Growth Characteristics of *Lactobacillus iners* that Promote Persistence in Bacterial Vaginosis

Margot Oliver, Emily Diekema, & Amy Wilstermann, Ph.D.

**Abstract**

Bacterial Vaginosis (BV) is the most common vaginal infection presented in medical practice. It is associated with a variety of negative effects regarding reproduction, including premature rupture of fetal membranes (PROM). Our research focuses on the shift between healthy and BV conditions. This shift is characterized by an increase in pH and a loss of the typically dominant *Lactobacillus*, with the exception of *L. iners*. Growth characteristics of the four most common *Lactobacillus* species, *L. gasseri*, *L. jensenii*, *L. crispatus*, and *L. iners*, were analyzed. Factors tested included environmental pH and interaction with the BV-associated bacterium *Gardnerella vaginalis*. The growth of *L. iners* was inhibited by the low pH associated with healthy environments but not by the presence of *G. vaginalis*. The inverse was found to be true for *L. gasseri* and *L. jensenii*. *L. crispatus* was found to be inhibited by both a low pH and factors produced by *G. vaginalis*.

**Introduction**

Healthy vaginal environments are often dominated by one or two species from the gram-positive genus *Lactobacillus* through the production of lactic acid, hydrogen peroxide, and bacteriocins (proteinaceous toxins). In BV conditions, most *Lactobacilli* are replaced with rod-type, gram-variable anaerobes and vaginal pH increases. *L. iners*, however, remains in large numbers. *L. iners* has been shown to produce less lactic acid and hydrogen peroxide than other common vaginal *Lactobacilli*—factors which help explain its inability to protect against BV bacteria despite its persistence. The ability of *L. iners* to persist in BV has been the subject of several studies; it was recently demonstrated that the binding mechanism used by *L. iners* allows stronger adhesion to vaginal mucosa than mechanisms used by other vaginal *Lactobacilli* at pH conditions consistent with BV. (McMillan, Reprod Sci, 2012) This study attempts to uncover additional differences between *L. iners* and other vaginal *Lactobacillus* species that may contribute to its unique ability to persist in BV communities. Specifically, this study explores growth rates under various pH conditions, susceptibility to factors secreted by BV organisms, and competitiveness in a mixed culture containing *G. vaginalis*, the most prevalent BV bacterium.

**Results**

**Analysis of Bacterial Growth:** *L. iners* and *L. crispatus* have significantly longer generation times than the other *Lactobacilli* studied when grown within a normal vaginal pH range (Fig. 1). As shown in the figure inset, growth rates of *L. iners* and *L. crispatus* increase dramatically as pH is shifted from normal (pH<4.5) to BV (pH>4.5) conditions. The generation time of *G. vaginalis* was less than that of *L. iners* and *L. crispatus*, but greater than that of *L. jensenii* and *L. gasseri* at pH<4.5 (Fig. 1).

**Supernatant Inhibition:** Neither the generation time nor the lag time of *L. iners* is inhibited by factors secreted by *G. vaginalis* (Figure not shown) as no significant differences are observed between the samples grown in *L. iners* supernatant (control) and *G. vaginalis* supernatant. The generation time of *L. jensenii* was increased with exposure to increasing volumes of *G. vaginalis* supernatant. In addition, the period before log phase of growth of the *Lactobacillus* species *crispatus, gasseri* was increased due to exposure to supernatant collected from a *G. vaginalis* culture.

**Methods**

**Bacterial Species:** Bacteria selected for testing were *Gardnerella vaginalis* and four common vaginal *Lactobacillus* species: *L. gasseri, L. jensenii, L. crispatus*, and *L. iners*. All bacteria were grown in NYIII media under anaerobic conditions.

**Analysis of Bacterial Growth:** Bacteria were grown in NY III media, pH 4.0–7.0. Absorbance at 600nm was measured for 8 hours using a microplate reader.

**Supernatant Inhibition:** Preparation of Supernatant from *G. vaginalis* and all *Lactobacillus* species cultures: cultures in were grown to late log phase and supernatant was removed.

**Inhibition Assay:** Each *Lactobacillus* species was grown in pH 5.51 NY III media and prepared *G. vaginalis* supernatant in 1:3, 1:1, and 1:5 ratios. Control wells contained supernatant obtained from the same *Lactobacillus* species.

**Head-to-Head Competition:** Preparation of Bacteria: Co-cultures at pH 4.27 or 5.51 were created by combining *Lactobacillus* culture with *G. vaginalis* culture. Pure culture was grown in parallel with the co-cultures.

**Experimental Procedure:** Immediately after preparation of co-cultures (t=0), both pure and co-cultures were plated. Plates containing 2 ug/ml of metronidazole were used to inhibit *G. vaginalis* growth. Bacteria were plated when the *G. vaginalis* pure culture reached an O.D. of 0.6 (t=1), and again one time interval later (approximately 4 hours).

**Head-to-Head Assay:** Supernatant suppression of Lactobacillus co-cultures was evaluated at each pH condition (4.27 and 5.51). Culture supernatants from *L. jensenii* at pH 5.51 were added to the respective cultures to see the alterations in competitive advantage. Inhibition was observed in the following results: *L. jensenii* at pH 5.51 showed no significant inhibition, whereas *L. gasseri* and *G. vaginalis* showed significant inhibition at both pH conditions.

**Conclusion:** *L. jensenii* and *L. gasseri* have a growth advantage in the healthy pH range (<4.5).

**Growth rates of *L. iners* and *L. crispatus* increase as environmental pH shifts from healthy to BV conditions.

**G. vaginalis* has the ability to directly inhibit the growth of several *Lactobacillus* species (*L. gasseri, L. crispatus, L. jensenii*)

**In mixed culture, *L. iners* is able to become dominant under BV conditions, but does not inhibit *G. vaginalis* at healthy conditions while the inverse is true for *L. jensenii* and *L. gasseri*.

The pH-dependent growth characteristics of *L. iners* may contribute to its persistence in BV communities, which is not observed among other common vaginal *Lactobacilli*. Clearly, however, this factor alone cannot account for *L. iners* persistence since *L. crispatus* shares the same growth characteristics and is not found in BV communities.

Growth inhibition of *Lactobacillus* species could account for the inability of probiotic *Lactobacillus* to successfully recolonize a reproductive tract that is inhabited by *G. vaginalis*.

Mixed culture results are consistent with microbial community composition observed in samples collected from women with BV.

**Remaining Questions:**

Is *L. iners* more resistant than other *Lactobacillus* to the competitive antagonists *G. vaginalis* produces? What is the role of the pH change in the shift from healthy to BV conditions?

**References**