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# *Legionella*-protozoa-nematode interactions in aquatic biofilms and influence of Mip on *Caenorhabditis elegans colonization*

Janine Rasch[a], Stefanie Krüger[a], Dominique Fontvieille[b], Can M. Ünal[a], Rolf Michel[c], Aurélie Labrosse[d], Michael Steinert[a,e,*]

[a] *Institut für Mikrobiologie, Technische Universität Braunschweig, Germany*
[b] *UMR CARRTEL, Universite de Savoie-INRA, Le Bourget du Lac, France*
[c] *Central Institute of the Federal Armed Forces Medical Services, Koblenz, Germany*
[d] *Thermes Nationaux dAix-les Bains, Aix-les Bains, France*
[e] *Helmholtz Center for Infection Research, Braunschweig, Germany*

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ABSTRACT

*Legionella pneumophila*, the causative agent of Legionnairesí disease, is naturally found in aquatic habitats. The intracellular life cycle within protozoa pre-adapted the "accidental" human pathogen to also infect human professional phagocytes like alveolar macrophages. Previous studies employing the model organism *Caenorhabditis elegans* suggest that also nematodes might serve as a natural host for *L. pneumophila*. Here, we report for the first time from a natural co-habitation of *L. pneumophila* and environmental nematode species within biofilms of a warm water spring. In addition, we identified the protozoan species *Oxytricha bifaria*, *Stylonychia mytilus*, *Ciliophrya* sp. which have never been described as potential interaction partners of *L. pneumophila* before. Modeling and dissection of the *Legionella*-protozoa-nematode interaction revealed that *C. elegans* ruptures *Legionella*-infected amoebal cells and by this means incorporate the pathogen. Further infection studies revealed that the macrophage infectivity potentiator (Mip) protein of *L. pneumophila*, which is known to bind collagen IV during human lung infection, promotes the colonization of the intestinal tract of L4 larvae of *C. elegans* and negatively influences the life span of the worms. The Mip-negative *L. pneumophila* mutant exhibited a 32-fold reduced colonization rate of the nematodes after 48 h when compared to the wild-type strain. Taken together, these studies suggest that nematodes may serve as natural hosts for *L. pneumophila*, promote their persistence and dissemination in the environment, and co-evolutionarily pre-adapt the pathogen for interactions with extracellular constituents of human lung tissue.



## 1. Introduction

It is an intriguing question why bacteria with a solely environmental life cycle can be very successful human pathogens. The Gram-negative bacterium *Legionella pneumophila* is a prominent example, since the reservoir and the evolution of the causative agent of Legionnairesí disease is restricted to environmental habitats (Amaro et al., 2015). *L. pneumophila* naturally inhabits freshwaters and biofilms, where it parasitizes intracellularly within different protozoa species. *L. pneumophila* also thrives in man-made water systems including air conditioning systems and cooling towers (Hilbi et al., 2011). Infection of humans is by inhalation of *L. pneumophila* contaminated aerosols, which can lead to a severe and life-threatening pneumonia. Upon transmission, the bacteria invade and replicate mainly within alveolar macrophages (Winn and Myerowitz, 1981). Hallmarks of advanced stages of infection are fibrinolysis, cellular infiltrations of neutrophils and macrophages, and alveolar damage followed by dispersal of extracellular bacteria to other organs (Jäger et al., 2014).

*L. pneumophila* only accidentally colonizes humans after transmission by technical vectors (Benin et al., 2002; Marston et al., 1994). In the light of evolution, interactions of *L. pneumophila* with protozoa may have led to a pre-adaptation, which enables the pathogen to also infect human phagocytes like alveolar macrophages (Greub and Raoult, 2004; Molmeret et al., 2005; Uenal et al., 2011). During intracellular infection of different protozoa species and human macrophages the *Legionella*-containing vacuole

* Corresponding author at: Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstr. 7, 38106, Braunschweig, Germany.
*E-mail address:* m.steinert@tu-bs.de (M. Steinert).

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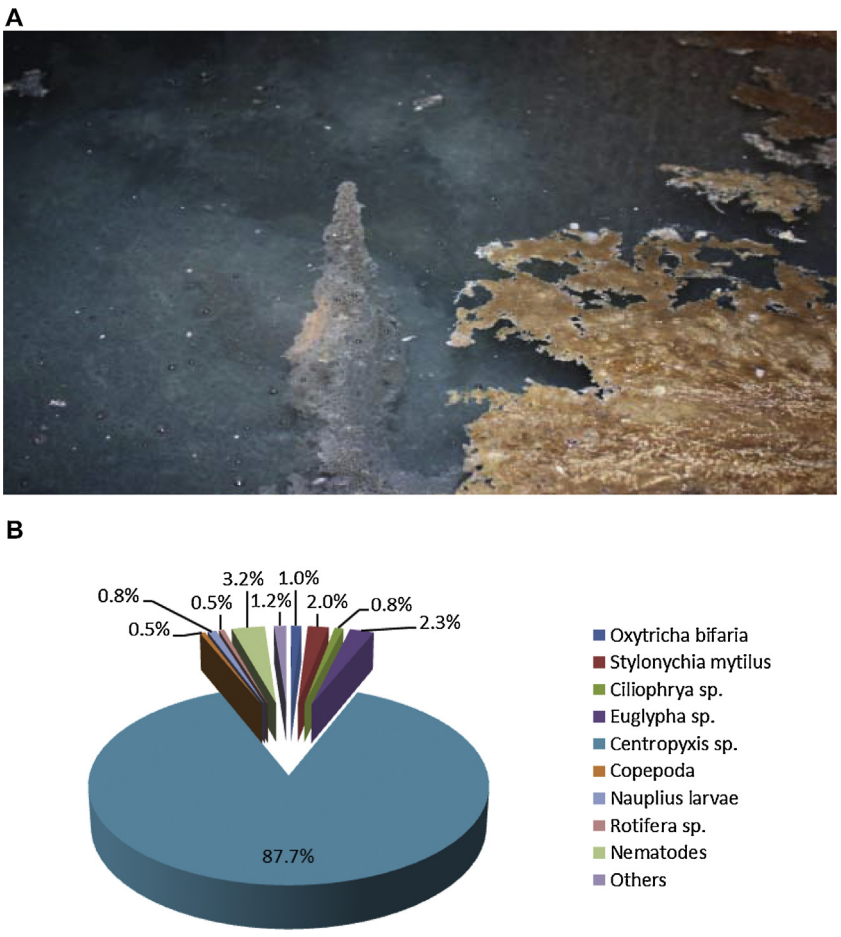


**Fig. 1.** Biofilms from the thermal spring Soufre (Lake Bourget tributaries, Aix-les Bains, France). (A) Floating biofilms from the air interface were collected for further microbial analyzes. The water temperature was 37° C and the cfu of *L. pneumophila* ranged from 1 to 12 per ml. (B) Species distribution of protozoans and metazoan invertebrates in isolated *Legionella*-positive biofilms. The frequency of encounters between the different species was determined microscopically.

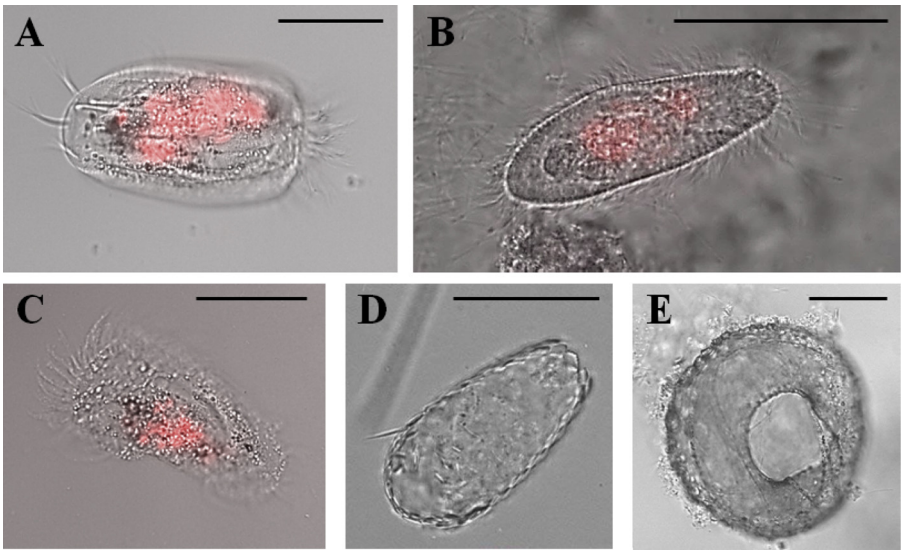


**Fig. 2.** Microscopic *in situ* detection of mCherry labeled *L. pneumophila* within protozoa after inoculation of natural biofilm samples. Red fluorescent bacteria were detected within the protozoa species (A) *Oxytricha bifaria*, (B) *Stylonychia mytilus*, and (C) *Ciliophrya* sp., but not in (D) *Euglypha* sp., and (E) *Centropyxis* sp. Size bars indicate 50 μm.

(LCV) recruits small GTPases, modulates the host phosphoinositide metabolism, modifies the host endocytic pathway, intercepts vesicle trafficking and avoids fusion with lysosomes (Hubber and Roy, 2010). Moreover, *L. pneumophila* uses the proteasome machinery to generate amino acids essential for bacterial growth within LCVs. The pivotal *L. pneumophila* virulence factor dominating the inter-