



The Flagellar Regulon of *Legionella* – A Review

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The *Legionella* genus comprises more than 60 species. In particular, *Legionella pneumophila* is known to cause severe illnesses in humans. Legionellaceae are ubiquitous inhabitants of aquatic environments. Some Legionellaceae are motile and their motility is important to move around in habitats. Motility can be considered as a potential virulence factor as already shown for various human pathogens. The genes of the flagellar system, regulator and structural genes, are structured in hierarchical levels described as the flagellar regulon. Their expression is modulated by various environmental factors. For *L. pneumophila* it was shown that the expression of genes of the flagellar regulon is modulated by the actual growth phase and temperature. Especially, flagellated *Legionella* are known to express genes during the transmissive phase of growth that are involved in the expression of virulence traits. It has been demonstrated that the alternative sigma-28 factor is part of the link between virulence expression and motility. In the following review, the structure of the flagellar regulon of *L. pneumophila* is discussed and compared to other flagellar systems of different *Legionella* species. Recently, it has been described that *Legionella micdadei* and *Legionella fallonii* contain a second putative partial flagellar system. Hence, the report will focus on flagellated and non-flagellated *Legionella* strains, phylogenetic relationships, the role and function of the alternative sigma factor (FliA) and its anti-sigma-28 factor (FlgM).

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Received: 27 June 2017

Accepted: 06 October 2017

Published: 20 October 2017

Citation:

Appelt S and Heuner K (2017) The
Flagellar Regulon of *Legionella* – A
Review.
Front. Cell. Infect. Microbiol. 7:454.
doi: 10.3389/fcimb.2017.00454

Keywords: *Legionella*, flagellar regulon, flagellin, virulence, FliA, FleQ, alternative sigma factor

INTRODUCTION AND OVERVIEW

The Legionellaceae family consists of a single genus: *Legionella* that comprises more than 60 species so far (Gomez-Valero et al., 2009; Bajrai et al., 2016; Khodr et al., 2016). New species are identified continuously (i.e., *Legionella drancourtii*, *Legionella gresilensis*, and *Legionella beliardensis*), extending the list of known *Legionella* species (Lo Presti et al., 2001; La Scola et al., 2004; Gomez-Valero et al., 2009; Rizzardi et al., 2015; Bajrai et al., 2016; Khodr et al., 2016). More than 20 pathogenic *Legionella* species are known today that differ in their ability to infect hosts and to cause severe to mild diseases in humans (Rizzardi et al., 2015). Human pathogens known to cause the Legionnaires' disease—an atypical pneumonia—are for instance *Legionella pneumophila*, *Legionella micdadei*, and *Legionella longbeachae* (Yu et al., 2002; Whiley and Bentham, 2011). *Legionella* known to cause the Pontiac fever—a mild flu-like disease—are for instance *Legionella feelei*, *L. micdadei* and *Legionella anisa*, but also *L. pneumophila*. Often *Legionella* strains of the same species and same serogroup cause one of the mentioned diseases (Swanson and Hammer, 2000; Fields et al., 2002; Wang et al., 2015).

Nevertheless, it was assumed that humans are accidental hosts of *Legionella* species within which the bacterium replicates (Horwitz and Silverstein, 1980; Cianciotto et al., 1989; Horwitz, 1992; Fields, 1996; Neumeister et al., 1997; Newton et al., 2010). Known natural hosts are protozoa, especially free-living amoebae: *Acanthamoeba* spp., *Naegleria* spp., or *Hartmannella vermiformis* (Barbaree et al., 1986; Rowbotham, 1986; Fields, 1996; Atlas, 1999; Fields et al., 2002; Greub and Raoult, 2004; Abdel-Nour et al., 2013; Richards et al., 2013; Cateau et al., 2014). Accordingly, in general, *Legionella* species are prevalent inhabitants of soil, mud and above all of aquatic environments (Fliermans et al., 1981; Fields, 1996; Atlas, 1999; Gomez-Valero et al., 2009; Declerck, 2010; Schalk et al., 2014; Currie and Beattie, 2015). The ability of *L. pneumophila* to grow within biofilms made by *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* in aquatic or wet environments raised questions about their host-free persistence (Stewart et al., 2012). In connection with favorable aquatic habitats and potential protozoa hosts, especially flagella-driven motility of some *Legionella* spp. is an important feature needed to move around, to find new hosts and to form maybe even biofilms (Kirov et al., 2004; Danhorn and Fuqua, 2007; Heuner and Albert-Weissenberger, 2008). For *Legionella* infecting humans, motility may also be crucial for spreading within lungs of patients, as flagellated forms of *L. pneumophila* were detected in alveolar spaces (Chandler et al., 1980; Jager et al., 2014). Recently, it was published that *L. feelei* strains that cause Legionnaires' disease are flagellated while *L. feelei* strains that cause the Pontiac fever are non-flagellated (Wang et al., 2015). The majority of *Legionella* species are flagellated (Elliott and Johnson, 1981; Bornstein et al., 1991; Bangsberg et al., 1995; Heuner et al., 1995), but not all pathogenic *Legionella* have a complete flagellar regulon (i.e., *L. longbeachae* and *Legionella oakridgensis*, see below) (Orrison et al., 1983; Heuner et al., 1995; Cazalet et al., 2010; Kozak et al., 2010; Brzuszkiewicz et al., 2013).

The assumption that the expression of flagella and virulence are linked was already made at an early stage (Rowbotham, 1986) and later on confirmed. It was shown that there is a regulatory link between the expression of a virulent phenotype and the flagellum (Pruckler et al., 1995; Byrne and Swanson, 1998; Hammer et al., 2002; Gal-Mor and Segal, 2003; Molofsky et al., 2005; Heuner and Albert-Weissenberger, 2008; Albert-Weissenberger et al., 2010; Schulz et al., 2012). The expression of flagellar genes is regulated on the flagellar regulon, extensively investigated in *L. pneumophila* due to its biphasic intracellular life cycle during which the bacterium undergoes a shape change. Within the host, after replication inside of *Legionella*-containing vacuoles (LCVs), when nutrients become limited, *L. pneumophila* differentiates into a flagellated, non-replicating form. The flagellated, transmissible, mature form is stress-resistant, virulent and metabolically resting as well as infectious (abbr. MIF) (Rowbotham, 1986; Byrne and Swanson, 1998; Heuner et al., 1999; Swanson and Hammer, 2000; Faulkner and Garduno, 2002; Garduno et al., 2002; Hammer et al., 2002; Molofsky and Swanson, 2004; Fonseca and Swanson, 2014; Eisenreich and Heuner, 2016). The actual release process of mature forms is still under discussion: either the bacteria are released from the LCV into the environment by lysis of the host

or the bacteria are released first into the cytosol of the host and then after an additional putative round of replication into the environment (Rowbotham, 1986; Molmeret et al., 2004). The latter hypothesis implies that the flagellum is produced inside the cytosol of the host and not in LCVs as proposed earlier. Furthermore, there is also a possibility that the bacteria are released by the host *via* a non-lytic mechanism (Chen et al., 2004; Bouyer et al., 2007; Berk et al., 2008). However, the released form is well-prepared to reinfect new hosts or to differentiate into a viable-but-nonculturable form (VBNC) meant to enable a long-term survival of the bacteria (Rowbotham, 1986; Steinert et al., 1997; Ohno et al., 2003; Molmeret et al., 2010; Al-Bana et al., 2014). VBNC forms can be resuscitated when they are taken up by amoebae (Steinert et al., 1997; Ohno et al., 2003; Al-Bana et al., 2014). Further, different morphological forms of *L. pneumophila* have been recently discussed (Robertson et al., 2014). Next to the biphasic intracellular life cycle it was shown that *L. pneumophila* exhibits also a life stage-specific bipartite metabolism, an area for further investigations (Schunder et al., 2014; Eisenreich and Heuner, 2016; Gillmaier et al., 2016; Hauslein et al., 2016).

MOTILITY OF *LEGIONELLA*

Different forms of bacterial motility are known including swarming, twitching and sliding. Notably the flagellum—next to pili—allows bacteria to move. Bacterial motility is often related to chemotactic behavior that enables a bacterium to locate special environmental conditions and to get closer to higher concentrations of attractants (Szurmant and Ordal, 2004; Hazelbauer et al., 2008; Micali and Endres, 2016). Some *Legionella* have a chemotaxis system (*L. longbeachae*, *Legionella parisiensis*, and *Legionella bozemanii*) but most *Legionella* do not have the corresponding genes (e.g., *L. pneumophila*, *L. micdadei*, and *L. oakridgensis*). Moreover, the ability of *Legionella* to swarm and to show a chemotaxis behavior has not yet been reported.

Twitching motility is based on a functional type IV pilus. The ability to move forward by twitching has been reported for *L. pneumophila* (Coil and Anne, 2009; Hoppe et al., 2017). In addition, sliding motility, a surfactant-mediated motility, has been described for *L. pneumophila* (Stewart et al., 2009).

THE FLAGELLUM AND THE FLAGELLAR REGULON

The Structure of the Flagellum and Flagellar Systems

Most *Legionella* species are motile due to a single polar flagellum (**Figure 1**) (Chandler et al., 1980; Elliott and Johnson, 1982; Heuner et al., 1995). More than 50 genes are involved in the expression of functional flagella, and due to high metabolic costs, a tight regulation is essential (Chilcott and Hughes, 2000; McCarter, 2006; Osterman et al., 2015). The flagellum of *Legionella* consists of a basal body, a hook structure and a filament (**Figure 2**; Heuner and Steinert, 2003; Heuner and Albert-Weissenberger, 2008). For the assembly of the flagellum, needed proteins (hook, rod and the filament forming proteins)

are exported out of the cell by a flagellum-specific export apparatus, a type III-like secretion system (T3SS) (Heuner and Albert-Weissenberger, 2008; Altegoer and Bange, 2015).

The basal body consists of a rod, three rings [“membrane/supramembrane” (MS), “peptidoglycan” (P), and “lipopolysaccharide” (L)] and a motor switch complex (MotAB). The MotAB is providing the energy for the rotation of the flagellum (Minamino and Imada, 2015). For the formation of the slightly curved hook structure, FlgE and FlgD are essential. The hook cap protein FlgD assists when FlgE is incorporated into the hook structure (Altegoer and Bange, 2015).

Interestingly, an uncommonly straight hook has been reported for *L. pneumophila* mutant strains ($\Delta fliA$, $\Delta fliD$, and $\Delta flaA$, i.e., a flagellin mutant) (Figure 1) (Schulz et al., 2012). The findings give credit to the assumption that *L. pneumophila* might have a straight hook that is hard to detect in wildtype strains. The filament consists mainly out of a single protein, the flagellin (FlaA or FliC) (Figure 2). The cap protein FliD is essential for the assembly of flagellin subunits into the filament. To assemble the filament, flagellin is exported through the filament structure by a flagellum-specific export apparatus (T3SS) and assembled at the tip of the filament (Heuner and Albert-Weissenberger, 2008; Altegoer and Bange, 2015).

More details about the flagellum structure can be found in dedicated review articles (Aldridge and Hughes, 2002; Heuner and Steinert, 2003; Macnab, 2003; Pallen et al., 2005; Heuner and Albert-Weissenberger, 2008; Altegoer and Bange, 2015).

Next to the regular flagellar system, a second putative flagellar system was suspected for two *Legionella* species: *Legionella fallonii* and *L. micdadei* (Gomez-Valero et al., 2014). Comparative genome analysis led to the suspicion that the strains do have homologs to flagellar genes of *L. pneumophila*. The identified genetic region is comprised of genes that encode a putative basal body, a secretion system, as well as a putative hook structure. No homologs to *flaA* or *fliD* were found in the predicted genomic region. Yet, further investigations are needed to find out if a T3SS or a putative second flagellum is encoded. Additionally, *in silico* investigation, performed on the draft genome sequence of *Legionella israelensis*, identified a similar operon. A BLAST search using the operon (10,041 bp, ctg_064, *L. israelensis* draft-genome; Burstein et al., 2016) as query identified similar genes in *L. drancourtii*, *L. fallonii*, *L. worsleiensis*, *L. quateirensis*, *L. birminghamensis*, and *L. drozanskii* (Heuner, unpublished results). Initial findings show that the operon is present in two out of three *Legionella* clades (Figure 3), leaving a margin for additional studies.

The Flagellar Regulon

The expression of flagellar genes of *L. pneumophila* is regulated in a hierarchical cascade (Figure 4) (Heuner et al., 1995, 2006; Heuner and Steinert, 2003; Jacobi et al., 2004; Albert-Weissenberger et al., 2010; Schulz et al., 2012). Their expression depends on growth phase, temperature, medium viscosity and nutrient availability (e.g., amino acids and fatty acids) (Ott et al., 1991; Byrne and Swanson, 1998; Heuner et al., 1999, 2006; Heuner and Albert-Weissenberger, 2008).

In short, intracellular alarmone accumulation—ppGpp, a signal molecule, produced when environmental conditions are unfavorable (e.g., limited nutrient supply)—is regulated by RelA and SpoT. RelA senses the amount of available intracellular amino acids and SpoT senses the amount of intracellular fatty acids (Hammer and Swanson, 1999; Dalebroux et al., 2009, 2010).

Alarmone accumulation triggers the activation of an alternative stationary-phase sigma factor (RpoS) and of the two component system LetA/S (Byrne and Swanson, 1998; Hammer et al., 2002; Zusman et al., 2002; Molofsky and Swanson, 2004; Dalebroux et al., 2009, 2010; Edwards et al., 2009; Rasis and Segal, 2009; Sahr et al., 2009) (Figure 4). RpoS and LetA/S promote the transcription of small regulatory RNAs (RsmX, RsmY, RsmZ). RsmX plays a role in the virulence of *L. pneumophila* (Sahr et al., 2012). The transcription of *rsmZ/rmsY* is also influenced by a quorum sensing system regulator, LqsR (Tiaden et al., 2007; Schell et al., 2016). The two regulatory RNAs are able to bind a number of carbon storage regulator molecules (CsrA) at once (Sahr et al., 2017). CsrA is a negative regulator and through the binding on RsmY or RsmZ, other targets of the regulatory RNAs can be expressed. The expression of transmissive traits starts and main activator proteins (e.g., FleQ) are produced and flagellar genes are expressed (Zusman et al., 2002; Molofsky and Swanson, 2003; Rasis and Segal, 2009; Sahr et al., 2009, 2017; Albert-Weissenberger et al., 2010). Notably, some parts of the function of the negative regulator CsrA (*flaA* expression and motility of *L. pneumophila*) can be “complemented” by ectopically expressed *csrT*, a CsrA-like regulatory gene associated with integrative conjugative elements (Abbott et al., 2015).

CsrA is also controlling a major regulator involved in the expression of flagella, FleQ (Sahr et al., 2017). FleQ is responsible for the expression of early flagellar genes belonging to class II and III genes in an RpoN-dependent and RpoN-independent pathway (Jacobi et al., 2004; Albert-Weissenberger et al., 2010; Schulz et al., 2012). RpoN is an enhancer-binding protein encoding an alternative sigma factor that initiates transcription when activator proteins like FleQ, FleR, and PilR (Jacobi et al., 2004) are present. When class II and III genes are expressed, the activity of FliA—the alternative sigma factor—leads to the expression of class IV genes and the assembly of the flagellum (Figure 4). RpoN and FleR seem to be responsible for a negative feedback loop on flagellar genes (Albert-Weissenberger et al., 2010). It was found that RpoS and FlaR (transcriptional regulator FlaR, LysR family member) are also involved in the expression of the flagellin gene (Heuner et al., 2000; Bachman and Swanson, 2001, 2004; Rasis and Segal, 2009; Sahr et al., 2009). The production of FlaA is also regulated by cyclic di-GMP, shown by the analysis of a gene (*cdgS13*) coding for a protein with diguanylate cyclase activity (Levi et al., 2011). The influence of cyclic di-GMP on flagellum-based motility has been shown for other bacteria than *Legionella* species (Wolfe and Visick, 2008).

THE FLAGELLUM AND VIRULENCE

Already early on, it has been hypothesized that virulence and flagellum expression are genetically linked with each other.

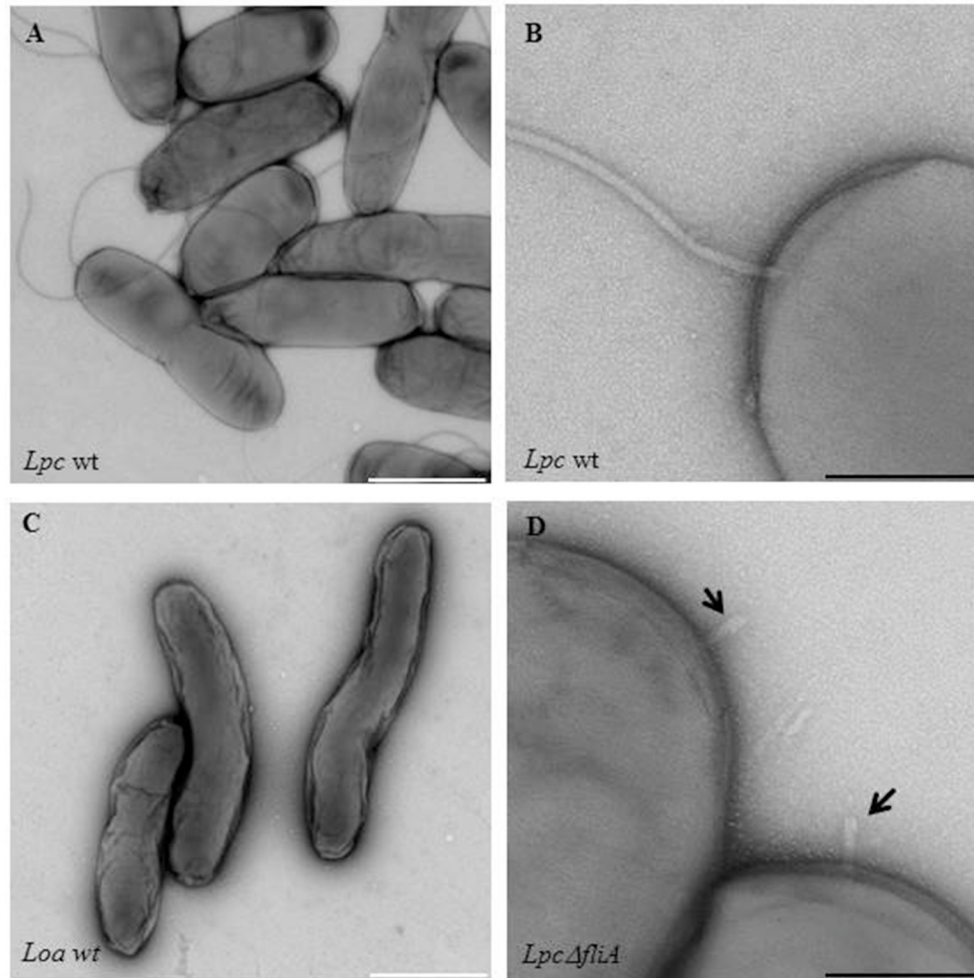
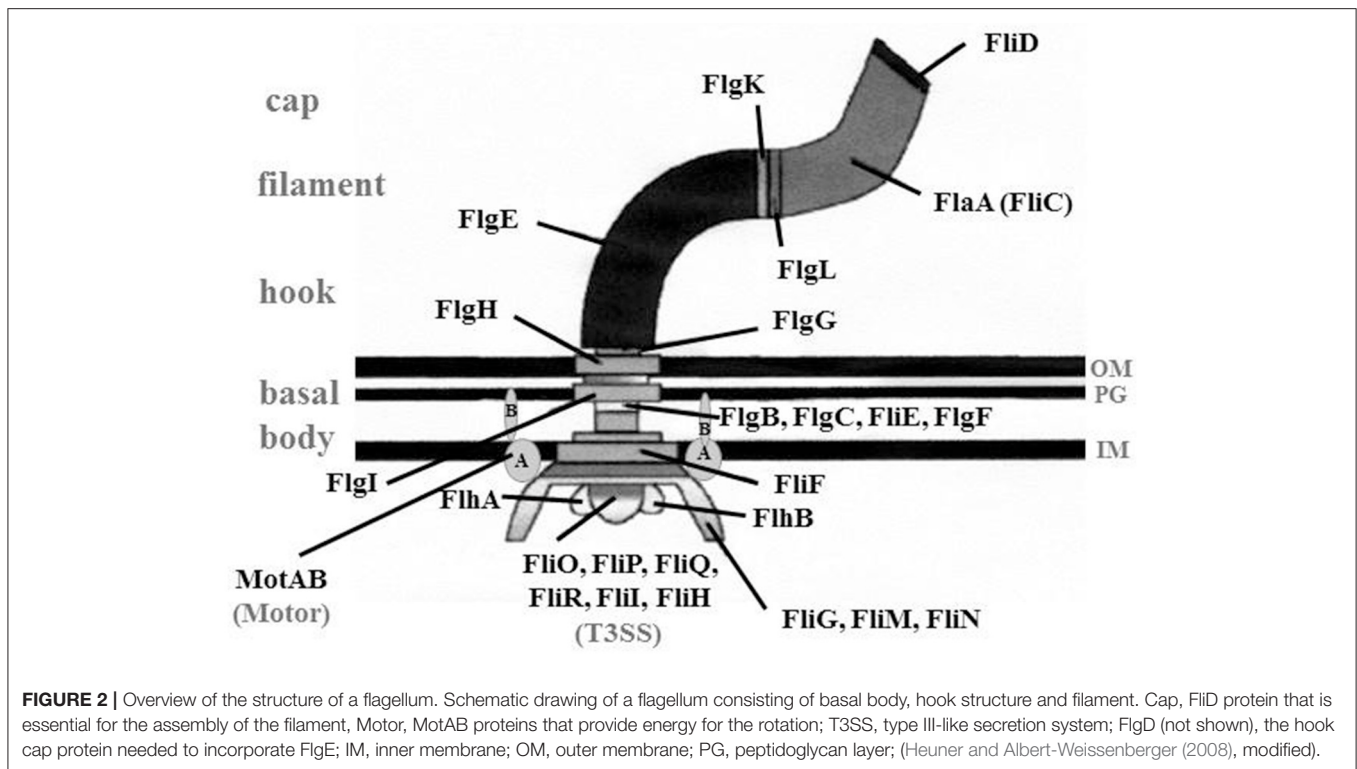


FIGURE 1 | Electron microscopic images of flagellated and non-flagellated *Legionella* wild-type strains as a flagella mutant strain. Shown are **(A,B)** *Legionella pneumophila* Corby (Lpc wt) and **(C)** *Legionella oakridgensis* (Loa wt) wild-type strains that are either representative of flagellated or non-flagellated *Legionella*. An electron microscopic image of a *fliA* *L. pneumophila* mutant strain (Lpc Δ *fliA*), is also shown **(D)**. The straight hook structure of the mutant strain is indicated by black arrows **(D)**. Bacteria were grown in AYE medium at 30°C and the samples were stained with 0.5% uranyl acetate. White scale bars: 1 μ m; black scale bars: 200 nm.

It has been shown that motile, transmissible *Legionella* were more infectious for amoebae than non-motile replicative phase *Legionella* (Rowbotham, 1986; Pruckler et al., 1995; Bosshardt et al., 1997; Byrne and Swanson, 1998; Hammer et al., 2002; Heuner et al., 2002; Molofsky et al., 2005; Heuner and Albert-Weissenberger, 2008). Different experiments could show that the motility but not the flagellin promotes the contact with host cells. Motility increases the infectivity and the fitness. Furthermore, it turned out that the flagellum is not necessary for intracellular replication (Pruckler et al., 1995; Dietrich et al., 2001; Polesky et al., 2001; Heuner et al., 2002; Jacobi et al., 2004; Molofsky et al., 2005; Schulz et al., 2012).

The four major regulators of the flagellar regulon (RpoN, FleQ, FleSR, FliA) seems to be involved in the invasion process of *L. pneumophila* into hosts. These findings point out the proposed link between virulence traits and flagellum expression (Dietrich et al., 2001; Hammer et al., 2002; Molofsky et al., 2005; Heuner

and Albert-Weissenberger, 2008; Albert-Weissenberger et al., 2010; Schulz et al., 2012). Especially the FliA regulon plays an important role (please, see the section: FliA and its implication in virulence below). *FliA*, but not the flagellin (*fliA*), is involved into the ability of *L. pneumophila* to form biofilm that allow bacteria to survive whenever environmental conditions are not favorable. (Mampel et al., 2006). Notably unwanted biofilms are a health issue causing a significant amount of nosocomial infections (Bryers, 2008). *Legionella* are known to survive within biofilm of other bacteria (e.g., *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) (Molofsky et al., 2005; Stewart et al., 2012). More recently, findings about *L. pneumophila*'s ability to form biofilms by itself in natural environments and on medical devices have attracted attention (Lau and Ashbolt, 2009; Abu Khweek et al., 2013). Biofilm formation is regulated by temperature, surface material and intracellular growth (Konishi et al., 2006; Piao et al., 2006; Bigot et al., 2013) and biofilm-derived *L. pneumophila* do



not express flagellin (Abu Khweek et al., 2013). More information about biofilms and *L. pneumophila* can be found in a recent review (Abdel-Nour et al., 2013).

The flagellum also affects the resistance of hosts to Legionnaires' disease and when *Legionella* do not produce flagellin they can evade the innate immune response in macrophages (Hawn et al., 2003; Molofsky et al., 2006; Ren et al., 2006; Abu Khweek et al., 2013). Resistance is mediated by the Naip5/Ipaf-dependent recognition of flagellin, which induces a protective immunity in non-A/J mouse models (Ricci et al., 2005). Detailed information about addressed points can be found in dedicated reviews (Fontana and Vance, 2011; Schell et al., 2016; Mascarenhas and Zamboni, 2017).

THE ALTERNATIVE SIGMA FACTOR 28

FliA Expression

One of the major regulators involved in the expression of the flagellum is FliA and an increased alarmone level leads to accumulation of functional FliA (Bruggemann et al., 2006; Heuner et al., 2006; Dalebroux et al., 2010). The alternative sigma factor (σ^{28}) is directly involved in the regulation and expression of the flagellin gene (*flaA*) and others (Figure 4). A *fliA* mutant of *L. pneumophila* does not produce flagellin and is consequently non-flagellated. Moreover, a Δ *fliA* mutant of *Escherichia coli* can be complemented with a *fliA* gene of *L. pneumophila* (Heuner et al., 1995, 1997, 2002; Bruggemann et al., 2006; Albert-Weissenberger et al., 2010; Schulz et al., 2012).

The expression of flagellar class III and IV genes is induced in a FleQ-dependent manner. The FliA-regulated class IV genes are involved in the assembly of the filament and flagella motility (*flgL*, *fliD*, *flaA*, *motY*). Both lead to the complete synthesis of the flagellum (Jacobi et al., 2004; Albert-Weissenberger et al., 2010). The *fliA* gene itself is expressed in a FleQ-dependent but RpoN-independent manner (Albert-Weissenberger et al., 2010). Nevertheless, FleQ and RpoN are not necessary for a basal expression of *fliA*. For a basal expression, *fliA* is transcribed from a putative sigma-70 promoter element and later, during the exponential phase, the expression of *fliA* is induced in a FleQ-dependent manner (Schulz et al., 2012). Accordingly, it was hypothesized that during the exponential phase the basal *fliA* promoter activity may be mediated by DksA independent of the ppGpp concentration, whereas during the post-exponential phase DksA cooperates with ppGpp to activate *fliA* (Dalebroux et al., 2010). The identification of the transcription start point of *fliA* corroborates the presence of a putative DksA binding site, an A/T rich discriminator site (Schulz et al., 2012).

FliA and Its Implication in Virulence

FliA is a regulator that is also involved in the expression of putative virulence genes (Bruggemann et al., 2006; Albert-Weissenberger et al., 2010; Tlapak et al., 2017).

Several investigations performed on a *fliA* mutant strain of *L. pneumophila* pointed out that the mutant (at low MOI) is not replicating in host cells anymore (*Dictyostelium discoideum*). The *fliA* mutant seems to be less infectious for macrophages and non-cytotoxic to bone marrow-derived macrophages. Moreover, the mutant has a reduced fitness

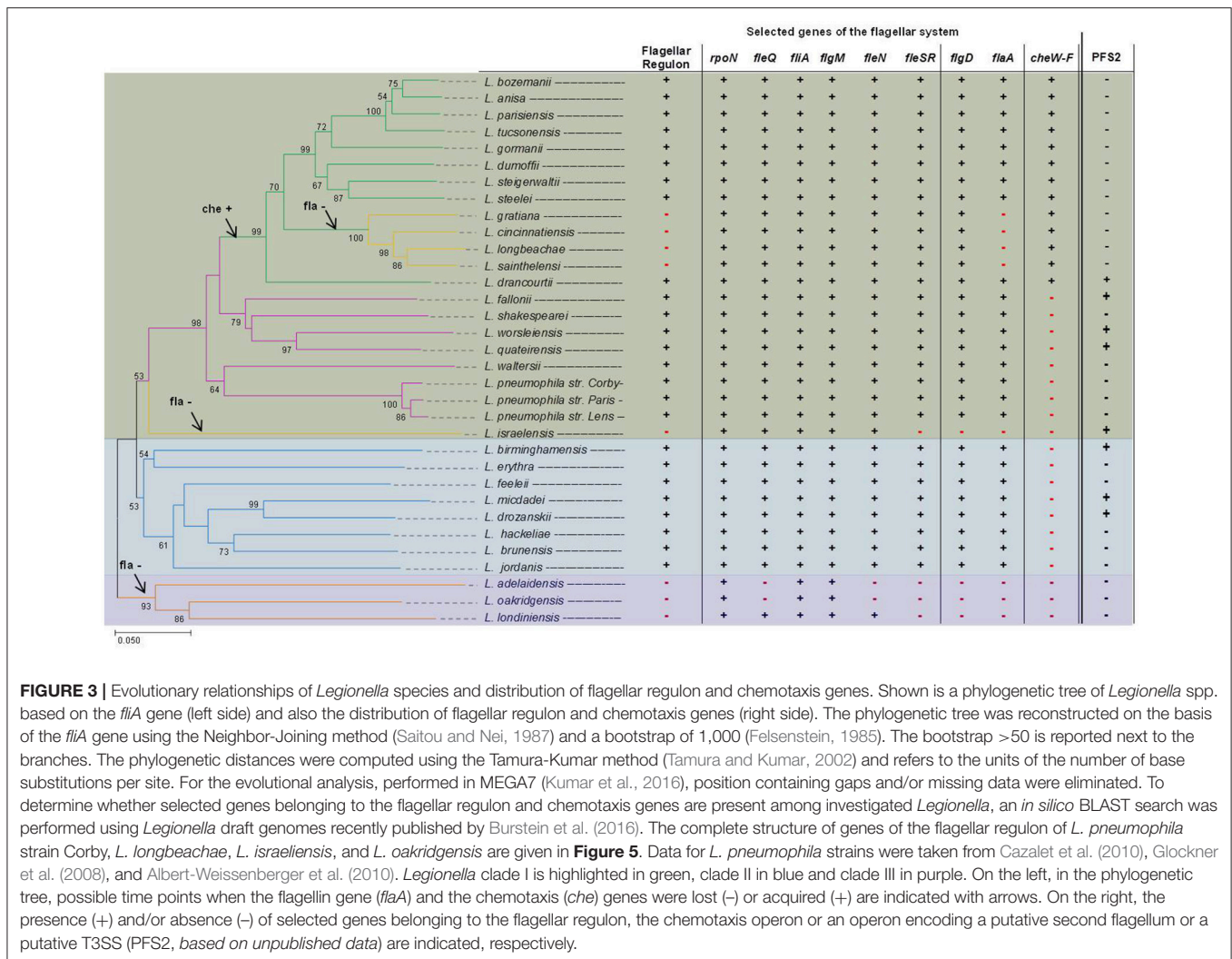


FIGURE 3 | Evolutionary relationships of *Legionella* species and distribution of flagellar regulon and chemotaxis genes. Shown is a phylogenetic tree of *Legionella* spp. based on the *fliA* gene (left side) and also the distribution of flagellar regulon and chemotaxis genes (right side). The phylogenetic tree was reconstructed on the basis of the *fliA* gene using the Neighbor-Joining method (Saitou and Nei, 1987) and a bootstrap of 1,000 (Felsenstein, 1985). The bootstrap >50 is reported next to the branches. The phylogenetic distances were computed using the Tamura-Kumar method (Tamura and Kumar, 2002) and refers to the units of the number of base substitutions per site. For the evolutionary analysis, performed in MEGA7 (Kumar et al., 2016), position containing gaps and/or missing data were eliminated. To determine whether selected genes belonging to the flagellar regulon and chemotaxis genes are present among investigated *Legionella*, an *in silico* BLAST search was performed using *Legionella* draft genomes recently published by Burstein et al. (2016). The complete structure of genes of the flagellar regulon of *L. pneumophila* strain Corby, *L. longbeachae*, *L. israeliensis*, and *L. oakridgensis* are given in **Figure 5**. Data for *L. pneumophila* strains were taken from Cazalet et al. (2010), Glockner et al. (2008), and Albert-Weissenberger et al. (2010). *Legionella* clade I is highlighted in green, clade II in blue and clade III in purple. On the left, in the phylogenetic tree, possible time points when the flagellin gene (*fliA*) and the chemotaxis (*che*) genes were lost (-) or acquired (+) are indicated with arrows. On the right, the presence (+) and/or absence (-) of selected genes belonging to the flagellar regulon, the chemotaxis operon or an operon encoding a putative second flagellum or a putative T3SS (PFS2, based on unpublished data) are indicated, respectively.

potential in amoebae (Dietrich et al., 2001; Hammer et al., 2002; Heuner et al., 2002; Jacobi et al., 2004; Molofsky et al., 2005; Heuner and Albert-Weissenberger, 2008; Schulz et al., 2012). Likewise, a *fliA* mutant strain of *L. oakridgensis* showed a reduced fitness in its host (*Acanthamoeba lenticulata*) (Tlapak et al., 2017). In addition, another *L. pneumophila* *fliA* mutant strain exhibited a reduced ability to form biofilms (Mampel et al., 2006).

FliA is obviously a virulence factor, and target genes of *fliA* were investigated to understand its implication for virulence (Bruggemann et al., 2006; Albert-Weissenberger et al., 2010; Tlapak et al., 2017). Target genes of *fliA* in *L. pneumophila* strains include genes of the flagellar regulon (e.g., *fliA* and *flgM*) and others (e.g., *enhA* and *lvrA*), illustrated in **Figure 4** and listed in **Table 1** (Bruggemann et al., 2006; Albert-Weissenberger et al., 2010; Schulz et al., 2012). Other identified FliA-dependent genes encode for putative virulence factors corroborating the involvement of FliA in the establishment of *Legionella* infections. Identified putative virulence factors are: *lpp0952*, *lpp1290*, and *lpp0972*. The first one, *lpp0952*, is coding for a GGDEF/EAL

and PAS/PAC domain protein (Bruggemann et al., 2006; Albert-Weissenberger et al., 2010). The *two* remaining genes are homologs of the enhanced entry proteins EnhA. Respective homologs were also found in *L. longbeachae* which is non-flagellated, putatively associated with the flagellar system (Kozak et al., 2010).

Recently, especially *L. oakridgensis* simplified the identification of *fliA* targets potentially involved in virulence (Tlapak et al., 2017). *L. oakridgensis* strains are non-flagellated, the entire flagellar regulon is missing and only homologs of FliA and FlgM are present (**Figure 5**). *L. oakridgensis* is less infectious than *L. pneumophila*, but still causes Legionnaires' disease. In addition, *L. oakridgensis* replicates in guinea pigs, in human cell lines, in *Acanthamoeba lenticulata* and for growth in media no additional cysteine is needed. (Orrison et al., 1983; Fields et al., 1986; O'Connell et al., 1996; Neumeister et al., 1997; Lo Presti et al., 2001; Brzuszkiewicz et al., 2013). Nevertheless, *L. oakridgensis* exhibits a functional T4SS, homologs of known virulence factors, as well as newly identified virulence factors (Brzuszkiewicz et al., 2013). *L. oakridgensis* is used to investigate

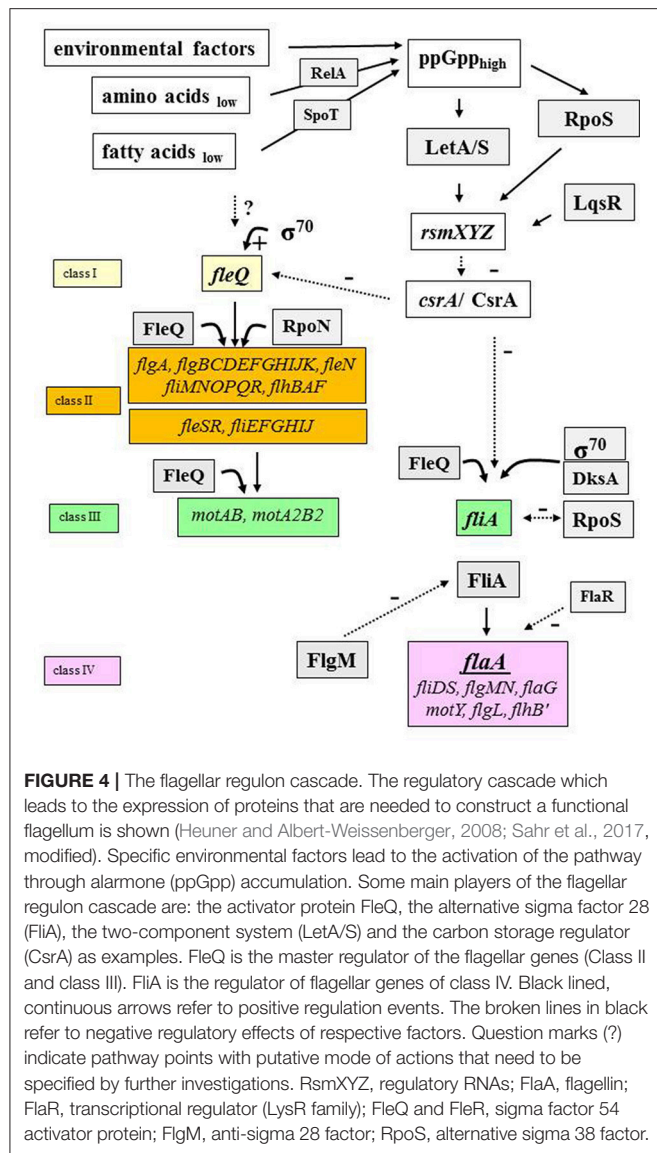


FIGURE 4 | The flagellar regulon cascade. The regulatory cascade which leads to the expression of proteins that are needed to construct a functional flagellum is shown (Heuner and Albert-Weissenberger, 2008; Sahr et al., 2017, modified). Specific environmental factors lead to the activation of the pathway through alarmonone (ppGpp) accumulation. Some main players of the flagellar regulon cascade are: the activator protein FleQ, the alternative sigma factor 28 (FliA), the two-component system (LetA/S) and the carbon storage regulator (CsrA) as examples. FleQ is the master regulator of the flagellar genes (Class II and class III). FliA is the regulator of flagellar genes of class IV. Black lined, continuous arrows refer to positive regulation events. The broken lines in black refer to negative regulatory effects of respective factors. Question marks (?) indicate pathway points with putative mode of actions that need to be specified by further investigations. RsmXYZ, regulatory RNAs; FlaA, flagellin; FlaR, transcriptional regulator (LysR family); FleQ and FleR, sigma factor 54 activator protein; FlgM, anti-sigma 28 factor; RpoS, alternative sigma 38 factor.

FliA since a *fliA* *L. oakridgensis* knockout will not cause the inactivation of the entire flagellar regulon and target genes of FliA can still be identified as well as genes involved in the expression of virulence traits. However, mutant strain analyses aimed at identifying target genes of FliA in *L. oakridgensis* yielded no results for putative FliA-dependent virulence genes yet (Table 1; Tlapak et al., 2017). Additional investigations are needed including phenotypic characterizations and deletion analyses of further target genes of FliA.

FliA-FlgM Interaction in *Legionella oakridgensis*

Flagellated bacteria regulate the FliA activity often post-transcriptionally. For *Salmonella*, *Escherichia*, and *Vibrio* species it is known that an anti-sigma-28 factor (FlgM) binds FliA, preventing the binding of FliA to FliA-dependent promoter sites, and FliA-dependent genes are consequently not translated. After

assembling of the hook-basal body structure, FlgM is exported and FliA is not repressed anymore (Gillen and Hughes, 1991; Ohnishi et al., 1992; Chilcott and Hughes, 2000; Aldridge et al., 2006). In *Helicobacter pylori*, the FlgM protein is inactivated instead of being exported out of the cells (Rust et al., 2009). FliA-FlgM interactions in *Legionella* are still unknown. Recent findings suggest that FliA-FlgM interaction might be different than in other flagellated bacteria, at least for *L. oakridgensis*. Respective species do not have a flagellum (Figure 1C), a flagellar regulon (Figure 5) and a basal body although *flgM* and *fliA* homologs are present which encode for functional FlgM and FliA proteins (Brzuszkiewicz et al., 2013; Tlapak et al., 2017); consequently, the mechanism controlling FliA-FlgM interactions must be different.

For *L. pneumophila* as well as for *L. oakridgensis* it was found that the expression of FlgM or homologs is sigma-28-dependent (Albert-Weissenberger et al., 2010; Tlapak et al., 2017). Although *L. oakridgensis* has no flagellar system, the expression of *fliA* dependent genes is growth phase- and temperature-dependent (Heuner et al., 1999; Tlapak et al., 2017). Moreover, for *L. oakridgensis* it was demonstrated that FlgM is a negative regulator of FliA-dependent genes and the protein itself seems to be degraded in a growth phase- and temperature-dependent manner (Tlapak et al., 2017). Thus, it seems likely that, as described for *H. pylori*, FlgM in *L. oakridgensis* is degraded by protease activity instead of being secreted. However, investigations are needed to show if FlgM in *L. pneumophila* is effectively secreted in a basal body-dependent manner.

DISTRIBUTION OF THE FLAGELLAR SYSTEM AMONG *LEGIONELLA* SPECIES

Phylogenetic reconstruction—based on concatenated amino acid alignment of 78 orthologous ORFs—divided the *Legionella* species into three major clades (clade I and clade II, clade III) (Burstein et al., 2016). Clade I is comprised of most *Legionella* species including *L. pneumophila*, *L. parisiensis*, *L. bozemanii*, and *L. longbeachae* (Burstein et al., 2016). Clade II comprises among others *L. feelei* and *L. micdadei* and clade III, a deep-branching clade, includes three members: *Legionella adelaidensis*, *L. oakridgensis* and *Legionella londiniensis* (Burstein et al., 2016). Phylogenetic reconstructions performed herein, yielded similar results that are given in Figure 3. As opposed to former investigations, the phylogenetic tree was reconstructed on the basis of the *fliA* gene.

The flagellar system can be found in *Legionella* species classified as clade I or II (Cazalet et al., 2004, 2010; Chen et al., 2004; Bruggemann et al., 2006; Glockner et al., 2008; Kozak et al., 2010; Brzuszkiewicz et al., 2013; Gomez-Valero et al., 2014; Burstein et al., 2016), but not in clade III *Legionella*. Clade III *Legionella* do not have a functional flagellar system and do not have most of the flagellar regulon genes, except for *fliA* and its anti-sigma factor *flgM* (*L. oakridgensis*, *L. adelaidensis*, and *L. londiniensis*) (Cazalet et al., 2010; Brzuszkiewicz et al., 2013; Tlapak et al., 2017) and two additional genes: *fleQ* and *fleN* (*L. londiniensis*) (Figures 3, 5). Also, some clade I *Legionella* species do not have a functional flagellar system

TABLE 1 | Genes belonging to the FliA regulon (FliA target genes) of *L. pneumophila* Paris (Lpp)*.

Gene name	Annotation	FC
§ <i>lpp1294</i> , <i>fliA</i>	Flagelline	0.003
<i>lpp1293</i> , <i>fliG</i>	Unknown	0.007
§ <i>lpp0972</i>	Similar to enhanced entry protein EnhA	0.010
#§ <i>lpp2282</i>	Unknown	0.024
<i>lpp1746</i> , <i>fliA</i>	Sigma factor 28	0.042
#§ <i>lpp2998</i>	Similar to conserved hypothetical protein	0.045
§ <i>lpp1292</i> , <i>fliD</i>	Flagellar capping protein	0.045
<i>lpp0197</i>	Similar to adenine specific DNA methylase	0.046
<i>lpp1291</i> , <i>fliS</i>	Similar to flagellar protein FliS	0.048
<i>lpp1745</i> , <i>motA</i>	Flagellar motor protein MotA	0.052
§ <i>lpp1290</i>	Similar to enhanced entry protein EnhA	0.053
§ <i>lpp1841</i>	Unknown	0.059
<i>lpp0968</i> , <i>flgN</i>	Hook-associated protein	0.068
# <i>lpp0969</i> , <i>flgM</i>	Anti-sigma-28 factor	0.081
<i>lpp0198</i>	Similar to Type III RM enzyme- helicase subunit	0.097
<i>lpp1050</i>	Unknown	0.114
§ <i>lpp3034</i> , <i>motY</i>	Similar to sodium-type flagellar protein MotY	0.116
<i>lpp1743</i>	Similar to hypothetical proteins	0.122
§ <i>lpp0952</i>	Regulatory protein (GGDEF and EAL domains)	0.122
<i>lpp2281</i>	Similar to membrane-associated metalloprotease proteins	0.133
<i>lpp2376</i>	Similar to <i>Legionella vir</i> region protein LvrA	0.165
<i>lpp0763</i>	Weakly similar to <i>L. pneumophila</i> lcnL protein	0.187
<i>lpp1941</i>	Unknown	0.202
<i>lpp2634</i>	Similar to hypothetical proteins	0.228
<i>lpp1568</i> , <i>plaB</i>	Phospholipase	0.232
§ <i>lpp2260</i>	Unknown	0.262
<i>lpp2635</i> , <i>flhB'</i>	Similar to FlhB protein	0.295
<i>lpp0010</i>	Similar to GTP-binding protein HflX	0.296
<i>lpp0009</i>	Similar to host factor-1 protein	0.366
<i>plpp0131</i>	Similar to alanyl tRNA synthetase	0.370
<i>lpp1742</i> , <i>prfB</i>	Highly similar to peptide chain release factor 2	0.371
<i>lpp2386</i>	Unknown	0.374
<i>lpp1234</i> , <i>flgL</i>	Flagellar hook-associated protein FlgL	0.379

*From Table S7 (Albert-Weissenberger et al., 2010), modified; #Homolog gene belonging to the FliA-regulon of *L. oakridgensis* (data from Tlapak et al., 2017); §Belonging to the FliA regulon of *L. pneumophila* Paris replicating in *A. castellanii* (data from Bruggemann et al., 2006); FC, fold-change values.

(clade I: *L. longbeachae*, **Figure 5**, *Legionella gratiana*, *Legionella cincinnatiensis*, *Legionella sainthelensi*, and *L. israelensis*). It has been hypothesized that the loss of flagellar genes has not happened recently (Kozak et al., 2010). This is corroborated by the finding that *L. longbeachae* and all subclade members are negative for the flagellar regulon but positive for genes coding for the sigma factor FliA, the regulator FleN and the two component system comprising of FleR and FleS, as well as FlgD (**Figure 3**) (Cazalet et al., 2010; Kozak et al., 2010). Also *L. israelensis* is negative for *fliA* (Heuner et al., 1995) and most flagellar regulon genes, except: *fleQ*, *fliA*, *fleN*, and *flgM* (**Figure 5**). The finding allows to assume that the flagellar system may have been lost at different time points during the evolution

of *Legionella* species (**Figure 3**). In addition, some genes that have regulatory functions outside of the flagellar system are still present (Albert-Weissenberger et al., 2010; Cazalet et al., 2010; Kozak et al., 2010; Tlapak et al., 2017). The same applies to *flgD* which is involved in the assembly of the hook structure of the flagellum with unassigned hypothetical alternative functions.

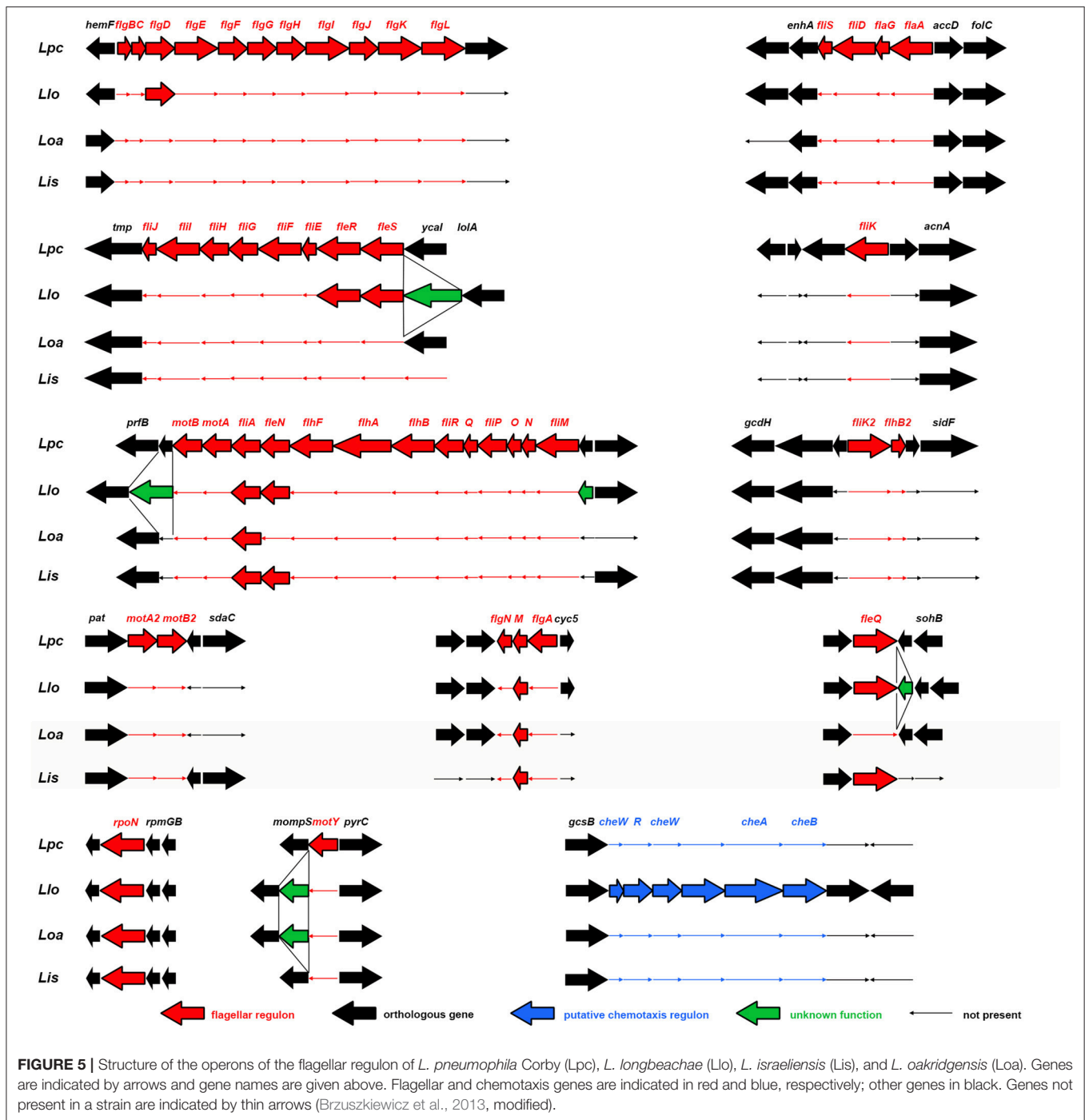
The investigated *Legionella* genomes were also screened for the presence/absence of genes of major regulators of the flagellar system as well as of the chemotaxis operon (**Figure 3**). It was found that the genes of the chemotaxis operon are only found in a subclade of the clade I *Legionella*. *L. longbeachae* is the first *Legionella* species described to exhibit chemotaxis genes (Cazalet et al., 2010; Kozak et al., 2010) that do not have flagellar genes. It seemed paradoxical that *L. pneumophila* is flagella positive but chemotaxis negative and *L. longbeachae* is flagella negative but chemotaxis positive. The distribution of the chemotaxis operon may indicate that the chemotaxis operon was acquired by a common 'ancestor' of this sub-tree clade (**Figure 3**).

CONCLUSION

The review aimed to summarize knowledge gained about flagella and the flagellar regulon of different *Legionella* species. The majority of *Legionella* species exhibit genes encoding for a functional flagellum and they are flagellated (Elliott and Johnson, 1981; Bornstein et al., 1991; Bangsberg et al., 1995; Heuner et al., 1995). Motility increases infectivity and fitness, helping the bacteria to reach new hosts after successful replication within protozoan host cells and release into aquatic environments.

Some *Legionella*—including some pathogenic species (e.g., *L. longbeachae*, and *L. oakridgensis*)—are not flagellated and most flagellar regulon genes are absent (Orrison et al., 1983; Heuner et al., 1995; Cazalet et al., 2010; Kozak et al., 2010; Brzuszkiewicz et al., 2013). With the advance in molecular techniques and the ability to produce and to process metagenomics datasets, it was found that some of the non-flagellated *Legionella* have still parts of the flagellar regulon, mainly genes with regulator functions.

However, in *Legionella* flagellum synthesis is associated with the expression of a virulence phenotype; and motility can be seen as a virulence and a fitness factor in *Legionella* and other bacteria. The alternative sigma factor FliA is also involved in the expression of virulence traits. FliA-dependent putative virulence genes were already identified by initial investigations that need to be extended. Also, additional investigations are needed to determine the role of FliA and molecular mechanisms of FliA-FlgM interactions in *Legionellae*. FlgM and FliA, two main players involved in the expression of the flagellum genes, are still present in non-flagellated *Legionella*, a promising take-off for future investigations. Nevertheless, the flagellum is not necessarily needed for an intracellular replication within host cells. Moreover, in some hosts the Naip5/Ipaf-dependent recognition of flagellin can cause an innate immune response leading to resistance against *Legionella* infections (Molofsky et al.,



2006; Ren et al., 2006). For example, it was reported that biofilm-derived *L. pneumophila* without flagellin expression evade the innate immune response in macrophages (Abu Khweek et al., 2013), as it was suggested for the non-flagellated *L. longbeachae* (Cazalet et al., 2010; Kozak et al., 2010). It seems that under certain conditions, the loss of the flagellum may increase the fitness of bacteria. For instance, *L. pneumophila* which can be found mainly in aquatic environments, is still flagellated whereas *L. longbeachae* which can be found predominantly

in soil, is non-flagellated (Kozak et al., 2010). Nevertheless, if the loss of the flagellar system from *Legionella* species depends on the habitat or environmental conditions remains unanswered.

As outlined, flagellated and non-flagellated *Legionella* are positive for genes belonging to the chemotaxis operon. The ability of *Legionella* to swarm and to show off a chemotaxis behavior has not yet been reported. Interestingly, some chemotaxis-positive and flagellar operon-negative *Legionella* (e.g., *L. longbeachae*)

give credit to the assumption that chemotaxis genes may not be involved in flagellum-mediated motility. Recent investigations could even show that chemotaxis sensory systems—different from those found in *E. coli*—in distinct bacteria (e.g., *Myxococcus* spp., *Geobacter* spp.) are not necessarily involved in bacterial flagellum-mediated motility (Kirby, 2009; Kozak et al., 2010). Chemotaxis-like systems seem to be involved among other things in type IV pilus-based motility and cell to cell interaction and/or social motility (Kirby, 2009; Kozak et al., 2010). Accordingly, additional experimentations are needed to investigate the role of the chemotaxis operon in flagellated and non-flagellated, chemotaxis-positive *Legionella*.

REFERENCES

Abbott, Z. D., Flynn, K. J., Byrne, B. G., Mukherjee, S., Kearns, D. B., and Swanson, M. S. (2015). *csrT* represents a new class of *csrA*-like regulatory genes associated with integrative conjugative elements of *Legionella pneumophila*. *J. Bacteriol.* 198, 553–564. doi: 10.1128/JB.00732-15

Abdel-Nour, M., Duncan, C., Low, D. E., and Guyard, C. (2013). Biofilms: the stronghold of *Legionella pneumophila*. *Int. J. Mol. Sci.* 14, 21660–21675. doi: 10.3390/ijms141121660

Abu Khweek, A., Fernandez Davila, N. S., Caution, K., Akhter, A., Abdulrahman, B. A., Tazi, M., et al. (2013). Biofilm-derived *Legionella pneumophila* evades the innate immune response in macrophages. *Front. Cell. Infect. Microbiol.* 3:18. doi: 10.3389/fcimb.2013.00018

Al-Bana, B. H., Haddad, M. T., and Garduno, R. A. (2014). Stationary phase and mature infectious forms of *Legionella pneumophila* produce distinct viable but non-culturable cells. *Environ. Microbiol.* 16, 382–395. doi: 10.1111/1462-2920.12219

Albert-Weissenberger, C., Sahr, T., Sismeiro, O., Hacker, J., Heuner, K., and Buchrieser, C. (2010). Control of flagellar gene regulation in *Legionella pneumophila* and its relation to growth phase. *J. Bacteriol.* 192, 446–455. doi: 10.1128/JB.00610-09

Aldridge, P. D., Karlinsey, J. E., Aldridge, C., Birchall, C., Thompson, D., Yagasaki, J., et al. (2006). The flagellar-specific transcription factor, sigma28, is the Type III secretion chaperone for the flagellar-specific anti-sigma28 factor FlgM. *Genes Dev.* 20, 2315–2326. doi: 10.1101/gad.380406

Aldridge, P., and Hughes, K. T. (2002). Regulation of flagellar assembly. *Curr. Opin. Microbiol.* 5, 160–165. doi: 10.1016/S1369-5274(02)00302-8

Altegoer, F., and Bange, G. (2015). Undiscovered regions on the molecular landscape of flagellar assembly. *Curr. Opin. Microbiol.* 28, 98–105. doi: 10.1016/j.mib.2015.08.011

Atlas, R. M. (1999). *Legionella*: from environmental habitats to disease pathology, detection and control. *Environ. Microbiol.* 1, 283–293. doi: 10.1046/j.1462-2920.1999.00046.x

Bachman, M. A., and Swanson, M. S. (2001). RpoS co-operates with other factors to induce *Legionella pneumophila* virulence in the stationary phase. *Mol. Microbiol.* 40, 1201–1214. doi: 10.1046/j.1365-2958.2001.02465.x

Bachman, M. A., and Swanson, M. S. (2004). Genetic evidence that *Legionella pneumophila* RpoS modulates expression of the transmission phenotype in both the exponential phase and the stationary phase. *Infect. Immun.* 72, 2468–2476. doi: 10.1128/IAI.72.5.2468-2476.2004

Bajrai, L. H., Azhar, E. I., Yasir, M., Jardot, P., Barrassi, L., Raoult, D., et al. (2016). *Legionella saoudiensis* sp. nov., isolated from a sewage water sample. *Int. J. Syst. Evol. Microbiol.* 66, 4367–4371. doi: 10.1099/ijsem.0.001357

Bangsborg, J. M., Hindersson, P., Shand, G., and Hoiby, N. (1995). The *Legionella micdadei* flagellin: expression in *Escherichia coli* K12 and DNA sequence of the gene. *APMIS* 103, 869–877. doi: 10.1111/j.1699-0463.1995.tb01446.x

Barbaree, J. M., Fields, B. S., Feeley, J. C., Gorman, G. W., and Martin, W. T. (1986). Isolation of protozoa from water associated with a legionellosis outbreak and demonstration of intracellular multiplication of *Legionella pneumophila*. *Appl. Environ. Microbiol.* 51, 422–424.

AUTHOR CONTRIBUTIONS

SA and KH contributed substantially to the conception and design of the work. SA and KH wrote the paper.

ACKNOWLEDGMENTS

We would like to thank Roland Grunow for assistance with different aspects of the presented work and for copy-editing. This work received financial support by grant HE2854/5-1+2/9-1 from the Deutsche Forschungsgemeinschaft DFG (Bonn, Germany) to KH.

Berk, S. G., Faulkner, G., Garduno, E., Joy, M. C., Ortiz-Jimenez, M. A., and Garduno, R. A. (2008). Packaging of live *Legionella pneumophila* into pellets expelled by *Tetrahymena* spp. does not require bacterial replication and depends on a Dot/Icm-mediated survival mechanism. *Appl. Environ. Microbiol.* 74, 2187–2199. doi: 10.1128/AEM.01214-07

Bigot, R., Bertaux, J., Frere, J., and Berjeaud, J. M. (2013). Intra-amoeba multiplication induces chemotaxis and biofilm colonization and formation for *Legionella*. *PLoS ONE* 8:e77875. doi: 10.1371/journal.pone.0077875

Bornstein, N., Marmet, D., Dumaine, M. H., Surgot, M., and Fleurette, J. (1991). Detection of flagella in 278 *Legionella* strains by latex reagent sensitized with anti-flagellum immunoglobulins. *J. Clin. Microbiol.* 29, 953–956.

Bosshardt, S. C., Benson, R. F., and Fields, B. S. (1997). Flagella are a positive predictor for virulence in *Legionella*. *Microb. Pathog.* 23, 107–112. doi: 10.1006/mpat.1997.0134

Bouyer, S., Imbert, C., Rodier, M. H., and Hechard, Y. (2007). Long-term survival of *Legionella pneumophila* associated with *Acanthamoeba castellanii* vesicles. *Environ. Microbiol.* 9, 1341–1344. doi: 10.1111/j.1462-2920.2006.01229.x

Bruggemann, H., Hagman, A., Jules, M., Sismeiro, O., Dillies, M. A., Gouyette, C., et al. (2006). Virulence strategies for infecting phagocytes deduced from the *in vivo* transcriptional program of *Legionella pneumophila*. *Cell. Microbiol.* 8, 1228–1240. doi: 10.1111/j.1462-5822.2006.00703.x

Bryers, J. D. (2008). Medical biofilms. *Biotechnol. Bioeng.* 100, 1–18. doi: 10.1002/bit.21838

Brzuskiewicz, E., Schulz, T., Rydzewski, K., Daniel, R., Gillmaier, N., Dittmann, C., et al. (2013). *Legionella oakridgensis* ATCC 33761 genome sequence and phenotypic characterization reveals its replication capacity in amoebae. *Int. J. Med. Microbiol.* 303, 514–528. doi: 10.1016/j.ijmm.2013.07.003

Burstein, D., Amaro, F., Zusman, T., Lifshitz, Z., Cohen, O., Gilbert, J. A., et al. (2016). Genomic analysis of 38 *Legionella* species identifies large and diverse effector repertoires. *Nat. Genet.* 48, 167–175. doi: 10.1038/ng.3481

Byrne, B., and Swanson, M. S. (1998). Expression of *Legionella pneumophila* virulence traits in response to growth conditions. *Infect. Immun.* 66, 3029–3034.

Cateau, E., Delafont, V., Hechard, Y., and Rodier, M. H. (2014). Free-living amoebae: what part do they play in healthcare-associated infections? *J. Hosp. Infect.* 87, 131–140. doi: 10.1016/j.jhin.2014.05.001

Cazalet, C., Gomez-Valero, L., Rusniok, C., Lomma, M., Dervins-Ravault, D., Newton, H. J., et al. (2010). Analysis of the *Legionella longbeachae* genome and transcriptome uncovers unique strategies to cause Legionnaires' disease. *PLoS Genet.* 6:e1000851. doi: 10.1371/journal.pgen.1000851

Cazalet, C., Rusniok, C., Bruggemann, H., Zidane, N., Magnier, A., Ma, L., et al. (2004). Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat. Genet.* 36, 1165–1173. doi: 10.1038/ng1447

Chandler, F. W., Thomason, B. M., and Hebert, G. A. (1980). Flagella on Legionnaires' disease bacteria in the human lung. *Ann. Intern. Med.* 93, 715–716. doi: 10.7326/0003-4819-93-5-715

Chen, J., de Felipe, K. S., Clarke, M., Lu, H., Anderson, O. R., Segal, G., et al. (2004). *Legionella* effectors that promote nonlytic release from protozoa. *Science* 303, 1358–1361. doi: 10.1126/science.1094226

- Chilcott, G. S., and Hughes, K. T. (2000). Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar typhimurium and *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* 64, 694–708. doi: 10.1128/MMBR.64.4.694-708.2000
- Cianciotto, N., Eisenstein, B. I., Engleberg, N. C., and Shuman, H. (1989). Genetics and molecular pathogenesis of *Legionella pneumophila*, an intracellular parasite of macrophages. *Mol. Biol. Med.* 6, 409–424.
- Coil, D. A., and Anne, J. (2009). Twitching motility in *Legionella pneumophila*. *FEMS Microbiol. Lett.* 293, 271–277. doi: 10.1111/j.1574-6968.2009.01532.x
- Currie, S. L., and Beattie, T. K. (2015). Compost and *Legionella longbeachae*: an emerging infection? *Perspect. Public Health* 135, 309–315. doi: 10.1177/1757913915611162
- Dalebroux, Z. D., Edwards, R. L., and Swanson, M. S. (2009). SpoT governs *Legionella pneumophila* differentiation in host macrophages. *Mol. Microbiol.* 71, 640–658. doi: 10.1111/j.1365-2958.2008.06555.x
- Dalebroux, Z. D., Yagi, B. F., Sahr, T., Buchrieser, C., and Swanson, M. S. (2010). Distinct roles of ppGpp and DksA in *Legionella pneumophila* differentiation. *Mol. Microbiol.* 76, 200–219. doi: 10.1111/j.1365-2958.2010.07094.x
- Danhorn, T., and Fuqua, C. (2007). Biofilm formation by plant-associated bacteria. *Annu. Rev. Microbiol.* 61, 401–422. doi: 10.1146/annurev.micro.61.080706.093316
- Declerck, P. (2010). Biofilms: the environmental playground of *Legionella pneumophila*. *Environ. Microbiol.* 12, 557–566. doi: 10.1111/j.1462-2920.2009.02025.x
- Dietrich, C., Heuner, K., Brand, B. C., Hacker, J., and Steinert, M. (2001). Flagellum of *Legionella pneumophila* positively affects the early phase of infection of eukaryotic host cells. *Infect. Immun.* 69, 2116–2122. doi: 10.1128/IAI.69.4.2116-2122.2001
- Edwards, R. L., Dalebroux, Z. D., and Swanson, M. S. (2009). *Legionella pneumophila* couples fatty acid flux to microbial differentiation and virulence. *Mol. Microbiol.* 71, 1190–1204. doi: 10.1111/j.1365-2958.2009.06593.x
- Eisenreich, W., and Heuner, K. (2016). The life stage-specific pathometabolism of *Legionella pneumophila*. *FEBS Lett.* 590, 3868–3886. doi: 10.1002/1873-3468.12326
- Elliott, J. A., and Johnson, W. (1981). Immunological and biochemical relationships among flagella isolated from *Legionella pneumophila* serogroups 1, 2, and 3. *Infect. Immun.* 33, 602–610.
- Elliott, J. A., and Johnson, W. (1982). Virulence conversion of *Legionella pneumophila* serogroup 1 by passage in guinea pigs and embryonated eggs. *Infect. Immun.* 35, 943–946.
- Faulkner, G., and Garduno, R. A. (2002). Ultrastructural analysis of differentiation in *Legionella pneumophila*. *J. Bacteriol.* 184, 7025–7041. doi: 10.1128/JB.184.24.7025-7041.2002
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791. doi: 10.1111/j.1558-5646.1985.tb00420.x
- Fields, B. S. (1996). The molecular ecology of legionellae. *Trends Microbiol.* 4, 286–290. doi: 10.1016/0966-842X(96)10041-X
- Fields, B. S., Barbaree, J. M., Shotts, E. B. Jr., Feeley, J. C., Morrill, W. E., Sanden, G. N., et al. (1986). Comparison of guinea pig and protozoan models for determining virulence of *Legionella* species. *Infect. Immun.* 53, 553–559.
- Fields, B. S., Benson, R. F., and Besser, R. E. (2002). *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin. Microbiol. Rev.* 15, 506–526. doi: 10.1128/CMR.15.3.506-526.2002
- Fliermans, C. B., Cherry, W. B., Orrison, L. H., Smith, S. J., Tison, D. L., and Pope, D. H. (1981). Ecological distribution of *Legionella pneumophila*. *Appl. Environ. Microbiol.* 41, 9–16.
- Fonseca, M. V., and Swanson, M. S. (2014). Nutrient salvaging and metabolism by the intracellular pathogen *Legionella pneumophila*. *Front. Cell. Infect. Microbiol.* 4:12. doi: 10.3389/fcimb.2014.00012
- Fontana, M. F., and Vance, R. E. (2011). Two signal models in innate immunity. *Immunol. Rev.* 243, 26–39. doi: 10.1111/j.1600-065X.2011.01037.x
- Gal-Mor, O., and Segal, G. (2003). The *Legionella pneumophila* GacA homolog (LetA) is involved in the regulation of icm virulence genes and is required for intracellular multiplication in *Acanthamoeba castellanii*. *Microb. Pathog.* 34, 187–194. doi: 10.1016/S0882-4010(03)00027-5
- Garduno, R. A., Garduno, E., Hiltz, M., and Hoffman, P. S. (2002). Intracellular growth of *Legionella pneumophila* gives rise to a differentiated form dissimilar to stationary-phase forms. *Infect. Immun.* 70, 6273–6283. doi: 10.1128/IAI.70.11.6273-6283.2002
- Gillen, K. L., and Hughes, K. T. (1991). Molecular characterization of flgM, a gene encoding a negative regulator of flagellin synthesis in *Salmonella typhimurium*. *J. Bacteriol.* 173, 6453–6459. doi: 10.1128/jb.173.20.6453-6459.1991
- Gillmaier, N., Schunder, E., Kutzner, E., Tlapak, H., Rydzewski, K., Herrmann, V., et al. (2016). Growth-related metabolism of the carbon storage poly-3-hydroxybutyrate in *Legionella pneumophila*. *J. Biol. Chem.* 291, 6471–6482. doi: 10.1074/jbc.M115.693481
- Glockner, G., Albert-Weissenberger, C., Weinmann, E., Jacobi, S., Schunder, E., Steinert, M., et al. (2008). Identification and characterization of a new conjugation/type IVA secretion system (trb/tra) of *Legionella pneumophila* Corby localized on two mobile genomic islands. *Int. J. Med. Microbiol.* 298, 411–428. doi: 10.1016/j.ijmm.2007.07.012
- Gomez-Valero, L., Rusniok, C., and Buchrieser, C. (2009). *Legionella pneumophila*: population genetics, phylogeny and genomics. *Infect. Genet. Evol.* 9, 727–739. doi: 10.1016/j.meegid.2009.05.004
- Gomez-Valero, L., Rusniok, C., Rolando, M., Neou, M., Dervins-Ravault, D., Demirtas, J., et al. (2014). Comparative analyses of *Legionella* species identifies genetic features of strains causing Legionnaires' disease. *Genome Biol.* 15, 505. doi: 10.1186/PREACCEPT-1086350395137407
- Greub, G., and Raoult, D. (2004). Microorganisms resistant to free-living amoebae. *Clin. Microbiol. Rev.* 17, 413–433. doi: 10.1128/CMR.17.2.413-433.2004
- Hammer, B. K., and Swanson, M. S. (1999). Co-ordination of *Legionella pneumophila* virulence with entry into stationary phase by ppGpp. *Mol. Microbiol.* 33, 721–731. doi: 10.1046/j.1365-2958.1999.01519.x
- Hammer, B. K., Tateda, E. S., and Swanson, M. S. (2002). A two-component regulator induces the transmission phenotype of stationary-phase *Legionella pneumophila*. *Mol. Microbiol.* 44, 107–118. doi: 10.1046/j.1365-2958.2002.02884.x
- Hauslein, L., Manske, C., Goebel, W., Eisenreich, W., and Hilbi, H. (2016). Pathway analysis using (13) C-glycerol and other carbon tracers reveals a bipartite metabolism of *Legionella pneumophila*. *Mol. Microbiol.* 100, 229–246. doi: 10.1111/mmi.13313
- Hawn, T. R., Verbon, A., Lettinga, K. D., Zhao, L. P., Li, S. S., Laws, R. J., et al. (2003). A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J. Exp. Med.* 198, 1563–1572. doi: 10.1084/jem.20031220
- Hazelbauer, G. L., Falke, J. J., and Parkinson, J. S. (2008). Bacterial chemoreceptors: high-performance signaling in networked arrays. *Trends Biochem. Sci.* 33, 9–19. doi: 10.1016/j.tibs.2007.09.014
- Heuner, K., and Albert-Weissenberger, C. (2008). “The flagellar regulon of *Legionella pneumophila* and the expression of virulence traits,” in *Legionella: Molecular Microbiology*, eds K. Heuner and M. S. Swanson (Norfolk, UK: Horizon Scientific Press), 249.
- Heuner, K., Bender-Beck, L., Brand, B. C., Luck, P. C., Mann, K. H., Marre, R., et al. (1995). Cloning and genetic characterization of the flagellum subunit gene (flaA) of *Legionella pneumophila* serogroup 1. *Infect. Immun.* 63, 2499–2507.
- Heuner, K., Brand, B. C., and Hacker, J. (1999). The expression of the flagellum of *Legionella pneumophila* is modulated by different environmental factors. *FEMS Microbiol. Lett.* 175, 69–77. doi: 10.1111/j.1574-6968.1999.tb13603.x
- Heuner, K., Dietrich, C., Sriwan, C., Steinert, M., and Hacker, J. (2002). Influence of the alternative σ^{28} factor on virulence and flagellum expression of *Legionella pneumophila*. *Infect. Immun.* 70, 1604–1608. doi: 10.1128/IAI.70.3.1604-1608.2002
- Heuner, K., Dietrich, C., Steinert, M., Gobel, U. B., and Hacker, J. (2000). Cloning and characterization of a *Legionella pneumophila*-specific gene encoding a member of the LysR family of transcriptional regulators. *Mol. Gen. Genet.* 264, 204–211. doi: 10.1007/s004380000310
- Heuner, K., Hacker, J., and Brand, B. C. (1997). The alternative sigma factor sigma²⁸ of *Legionella pneumophila* restores flagellation and motility to an *Escherichia coli* fliA mutant. *J. Bacteriol.* 179, 17–23. doi: 10.1128/jb.179.1.17-23.1997
- Heuner, K., Jacobi, S., Albert, C., Steinert, M., Brüggemann, H., and Buchrieser, C. (2006). “Gene expression and virulence in *Legionella*: the flagellar regulon,” in *Legionella: State of the Art 30 Years After its Recognition*, eds N. Cianciotto, Abu, Y. Kwaik, Edelstein, P. Fields, B. Geary, D. Harrison, T. Joseph, C. Ratcliff, R. J. Stout, and Swanson (Washington, DC: ASM), 327–332.

- Heuner, K., and Steinert, M. (2003). The flagellum of *Legionella pneumophila* and its link to the expression of the virulent phenotype. *Int. J. Med. Microbiol.* 293, 133–143. doi: 10.1078/1438-4221-00259
- Hoppe, J., Unal, C. M., Thiem, S., Grimpe, L., Goldmann, T., Gassler, N., et al. (2017). PLY1 Promotes *Legionella pneumophila* infection of human lung tissue explants and contributes to bacterial adhesion, host cell invasion, and twitching motility. *Front. Cell. Infect. Microbiol.* 7:63. doi: 10.3389/fcimb.2017.00063
- Horwitz, M. A. (1992). Interactions between macrophages and *Legionella pneumophila*. *Curr. Top. Microbiol. Immunol.* 181, 265–282. doi: 10.1007/978-3-642-77377-8_10
- Horwitz, M. A., and Silverstein, S. C. (1980). Legionnaires' disease bacterium (*Legionella pneumophila*) multiples intracellularly in human monocytes. *J. Clin. Invest.* 66, 441–450. doi: 10.1172/JCI109874
- Jacobi, S., Schade, R., and Heuner, K. (2004). Characterization of the alternative sigma factor sigma54 and the transcriptional regulator FleQ of *Legionella pneumophila*, which are both involved in the regulation cascade of flagellar gene expression. *J. Bacteriol.* 186, 2540–2547. doi: 10.1128/JB.186.9.2540-2547.2004
- Jager, J., Marwitz, S., Tiefenau, J., Rasch, J., Shevchuk, O., Kugler, C., et al. (2014). Human lung tissue explants reveal novel interactions during *Legionella pneumophila* infections. *Infect. Immun.* 82, 275–285. doi: 10.1128/IAI.00703-13
- Khodr, A., Kay, E., Gomez-Valero, L., Ginevra, C., Doublet, P., Buchrieser, C., et al. (2016). Molecular epidemiology, phylogeny and evolution of *Legionella*. *Infect. Genet. Evol.* 43, 108–122. doi: 10.1016/j.meegid.2016.04.033
- Kirby, J. R. (2009). Chemotaxis-like regulatory systems: unique roles in diverse bacteria. *Annu. Rev. Microbiol.* 63, 45–59. doi: 10.1146/annurev.micro.091208.073221
- Kirov, S. M., Castrisios, M., and Shaw, J. G. (2004). *Aeromonas* flagella (polar and lateral) are enterocyte adhesins that contribute to biofilm formation on surfaces. *Infect. Immun.* 72, 1939–1945. doi: 10.1128/IAI.72.4.1939-1945.2004
- Konishi, T., Yamashiro, T., Koide, M., and Nishizono, A. (2006). Influence of temperature on growth of *Legionella pneumophila* biofilm determined by precise temperature gradient incubator. *J. Biosci. Bioeng.* 101, 478–484. doi: 10.1263/jbb.101.478
- Kozak, N. A., Buss, M., Lucas, C. E., Frace, M., Govil, D., Travis, T., et al. (2010). Virulence factors encoded by *Legionella longbeachae* identified on the basis of the genome sequence analysis of clinical isolate D-4968. *J. Bacteriol.* 192, 1030–1044. doi: 10.1128/JB.01272-09
- Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. doi: 10.1093/molbev/msw054
- La Scola, B., Birtles, R. J., Greub, G., Harrison, T. J., Ratcliff, R. M., and Raoult, D. (2004). *Legionella drancourtii* sp. nov., a strictly intracellular amoebal pathogen. *Int. J. Syst. Evol. Microbiol.* 54(Pt 3), 699–703. doi: 10.1099/ijs.0.02455-0
- Lau, H. Y., and Ashbolt, N. J. (2009). The role of biofilms and protozoa in *Legionella pathogenesis*: implications for drinking water. *J. Appl. Microbiol.* 107, 368–378. doi: 10.1111/j.1365-2672.2009.04208.x
- Levi, A., Folcher, M., Jenal, U., and Shuman, H. A. (2011). Cyclic diguanylate signaling proteins control intracellular growth of *Legionella pneumophila*. *MBio* 2:e00316–10. doi: 10.1128/mBio.00316-10
- Lo Presti, F., Riffard, S., Meugnier, H., Reyrolle, M., Lasne, Y., Grimont, P. A., et al. (2001). *Legionella gresilensis* sp. nov. and *Legionella beliardensis* sp. nov., isolated from water in France. *Int. J. Syst. Evol. Microbiol.* 51(Pt 6), 1949–1957. doi: 10.1099/00207713-51-6-1949
- Macnab, R. M. (2003). How bacteria assemble flagella. *Annu. Rev. Microbiol.* 57, 77–100. doi: 10.1146/annurev.micro.57.030502.090832
- Mampel, J., Spirig, T., Weber, S. S., Haagensen, J. A., Molin, S., and Hilbi, H. (2006). Planktonic replication is essential for biofilm formation by *Legionella pneumophila* in a complex medium under static and dynamic flow conditions. *Appl. Environ. Microbiol.* 72, 2885–2895. doi: 10.1128/AEM.72.4.2885-2895.2006
- Mascarenhas, D. P., and Zamboni, D. S. (2017). Inflammasome biology taught by *Legionella pneumophila*. *J. Leukoc. Biol.* 101, 841–849. doi: 10.1189/jlb.3MR0916-380R
- McCarter, L. L. (2006). Regulation of flagella. *Curr. Opin. Microbiol.* 9, 180–186. doi: 10.1016/j.mib.2006.02.001
- Micali, G., and Endres, R. G. (2016). Bacterial chemotaxis: information processing, thermodynamics, and behavior. *Curr. Opin. Microbiol.* 30, 8–15. doi: 10.1016/j.mib.2015.12.001
- Minamino, T., and Imada, K. (2015). The bacterial flagellar motor and its structural diversity. *Trends Microbiol.* 23, 267–274. doi: 10.1016/j.tim.2014.12.011
- Molmeret, M., Bitar, D. M., Han, L., and Kwaik, Y. A. (2004). Disruption of the phagosomal membrane and egress of *Legionella pneumophila* into the cytoplasm during the last stages of intracellular infection of macrophages and *Acanthamoeba polyphaga*. *Infect. Immun.* 72, 4040–4051. doi: 10.1128/IAI.72.7.4040-4051.2004
- Molmeret, M., Jones, S., Santic, M., Habyarimana, F., Esteban, M. T., and Kwaik, Y. A. (2010). Temporal and spatial trigger of post-exponential virulence-associated regulatory cascades by *Legionella pneumophila* after bacterial escape into the host cell cytosol. *Environ. Microbiol.* 12, 704–715. doi: 10.1111/j.1462-2920.2009.02114.x
- Molofsky, A. B., Byrne, B. G., Whitfield, N. N., Madigan, C. A., Fuse, E. T., Tateda, K., et al. (2006). Cytosolic recognition of flagellin by mouse macrophages restricts *Legionella pneumophila* infection. *J. Exp. Med.* 203, 1093–1104. doi: 10.1084/jem.20051659
- Molofsky, A. B., Shetron-Rama, L. M., and Swanson, M. S. (2005). Components of the *Legionella pneumophila* flagellar regulon contribute to multiple virulence traits, including lysosome avoidance and macrophage death. *Infect. Immun.* 73, 5720–5734. doi: 10.1128/IAI.73.9.5720-5734.2005
- Molofsky, A. B., and Swanson, M. S. (2003). *Legionella pneumophila* CsrA is a pivotal repressor of transmission traits and activator of replication. *Mol. Microbiol.* 50, 445–461. doi: 10.1046/j.1365-2958.2003.03706.x
- Molofsky, A. B., and Swanson, M. S. (2004). Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. *Mol. Microbiol.* 53, 29–40. doi: 10.1111/j.1365-2958.2004.04129.x
- Neumeister, B., Schoniger, S., Faigle, M., Eichner, M., and Dietz, K. (1997). Multiplication of different *Legionella* species in Mono Mac 6 cells and in *Acanthamoeba castellanii*. *Appl. Environ. Microbiol.* 63, 1219–1224.
- Newton, H. J., Ang, D. K., van Driel, I. R., and Hartland, E. L. (2010). Molecular pathogenesis of infections caused by *Legionella pneumophila*. *Clin. Microbiol. Rev.* 23, 274–298. doi: 10.1128/CMR.00052-09
- O'Connell, W. A., Dhand, L., and Cianciotto, N. P. (1996). Infection of macrophage-like cells by *Legionella* species that have not been associated with disease. *Infect. Immun.* 64, 4381–4384.
- Ohnishi, K., Kutsukake, K., Suzuki, H., and Lino, T. (1992). A novel transcriptional regulation mechanism in the flagellar regulon of *Salmonella typhimurium*: an antisigma factor inhibits the activity of the flagellum-specific sigma factor, sigma F. *Mol. Microbiol.* 6, 3149–3157. doi: 10.1111/j.1365-2958.1992.tb01771.x
- Ohno, A., Kato, N., Yamada, K., and Yamaguchi, K. (2003). Factors influencing survival of *Legionella pneumophila* serotype 1 in hot spring water and tap water. *Appl. Environ. Microbiol.* 69, 2540–2547. doi: 10.1128/AEM.69.5.2540-2547.2003
- Orrison, L. H., Cherry, W. B., Tyndall, R. L., Fliermans, C. B., Gough, S. B., Lambert, M. A., et al. (1983). *Legionella oakridgensis*: unusual new species isolated from cooling tower water. *Appl. Environ. Microbiol.* 45, 536–545.
- Osterman, I. A., Dikhtyar, Y. Y., Bogdanov, A. A., Dontsova, O. A., and Sergiev, P. V. (2015). Regulation of flagellar gene expression in bacteria. *Biochem. Mosc.* 80, 1447–1456. doi: 10.1134/S000629791511005X
- Ott, M., Messner, P., Heesemann, J., Marre, R., and Hacker, J. (1991). Temperature-dependent expression of flagella in *Legionella*. *J. Gen. Microbiol.* 137, 1955–1961. doi: 10.1099/00221287-137-8-1955
- Pallen, M. J., Penn, C. W., and Chaudhuri, R. R. (2005). Bacterial flagellar diversity in the post-genomic era. *Trends Microbiol.* 13, 143–149. doi: 10.1016/j.tim.2005.02.008
- Piao, Z., Sze, C. C., Barysheva, O., Iida, K., and Yoshida, S. (2006). Temperature-regulated formation of mycelial mat-like biofilms by *Legionella pneumophila*. *Appl. Environ. Microbiol.* 72, 1613–1622. doi: 10.1128/AEM.72.2.1613-1622.2006
- Polesky, A. H., Ross, J. T., Falkow, S., and Tompkins, L. S. (2001). Identification of *Legionella pneumophila* genes important for infection of amoebas by signature-tagged mutagenesis. *Infect. Immun.* 69, 977–987. doi: 10.1128/IAI.69.2.977-987.2001
- Pruckler, J. M., Benson, R. F., Moyenuddin, M., Martin, W. T., and Fields, B. S. (1995). Association of flagellum expression and intracellular growth of *Legionella pneumophila*. *Infect. Immun.* 63, 4928–4932.
- Rasis, M., and Segal, G. (2009). The LetA-RsmYZ-CsrA regulatory cascade, together with RpoS and PmrA, post-transcriptionally regulates stationary phase

- activation of *Legionella pneumophila* Icm/Dot effectors. *Mol. Microbiol.* 72, 995–1010. doi: 10.1111/j.1365-2958.2009.06705.x
- Ren, T., Zamboni, D. S., Roy, C. R., Dietrich, W. F., and Vance, R. E. (2006). Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog.* 2:e18. doi: 10.1371/journal.ppat.0020018
- Ricci, M. L., Torosantucci, A., Scaturro, M., Chiani, P., Baldassarri, L., and Pastoris, M. C. (2005). Induction of protective immunity by *Legionella pneumophila* flagellum in an A/J mouse model. *Vaccine* 23, 4811–4820. doi: 10.1016/j.vaccine.2005.05.013
- Richards, A. M., Von Dwingelo, J. E., Price, C. T., and Abu Kwaik, Y. (2013). Cellular microbiology and molecular ecology of *Legionella*-amoeba interaction. *Virulence* 4, 307–314. doi: 10.4161/viru.24290
- Rizzardi, K., Winiecka-Krusnell, J., Ramliden, M., Alm, E., Andersson, S., and Byfors, S. (2015). *Legionella norrlandica* sp. nov., isolated from the biopurification systems of wood processing plants. *Int. J. Syst. Evol. Microbiol.* 65(Pt 2), 598–603. doi: 10.1099/ij.s.0.068940-0
- Robertson, P., Abdelhady, H., and Garduno, R. A. (2014). The many forms of a pleomorphic bacterial pathogen—the developmental network of *Legionella pneumophila*. *Front. Microbiol.* 5:670. doi: 10.3389/fmicb.2014.00670
- Rowbotham, T. J. (1986). Current views on the relationships between amoebae, legionellae and man. *Isr. J. Med. Sci.* 22, 678–689.
- Rust, M., Borchert, S., Niehus, E., Kuehne, S. A., Gripp, E., Bajceta, A., et al. (2009). The *Helicobacter pylori* anti-sigma factor FlgM is predominantly cytoplasmic and cooperates with the flagellar basal body protein FlhA. *J. Bacteriol.* 191, 4824–4834. doi: 10.1128/JB.00018-09
- Sahr, T., Bruggemann, H., Jules, M., Lomma, M., Albert-Weissenberger, C., Cazalet, C., et al. (2009). Two small ncRNAs jointly govern virulence and transmission in *Legionella pneumophila*. *Mol. Microbiol.* 72, 741–762. doi: 10.1111/j.1365-2958.2009.06677.x
- Sahr, T., Rusniok, C., Dervins-Ravault, D., Sismeiro, O., Coppee, J. Y., and Buchrieser, C. (2012). Deep sequencing defines the transcriptional map of *L. pneumophila* and identifies growth phase-dependent regulated ncRNAs implicated in virulence. *RNA Biol.* 9, 503–519. doi: 10.4161/rna.20270
- Sahr, T., Rusniok, C., Impens, F., Oliva, G., Sismeiro, O., Coppee, J. Y., et al. (2017). The *Legionella pneumophila* genome evolved to accommodate multiple regulatory mechanisms controlled by the CsrA-system. *PLoS Genet.* 13:e1006629. doi: 10.1371/journal.pgen.1006629
- Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.
- Schalk, J. A., Euser, S. M., van Heijnsbergen, E., Bruin, J. P., den Boer, J. W., and de Roda Husman, A. M. (2014). Soil as a source of *Legionella pneumophila* sequence type 47. *Int. J. Infect. Dis.* 27, 18–19. doi: 10.1016/j.ijid.2014.05.009
- Schell, U., Simon, S., and Hilbi, H. (2016). Inflammation recognition and regulation of the *Legionella* flagellum. *Curr. Top. Microbiol. Immunol.* 397, 161–181. doi: 10.1007/978-3-319-41171-2_8
- Schulz, T., Rydzewski, K., Schunder, E., Holland, G., Bannert, N., and Heuner, K. (2012). FliA expression analysis and influence of the regulatory proteins RpoN, FleQ and FliA on virulence and *in vivo* fitness in *Legionella pneumophila*. *Arch. Microbiol.* 194, 977–989. doi: 10.1007/s00203-012-0833-y
- Schunder, E., Gillmaier, N., Kutzner, E., Eisenreich, W., Herrmann, V., Lautner, M., et al. (2014). Amino acid uptake and metabolism of *Legionella pneumophila* hosted by *Acanthamoeba castellanii*. *J. Biol. Chem.* 289, 21040–21054. doi: 10.1074/jbc.M114.570085
- Steinert, M., Emody, L., Amann, R., and Hacker, J. (1997). Resuscitation of viable but non-culturable *Legionella pneumophila* philadelphia JR32 by *Acanthamoeba castellanii*. *Appl. Environ. Microbiol.* 63, 2047–2053.
- Stewart, C. R., Muthye, V., and Cianciotto, N. P. (2012). *Legionella pneumophila* persists within biofilms formed by *Klebsiella pneumoniae*, *Flavobacterium* sp., and *Pseudomonas fluorescens* under dynamic flow conditions. *PLoS ONE* 7:e50560. doi: 10.1371/journal.pone.0050560
- Stewart, C. R., Rossier, O., and Cianciotto, N. P. (2009). Surface translocation by *Legionella pneumophila*: a form of sliding motility that is dependent upon type II protein secretion. *J. Bacteriol.* 191, 1537–1546. doi: 10.1128/JB.01531-08
- Swanson, M. S., and Hammer, B. K. (2000). *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. *Annu. Rev. Microbiol.* 54, 567–613. doi: 10.1146/annurev.micro.54.1.567
- Szurmant, H., and Ordal, G. W. (2004). Diversity in chemotaxis mechanisms among the bacteria and archaea. *Microbiol. Mol. Biol. Rev.* 68, 301–319. doi: 10.1128/MMBR.68.2.301-319.2004
- Tamura, K., and Kumar, S. (2002). Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Mol. Biol. Evol.* 19, 1727–1736. doi: 10.1093/oxfordjournals.molbev.a003995
- Tiaden, A., Spirig, T., Weber, S. S., Bruggemann, H., Bosshard, R., Buchrieser, C., et al. (2007). The *Legionella pneumophila* response regulator LqsR promotes host cell interactions as an element of the virulence regulatory network controlled by RpoS and LetA. *Cell. Microbiol.* 9, 2903–2920. doi: 10.1111/j.1462-5822.2007.01005.x
- Tlapak, H., Rydzewski, K., Schulz, T., Weschka, D., Schunder, E., and Heuner, K. (2017). Functional analysis of the alternative sigma-28 factor FliA and Its Anti-sigma factor FlgM of the non-flagellated *Legionella* species, *L. oakridgensis*. *J. Bacteriol.* 199:e00018-17. doi: 10.1128/JB.00018-17
- Wang, C., Saito, M., Tanaka, T., Amako, K., and Yoshida, S. (2015). Comparative analysis of virulence traits between a *Legionella feeleyi* strain implicated in Pontiac fever and a strain that caused Legionnaires' disease. *Microb. Pathog.* 89, 79–86. doi: 10.1016/j.micpath.2015.09.004
- Whiley, H., and Bentham, R. (2011). *Legionella longbeachae* and legionellosis. *Emerg. Infect. Dis.* 17, 579–583. doi: 10.3201/eid1704.100446
- Wolfe, A. J., and Visick, K. L. (2008). Get the message out: cyclic-Di-GMP regulates multiple levels of flagellum-based motility. *J. Bacteriol.* 190, 463–475. doi: 10.1128/JB.01418-07
- Yu, V. L., Plouffe, J. F., Pastoris, M. C., Stout, J. E., Schousboe, M., Widmer, A., et al. (2002). Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. *J. Infect. Dis.* 186, 127–128. doi: 10.1086/341087
- Zusman, T., Gal-Mor, O., and Segal, G. (2002). Characterization of a *Legionella pneumophila* relA insertion mutant and roles of RelA and RpoS in virulence gene expression. *J. Bacteriol.* 184, 67–75. doi: 10.1128/JB.184.1.67-75.2002

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