Identification of Putative Secretion Effectors in *Rickettsia prowazekii*

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Abstract

The obligate intracellular, gram-negative bacterium *Rickettsia prowazekii*, the louse-borne, causative agent of epidemic typhus, is a historically significant pathogen that has caused millions of deaths during periods of war and famine. It is also identified as a potential bioterrorism weapon. Because *R. prowazekii* only grows within the cytosol of host cells, it has evolved mechanisms that aid its infection, intracellular growth, and ability to evade host cell defense. The rickettsial genome contains several secretion systems that may support the delivery of secreted effectors that would interact with its host. To identify putative effectors, a shuttle vector was constructed containing an expression cassette that incorporates a tandem FLAG and glycogen synthase kinase (GSK) tag that can be fused to a protein of interest. The FLAG-GSK tag allows the proteins to be identified within the eukaryotic cytosol, confirming protein secretion from the pathogen and supporting its role as an effector molecule. Finally, incorporation of an RpCherry-Arr2 fusion protein into the plasmid allows for fluorescence detection and selection of the desired transformants using the antibiotic rifampin. This system permits the screening of hypothesized secreted effectors in *Escherichia coli*, a surrogate host. Targets secreted can then be identified prior to validating the secretion of the effectors in BSL-3 agent *R. prowazekii*. The identification of potential secreted effectors that allow rickettsiae to enter, manipulate, and exit its host will reveal crucial information regarding rickettsial obligate intracellular growth and ability to cause human disease.

Background

Secretion of effector proteins may be involved in several aspects of rickettsial growth, including invasion, intracellular replication, and ability to evade host cell defense mechanisms. Investigating potential secreted effectors of *R. prowazekii* is crucial to understanding how rickettsiae manipulate its host and cause disease. As many as 40 proteins are hypothesized to be putative secreted effectors based on bioinformatics. Based on their G/C content, signal sequences, presence of eukaryotic domains, and homology to effectors in other pathogens, five genes (RP016, RP290, RP621, RP787, and RP807) were selected for this initial study. To investigate putative effectors, a shuttle vector was constructed containing an expression cassette that contains a FLAG-GSK tag and RpCherry-Arr2 fusion protein. The FLAG tag was selected because cytoplasmic expression and secretion of recombinant fusion proteins can be easily detected using antibody recognition in cellular localization studies and immunofluorescence. The incorporation of the GSK tag is imperative because only a eukaryotic kinase, located in the cytoplasm, can phosphorylate GSK, validating the secretion of proteins into the cytosol of the host. The plasmid construct also contains RpCherry-Arr2 fusion protein, permitting fluorescence detection and selection of the desired transformants using the antibiotic rifampin and presence of “pink” colonies. These putative effectors are initially expressed in *Escherichia coli* and Biosafety Level-2 *Rickettsia* sp. as surrogates to identify the potential secreted effectors. Protein targets will then be analyzed in secretion assays using *R. prowazekii*, a Biosafety Level-3 pathogen.

Rickettsial Secretion Systems

![Diagram of Rickettsial Secretion Systems](image)

Methodology

Selection of Secreted Effectors

- Signal sequences
- Increased G/C content
- Eukaryotic domains
- Homologous to effectors in other pathogens

**Potential Secretion**

<table>
<thead>
<tr>
<th>Potential Secretion Effector</th>
<th>Proposed Function</th>
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<tbody>
<tr>
<td>RP016</td>
<td>Possible Cell Surface Antigen</td>
</tr>
<tr>
<td>RP290</td>
<td>Type IV Sec Pathway</td>
</tr>
<tr>
<td>RP621</td>
<td>Zinc Uptake Mechanism</td>
</tr>
<tr>
<td>RP807</td>
<td>Penicillin Binding Protein</td>
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</tbody>
</table>

Selected Genes and their Proposed Functions

Plasmid Elements

- FLAG tag
- GSK tag
- RpCherry-Arr2 fusion

Fusion Cassette

![Image of Fusion Cassette](image)

Fusion Plasmid Construction

**Plasmid Elements**

- FLAG tag
- GSK tag
- RpCherry-Arr2 fusion

**Fusion Cassette**

![Image of Fusion Cassette](image)

**Verified Insert Orientation**

![Image of Verified Insert Orientation](image)

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References