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Molecular Biology of a Surface-Exposed Protein Family of Bartonella Bacilliformis

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MOLECULAR BIOLOGY OF A SURFACE-EXPOSED PROTEIN FAMILY OF BARTONELLA BACILLIFORMIS

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Bartonella bacilliformis (Bb) is the bacterial agent of Carrión's disease and is presumed to be transmitted between humans by phlebotomine sand flies, most notably *Lutzomyia verrucarum*. Carrión's disease is endemic to high-altitude valleys of the South American Andes, and the first reported outbreak (1871) resulted in over 4,000 casualties. Since then, numerous outbreaks have been documented in endemic regions, and over the last two decades, outbreaks have occurred at atypical elevations, strongly suggesting that the area of endemicity is expanding. Approximately 1.7 million South Americans are estimated to be at risk for the disease in an area covering roughly 56,000 square miles of Ecuador, Colombia and Peru. Although disease manifestations vary, two disparate syndromes can occur independently or sequentially. The first, Oroya fever (OF), occurs ~60 days following the bite of an infected sand fly where infection of nearly all erythrocytes results in an acute hemolytic anemia with attendant symptoms of fever, jaundice and myalgia. This phase of Carrión's disease often includes secondary infections and is fatal in up to 88% of patients without antimicrobial intervention. The second syndrome, verruga peruana (VP), describes the endothelial cell-derived blood-filled tumors that develop on the surface of the skin. VP is rarely fatal, but can bleed and scar the patient. Moreover, persistently-infected individuals with VP provide a reservoir for infecting sand flies and thus maintaining Bb in nature. Little is known about the epidemiology and pathogenesis of *B. bacilliformis*. The overall objective of this research is to investigate a remarkable and novel, 15-member "Imp" protein family of Bb. Recent research done by UC San Diego found this paralogous gene family is shared only by the *Bartonella* and *Leptospira* genera. This is very intriguing since they are very far apart from each other phylogenetically. The gene family is suspected to affect the virulence of the bacterium, based on the findings that it was one of a handful of genes that mutated during attenuation. Moreover, during an infection of *Leptospira* in a mouse, all members of the gene family were up-regulated considerably. The overall objective of our research is to investigate what the paralogous gene family does and why both *Leptospira* and *Bartonella* have both maintained it. This will lead to a better understanding of *B. bacilliformis'* pathological processes and indicate if the proteins are bona fide virulence factors. Determining where in the cell the protein is located will give us a better understanding of its function. One member of the paralagous gene family of *B. bacilliformis* (gene Bb0452) has been cloned into an expression vector to generate a fusion protein with a His_6 tag. This construct has been transformed into *E. coli.* We subsequently purified recombinant Bb0452 protein from the *E. coli* using Ni-resin affinity purification, produced polyclonal anti-Bb0452 antibodies in a rabbit, and are currently working with the antibodies to localize Bb0452 in the *B. bacilliformis* cell.

The specific aims of our research were:

1) Purify recombinant Bb0452 (rBb0452) protein from *E. coli* using nickel-resin affinity chromatography.

2) Generate rabbit polyclonal anti-Bb0452 antibodies.

3) Localize the Bb0452 protein in the Bb cell using bacterial cell fractionation techniques and anti-Bb0452 antibodies as a tool to detect the protein.

Our first aim- purify recombinant Bb0452 (rBb0452) protein from *E. coli* using nickel-resin affinity chromatography- took approximately 4 months. Our plasmid was introduced into *E. coli* and induced with IPTG to overexpress the protein (Bb0452). A BCA assay was done to determine the protein preparation's concentration. A SDS-PAGE gel was run to analyze the protein profile of the original strain of *E. coli* and the strain expressing the recombinant protein. A western blot was also done as a more sensitive way to detect if our protein was present, using antibodies generated against the Leptospira proteins. It was, although not as strongly as hoped. Purification of the protein was then done with NiNTA affinity purification, and examined by a silver-stained gel for extra sensitivity. The protein did not show up definitively. Thus, we had to shorten our initial gene construct in the hopes that a shorter protein product would be easier for *E. coli* to tolerate. The initial plasmid was purified from *E. coli* and treated with the restriction enzyme HindIII. This cut out a large portion of the gene and created 2 fragments. These were isolated by DNA agarose gel and the correct band was cut out and saved. The DNA was then extracted from the gel, ligated, and then transformed back into *E. coli*. This strain was then cultured, its plasmid purified and then examined by agarose gel electrophoresis to see if the correct band was present. After confirming the plasmid content, the corresponding strain was used to express the truncated Bb0452 protein. A protein gel was run to determine if the 13-kDa protein was there and it was. A Ni-NTA purification of the protein was done. The resulting protein was isolated further by SDS-PAGE gel and the bands were cut out and stored at -20^{o}C until used. This was our pure recombinant Bb0452 protein.

Our second aim- To generate rabbit polyclonal anti-Bb0452 antibodies took another 4 months. The pure protein was used to maketwo vaccines for our New Zealand white rabbit (Oliver). The first vaccine was administered after a pre-immune serum sample was taken. Four weeks later the second vaccination (booster) was administered. Every week after that, a blood sample was taken. The samples were spun down and the serum was collected and saved. The antibodies were then tested to see if they were indeed anti-Bb0452. This was done with a series of western blots to test the sensitivity and the levels of each week's blood serum. This is still in process. The antibodies do recognize the isolated,

truncated protein but it is still being determined if they can recognize and bind to the full-lengthprotein in the cell fractionation.

We will continue with the second aim and on to our third- localize the Bb0452 protein in the Bb cell using bacterial cell fractionation techniques and anti-Bb0452 antibodies as a tool to detect the protein- at the beginning of next semester. To this end, we have prepared cytosol, periplasm and membrane fractions, and these are archived in the freezer. Once we determine where in the cell the protein is being expressed, we can work on determining if the entire gene family is a part of causing the disease (by genetic manipulation) and even on to identifying a target for a potential vaccine, and the vaccine itself.