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Thesis abstract

Mycoplasma hyopneumoniae proteases: Investigating their role in pathogenesis and chronic infection

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Proteases are enzymes that cleave peptide bonds in polypeptides thus influencing protein shape, size, composition, function, cell localisation, turnover, and degradation. In bacteria, in addition to being responsible for a myriad of physiological processes, proteases are also secreted as toxins and other virulence factors. Hence proteases have been identified as potential therapeutic targets in a range of microbial pathogens and have successful applications in treating viral and fungal infections.

The genome-reduced, and economically significant, swine respiratory pathogen, *Mycoplasma hyopneumoniae*, is predicted to encode ten proteases. So far, a glutamyl aminopeptidase (GAP) has been characterised as a moonlighting protein with adhesive functions, and signal peptidase I was found to be cytotoxic to mammalian cells. In this thesis, four proteases (loci: MHJ_0522, MHJ_0659, MHJ_0461, MHJ_0169) were expressed as polyhistidine tagged recombinant proteins, and their activities, both canonical and moonlighting, were characterised. Further substrate characterisation of GAP was also achieved.

MHJ_0522, MHJ_0659, and MHJ_0461 were characterised as functional oligopeptidase F (PepF), xaa-pro aminopeptidase (PepP), and leucine aminopeptidase (LAP), respectively. All three proteases were predicted to be cytosolic, yet all three were identified on the surface of *M. hyopneumoniae* by both proteomic methodologies and immunofluorescence microscopy. All three proteases were found to possess moonlighting adhesive properties by binding heparin, and LAP was found to additionally bind exogenous DNA and plasminogen. Furthermore, LAP binding plasminogen enhanced its conversion to plasmin.

Collectively, PepF and PepP are described here as possessing the ability to deactivate four important mediators of inflammation. Using a matrix-assisted laser desorption/ionisation (MALDI) — time-of-flight (TOF) — mass spectrometry (MS) assay, PepF and PepP were shown to cleave bradykinin, substance P, neurokinin A, and neuropeptide Y in ways that would disable receptor binding. This discovery may help explain how *M. hyopneumoniae* is able to establish chronic infections and avoid host innate immune system clearance.

M. hyopneumoniae is known to proteolytically process, often extensively, proteins that reside on its cell surface. By mining N-terminiome data, this thesis also provides an *in silico* analysis of *M. hyopneumoniae* generated protein fragments, demonstrating an increase in disorder and availability of protein:protein interaction sites. This observation suggests that genome-reduced *M. hyo-*

pneumoniae uses proteolytical processing to increase its proteins functional repertoire. An observed N-terminal methionine excision (NME) peculiarity, that is, NME occurring when the P1' residue is large and charged, is explored by expressing and characterising recombinant methionine aminopeptidase (MAP; MHJ_0169). Ultimately, the activity is assigned to surface exposed GAP and LAP using peptides mimicking the N-termini of offending proteins and MALDI-TOF-MS.

Lastly, formylated bacterial peptides are known to be potent chemo-attractants for innate immune cells, particularly white blood cells. In bacteria, a formyl group is added to methionine to initiate protein synthesis. This thesis provides evidence that *M. hyopneumoniae,* and fourteen other mycoplasmas, lack the enzymes required to generate and attach formyl groups. It is proposed that these mycoplasmas have evolved an alternative NME process that may be a means to escape host recognition.

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