Investigating penicillin binding proteins in *Borrelia burgdorferi:* searching for answers in the treatment of Lyme disease

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Introduction/Background

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.

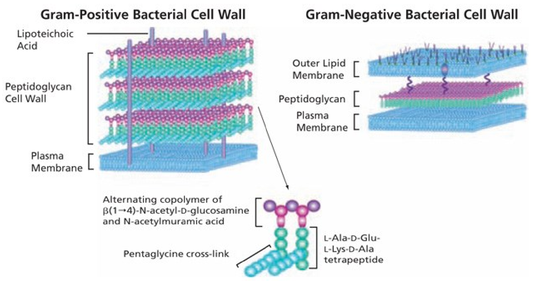

Figure 1. Structures of Gram-positive and Gram-negative bacterial cell walls. Figure courtesy of Sigma-Aldrich.

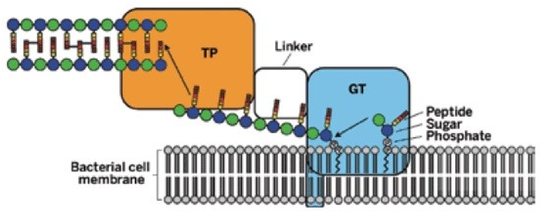

Figure 2. The action of the glycosyltransferase (GT) domain of PBPs in the construction of peptidoglycan component of bacterial cell walls. The GT domain polymerizes peptidyl lipid sugars from the membrane, creating a peptidyl sugar backbone. The backbone is passed to the transpeptidase (TP) domain, which cross-links the peptides to construct the peptidoglycan (Strynadka and Borman, 2007).

β-lactam antibiotics bind to the PBPs because of their structural resemblance to the building blocks of peptidoglycan. The binding of a β-lactam antibiotic to the PBP results in the formation of a covalent bond between the antibiotic and the catalytic serine residue at the active site. This binding results in permanent deactivation of the PBP.

Borrelia burgdorferi and Lyme Disease

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.

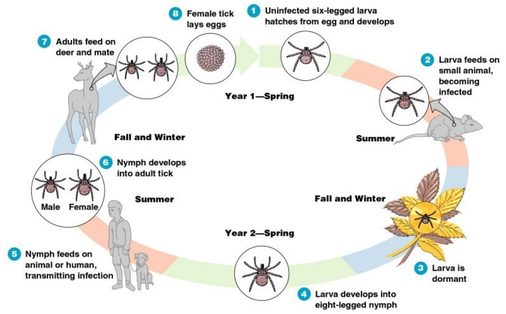

Figure 3. The life cycle of the deer tick (*Ixodes scapularis*) divided into seasons. The tick has a two year life cycle and requires three blood meals during this time. If the tick picks up *Borrelia burgdorferi* during its first meal, it can pass the bacteria to a human in its second meal (Figure courtesy of Pearson Education).

If the infection is caught early, Lyme disease is typically treated with oral administration of doxycycline (except in children under 8 years of age or pregnant/breastfeeding women; the alternative is usually amoxicillin or azithromycin). Doxycycline is used because of its effectiveness against *B. burgdorferi* along with other bacteria the tick may have been carrying. In late stage infections, patients may begin having cardiac, arthritic, and neurological symptoms and are treated with intravenous administration of the β-lactam antibiotic ceftriaxone.

Despite treatment, some patients have lasting symptoms that persist for years after infection. Sometimes called Post-treatment Lyme Disease Syndrome, patients often suffer from fatigue, joint and muscle pain, and neurocognitive symptoms.

The exact cause of the Post-Treatment Lyme Disease Syndrome is unknown. One theory is that the bacteria, when exposed to a stressor like an antibiotic, go into a dormant cyst state that allows them to survive treatment. After the stressor is removed, they return to the active spirochete form and the infection continues. Another possibility is that the bacteria have become resistant to the antibiotic.

Goals and Approach

Treatment failure could result from the *Borrelia* developing resistance to the drug. This could occur due to a change in the base pairs within the 16S ribosomal subunit where DNA binds or within the penicillin binding protein sequences where ceftriaxone binds. By studying this region as well as the PBPs and their binding affinities to various β-lactam antibiotics, we can answer questions regarding treatment failure.

Materials and Methods

PCR and Bioinformatics

Donations of *Ixodes scapularis*, the primary vector, were acquired from various regions of Minnesota. A nested PCR assay was created to amplify the region of the *B. burgdorferi* genome known to interact with doxycycline and penicillin binding protein 3 predicted to interact with ceftriaxone. After gel electrophoresis, the products were sent for sequencing. Using DNA sequence validation through the BLAST database, we were then able to compare strains in MN for molecular modifications leading to any potential resistance.

Detection of PBPs

BOCILLIN-FL (penicillin V conjugated to BODIPY-FL dye) is a fluorescent β-lactam used to identify PBPs and visualize them in living cells. In this experiment, *B. burgdorferi* cells were treated with BOCILLIN-FL and the cell membranes were isolated through ultracentrifugation. The resulting protein was then run on an SDS-PAGE gel for quantification via Western blotting.

Results

PCR and Bioinformatics

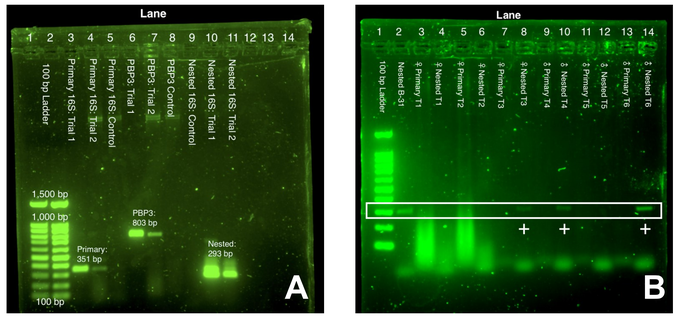

Figure 4. Results of primary and nested PCR on a 2% agarose gel. (A) DNA extraction and PCR targeting the 16S ribosomal region and penicillin binding protein 3 of a lab culture of *Borrelia burgdorferi.* This was done to ensure the assay was working properly before testing the *Ixodes scapularis* for the presence of the bacteria. (B) DNA extraction and PCR targeting the 16S ribosomal tetracycline binding region of *B. burgdorferi* obtained from inside *I. scapularis* samples collected across Minnesota. Three of the six ticks showed a positive result, indicating the presence of *B. burgdorferi.*

Detection of PBPs

Due to several unexpected problems with the assay, numerous attempts with the Western blotting have resulted in blank blots. This is likely due to a lack of sufficient protein extracted in the form of cell membranes from the bacteria, so the signal is undetectable. However, dot blot analysis of pure lysate indicated binding of the Bocillin and responsiveness to the antibody, so measures are currently being taken to enrich for the cell membrane and get more protein for the SDS-PAGE analysis.

Future Work

PCR and Bioinformatics

In addition to the amplification of the 16S ribosomal region and waiting on the sequencing from the PCR, we are also searching for methods that allow us to culture the bacteria from the ticks in order to carry out future experiments testing for antibiotic resistance.

Detection of PBPs

The first goal for future work is to get the assay working successfully, which likely is a matter of using more *B. burgdorferi* for the lysates in order to gain more protein for the Western blot. The second goal is to perform competition assays using various β-lactam antibiotics to ascertain the binding affinities of the antibiotics to the PBPs.

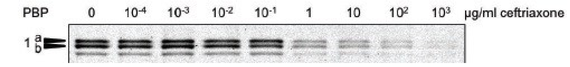

Figure 5. Sample blot of a competition assay using BOCILLIN-FL. A culture of *Escherichia coli* was treated with varying concentrations of ceftriaxone followed by a fixed amount of BOCILLIN-FL. The bands show the binding of BOCILLIN-FL; darker bands indicate more binding by BOCILLIN-FL and less binding by the antibiotic (Kocaoglu and Carlson, 2015).

Acknowledgements

We would like to thank our mentor, Dr. Amy Prunuske, for her endless help and dedication to our success.

We also would like to thank Dr. Ben Clarke for allowing us to use his lab space for the majority of our work. Additional thanks go to Dr. Andrew Skildum for allowing space in his lab for our work.

Finally, thank you to the entire Lyme disease research team at the University of Minnesota-Duluth Medical School for their support. http://d.umn.edu/lyme/

This research was made possible by the Undergraduate Research Opportunities Program (UROP) at UMD.

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