Analysis of Biofilm Communities for *Legionella pneumophila*

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Abstract

Legionella pneumophila is a pathogenic bacterium that causes Legionnaires' disease, which presents as a form of pneumonia. *L. pneumophila* occurs naturally in the environment but persists in biofilms in indoor water-based environments such as air conditioners and cooling towers. Cooling towers are a known source of outbreaks of the disease. Records of *Legionella* presence in cooling towers show that some towers are continually contaminated with the bacteria while in others it is never detected. We hypothesize that the microbial community present in the towers affects the ability of Legionella to colonize and/or persist within tower environments. For this study, cooling towers that are *L. pneumophila* positive or *L. pneumophila* negative will be sampled and the associated biofilm communities analyzed for fungi, bacteria, and archaea. gDNA from samples will be extracted using the ZR Soil Microbe DNA MiniPrep Kit. PCR using general fungal, 16S bacterial, and archaeal primers will be run to determine the general community makeup. PCR with *Legionella* specific primers, which amplify the 16S RNA region of the *Legionella* genus, will be conducted to verify presence of *Legionella*. Finally, DGGE will be conducted to examine total community profile. In preliminary analyses of three towers, *Legionella*, fungi, bacteria, and archaea were found to be present in samples from all three towers. From continued community analysis, we will attempt to identify cooling tower biofilm community members that have prevented colonization by *L. pneumophila*. Identification of members antagonistic to Legionella will add in development of improved prevention strategies.

Hypothesis:

We hypothesize that community members play a role in the persistence of *L. pneumophila* within biofilms in cooling towers and fungi within the community may be capable of preventing colonization of *L. pneumophila* through bacteriocidal properties.

Objectives:

– Determine presence of Legionella in cooling tower samples.

– Characterize the biofilm community from the cooling tower samples

– Compare communities across towers and across time in same towers.

– Identify the community members affecting *L. pneumophila* presence

Materials

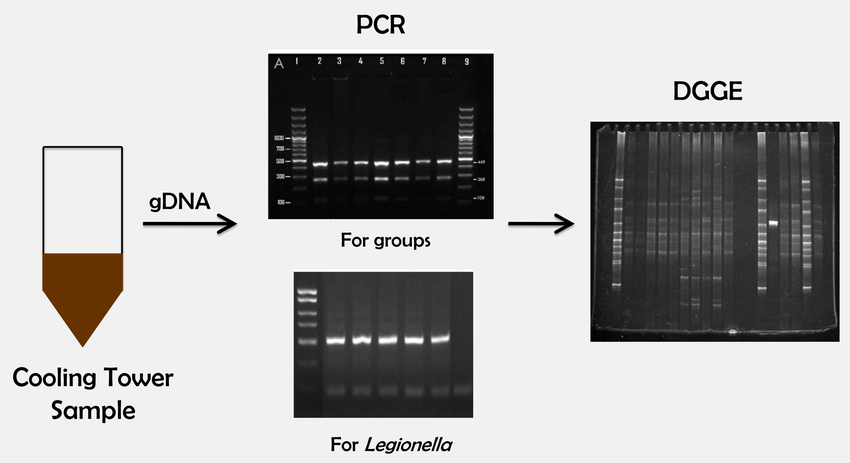
–Biofilm, filter, and retentate samples from cooling towers (Savannah River Site)

–ZR Soil Microbe DNA Miniprep Kit

–PCR primers
- –16S Bacterial: 8 F / 1492 R
- –Fungal: ITS1 F / ITS4 R
- –Archeal: 69 F / D30 R
- –Legionella 16S RNA

–DGGE primers
- –Bacterial: PRBA968 F / PRBA1406 R
- –Fungal: DGGE-nu-SSU-0817 / nu-ssu-1196
- –Initial Archaeal: PRA46 F / PREA1100 R
- –Final Archaeal: PARCH340 F / PARCH519 R

Methods



Experiment Schematic

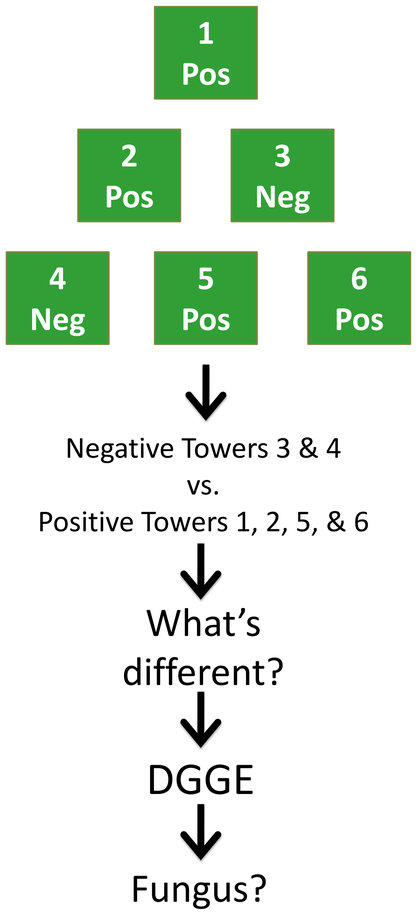


Figure 1. Cooling tower sampling and testing schematic based on presence of *Legionella pneumophila*

PCR Results

Table 1. PCR results for each cooling tower biofilm using 16S Bacterial, Fungal, Archaeal, and *Legionella* primers

Biofilm Samples	16S Bacterial	Fungal	Archaeal	Legionella
Tower 1	+	+	+	+
Tower 2	+	+	+	+
Tower 3	+	+	+	+

Table 2. PCR results for filter samples from each cooling tower

Filter Samples	Method of ID	Legionella
Tower 1	Culture,PCR	+
Tower 2	Culture,PCR	+
Tower 3	Culture,PCR	+

Table 3. PCR results for retentate samples from each cooling tower

Retentate Samples	Method of ID	Legionella
Tower 1	PCR	+
Tower 2	PCR	+
Tower 3	PCR	+

DGGE Results

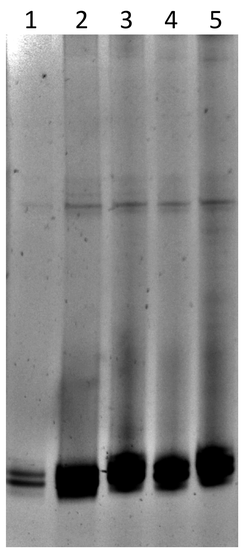


Figure 2. DGGE results for cooling tower biofilms using Bacterial primers

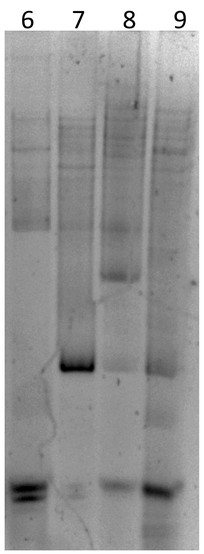


Figure 3. DGGE results for cooling tower biofilms using Archaeal primers

Table 4. Samples in each lane of DGGE

Lane	Type	Sample	Collection Date
1	Bacteria	Tower 1	4.19.12
2	Bacteria	Tower 2	1.31.12
3	Bacteria	Tower 3	1.31.12
4	Bacteria	Tower 3	4.19.12
5	Bacteria	Tower 3	7.11.12
6	Archaea	Tower 1	1.31.12
7	Archaea	Tower 3	1.31.12
8	Archaea	Tower 3	4.19.12
9	Archaea	Tower 3	7.11.12

Expected Outcome

Identification of members of the microbial communities within biofilms of cooling towers.

Future Directions

- Develop most efficient method for DGGE preparation and imaging
- Compare biofilm communities at different times of the year
- Identify agents that inhibit *Legionella pneumophila* colonization

Acknowledgements

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