Intracellular life of *Francisella* and *Legionella* within amoebae cells

Unutarstanični život bakterija *Francisella* i *Legionella* u amebama

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Abstract. free-living amoebae are present in the nature, feeding mainly with bacteria, fungi, and algae. Some microorganisms have evolved different mechanisms to resist the digestion by amoebae and they are called "amoeba-resistant microorganisms". Some of the important human bacterial pathogens belong to this category including *Cryptococcus neoformans, Chlamydophila pneumoniae*, *Mycobacterium avium*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Legionella* spp., and *Francisella tularensis*. *Francisella* and *Legionella* are gram negative facultative intracellular bacteria. Although the diseases they cause are completely different, they share some of the unique features in intracellular lifestyle within amoeba cells.

Key words: amoeba; *Francisella*; *Legionella*

Sažetak. Slobodno-živuće amebe prisutne su u prirodi, a hrane se uglavnom bakterijama, gljivama i algama. Neki mikroorganizmi razvili su različite mehanizme kojima izbjegavaju razgradnju unutar stanica ameba te se nazivaju „ameba-otporni mikroorganizmi". Bakterije koje pripadaju toj skupini uključuju *Cryptococcus neoformans, Chlamydophila pneumoniae*, *Mycobacterium avium*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Legionella* spp. i *Francisella tularensis*. *Francisella* i *Legionella* su gram negativne fakultativno unutarstanične bakterije. Iako dovode do potpuno drugačijih oblika bolesti, te bakterije pokazuju sličan životni ciklus unutar stanica ameba.

Ključne riječi: ameba; *Francisella*; *Legionella*

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INTRODUCTION

Nonpathogenic bacteria are taken up by the cells into vacuoles or phagosomes that are processed into endocytic pathway during which the vacuoles matures into the lysosomes where the bacterium is degraded. Phagocytosis and degradation of bacteria within the phagolysosomes is our first line of defense against microorganisms. To avoid this killing within phagocytic cells intracellular pathogens have evolved different strategies to survive and evade phagosome lysosome fusion: (i) escape from the phagosome into the cytoplasm such as the case for *Listeria* and *Shigella*[1,2]; (ii) adapt to the acidic harsh environment within the phagolysosomes, such as the case of *Coxiella*[3]; and (iii) the more common strategy is to modulate biogenesis of the phagosomes into niches permissive for intracellular replication, such as the case of *Mycobacterium, Chlamydia, Brucella, Salmonella* and *Legionella*[4-8]. Understanding the mechanisms by which pathogens explore the vesicle trafficking in different host is extremely important for understanding the ability of certain microbe to cause disease and is essential for designing novel strategies for prevention and treatment.

Most of the bacteria use similar mechanisms to adapt to different niches such as the case for *Legionella.* The life cycle of *Legionella* in amoebae and macrophages is very similar. Why *Francisella* uses different cycle in these pahagocitic cells?

Free-living amoebae are ubiquitous in natural sources such as soil, freshwater and dust, providing multiple opportunities for contact with humans[9-11]. They are also frequently isolated from anthropogenic ecosystems such as tap water, air conditioning units, cooling towers, jacuzzi tubs and hydrotherapy pools in hospitals, feeding on the microbial biofilm present in those systems[11-14]. Free-living amoebae such as *Acanthamoeba* and *Hartmannella* have been found in large variety of natural and anthropogenic habitats. Free-living amoebae feed mainly on bacteria, fungi, and algae by phagocytosis. In contrast, some bacteria can act like real amoeba pathogens able to lyse the amoeba cells before or after completing an intra-amoeba replication cycle. In addition, some microorganisms have shown the ability to survive the uptake by free-living amoeba and prevent intracellular destruction. These "amoeba-resistant microorganisms" include bacteria, viruses, and fungi. Bacteria are considered as symbionts when they manage to live in association with amoeba for a specific period of their lifetime[15,16]. Amoeba can serve as hosts for a large number of pathogenic bacteria, including *Francisella tularensis*, *Legionella pneumophila*, *Coxiella burnetii*, *Listeria monocytogenes*, many *Mycobacterium spp*., *Chlamydia* related bacteria, *Escherichia coli* serotype O157[11,17-23]. Taken together, both amoebal symbionts and amoebal pathogens may use amoeba as their replicative niche[24]. Amoeba play an important role for transmission of human pathogen bacteria to humans and they could be described like ''Trojan horse'' in the world of microbes[13,25,26]. Understanding the intracellular lifestyle of bacteria within amoeba cells is important to understanding of bacterial ecology and transmission to humans.

The intracellular life of *Francisella* and *Legionella* within amoebae cells will be discussed in this paper.

INTRACELLULAR LIFE OF *FRANCISELLA TULARENSIS*

F. tularensis is a gram negative, highly infectious, facultative intracellular bacterium that causes fulminating disease tularemia. The genus *Francisella* includes four organisms: *F. tularensis* subsp. *tularensis* (type A), *F. tularensis* subsp. *holarctica* (type B), *F. tularensis* subsp. *mediasiatica* and *Francisella novicida*. The ability of *F. tularensis* to envade and proliferate within cells was shown to be of great relevance for the development of tularemia[27-30]. *F. novicida* is able to survive and replicate within various cell types, macrophages, dendritic cells, epithelial cells, including amoebae cells[31-35]. *F. tularensis* subsp. *holarctica* and *F. novicida* have a strong association with fresh water environments, free living amoeba and biofilms[36,37]. It is assumed that the bacterium survives in such waters by different

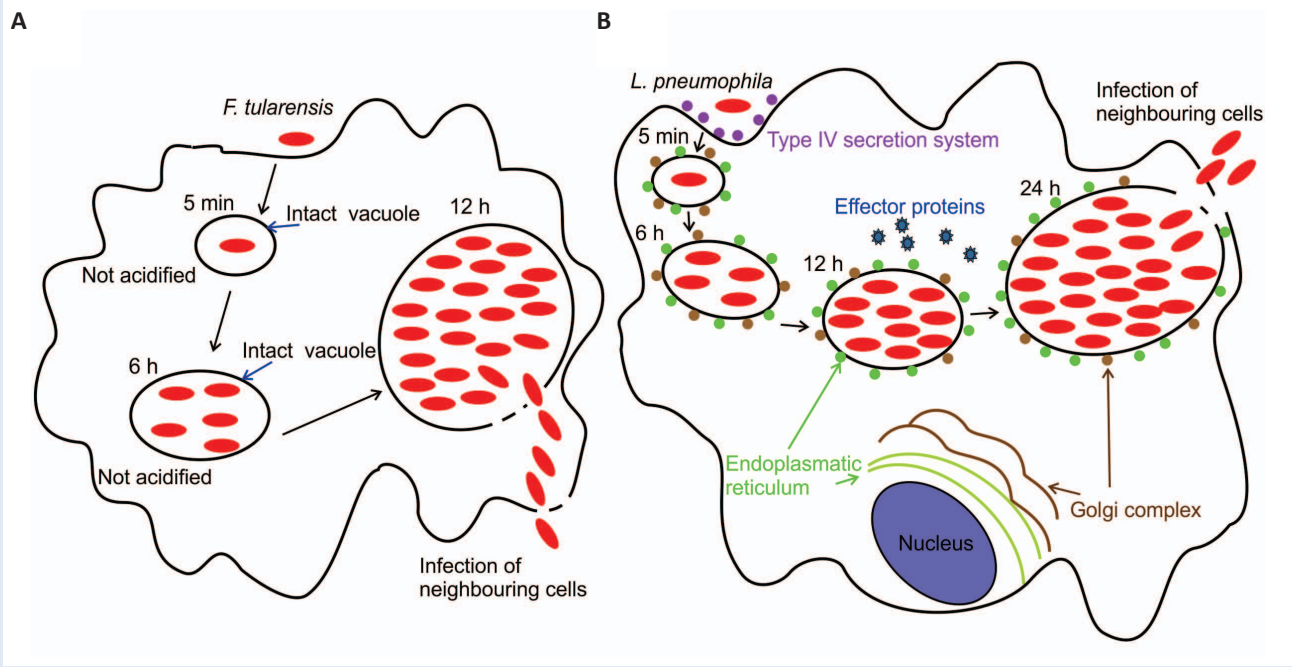

Figure 1. Intracellular life style of *F. tularensis* and *L. pneumophila* within amoebae cells. After phagocytosis by amoeba, bacteria are localized in intact vacuoles. Bacteria reside in the vacuoles and replicates. Purple circle – Type IV secretion system, Green circle – Endoplasmatic reticulum, Brown circle – Golgi complex, Blue star – effector protein.

models; i) persists in open water in a viable but not cultivable state in which bacteria are not infectious[38]; ii) lives in biofilm which is considered as an important niche for environmental survival and persistence of *Francisella*[39]; and iii) associated with protozoa.

Previous *in vitro* studies showed that *F. tularensis subsp. holarctica, tularensis,* and *F. novicida* are able to enter and multiply within *A. castellanii*[20,33,35,40-42], *H. vermiformis*[35] and *Dictyostelium discoideum* (unpublished data) cells. Once inside the cells the bacterium resides and replicates within membrane-bound vacuoles known as *Francisella*-containing vacuoles[35] (FCVs) (Figure 1A). In mammalian cells bacteria escape from *Francisella*-containing vacuoles (FCVs) and proliferate in cytosol, which is the major difference between these two host cells. In addition, imaging studies using the lysosomotropic agent LysoTracker Red DND-99, which concentrates in acidified vesicles and compartments, have shown that FCVs did not acquire this dye at any time point during the infection of amoebae with *F. tularensis* subsp. *novicida*[43]. It has also been shown that *F. novicida* blocks lysosomal fusion within *A. castellanii*[33]. In contrast, in mammalian cells the transient acidification of the FCV is essential for subsequent bacterial escape and replication of *Francisella* in the macrophage cytosol[44-47]. Therefore, *Francisella* escapes from acidified vacuoles in human and arthropod-derived cells, but replicates within non-acidified FCV in *H. vermiformis*[35].

The ability of *Francisella* to survive and replicate inside the protozoa cell seems to be of great importance in sustaining the life cycle of *Francisella* in aquatic environment.

INTRACELLULAR LIFE OF *LEGIONELLA PNEUMOPHILA*

L. pneumophila is an intracellular gram-negative bacterium, ubiquitous in the aquatic environment and important causative agent of community-acquired and nosocomial bacterial pneumonia. At present, 52 characterized species belongs to genus *Legionella* of which more than a half has been implicated in human disease[48]. However, *L. pneumophila* accounts for over 90% of legionellosis. Two distinct syndromes of the disease are known in the infection caused by *L. pneumophila;* Legionnaires' disease, a severe, acute pneumonia and a self-limiting flu-like infection termed Pontiac fever.

The bacterium enters the human body via inhalation of aerosol droplets. Once in the lungs, *L. pneumophila* invades and replicate mainly in alveolar macrophages[49,50]. The pathogenesis of legionellosis depends also on prior adaptation of *L. pneumophila* in the natural water environment. In freshwater, *Legionella* survive and replicate within free-living protozoa including ciliates *Tetrahymena* and *Cyclidium spp.* as well as amoeba species belonging to *Acanthamoeba, Hartmanella, Valkampfia, Naegleria and Dictyostelium.* In contrast to protozoa macrophages are not „natural" host cells for *Legionella*, although they utilize the same mechanism to avoid the degradation by these cells.

Free-living amoeba could serve as a good model in studying environmental existence and adaptation of *Francisella*.

Upon phagocytosis by protozoa *Legionella* utilizes the Icm/Dot type IV secretion system (T4SS) which plays important role in hijacking the normal phagocyte-lysosome pathway[17,51]. The bacterium resides in the *Legionella*-containing vacuole (LCV) and avoids the degradation by the lysosomes[52]. Within a few minutes, mitochondria and endoplasmic reticulum (ER)-derived vesicle are recruited to the LCV which becomes remodeled into an ER-derived compartment. The bacterium replicate extensively, disrupts phagosomal membrane escapes into the host cell cytosol, lyses and exit the cells to get ready for the new infection cycle[53].

Free-living protozoa represent valuable experimental model to study *Legionella* ecology and pathogenesis, as its natural hosts and as a paradigm for infection of human macrophages.

CONCLUSION

F. tularensis has shown a different lifestyle within macrophages and protozoa cells. Within macrophages *F. tularensis* escape from FCV and replicate in the cytosol, while in *H. vermiformis* bacteria replicate in intact vacuoles. However, the molecular and cellular aspects of infection by *L. pneumophila* in both protozoa and mammalian phagocytes are similar. Once engulfed by phagocytic cell, bacteria replicate extensively within phagosome. It is followed by lyses of host cell and proliferation of bacteria into the environment.

F. tularensis and *L. pneumophila* shares very similar life style in protozoa cells. It is likely that association of these bacteria with protozoa is a major factor in the continuous persistence of bacteria in the environment, as well as transmission of bacteria to humans. In addition, the human's cells seem to be the dead-end from the bacterial point of view, as no human-to-human transmission has been reported for *Francisella* or *Legionella.*

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