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UROP Final Report  
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UROP Final Report: Investigating Penicillin Binding Proteins in *Borrelia burgdorferi*

*Borrelia burgdorferi* is a spirochete bacteria found in North America and Europe, and it is the primary cause of Lyme disease. It is a unique bacterial species because it is neither Gram-positive nor Gram-negative; instead, like other members of the phylum, it is a diderm (double membrane) species. *B. burgdorferi* is spread by ticks of the *Ixodes* genus—specifically *Ixodes scapularis* in the Midwest and northeastern regions of the United States. Not surprisingly, Lyme disease is much more common in these regions than the rest of the country.

Lyme disease is treated orally with doxycycline if caught early (except in young children and pregnant/breastfeeding women—the alternative is amoxicillin or azithromycin). If the disease is caught later in its progression, patients are often treated intravenously with ceftriaxone. All of the antibiotics used to treat Lyme disease are part of the β-lactam family of antibiotics that work to inhibit peptidoglycan synthesis in the bacterial cell walls by binding to penicillin binding proteins.

Penicillin binding proteins (PBPs) are found in many bacteria and are characterized by their affinity for and binding of penicillin and other β-lactam antibiotics. Most bacteria have several PBPs, and these proteins can be membrane-bound or cytoplasmic. PBPs are involved in the final steps of peptidoglycan synthesis. Peptidoglycan is a major component of bacterial cell walls, so the action of PBPs is absolutely essential for the survival and proliferation of bacteria. If the action of PBPs is inhibited, the structure of the cell wall suffers and the cell often undergoes lysis.

In most cases, the β-lactams used to treat Lyme disease are able to eradicate the infection. However, some patients develop a condition known as Post-Treatment Lyme Disease Syndrome in which they experience lasting symptoms of the disease that persist for years after infection. These patients often suffer from fatigue, joint and muscle pain, and neurocognitive symptoms. The cause of this is unknown, but some theories exist as to why this occurs. Normally, *Borrelia* exist as spirochete bacteria. However, when exposed to environmental stressors (such as antibiotics), they enter a dormant cyst form. It is hypothesized that, upon treatment, some of the bacteria enter this form and lie dormant until the treatment concludes. At this point, the bacteria can become active again. The other theory is that the bacteria are simply becoming resistant to the antibiotics used for treatment.

The main focus of my project was to investigate the PBPs in *Borrelia burgdorferi*. Very little is known about the PBPs in this bacteria, and I wanted to find out how many there are, their molecular weights, and their binding affinities to various β-lactams. In order to do this, I cultured *Borrelia burgdorferi*, isolated the cell membrane (where the PBPs are) via ultracentrifugation,
and ran a Western blot to discern the number of PBPs and their approximate molecular weights. As of the writing of this paper, I have not been able to successfully get distinct protein bands on the blot. However, I performed a crude dot blot in order to ensure that protein is present, and my results indicated that there is protein and the antibodies for the blot are working appropriately. I suspect the problem is that I need more concentrated samples. In order to do this, I will culture more *Borrelia*.

Once the assay is working consistently, I plan to continue my work by determining the affinity of various \(\beta\)-lactams by running competition assays with a fluorescently labeled penicillin derivative that can be detected via Western blot. The bacteria will be first treated with the antibiotic before being treated with the labeled penicillin. By quantifying the amount of the penicillin derivative that bound to the PBPs (for it will occupy any PBPs left open after treatment with the antibiotic), I can ascertain which antibiotics bind more strongly. This could provide more information on which antibiotics are most effective against these bacteria. If I have the time, I would also like to run these competition assays with other species of *Borrelia* known to cause Lyme disease in other parts of the world. Combined with bioinformatics, we can compare the species to understand more about the organism’s evolution and the disease presentation.

I had a lot of problems getting the assay to work effectively over the past several months; I had a UROP in the spring, and I ended up getting an extension because I needed to change my project idea to be able to complete it in an appropriate amount of time. However, I feel I put in a solid effort and spent plenty of time in the lab. In order to have enough to present for the UROP showcase, I combined my work with that of a colleague’s. The projects complement each other well, and we plan to work more on our individual ventures before attempting a publication. When that time comes, we will be combining our work with others from the Lyme disease research team in the medical school.

Because my research advisor was able to get a grant to fund more research and outreach, I will be working for a few months after I graduate in December. I will be continuing the work I am doing and hopefully getting solid results. In addition to this, I will be a part of a team doing outreach in the community. Our goal is to raise awareness of Lyme disease as well as the research we are doing to try and aid in the treatment of it. We will be speaking primarily at schools, but we are also considering other ventures.

Overall, my experience with UROP was very positive. Because I was already a member of the lab prior to applying for funding through UROP, I could have gotten research experience without the program. However, I do not think I would have achieved nearly as much or been able to be compensated for my work. The funding that UROP gave certainly helped get this project off the ground, and I believe it will lead to new findings in the field of Lyme disease research. Additionally, I suspect I will be able to be a part of a publication in a scientific journal when combining my work with the other great research going on within our team. I could not have made this progress without this program.