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ScienceDirect
International Journal of Medical Microbiology 297 (2007) 577–587
IJMM
www.elsevier.de/ijmm

Legionella pathogenicity: Genome structure, regulatory networks and the host cell response

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Received 17 January 2007; received in revised form 6 March 2007; accepted 7 March 2007

Abstract

Legionella spp. the causative agent of Legionnaires' disease is naturally found in fresh water where the bacteria parasitize intracellularly within protozoa. Upon aerosol formation via man-made water systems, *Legionella* can enter the human lung and cause a severe form of pneumonia. Here we review results from systematic comparative genome analysis of *Legionella* species with different pathogenic potentials. The complete genomes reveal that horizontal gene transfer has played an important role during the evolution of *Legionella* and indicate the importance of secretion machineries for the intracellular lifestyle of this pathogen. Moreover, we highlight recent findings on the in vivo transcriptional program of *L. pneumophila* and the regulatory networks involved in the biphasic life cycle. In order to understand how *Legionella* effectively subvert host cell functions for its own benefit the transcriptional host cell response upon infection of the model amoeba *Dictyostelium discoideum* was studied. The use of this model organism made it possible to develop a roadmap of host cell factors which significantly contribute to the uptake of *L. pneumophila* and the establishment of an ER-associated replicative vacuole.

Keywords: *Legionella*; Genome; Horizontal gene transfer; Secretion machineries; Transcriptome; Host cell response; *Dictyostelium discoideum*

Introduction

Legionella species are fastidious Gram-negative bacteria capable of replicating intracellularly within a variety of eukaryotic host cells. Ubiquitously present in aquatic habitats legionellae exploit different species of free-living protozoa. These natural host cells provide nutrients, protect the bacteria from adverse conditions and serve as a vehicle for the colonization of new habitats. Over evolutionary time the protozoa–*Legionella* interaction may have generated a pool of virulence traits which preadapted this pathogen for human infection (Steinert et al., 2002).

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doi:10.1016/j.ijmm.2007.03.009

Since legionellae colonized engineered hot water environments, these bacteria, which belong to the gamma-subgroup of proteobacteria, represent a hygienic problem. Many outbreaks and sporadic cases of nosocomial and community-acquired legionellosis have been identified worldwide. Epidemiologic studies revealed that elderly, smokers and immuno-compromised persons are most susceptible. Chest X-rays of patients with Legionnaires' disease commonly show interstitial pneumonia (Winn, 1988). Although the genus *Legionella* comprises over 48 species with 65 serogroups (Fields et al., 2002; Benson and Fields, 1998) the vast majority of *Legionella* infections is due to *L. pneumophila* (98%), and about 95% of the cases are due to serogroup 1. Other *Legionella* species only rarely or never cause disease although they are frequently found in man made water systems (Yu et al., 2002; Muder and Yu, 2002). This has led to the suggestion that comparative genomic and transcriptomic analyses may provide clues to understand this interesting phenomenon.

The transmission of *L. pneumophila* to humans takes place by inhalation of aerosols from contaminated water sources. During the course of infection the bacteria are phagocytosed by alveolar macrophages and multiply within a reprogrammed *Legionella*-specific vacuole. Experiments with several cellular infection models including the soil amoeba *Dictyostelium discoideum* have shown that this *Legionella*-specific vacuole resists lysosomal fusion, recruits organelles and develops into an endoplasmic reticulum (ER)-like compartment (Steinert and Heuner, 2005). The modulation of host cell markers is known to be important for the biogenesis of the replicative vacuole. The specifically altered morphology of this compartment is similar in protozoan and mammalian cells and requires the bacterial Dot/Icm type IV secretion system (Molofsky and Swanson, 2004).

Interestingly, the described cellular infection cycle of *L. pneumophila* corresponds to sequential growth phases of the bacterium where two distinct phenotypes of *Legionella* can be differentiated (Steinert et al., 2002). The switch from replicative, non-flagellated bacteria to the cytotoxic transmissive form is triggered by amino acid starvation at the end of the infection cycle and has been shown to be tightly regulated (Molofsky and Swanson, 2004). Moreover, this process involves a sophisticated pathogen–host cross talk.

As *L. pneumophila* naturally grows in protozoa, and *D. discoideum* is a well-studied model organism, its use as host model for *L. pneumophila* is obvious. Important applications of this haploid model organism are the use of *Dictyostelium* cells as screening system for evaluating bacterial virulence, the use of *Dictyostelium* mutant cells to identify genetic host determinants of susceptibility and resistance, and experiments which allow the dissection of the complex cross talk with infectious agents. In this review we will focus on the transcriptional host cell response upon infection with *L. pneumophila*.

The genome reflects the history and lifestyle of *Legionella*

Deciphering a genome is a valuable basis for the understanding of an organism. Furthermore, genomic comparisons reveal genetic differences that are important for the host range, pathogenic lifestyle and certain disease manifestations. Although the genomics era started late in *Legioenlla* research, we are now in the advantageous situation to have the complete genome sequence of three different *L. pneumophila* isolates available (Cazalet et al., 2004; Chien et al., 2004). One is *L. pneumophila* ssp. *pneumophila* Philadelphia (*L. pneumophila* Philadelphia), an isolate from the first reported outbreak of the disease in 1976 (Chien et al., 2004). The others are *L. pneumophila* strain Paris, an epidemic and endemic strain isolated in France, and *L. pneumophila* strain Lens, an isolate responsible for a large outbreak in France (Cazalet et al., 2004). The genomes of this subspecies consist of 3.4–3.5 mega base pairs (mbp) with a composition of 38% G and C nucleotides in average. Of the total gene complement roughly 60% have detectable similarities to genes in other fully sequenced species. Thus, 40% of the genes seem to be species-specific or at least genus-specific (see below).

As many other bacterial genomes the *L. pneumophila* genomes are subject to horizontal gene transfer. Around 2.4% of the genome are phage derived or are insertion sequences. Those sequences contribute to the plasticity to the genome via rearrangements but it is not known yet, whether they also add new functions to *L. pneumophila*. In contrast, certain protein-encoding genes with obvious foreign ancestry like the *ralF* gene (Nagai and Roy, 2003), which is probably derived from a eukaryotic host, clearly added new functions to *Legionella* as it was shown to be important in establishing the *Legionella*-specific vacuole. Furthermore, genome analysis identified also larger regions or 'islands' in the genome sequence, which partly exhibit a deviating G + C content from the mean value of the whole genome. One of these regions encodes a type IV secretion system named *lvh* (see below). A further region was suggested to have been acquired from a foreign source by the fact that it is surrounded by phage-related sequences and transposases. In addition, this region has a mosaic structure when compared among the three completely sequenced genomes (Cazalet et al., 2007). This region encodes efflux transporters, which might thus contribute to *L. pneumophila* fitness in adverse environments. Comparison with the genome of Q fever-causing *Coxiella*