Medical Education, TTUHSC, Lubbock; ⁵School of Medicine at the Permian Basin, TTUHSC, Odessa

Abstract

Bacterial vaginosis (BV) is the most common vaginal infection in women of reproductive age. Previous studies showed that in women diagnosed with BV, a shift in the population of Lactobacillus and Gardnerella species occurs. The exact mechanism for this phenomenon is not clearly defined. Gardnerella vaginalis (GV) exists within the vaginal microflora of healthy women, but there is awareness that it is not as healthy as Lactobacillus. Prior to the onset of BV, either host or bacterial factors, or both, increase the vaginal pH to 4.5. We hypothesize that environmental changes within the vagina, specifically the availability of nutrients and pH variation, influence the microbial population. The vaginal pH of healthy women is 3.5-4.2 favoring the growth of lactobacilli while BV women with BV > 4.5 or above, favoring the growth of GV. Metabolism of free glycogen within the vaginal fluid is likely to be essential for the colonization and growth of vaginal microflora. In this study, we used the medium simulating vaginal fluid (MSVF) to assess the growth of the Lactobacillus strains L. jensenii, L. gasseri, and L. crispatus as well as GV in the presence of different glycogen concentrations and at pH 4.4, 4.5, and 5. MSVF contains glucose and glycogen as carbon sources. Only L. gasseri grew in the absence of glycogen, while only L. jensenii survived at all pH conditions. L. crispatus was the most restricted, surviving only at pH 5 in 5 and 10 g/L glycogen. Of the lactobacilli, L. jensenii was the most versatile. GV showed the highest growth (3 logs over starting CFU). These results suggest that the presence of glycogen is of significant importance to the growth of different Lactobacillus spp. as well as for GV, and 2) vaginal pH influences the ability of the strains to utilize glycogen.

Introduction

Bacterial vaginosis (BV) is the most common vaginal infection in women of reproductive age (14-49 years of age). In the U.S., about 21 million women in this range suffer from BV (30%). Of those women, it is estimated that 84% will remain asymptomatic while others exhibit symptoms, including thin grayish vaginal discharge, foul-smelling fishy odor, vaginal itching, and burning sensation during urination. BV is also associated with various health complications such as an increased risk of premature delivery, miscarriage, pelvic inflammatory disease, and increased susceptibility to sexually transmitted diseases. In healthy women, the vaginal microflora is primarily composed of Lactobacillus species (dominant flora). BV is accompanied by a shift in the vaginal microbial population; a decrease in Lactobacillus and an increase in anaerobic bacteria, specifically G. vaginalis (GV). This shift in the bacterial population is associated with an increase in pH of the vaginal environment to 4.5 or higher.

Despite the numerous studies, the exact mechanism through which the shift in the vaginal microflora occurs during BV is not defined. GV exists within the vaginal microflora of healthy women, but their numbers are extremely low compared with that of Lactobacillus. Prior to the onset of BV, either host or bacterial factors, or both, increase the vaginal pH to 4.5 or above. This pH change significantly increases the GV population while reducing the population of Lactobacillus. To address the role of pH in the population shift it is essential to grow both lactobacilli and G. vaginalis in one medium and assess the effect of a pH change on their growth. For diagnostic and research purposes. Lactobacillus spp. are grown in de Man, Rogosa and Sharpe (MRS) medium while GV is grown in New York City (NYC) medium. Both organisms are grown at 37°C under 5% CO2. We recently showed that the previously described medium simulating vaginal fluid (MSVF) supports the growth of lactobacilli and GV. MSVF contains many of the components of vaginal fluid, including glycogen, mucin, albumin, acid, and lactic acid.

Results

Glycogen is essential for the growth of GV under starting pH conditions of 4.5 or 5.0

At a glycogen concentration of 10 g/L, L. jenseni maintained growth in MSVF at all three pH conditions.

Conclusions

> Vaginal pH influences the ability of lactobacilli and GV to grow in the presence of variable glycogen concentrations.
> Under higher pH conditions (5.0), GV competes with lactobacilli in utilizing available glycogen.
> Under normal glycogen levels, L. jenseni is an essential component of healthy vaginal microflora at pH 4, a condition unfavorable for the growth of GV, L. crispatus, and L. gasseri.
> L. gasseri utilizes carbon sources, other than glycogen, in the MSVF at pH 5.