Biofilms: the environmental playground of *Legionella*

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Summary
*Legionella pneumophila*, the aetiologic agent of 90% of legionellosis cases, is a common inhabitant of natural and anthropogenic freshwater environments, where it resides in biofilms. Biofilms are defined as complex, natural assemblages of microorganisms that involve a multitude of trophic interactions. A thorough knowledge and understanding of *Legionella* ecology in relation to biofilm communities is of primary importance in the search for innovative and effective control strategies to prevent the occurrence of disease cases. This review provides a critical update on the state-of-the-art progress in understanding the mechanisms and factors affecting the biofilm life cycle of *L. pneumophila*. Particular emphasis is given to discussing the different strategies this human pathogen uses to grow and retain itself in biofilm communities. Biofilms develop not only at solid-water interfaces (substrate-associated biofilms), but also at the water-air interface (floating biofilms). Disturbance of the water surface can lead to liberation of aerosols derived from the floating biofilm into the atmosphere that allow transmission of biofilm-associated pathogens over considerable distances. Recent data concerning the occurrence and replication of *L. pneumophila* in floating biofilms are also elaborated and discussed.

Introduction
*Legionellaceae* are Gram-negative bacilli that belong to the gamma-2 subdivision of the *Proteobacteria* and comprise *Legionella* as sole genus (Fry et al., 1991; Benson and Fields, 1998). To date, more than 50 different *Legionella* species have been described (Diederen, 2008) and approximately half of them are associated with human illness, better known as legionellosis (Muder and Yu, 2002). Legionellosis comprises two distinct entities, namely: Legionnaires’ disease, an often fatal pneumonia, if not promptly and correctly diagnosed, and Pontiac fever, a milder, flu-like disease (reviewed by Diederen, 2008). *Legionella pneumophila* accounts for 90% of reported disease cases (Yu et al., 2002) and was recognized as being pathogenic to humans for the first time after an outbreak of acute pneumonia at a convention of the American Legion in Philadelphia, USA in July 1976 (Fraser et al., 1977).

*Legionellae* are ubiquitously present in both natural and anthropogenic freshwater environments, where they can withstand temperatures of 5.0°C-63°C and a pH range of 5.0–9.2 (Fliermans et al., 1981; Atlas, 1999). This shows that the conditions under which *Legionella* spp. occur in the environment are not stringent. Using PCR-based techniques, Sheehan and colleagues (2005) recently detected at least four *Legionella* species in an extremely acidic (pH 2.7), predominately eukaryotic algal biofilm community in Yellowstone National Park. Natural environments are rarely related to legionellosis because habitat conditions do not support extensive *Legionella* spp. growth. The only natural waters considered a source of legionellosis are hot springs, where temperatures generally range between 35°C and 40°C (Mashiba et al., 1993). Upon transfer from natural freshwater habitats into anthropogenic systems, where temperatures are generally higher than ambient temperature, *L. pneumophila* colonizes existing biofilms and proliferates to high numbers (Rogers et al., 1994). Also, it has the ability to parasitize protozoans, which commonly graze on biofilm communities (Greub and Raoult, 2004; Kuiper et al., 2004; Declerck et al., 2005; 2007a). As legionellosis is generally considered a preventable illness, efficient control measures of the bacterium in the water concerned will lead to a significant decrease in disease cases. In this context, exhaustive effort is directed towards uncovering the ecology of biofilm-associated *L. pneumophila*.

Biofilms: a safe harbour for *L. pneumophila* in the cruel oligotrophic aquatic environment

In order to grow as a pure culture under lab conditions, *L. pneumophila* is remarkably fastidious and requires several different nutrients including iron salts and a number of amino acids such as L-cysteine, which are used as carbon, nitrogen and energy sources (George et al., 1980; Edelstein, 1982; Keen and Hoffman, 1984). The fact that *L. pneumophila* is commonly detected in oligotrophic aquatic environments (low nutrient content), despite its fastidious nature implies that the bacterium is able to obtain its necessary supply of amino acids and organic carbon from that same environment, more specifically from the microbial consortium located in biofilms. Biofilms are defined as complex microbial communities characterized by cells that are attached to a substrate or phase boundary and to each other by means of a matrix of extracellular polymeric substances (EPS) (Donlan and Costerton, 2002). Microcolonies of bacterial cells encased in the EPS matrix are separated from each other by interstitial water channels, allowing transport of nutrients, oxygen, genes and even antimicrobial agents (Prakash et al., 2003). Because of their dynamic character, biofilm communities can continuously change in time and space, providing better survival and growth of the associated microorganisms. For this reason, it is easy to understand that in most natural environments biofilms are the prevailing microbial lifestyle (Watnick and Kolter, 2000). Generally, there are three distinct phases in the ‘biofilm life cycle’ of bacteria: (i) bacterial attachment to a substratum, (ii) biofilm maturation and (iii) detachment from the biofilm and subsequent dispersal in the environment (reviewed by Donlan, 2002).