Exploring the host range of *Listeria monocytogenes* via the interaction of listerial internalin with E-cadherin domains from various mammalian hosts

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Introduction: Listeriosis is a human disease characterised by sporadic food-related outbreaks and a high mortality rate especially in immunocompromised patients such as the elderly, pregnant women, or young children. In 2017 to 2018 the largest-ever outbreak of listeriosis occurred in South Africa due to contaminated meat products. Listeriosis is caused by the gram-positive, mostly saprophytic bacterium *Listeria monocytogenes* (*Lm*). *Lm* has a unique infection strategy that involves adhering to and invading individual cells and spreading from cell to cell. To achieve this, the bacterium induces its own internalization into host epithelial cells using its virulence factor internalin (InlA) to bind to host e-cadherin. In addition to humans, listeriosis also affects various animals. However, incompatible E- cadherin molecules prevent listeriosis being spread by the oral route in some animals including mice. We aim to investigate the potential host range of *Listeria monocytogenes* by analysing the interaction of InlA with E-cadherinfrom cattle, sheep, pigs and chicken at the molecular level. This will include structural analyses by x-ray crystallography.

Initially, *in silico* analysis of InlA/E-cadherin interactions from different hosts were undertaken modelled on the known complex of InlA with human E-cadherin. Only hosts for which a complex was possible were further pursued. Proteins were prepared as GST-fusion proteins in *E. coli* and purified by affinity and anion exchange chromatographies. Protein-protein interaction studies are then undertaken by using purified proteins. Techniques include size exclusion chromatography, dynamic light scattering, microscale thermophoresis (MST) and X-ray crystallography.

Protein production and purification have been successfully optimized. Preliminary protein-protein interaction studies are confirming a possible interaction between InlA and ovine E-cadherin. Interactions between InlA and human and murine e-cadherins have been used as positive and negative controls, respectively, and the observed results are in line with literature. InlA has been crystallized. Crystals of InlA/E-cadherin complexes have been prevented by E-cadherin dimerization. This will hopefully be overcomed by speeding up the production methodology.

In conclusion, listerial InlA mediates bacterial internalization into host epithelial cells by binding to E-cadherin. We are studying the ability of InlA to bind the E-cadherin from sheep, cattle, pig, and chicken. Dimerization of E-cadherin N-terminal domains are currently preventing progress, but this will hopefully be rapidly overcome.

Keywords: Infectious bacterial diseases, Structural biology, Protein-protein interaction studies.