Bacterial vaginosis (BV) is the most common vaginal infection in women of reproductive age. While the vaginal microbiota of health women is dominated by lactobacilli, it shifts to favor other bacteria, specifically Gardnerella vaginalis (Gv), in women with BV. Lactobacilli produce antimicrobial factors including hydrogen peroxide, lactic acid, and bacteriocins. We hypothesize that these factors, lactobacilli, and Gv influence each other's growth within the vaginal environment through additional unidentified factors. To assess the interaction of Gv with Lactobacillus jensenii (Lj), one of the vaginal lactobacilli, we grew the organisms in transwell co-culture in medium simulating vaginal fluid. Lj significantly reduced Gv growth at 20 h post inoculation (hpi) and eliminated Gv at 24 hpi. Cell-free culture supernatant (CFS) of Lj harvested at 16 h did not affect Gv growth while CFS harvested at 24 hpi eliminated Gv growth entirely. Growth of Lj was unaffected by either 16h- or 24h-CFS. Additionally, 24h-CFS did not affect growth of other vaginal lactobacilli (L. gasseri and L. crispatus), Enterococcus faecalis, Staphylococcus epidermidis, or Escherichia coli. Co-culture experiments using 24h-CFS showed that elimination of Gv began at 2 h and was complete by 4 h. To rule out hydroxide peroxide and D-lactic acid as the responsible agents, we tested concentrations double that produced by Lj, neither inhibited Gv growth. Fractionation of 24h-CFS using 100-300, 30-10, and 5-2 kDa molecular weight cutoff columns revealed that the fraction containing ≤ 30-kDa proteins retained the inhibitory effect while the fraction containing less than ≤ 10-kDa proteins had no effect. These results suggest that: 1) a novel 10- to 30-kDa Lj secreted protein eliminates Gv and 2) the effect of this factor is unique to Gv.

In healthy women, the vaginal ecosystem is dominated by the Lactobacillus spp. L. crispatus, L. gasseri, L. iners, and L. jensenii. Through their production of antimicrobial compounds, including lactic acid, hydrogen peroxide, and bacteriocins, lactobacilli form a critical line of defense against potential pathogens. Lactobacilli provide additional protection through coaggregation, competitive exclusion, and immune-modulation. Disruption of the population of lactobacilli along with an increase in vaginal pH to 4.5 or higher allows overgrowth of pathogenic microorganisms. Specifically, BV is associated with a decrease in the numbers of lactobacilli and an increase in anaerobic bacteria, especially Gardnerella vaginalis (Gv), the most common microorganism identified from the vaginal samples of women with BV. Gv harbors a variety of virulence factors including sialidase and vaginolysin.

The rod-shaped, anaerobic gram-positive L. jensenii (Lj) is the second most common species of lactobacilli indigenous to the vaginal tract of healthy women. Lj accounts for 23% of all vaginal colonies, following L. crispatus at 32%. Research has shown that Lj also inhibits Neisseria gonorrhoeae adherence to and invasion of epithelial cells. Additional studies suggested that Lj contain at least two constitutively produced inhibitory proteins that target N. gonorrhoeae. Considering the complexity of the microbial interaction within the vaginal environment, Lj may target pathogenic bacteria such as Gv through additional yet to be identified mechanisms. While previous in vitro and clinical studies investigated the complex interaction between lactobacilli and Gv, a detailed and an accurate assessment of such interaction requires growth medium that closely mimics the conditions within the vagina and supports the growth of both lactobacilli and Gv. One such medium is the medium simulating vaginal fluid (MSVF) that contains many of the components of vaginal fluid, including glycosgen, mucin, albumin, acetic acid, and lactic acid.

In this study, we examined the possibility that upon growth in MSVF, Lj produces secreted factor(s) that inhibits or eliminates the growth of Gv.

Hypothesis

In addition to H₂O₂ and lactic acid, lactobacilli and Gv influence each other's growth within the vaginal environment through unidentified factors.

Funding

This project was funded in part by the Department of Obstetrics and Gynecology, TTUHSC-Permian Basin, Odessa, TX.