Potential regulation of deadly water-borne *Shigella* bacteria pathogenesis through the *Shigella* infection protein Spa47

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Shigella (Figure 1) is a gram-negative, bacterial pathogen typically found in contaminated water sources. Each year, Shigella is responsible for over 90 million infections and 100,000 deaths stemming from symptoms of severe dysentery, fever, nausea and vomiting. A needle-like apparatus (Figure 2) found on the surface of Shigella allows the bacterium to infect host cells. Each needle-apparatus has an associated ATPase, a protein that can hydrolyze ATP into ADP and Pi. The Shigella needle-apparatus ATPase Spa47 (Figure 2, circled red) is predicted to provide the energy for infection. Spa47 has been shown to be essential for infection – without Spa47, no infection will occur. We were able to recombinantly express, purify, and characterize Spa47 for the first time.

Figure 1 – A depiction of Shigella, a deadly water-borne pathogen

Figure 2 – A cartoon rendition of the Shigella needle-like infection system

Figure 3 – The Spa47 oligomer species is 10-fold more active than monomeric or mutant Spa47

I. Introduction

- Shigella (Figure 1) is a gram-negative, bacterial pathogen typically found in contaminated water sources.
- Each year, Shigella is responsible for over 90 million infections and 100,000 deaths stemming from symptoms of severe dysentery, fever, nausea and vomiting.
- A needle-like apparatus (Figure 2) found on the surface of Shigella allows the bacterium to infect host cells.
- Each needle-apparatus has an associated ATPase, a protein that can hydrolyze ATP into ADP and Pi.
- The Shigella needle-apparatus ATPase Spa47 (Figure 2, circled red) is predicted to provide the energy for infection.
- Spa47 has been shown to be essential for infection – without Spa47, no infection will occur.
- We were able to recombinantly express, purify, and characterize Spa47 for the first time.

II. Methods

- Shigella protein Spa47 was recombinantly expressed in E. coli expression strains with a chitin binding domain tag.
- Spa47 was purified based on protein affinity and size.
- Activity of Spa47 was measured using a radioactive ATP hydrolysis assay protocol to determine the rate of ATP hydrolysis.
- Several mutations of Spa47 were made at key amino acid residues and ATP hydrolysis activity is measured.

III. Results

- Spa47 purifies are two separate species, a monomer and a trimer (three associated Spa47 proteins) species.
- The ATP hydrolysis activity of the Spa47 trimer species is 10-fold higher than the Spa47 monomeric species (Figure 3).
- There is no ATP hydrolysis activity in Spa47 with a single amino acid mutation at the predicted active site of the protein.
- Activity of Spa47 appears to be oligomer dependent.

IV. Conclusions

- Spa47 has been successfully purified using recombinant protein expression.
- Single point mutations at key residues in Spa47 eliminate ATP hydrolysis activity.
- The trimeric Spa47 oligomer species was greater than 10 times that of the monomer, suggesting oligomerization may play a role in infection regulation.

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http://www.cdc.gov/shigella/