Induction of Changes in the *C. burnetii* Type IVB Secretion System During Axenic Growth

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*Coxiella burnetii* is an intracellular pathogen in nature and the causative agent of the zoonotic disease Q-fever. Maturation of a replicative niche occurs through the trafficking of the nascent *C. burnetii* containing vesicle along the endocytic pathway and results in a parasitophorous vacuole (PV) that is reminiscent of an autophagolysosome. From the PV, *C. burnetii* modulates the host cell using effector proteins secreted by an essential type IVB secretion system (T4BSS). It has been shown that the virulence secretion systems of a number of pathogens undergo physical changes based on signals from the host cell prior to secretion of effector proteins. However, it is not clear what signals may induce the *C. burnetii* T4BSS to become active and deliver effector proteins across the PV membrane. We have previously shown that DotA and IcmX are released from *C. burnetii* during growth in the first generation acidified citrate cystine medium (ACCM-1), and that DotA localizes to the PV membrane as well as to vesicles in the cytoplasm of the host cell during infection. Using immunoblot assays, we show that DotA and IcmX are not released during *C. burnetii* growth in the second generation ACCM (ACCM-2). To identify the components signaling DotA and IcmX release in ACCM-1, supplements were added to *C. burnetii* growing in ACCM-2. These assays revealed that lipid (fatty acid micells) recovered the release of DotA and the release of more limited amounts of IcmX. The addition of BSA on the other hand induced the release of larger amounts of IcmX and no DotA suggesting a series of signals may induce changes to the *C. burnetii* T4BSS during infection of host cells. Work to further define these interactions may reveal the nature of the signals present on host cell membranes/PV that induce *C. burnetii*’s T4BSS during infection.

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Characterization of an Anaplasma marginale mutant generated by transposon mutagenesis

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Transposon mutagenesis of A. marginale is achievable with a single plasmid carrying the Himar1 transposon and the A7 hyperreactive transposase. This resulted in isolation of spectinomycin-resistant organisms expressing a red fluorescent protein marker. Junctions between Himar1 inverted repeats and the A. marginale genome were determined with Next-Generation Sequencing technologies, Roche 454 and Illumina. Sequences obtained were analyzed using GALAXY, a web-based platform. A useful feature of this method is the ability to determine the ratio of mutated to non-mutated sites at any genome location. Approximately 50% of the Illumina reads showed an insertion of the transposon within either the Omp10 or Omp6 genes, and the other 50% of reads suggested that these sites were unmutated. This could be explained because Omp10 and Omp6 share a large stretch of identity (456/459 nt, 99%) and by the length of the reads obtained with this method (~100 nt). A similar analysis using longer sequencing reads obtained on the 454 platform allowed identification of reads that contained the transposon-chromosome insertion site followed by a region of the Omp10 gene that is not shared with the Omp6 gene, suggesting that the transposon was integrated within Omp10. Subsequent PCR amplifications using DNA templates from wild-type and transformed A. marginale and Omp6- and Omp10- specific primers confirmed the insertion within Omp10. Omp10 is arranged within an operon, with Omp9, 8, 7, and 6 arranged in tandem. Omp10 through 7 are differentially expressed in infected mammalian and tick cells. We characterized this A. marginale mutant to determine if insertion of the Himar1 transposon within Omp10 altered its expression and if insertion would alter transcription of the downstream genes. Inactivation or changes in expression of these outer membrane protein genes may result in altered phenotypes that can be evaluated in natural host-vector systems.
Description of *Candidatus* Bartonella ancashi isolated from the blood of two patients with verruga Peruana

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The genus *Bartonella* contains an increasing number of vector-borne, fastidious, small, Gram-negative bacteria. The original member of the genus, *Bartonella bacilliformis*, infects humans and is known to cause a biphasic illness (Carrion’s disease), consisting of an acute phase (Oroya fever) and a chronic phase (verruga Peruana). Verruga Peruana presents with benign, yet persistent, skin nodules. *B. bacilliformis* is endemic in the Andes mountain range- occurring between 2,500 and 8,000 feet above sea level. In 2003, a clinical treatment trial was conducted in Caraz, Ancash, Peru to test the efficacy of azythromycin as a treatment for verruga Peruana caused by *B. bacilliformis*. Two patients enrolled in the study were found to have infections with *Bartonella* species disparate from *B. bacilliformis* based on sequencing of *gltA*. Subsequent genome sequencing and whole genome mapping confirmed the isolates to be unique. Whole genome mapping (WGM) also confirmed the high similarity between the isolates and their distance from other *in-silico* Bartonella species. Interestingly, WGM also revealed major genomic rearrangements between the isolates. Additionally, phenotypic, microscopic, colonial morphology, and growth characteristics were observed among the three isolates and, these characteristics, were consistent with those found among members of the genus *Bartonella*. Gram-staining and transmission electron microscopy showed the isolates to be small (1.27-0.54 µm), Gram-negative bacilli with variable expression of flagella, while biochemical testing (oxidase, catalase, and the RapID ANA II system) provided a single phenotype for all three isolates, which is consistent with other *Bartonella* species. Based on these results the isolates are considered to be a unique *Bartonella* agent, provisionally named, *Candidatus* Bartonella ancashi, which is associated with the disease syndrome verruga Peruana.