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"A Comparison of Mutated Versus Wild Type *Scc4* Phenotype in Chlamydia Infected Cells"

Chlamydia trachomatis is a type of obligate intracellular bacteria with a wide variety of attributes that make studying this pathogen difficult. *C. trachomatis* has evolved a small genome and experiences a unique developmental cycle that rotates between an infectious but non-dividing Elementary Body (EB) and a non-infectious but dividing Reticulate Body (RB). Most genes in the chromosome are likely essential to *Chlamydia* function and pathogenesis. The primary method of *C. trachomatis* infection of a host cell is carried out by a Type III Secretion System (T3SS), which allows the bacterial effector proteins to directly enter the host cytosol. Scc4 is an important protein involved in this system that functions in cooperation with Scc1 to chaperone the effector CopN into host cells during infection. In addition to its role in the T3SS, Scc4 is also known to have a regulatory effect on bacterial transcription. Both of these functions, secretion and transcription, are necessary for chlamydial infection which makes studying Scc4 difficult because infection cannot occur in its absence. As a result, the exact mechanisms by which Scc4 carries out its functions are unclear. To examine Scc4's effects on *C. trachomatis* infection, we utilized a recently developed chlamydial genetic system and performed mutational analysis of *scc4*. The plasmids expressing Scc4 mutants were created using pBOMB-Scc4 as a backbone. Scc4 mutant Scc4_6A was created by introducing six alanine substitutions into the amino acid sequence. Mutant Scc4_I49V was created by changing amino acid 49 in the sequence from isoleucine (I) to valine (V). Each plasmid was transformed into *C. trachomatis* L2, resulting in strains L2/6A and L2/I49V. These strains were used to infect human cervical HeLa epithelial cells, followed by analyses of the chlamydial growth patterns using microscopy. Genes of interests were examined using Polymerase chain reaction (PCR), then with gel electrophoresis to determine if the correct fragment is present. We observed that both strains, L2/6A and L2/I49V, exhibited slow growing phenotypes which differed from those containing the wild type *scc4*. Unexpectedly, we failed to detect *scc4* gene from either plasmid or chromosome in the L2/I49V, suggesting a null *scc4.* On the other hand, strain L2/6A had the plasmid-encoded *bla* gene, yet retained replication and differentiation abilities in the presence of ampicillin. These surprising findings may reflect the ability of *C. trachomatis* in DNA exchanges between plasmid and chromosome. Further phenotypic and genotypic characterization of these recombinant strains is ongoing to better understand how Scc4 contributes to growth and infection progression of the host cells.