Margot Oliver 2012 Summer Research Summary

**Project:** Professor Wilstermann’s project “Investigating the Dynamics of Bacterial Communities in the Female Reproductive Tract”

Bacterial vaginosis, or BV, is the most common vaginal infection presented in medical practice among women of childbearing age. The current treatments for this infection often provide only temporary relief from BV bacteria. BV is associated with a variety of negative effects regarding reproduction and pregnancy including premature rupture of fetal membranes (PROM) and a higher risk of contracting STIs. Most healthy vaginal environments are dominated by one or two species from the gram-positive genus *Lactobacillus*. *Lactobacilli* are thought to play a protective role against infection by producing lactic acid, hydrogen peroxide, and bacteriocins (proteinaceous toxins). Due to the lactic acid production, a healthy vaginal environment has an acidity around pH 4. BV is characterized by a loss of *Lactobacilli* along with a decrease in acidity (pH >4.5). The *Lactobacilli* are replaced with rod-type, gram-variable anaerobes, with the most prevalent of these being *Gardnerella vaginalis*. Our research is especially focused on this pH and microbial shift between healthy and BV conditions. By better understanding these dynamics, we hope to gain insights for more effective treatments of BV.

We used *Gardnerella vaginalis* in our experiments, along with some of the most common *Lactobacillus* species found in the vaginal tract: *L. gasseri*, *L. jensonii*, *L. crispatus*, and *L. iners*. This summer, we grew all of these bacteria in broth media at various levels of acidity, in a pH range from 4 to 7. While all the bacteria grew similarly well at the higher pH levels, *G. vaginalis*, *L. crispatus*, and *L. iners* all had slower growth at the pH levels of a healthy vaginal environment, pH 4-4.5. While there is a large loss of most of these *Lactobacillus* species in the transition between healthy microbial environments to BV environments, *Lactobacillus iners* tends to remain in large numbers. These results may explain why *G. vaginalis* and *L. iners* thrive in the higher pH levels of BV conditions, but raises the question of how *L. crispatus* and *L. iners* can dominate the microflora of healthy conditions.

The question of the difference between *L. iners* and the other common *Lactobacillus* species is an important question in the field of BV research. One advantage of *L. iners* that could explain its persistence in BV is that it has better adhesion to epithelial cells. Additionally, *L. iners* has been shown to produce less lactic acid and less hydrogen peroxide than the other dominant *Lactobacilli* – factors that could explain its inability to fully protect against infection on its own. This summer we are also performing experiments to look at the differences in Lactobacilli with regards to their reaction to any bacteriocins produced by *Gardnerella vaginalis*, by growing them in broth media mixed with *G. vaginalis* supernatant. In another experiment, we are comparing the ability of the different Lactobacillus species to compete with *G. vaginalis* for broth media nutrients at healthy (pH 4) and BV (pH 5.5) conditions. This involves growing equal densities of *G. vaginalis* and a Lactobacillus species in the same broth media culture. At a couple of time points, we plate all of the bacteria at the appropriate dilutions and compare the numbers of colony forming units with those from the tubes with only one species growing by itself. We used replicate plates infused with the antibiotic Metronidazole (a current BV treatment) to distinguish between the *G. vaginalis* and Lactobacillus species, since Lactobacilli are resistant to that particular antibiotic.
This has been my second summer doing research at Calvin, in addition to some work during January term, and I am extremely thankful for the experience. Through it I’ve gained an appreciation for the process and problem solving of research and discovery in the scientific field. I’m certain that the critical thinking processes I’ve developed through this project will aid me in my future learning at medical school. Once I better understood the project’s questions at the end of my first summer, my curiosity took hold and I came back twice to keep searching for the answers. Even as I move away from Calvin at the end of this year, I’m sure I’ll keep track of all developments in the BV research field.