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CU and the CDC

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Abstract

Legionella is a gram-negative genus of bacteria that is the causative agent of Legionnaires' disease. Currently, 50 species and 70 serogroups of *Legionella* have been identified from both environmental and clinical samples. The Center for Disease Control (CDC) in Atlanta, Georgia maintains a bank of both previously identified and unidentified *Legionella* samples. The availability of sequencing technologies has greatly increased in the time since many of the samples were collected, allowing us to identify many of the previously unidentifiable isolates. We received 68 isolated, unidentified samples from the CDC with the goal to sequence and characterize them in the search for identification of novel species. A sequence based typing scheme designed for *Legionella* was used for initial characterization. Genomic DNA was extracted from each sample and then PCR was performed on the 16S and the *mip* genes. These samples were then sequenced at CUGI. Currently, we have identified several samples which were previously undescribed. Once a sample is identified as novel, further characterization through sequencing of additional genes along with morphological and biochemical assays will be conducted. As a collaborative project, regular meetings occur with scientists from the *Legionella* Lab at the CDC. Characterization of novel strains expands the ability of this lab in conducting outbreak analysis and risk assessment along with expanding our knowledge of the pathogen.

Hypothesis

Unidentified samples of *Legionella* can be identified based on sequencing of the *mip* and16S genes. Novel samples can be further characterized using sequencing by synthesis guidelines

Objectives

1. Identify unknown isolates collected by the CDC
2. Use bioinformatic tools to analyze sequence data and identify *Legionella* species
3. Conduct further biochemical assays and genetic sequencing to characterize novel species

Materials

Buffered Charcoal Yeast Extract agar (BCYE)

PCR protocol (CDC)

DNeasy Blood and Tissue Kit for DNA extraction

PCR mini extract agarose kit

MEGA

BioEdit

NCBI BLAST Database

Experiment Design

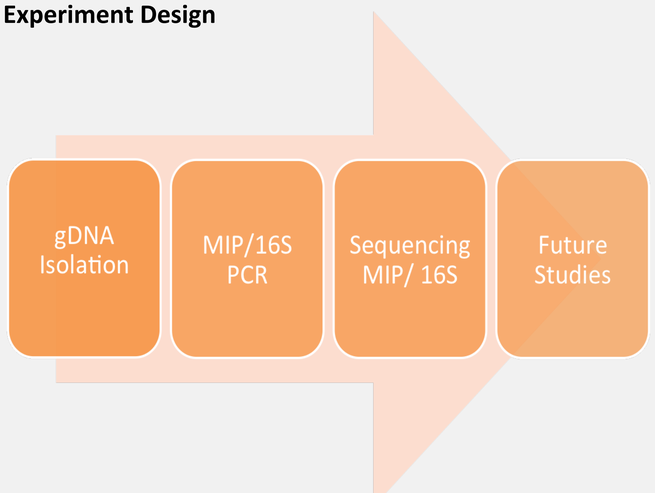

Figure 1. Red fluorescence of *L.erythra*

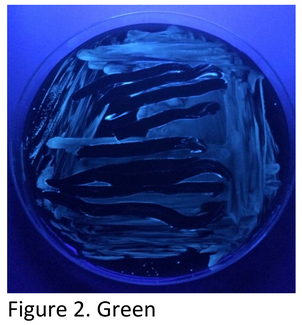

Figure 2. Green fluorescence of CU 21

CU #	Origin	Source	Identification
1	Arizona	Environmental	*L. erythra* based on mip gene
2	Wisconsin	BAL	*L. erythra* based on mip gene
3	Iowa	Environmental	*L. pneumophila* based on mip gene
4	Kentucky	Bronchial	*L. pneumophila* based on 16S and mip genes
5	New York	Environmental	*L. pneumophila* based on 16S and mip genes
6	Ohio	Bronch Wash	Novel species based on 16S and mip genes
7	*Georgia*	Bronch Wash	*L. wadsworthii based on 16S gene*
8	Japan	Unknown	*L. busanensis based on mip gene*
9	Japan	Unknown	*L. erythra based on mip gene*

CU #	Origin	Source	Identification
10	California	Environmental	*L. steelei* based on *mip* gene
11	Ohio	Lung Lobe	Needs Further Identification
12	Ohio	Environmental	*L. erythra* based on *mip* gene
13	Sweden	Unknown	Needs Further Identification
14	Canada	Unknown	Needs Further Identification
15	Canada	Unknown	Needs Further Identification
16	Ohio	Environmental	*L. bozemanii* based on *mip* gene
17	Missouri	Environmental	Needs Further Identification
18	New York	Environmental	Needs Further Identification

Conclusion

- CU samples 1, 2 and 9 identified as *L. erythra*
- CU sample 3, 4 and 5 identified as *L. pneumophila*
- CU sample 8 and identified as *L. busanensis*
- CU sample 10 identified as *L. steelei*

Future Directions

Future directions will include continued identification of unknown samples by genetic sequencing. of the *mip* and 16s genes. Sequenced based typing of several other *Legionella* genes will be performed on unique or novel strains as outlined by the Center for disease control. These strains will be further characterized based on their biochemical properties.

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