1. Summary

1.1 Summary (English)

Legionella pneumophila is the causative agent of Legionnaire's disease, a potentially fatal pneumonia. The bacterium possesses secreted and cell-associated lipolytic activities, in particular phospholipase A activity. Bacterial phospholipases are potential virulence factors as they facilitate lysis of host cell membranes and interference with host signal transduction pathways by the release of second messengers. *Pseudomonas aeruginosa* for example, also a bacterial lung pathogen, secretes an enzyme named ExoU with phospholipase A (PLA) and lysophospholipase A (LPLA) activities which lead to lung injury due to cytotoxicity. The secreted PLAs of *L. pneumophila* cause hydrolysis of lung surfactant, a phospholipid monolayer in the alveoli that is essential for lung stability, which implies a direct role for phospholipase A in *Legionella*-mediated pneumonia. Since the proteins responsible for this activity were still unknown, it was of particular interest to identify them and investigate their role during *L. pneumophila* infection.

In the present investigation seven proteins were identified as PLA candidate proteins either by screening the *L. pneumophila* genomic database for proteins with lipase motifs or by protein purification followed by N-terminal sequencing. These new proteins were designated as follows: PlaB, PlaC, PlaD, PatA, Unk1, LvrE, and Aas. The analysis of corresponding *L. pneumophila* mutants and expression of the corresponding genes in *Escherichia coli* revealed that five of the seven proteins indeed possessed phospholipase A and lysophospholipase A activities. These five proteins, PlaB, PlaC, PlaD, PatA, and Aas, belong to four different groups of lipolytic enzymes. PlaB displays a modified lipase motif, PlaC and PlaD belong to the family of GDSL hydrolases, PatA is one of eleven *L. pneumophila* Philadelphia-1 proteins with a patatin domain, and Aas belongs to the group of 2-acylglycerophospholipid acyltransferases.

Three of the five new *L. pneumophila* lipolytic enzymes, namely PlaC, PlaD, and PatA were secreted into the bacterial culture supernatant. The GDSL hydrolase PlaC was shown to be a glycerophospholipid:cholesterol acyltransferase (GCAT), i.e. an enzyme which abstracts a fatty acid residue from a phospholipid and transfers it to cholesterol. In addition to its GCAT activity, PlaC also exhibited PLA and LPLA activities in the absence of cholesterol. Notably, PlaC was

responsible for all the secreted GCAT activity of *L. pneumophila*, was exported by the type II secretion system into the bacterial culture supernatant, and required the type II secreted zinc metalloprotease ProA for its GCAT activity. The other GDSL enzyme, PlaD, possessed LPLA activity when expressed in *E. coli* and *L. pneumophila plaD* mutants displayed reduced PLA and LPLA activities in their culture supernatant indicating that PlaD was a secreted phospholipase A as well. The third secreted PLA/ LPLA, PatA, displayed conserved regions to potato patatin and additionally showed homology to the cytotoxic *Pseudomonas aeruginosa* ExoU. In contrast to *P. aeruginosa* ExoU, PatA did not require activation by a eukaryotic factor. PatA has ten paralogs encoded in the *L. pneumophila* Philadelphia-1 genome all of which have a patatin domain. Since patatin-like proteins were considered to be typical for plant cells, other bacterial genomes were also screened for patatin-like proteins and especially pathogens or symbionts of eukaryots were found to encode several patatin homologs.

The other two lipolytic proteins, PlaB and Aas, were associated with the bacterial cell. *L. pneumophila* PlaB was identified as the major cell-associated phospholipase A and lysophospholipase A. The PlaB protein sequence revealed conserved regions to the *L. pneumophila* lipase LipB and to the human inositol deacylase PGAP1. The second cell-associated protein, Aas, is involved in the regulation of the bacterial membrane lipid composition, because compared to the wild type, *L. pneumophila aas* mutants possessed increased amounts of lysophosphatidylcholine in their membrane while the contents of the membrane diacylphospholipids were reduced. Aas was furthermore required for the protection of the bacterium from cytolytic lysophosphatidylcholine.

The virulence of the *L. pneumophila plaC*, *plaD*, *patA,* and *aas* mutants was assayed in infection models. Both of the GDSL hydrolases PlaC and PlaD were found to be dispensable for intracellular replication which was also reported to be the case for the third *L. pneumophila* GDLS enzyme, PlaA, which is a lysophospholipase A. Since this study showed that all three GDSL enzymes have overlapping activities, they might replace each other during the infection process. The secreted patatin-like protein PatA is essential for the infection of amoebae and macrophages, as the corresponding mutant showed a severe defect to grow inside these host cells. Thus, PatA is the first *L. pneumophila* lipolytic enzyme with a role in bacterial virulence. The cell-associated protein Aas was not required for the infection of amoebae, macrophages, or epithelial cells. As Aas is important for the protection of the bacterium from cytolytic agents, the

protein might still be essential for the actual infection process in the human lung where lysophosphatidylcholine can be present.

In conclusion, this study identified five new proteins which cause most of the secreted and cell-associated phospholipase A activities of *L. pneumophila* and showed that one of them contributes to virulence of the lung pathogen.