



**HAL**  
open science

## Legionella Effectors Explored with INSeq: New Functional Insights

Monica Rolando, Carmen Buchrieser

► **To cite this version:**

Monica Rolando, Carmen Buchrieser. Legionella Effectors Explored with INSeq: New Functional Insights. Trends in Microbiology, 2018, 26 (3), pp.169-170. 10.1016/j.tim.2018.01.003. pasteur-02552011

**HAL Id: pasteur-02552011**

**<https://pasteur.hal.science/pasteur-02552011>**

Submitted on 16 Jun 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***Legionella* effectors explored with INSeq: new functional insights**

2  
3 Monica Rolando<sup>1,2</sup> and Carmen Buchrieser<sup>1,2,\*</sup>

4  
5  
6 <sup>1</sup>Institut Pasteur, Biologie des Bactéries Intracellulaires, Paris, France

7 <sup>2</sup>CNRS UMR 3525, Paris, France

8  
9  
10  
11  
12  
13  
14 **Keywords**

15 *Legionella pneumophila*, bacterial effectors, transposon insertion sequencing, virulence  
16 factors

17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29 \*For correspondence:

30 Carmen Buchrieser

31 Biologie des Bactéries Intracellulaires, Institut Pasteur

32 28, rue du Dr. Roux, 75724 Paris Cedex 15, France

33 Tel: +33.1.45.68.83.72

34 E-mail:cbuch@pasteur.fr

35 **Abstract**

36

37 *Legionella pneumophila* secretes over 300 effector proteins that manipulate host cells. This  
38 multiplicity of effectors hampers the characterization of their individual roles. Shames *et al.*  
39 report a new approach to solve the enigma of *Legionella* effector function by using INSeq to  
40 analyse effector functions in the context of infection.

41

42

43

44

45

46           Understanding how bacterial pathogens modulate host pathways and hence counteract  
47 host defence and cause disease is fundamental in our fight against infectious diseases. The  
48 intracellular pathogen *Legionella pneumophila*, the causative agent of Legionnaires' disease,  
49 is a unique model to study pathogen manipulation of host pathways during infection, with its  
50 sophisticated arsenal of molecular weapons that are secreted into the host cell to counteract  
51 host defences.

52           *L. pneumophila* is primarily an environmental bacterium that replicates within  
53 protozoan hosts (amoeba). When *Legionella*-contaminated aerosols are inhaled the bacterium  
54 can replicate within alveolar macrophages and cause an atypical pneumonia in susceptible  
55 individuals. *L. pneumophila* utilizes the same processes to parasitize human or protozoan cells  
56 as the *L. pneumophila* life cycle has been shown to be identical in all host cell types  
57 examined: the bacterium is internalized in a membrane-bound compartment called the  
58 *Legionella* containing vacuole (LCV) that escapes from lysosomal degradation, with the LCV  
59 remodeled through recruitment of host endoplasmic reticulum-derived vesicles into a  
60 protective vacuole for bacterial replication [1].

61           All key steps of the establishment of the LCV and the ability to replicate  
62 intracellularly are dependent on a functional Dot/Icm type IV secretion system (T4SS). This  
63 highly conserved machinery is essential for virulence as it secretes over 300 effector proteins  
64 into the host cell [2]. Genome sequencing has revealed a considerable plasticity and diversity  
65 in the effector repertoires encoded by different *L. pneumophila* strains, suggesting that this  
66 diversity allows adaptation to the different hosts *L. pneumophila* can parasitize [3]. Uniquely,  
67 among the over 300 T4SS effectors are an abundance of proteins with high similarity to  
68 eukaryotic proteins or proteins encoding eukaryotic-like domains, suggesting that molecular  
69 mimicry of eukaryotic proteins is a main virulence strategy of *L. pneumophila* [4]. Thus, the  
70 broad spectrum of tools employed for bacterial survival and replication in eukaryotic cells  
71 seems to have evolved during co-evolution of *L. pneumophila* and its protozoan hosts by  
72 horizontal gene transfer among domains of life, allowing this bacterium to parasitize a wide  
73 variety of hosts.

74           Although the T4SS is strictly necessary to initiate and sustain intracellular replication  
75 of *L. pneumophila*, the contribution of individual effectors remains minor [5]. Indeed, after  
76 the first excitement of scientists that *L. pneumophila* may encode as many functions as  
77 effectors, it soon became evident that high functional redundancy was present, and that most  
78 of the effectors did not show a virulence phenotype in the standard infection models used.

79 Furthermore, although a few effectors analyzed individually have been shown to play a role in  
80 intracellular replication of *L. pneumophila*, an efficient method to systematically assess the  
81 contribution of each effector was missing.

82 Shames and colleagues recently developed a new approach to define the contribution  
83 of the many *L. pneumophila* effector proteins during infection using a high-throughput  
84 transposon insertion sequencing technique called INSeq [6]. Guided by the principle of  
85 signature tagged mutagenesis, a number of groups have previously developed multiplex  
86 screening systems that combined random transposon mutagenesis and next generation  
87 sequencing (TnSeq) [7]. These comprehensive approaches allow high-resolution functional  
88 screening of whole genomes to identify key genes across various conditions *in vitro* or during  
89 host colonization. However, a limitation of *in vivo* experiments is that during the  
90 establishment of infection a considerable number of bacteria can be killed or removed  
91 stochastically; thus one cannot distinguish whether the bacteria were eliminated by chance or  
92 because they were indeed less fit. The approach of Shames *et al.* to circumvent such  
93 bottlenecks was to reduce the complexity of the input pool, establishing an effector mutant  
94 pool (EMP) library where 297 of the 315 genes predicted to encode effector proteins were  
95 represented. Subsequently they used the INseq technique to study the effects of this EMP on  
96 virulence phenotypes in amoeba, bone marrow-derived macrophages (BMDMs), and mice.  
97 This comprehensive approach allowed them to cluster individual mutants in distinct  
98 categories depending on their fitness phenotype. Mutants showing a universal defect, a  
99 mammalian-, lung-, BMDM-, or amoeba-specific virulence defect were identified. Indeed,  
100 effectors already known to play a role in *L. pneumophila* virulence were selected (*e.g. mavN*  
101 [8]), confirming the power of the technique to identify proteins implicated in virulence.  
102 Moreover, virulence phenotypes were detected for a large number of uncharacterized effector  
103 mutants, opening new vistas on the characterization of secreted *L. pneumophila* proteins. Due  
104 to the unbiased approach, effector proteins that contributed both positively and negatively to  
105 bacterial fitness could also be identified.

106 Another strength of the study was that the effector mutants were analyzed in three  
107 different host systems, which opened the possibility of evaluating the contribution of  
108 individual effectors in specific host contexts. An example is LegC4, an effector that, when  
109 deleted, conferred a competitive advantage on the mutant during *in vivo* murine infections,  
110 but not for *ex vivo* cellular infections. This was likely due to a role of LegC4 in stimulating  
111 pro-inflammatory responses *in vivo* that drive enhanced host control of the infection.

112 Furthermore, new metaeffectors were identified. First described by Kubori and  
113 colleagues [9], metaeffectors are defined as effectors that act on another effector to modulate  
114 its function in the host cell. The characterization of metaeffectors is an important step in  
115 understanding *L. pneumophila*-host interactions as uncovering their spatiotemporal regulation  
116 provides insight on how the bacterium modulates diverse host cell functions at discrete stages  
117 of infection. Shames and colleagues showed that Lpg2505 is a metaeffector that regulates the  
118 previously characterized effector SidI [10]. Interestingly, as with all metaeffectors identified  
119 to date, *lpg2505* constitutes an operon with *sidI*, and suppresses SidI toxicity to prevent host  
120 damage that would limit *L. pneumophila* replication efficiency.

121 In summary, INSeq analyses of a defined effector mutant pool in three different  
122 infection models allowed virulence phenotypes to be assigned to multiple effectors for which  
123 functions were previously unknown. Furthermore, effectors having distinct impact in different  
124 host models, or metaeffectors and effectors that act negatively on fitness can be identified.  
125 This new approach to identifying previously cryptic effector functions can now be applied  
126 across multiple bacterial pathogens and infection systems, opening the door to systematic  
127 deconvolution of host-pathogen interactions.

128 **References**

129

- 130 1. Isberg, R.R. et al. (2009) The *Legionella pneumophila* replication vacuole: making a cosy  
131 niche inside host cells. *Nat Rev Microbiol* 7 (1), 13-24.
- 132 2. Ensminger, A.W. (2016) *Legionella pneumophila*, armed to the hilt: justifying the largest  
133 arsenal of effectors in the bacterial world. *Curr Opin Microbiol* 29, 74-80.
- 134 3. Gomez-Valero, L. and Buchrieser, C. (2013) Genome dynamics in *Legionella*: the basis of  
135 versatility and adaptation to intracellular replication. *Cold Spring Harb Perspect Med* 3 (6),  
136 a009993.
- 137 4. Cazalet, C. et al. (2004) Evidence in the *Legionella pneumophila* genome for exploitation  
138 of host cell functions and high genome plasticity. *Nat Genet* 36 (11), 1165-1173.
- 139 5. O'Connor, T.J. et al. (2011) Minimization of the *Legionella pneumophila* genome reveals  
140 chromosomal regions involved in host range expansion. *Proc Natl Acad Sci U S A* 108  
141 (36), 14733-40.
- 142 6. Shames, S.R. et al. (2017) Multiple *Legionella pneumophila* effector virulence phenotypes  
143 revealed through high-throughput analysis of targeted mutant libraries. *Proc Natl Acad Sci*  
144 *U S A* 114 (48), E10446-E10454.
- 145 7. van Opijnen, T. and Camilli, A. (2013) Transposon insertion sequencing: a new tool for  
146 systems-level analysis of microorganisms. *Nat Rev Microbiol* 11 (7), 435-442.
- 147 8. Portier, E. et al. (2015) *IroT/mavN*, a new iron-regulated gene involved in *Legionella*  
148 *pneumophila* virulence against amoebae and macrophages. *Environ Microbiol* 17 (4),  
149 1338-50.
- 150 9. Kubori, T. et al. (2010) *Legionella* metaeffector exploits host proteasome to temporally  
151 regulate cognate effector. *PLoS Pathog* 6 (12), e1001216.
- 152 10. Shen, X. et al. (2009) Targeting eEF1A by a *Legionella pneumophila* effector leads to  
153 inhibition of protein synthesis and induction of host stress response. *Cell Microbiol* 11 (6),  
154 911-926.
- 155