

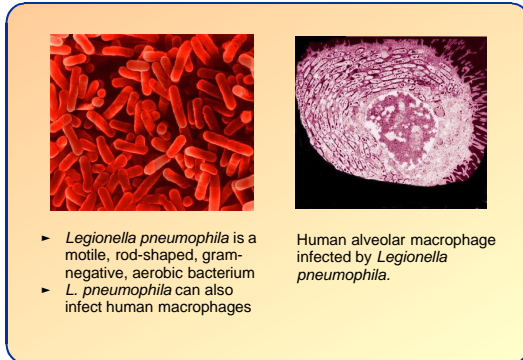
Structural Infectomics:

Identification and Characterization of Potential Virulence Factors in *Legionella pneumophila*

S. Heyne, M. Mann, D. Pal, A. Baharuddin, A. Vogel & R. Hilgenfeld

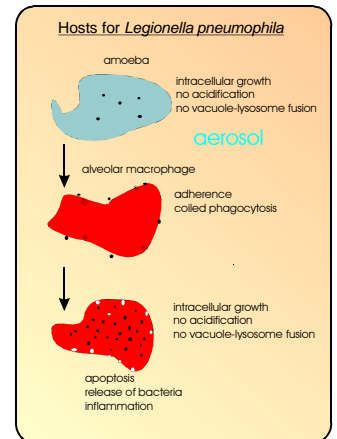
sheyne@imb-jena.de, mmann@imb-jena.de, dpal@imb-jena.de, aidabaha@imb-jena.de, avogel@imb-jena.de & hilgenfd@imb-jena.de

Department of Structural Biology & Crystallography, Institute of Molecular Biotechnology (IMB), Beutenbergstrasse 11, D-07745 Jena, Germany



Legionella pneumophila is a parasitic bacterium

- ♦ Air-conditioning units, jacuzzis and saunas provide ideal growth conditions for amoebae and ciliated protozoa, the natural hosts for *L. pneumophila* [1]
- ♦ *L. pneumophila* is able to infect humans through inhalation of contaminated aerosols
- ♦ Infection begins with binding of bacteria to human alveolar macrophages, followed by coiling phagocytosis and localization in a unique phagosomal vacuole [1]
- ♦ The bacteria multiply inside the macrophages to large numbers, until the host cell bursts
- ♦ Infection causes Legionnaires' Disease or Pontiac Fever

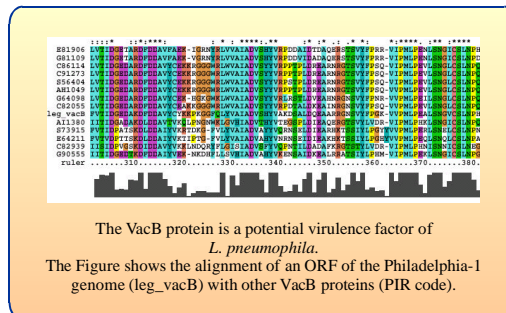


Goals

- ▶ Identification of *L. pneumophila* virulence proteins from genomic
- ▶ assignment of probable function
- ▶ cloning and expression
- ▶ X-ray structure analysis
- ▶ target validation

Legionnaires' Disease

- ♦ First outbreak reported in 1976 in Philadelphia during a convention of the American Legion, 34 people died
- ♦ Further serious outbreaks occur year by year; recent outbreaks have been reported from Spain and the UK (August 2002)
- ♦ Up to 15% of infected people do not survive the disease



Identification of virulence factors

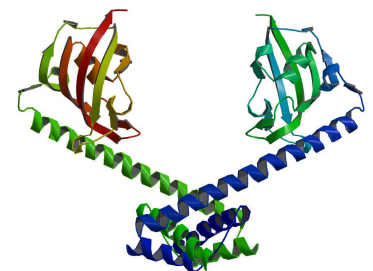
- ♦ The genome of *Legionella pneumophila* Philadelphia-1 has been almost completely sequenced at Columbia University [2]
- ♦ Using these data, we predicted potential virulence factors in *L. pneumophila* by sequence alignment with known virulence proteins from other pathogens
- ♦ These sequences were extracted from the PIR database (<http://pir.georgetown.edu/pirwww/>) and aligned against the translated *L. pneumophila* genome by using ClustalW (<http://clustalw.genome.ad.jp/>)
- ♦ The aligned sequences were scored for similarity by visual inspection and by software tools, some of which were developed by us

Current results

- ♦ About 500 virulence-associated proteins from different organisms, mostly gram-negative bacteria, were aligned against the Philadelphia-1 genome [2]
- ♦ Over 150 regions with a sequence identity of above 25% to the known virulence factors were identified
- ♦ 60 ORFs were found with a proper start and stop codon that might be possible virulence factors
- ♦ For example, we found a region coding for the virulence factor MviN, with a high sequence identity of 45% to *Salmonella enterica* MviN; the function of this transmembrane protein is still unknown
- ♦ We also identified the possible genes for VacB (an RNase), PhoP and PhoQ (a 2-component sensory system) and for a virulence ATPase

A major virulence factor: Mip

- ♦ 24 kD protein located on the outer membrane of *L. pneumophila*
- ♦ Mip is essential for the virulence of the pathogen [3]
- ♦ We have determined the three-dimensional structure by X-ray crystallography [4]
- ♦ A highly unusual fold was revealed
- ♦ Mip belongs to the FKBP subfamily of the peptidyl prolyl *cis/trans* isomerases (PPIases), which catalyze the *cis/trans* isomerization of peptide bonds N-terminal to proline residues
- ♦ The FKBP inhibitor FK506 was co-crystallized with Mip: starting point for drug design [4]



The structure of the macrophage infectivity potentiator protein (Mip) [4]

References:
[1] Y. Abu Kwaik: Mol. Microbiol. 30, 689-696 (1998).
[2] Columbia sequencing group from: (<http://genome3.cpmc.columbia.edu/~legion/>).
[3] M.S. Swanson & B.K. Hammer: Annu. Rev. Microbiol. 54, 567-613 (2000).
[4] A. Riboldi-Tunnicliffe, B. König, S. Jessen, M.S. Weiss, J. Rahfeld, J. Hacker, G. Fischer & R. Hilgenfeld: Nature Struct. Biol. 8, 779-783 (2001).