**Introduction**

Lactobacillus iners is a bacteria found in the vaginal microbiome. This bacteria differs from the other vaginal-dominating Lactobacilli in that is able to exist in both healthy vaginal ecosystems as well as those which exhibit symptoms of bacterial vaginosis. The vaginal communities of women who have bacterial vaginosis are comprised of gram-negative and anaerobic bacteria as opposed to the Lactobacilli-dominated communities of healthy women. Lactobacillus iners is able to survive in these drastically different conditions by expressing different genes in each environment. Macklaim et al found that one of the genes upregulated 6-fold by L. iners in women with bacterial vaginosis is a cholesterol-dependent cytolysin (1). Another recent study by ? determined that the protein family ReIEB, a toxin-antitoxin system, is found in Lactobacillus iners but not in any of the other Lactobacilli found in the vaginal microbiome (2).

The ReIEB toxin-antitoxin genes are found next to each other in the genome and expressed simultaneously. The two proteins bind together, and the antitoxin prevents the toxin from negatively impacting the cell. When a cell is under duress, the toxin-antitoxin complex degrades and the toxin acts against the cell. The ReIE toxin causes cells to enter a persistent state by cleaving the mRNA on ribosomes, and the cell is able to conserve energy and survive otherwise adverse conditions.

Keren et al explored this persister cell state induced by ReIE in Escherichia coli. They expressed the toxin in Escherichia coli cells that were in mid-exponential phase. After the exponential phase was finished, they introduced the antibiotics cefotaxime, ofloxacin, mitomycin C, and tobramycin to the cells and spotted them on an agar plate which contained IPTG; this initiated translation of the Re1B antitoxin. The persister cell colonies were counted. Persister cells survived in quantities 10 to 10,000 times higher when ReIE was expressed before exposing the cells to the antibiotics cefotaxime, ofloxacin, and tobramycin. While the experiment I am proposing is not going to be looking at antibiotics, these results are pertinent because they show that, in times of stress, Lactobacillus iners is able to enter into a state that allows it to survive. The goal of this experiment is to see if the ReIE toxin component of the toxin-antitoxin complex in Lactobacillus iners plays a part in the ability of this bacteria to survive in women with bacterial vaginosis.

 **Experiment**

To determine the effect of ReIBE on L. iners, I will be creating an artificial vaginal microbiome using bacteria found in healthy women and women affected by bacterial vaginosis. There will be three experimental groups: one with the toxin-antitoxin gene knocked out completely, one with a toxin and antitoxin expression vector, and one wild-type. Expression of the toxin will be induced, and after growth has stopped the relative abundance of each bacteria in the microbiome will be determined using 16s rRNA analysis.

1. Making the artificial microbiome

 Eight bacteria associated with bacterial vaginosis will be cultured and added to vaginal epithelial cells in equal concentrations (along with L. iners). These bacteria are G. vaginalis, A. vaginae, M. mulieris, P. bivia, Veillonella sp., Peptostreptococcus sp., Peptoniphilus sp., and F. nucleatum (6). The epithelial cultures will be incubated at 37 degrees Celsius for 48 hours to allow the bacteria to attach to the cells. After the incubation process, the cultures will be centrifuged for 2 minutes to increase attachment and then washed with PBS to remove bacterial cells that didn’t attach.

B. Creating Lactobacillus iners strains

 Toxin-antitoxin knockout

 Toxin-antitoxin expression vector

 (Am having a hard time with figuring out exactly how to do this in Lactobacillus iners - am going

 To meet with my mentor this week to discuss it)

Rapid induction and reversal of a bacteriostatic condition by controlled expression of toxins and antitoxins.

C. Inducing expression of toxin

Arabinose is added to the epithelial cells which will induce expression of the toxin. After three hours, the relative concentrations of bacterial cells will be determined using 16s rRNA analysis.

D. 16s rRNA analysis

Using PCR, the V1-V3 hypervariable regions of the 16s rRNA genes of the bacteria found in the epithelial cultures will be amplified. PCR products will then be sequenced and classified.

(Need to learn more about all the experimental techniques so I can speak about them intelligently)

**Discussion**

The hope is that expression of ReIE toxin leads to increased abundance of Lactobacillus iners relative to the strain that had the toxin gene removed. If this is the case, I will be able to conclude that the toxin-antitoxin complex plays a role in the increased survivability of Lactobacillus iners in women with bacterial vaginosis. It is possible that the toxin-antitoxin complex as a whole has a detrimental effect on the survival of Lactobacillus iners. That would mean that, when this complex is expressed, it degrades in the conditions associated with bacterial vaginosis. Another prospect is that the toxin-antitoxin or any combination thereof will have no effect on the survivability. This would be surprising considering this protein family is not found in any other Lactobacilli.

Antibiotics are not a good choice for treating bacterial vaginosis. While they are effective in killing off the bacteria causing harm, they also kill off all of the good bacteria, leaving the vagina susceptible to recolonization of the harmful bacteria, especially if these noxious bacteria have toxin-antitoxin complexes inducing persistent states. If the toxin portion of the complex does lead to higher numbers of surviving Lactobacillus iners, these genes could be incorporated into the genomes of other Lactobacilli that aren’t usually able to survive in women with bacterial vaginosis. If these altered Lactobacilli were able to survive and somehow overcome these conditions, their populations may be able to rebound and outcompete the other bacteria causing harm to the host.. The vaginal microbiome’s diversity is still being catalogued and understood. When it comes to bacterial vaginosis, better treatment options are needed that allow the beneficial bacteria to persist. The toxin-antitoxin complex may be a good place to start.

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