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

D. Limbaugh
T. McNealy
A study of gene expression in *Legionella pneumophila* biofilms through the use of confocal microscopy

David Limbaugh, Terri Bruce and Tamara L. McNealy

Department of Biological Sciences
Clemson University, Clemson, SC

microbesadapt.com

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 (green) while the biofilm is stained with Syto 59 (red). 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)

**Microorganisms:**

*Legionella pneumophila* Lp02 pCsrA
*Legionella pneumophila* Lp02 pFlaA

**Microscopes:**

Leica S8XM-CL MP

**Acknowledgements**

The authors would like to thank the Creative Inquiry program and the Calhoun Honors college for funding support.

**Biofilm Schematic**

[Image of biofilm schematic]

**Future Directions**

- Quantify data and analyze to characterize gene expression in *L. pneumophila* biofilms
- Characterize the gene expression of FlaA and CsrA in *L. pneumophila* biofilms

**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

**References**


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