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A study of gene expression in Legionella pneumophila biofilms through the use of confocal microscopy

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Abstract

Legionella pneumophila is the causative agent of Legionnaires' Disease. *L. pneumophila* is ubiquitous in freshwater environments as well as in man-made water systems such as air conditioners and cooling towers. *Legionella* biofilms in these systems have been identified as the source of a number of outbreaks. Gene expression in planktonic phase *L. pneumophila* has been well characterized but little analysis has been conducted within biofilms. We hypothesize that gene expression in *Legionella* biofilms will exhibit unique expression patterns as compared to planktonic cells. To test this hypothesis *Legionella* were transformed with reporter gene vectors and biofilms grown on glass slides and imaged using confocal microscopy. Characterization of biofilm stages was conducted from attachment through dispersal. Gene expression of the global regulatory protein, CsrA, and the flagellar gene, FlaA, was quantified over 120hr of biofilm growth. Biofilms were imaged at five key time points in the biofilm development: 12 hr (initial attachment), 24hr (irreversible attachment), 48hr (early maturation), 72hr (late maturation), 96hr (mature biofilm) and 120hr (mature biofilm with dispersal). Whole biofilm fluorescence was measured with syto59 staining and compared to the percentage of cells that demonstrated GFP fluorescence from the reporter gene. DIC images clearly demonstrate that Legionella biofilms follow the typical biofilm developmental stages. Analysis of the CsrA expression showed upregulation in early biofilms but little to no CsrA expression in mature biofilms. FlaA was expressed in early biofilms and during late biofilms where dispersal was occurring. Planktonic cultures are often used to characterize cycles of gene expression which are often not identical to the patterns seen in biofilms. *Legionella* biofilms are not well characterized molecularly and here we present the first evidence showing gene expression patterns of essential genes over time within biofilms. Use of confocal microscopy for such assays provides a high resolution, specific image that allows for quantification and detailed analysis of gene expression. This research begins the opportunity to better understand biofilm gene expression that can lead to improved prevention and control of infectious biofilms.

Hypothesis:
We hypothesize that gene expression in *Legionella* biofilms will exhibit unique expression patterns as compared to planktonic cells.

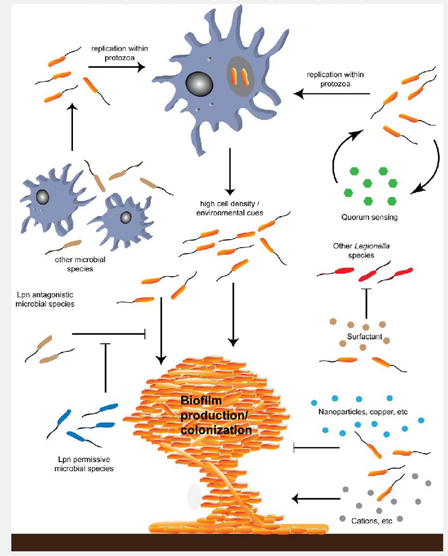
Objectives:
1. Understand the development of *Legionella* biofilms
2. Characterize the gene expression of CsrA in *Legionella* biofilms
3. Characterize the gene expression of FlaA in *Legionella* biofilms

Materials:
Buffered Charcoal Yeast Extract agar (BCYE)
ACES-buffered Yeast Extract broth (AYE)
Kanamycin (20 µg/mL)
Syto 59 DNA stain (3µM)
pCsrA and pFlaA – M. Swanson, U Michigan

Microorganisms:
Legionella pneumophila Lp02 pCsrA
Legionella pneumophila Lp02 pFlaA

Microscopes:
Leica SP8X MP

Biofilm Schematic



Abdel-Nour, M., Duncan, C., Low, D. & Guyard, C. Biofilms: The Stronghold of Legionella pneumophila. *International journal of molecular sciences* **14**, 21660–75 (2012).

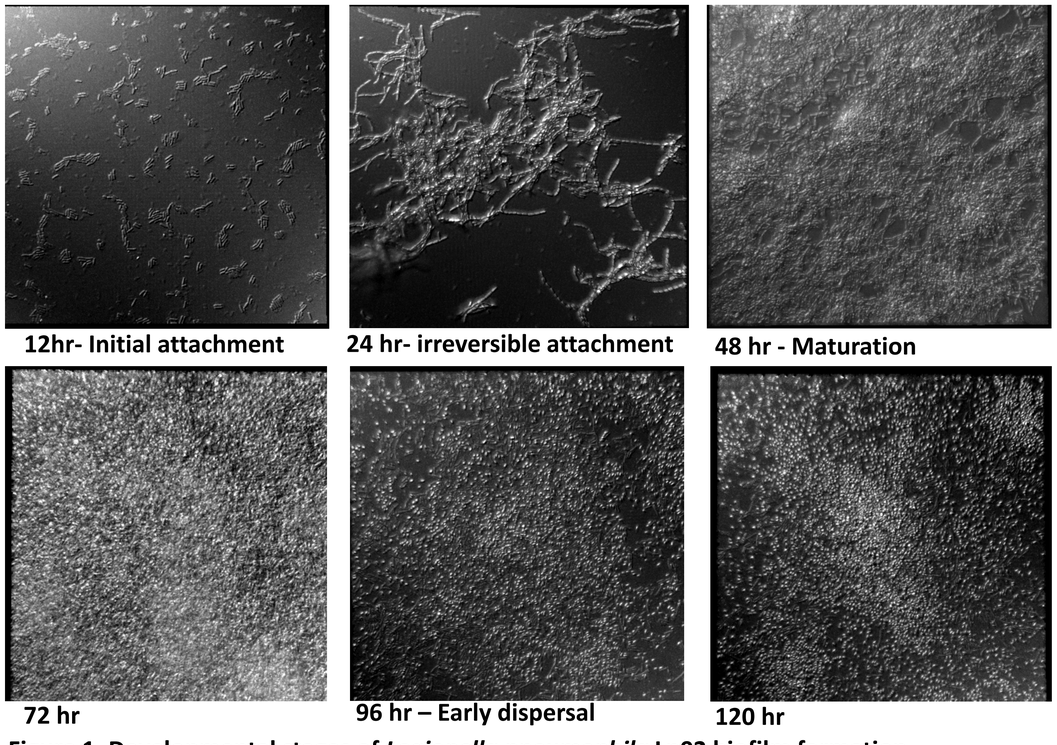


Figure 1. Developmental stages of *Legionella pneumophila* Lp02 biofilm formation.

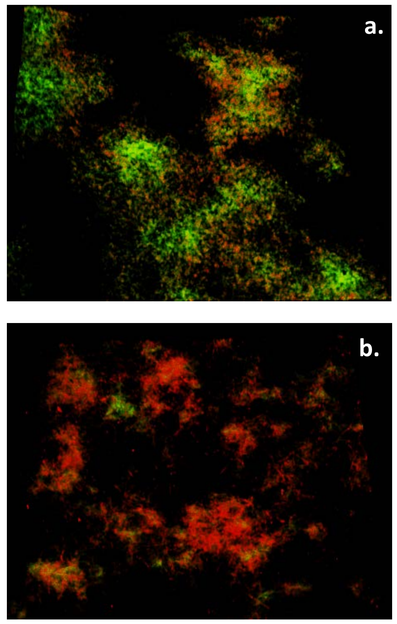


Figure 2. FlaA expression during biofilm growth at a) 72h – maturation and b) 120h – after dispersal event. FlaA activity is seen as GFP expression (green) while the biofilm is stained with Syto 59 (red).

Conclusion

- *Legionella pneumophila* biofilms show attachment at 12hours and exhibit maturation at around 96hours
- CsrA expression was seen in early development and decreased as biofilm maturation progressed
- FlaA was expressed both in early – mid developmental stages and lost after dispersal

Future Directions

- Quantify data and analyze to characterize gene expression in Lp02
- Grow and image *L. pneumophila Δ2107 pFlaA* and *pCsrA* biofilms and characterize their gene expression

Acknowledgements
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