Isolating *Aeromonas* from Rainwater

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Abstract

Members of the genus *Aeromonas* are ubiquitous aquatic bacteria that play several environmental roles and are associated with a number of opportunistic infections in humans. These organisms have been isolated from a variety of aquatic samples worldwide, as well as found in food and soil. Rainfall samples were taken at multiple sampling points to test for the presence of *Aeromonas*. Preliminary results showed that *Aeromonas* species were present in rainwater and can be isolated using *Aeromonas* Blue Medium. A positive test presents as creamy yellow colonies that are gram-negative, facultatively anaerobic rod-shaped organisms. After the bacterial cells were cultured, colonies of interest were tested for a positive output using oxidase strips. Polymerase chain reaction (PCR) and DNA sequencing were methods used to identify the suspected *Aeromonas* species. The positive existence of *Aeromonas* in rainwater would provide insight into the ubiquitous nature of this genus and the potential role of rainfall in the transmission of the bacteria.

Introduction

Over the past ten years, the identification of proposed *Aeromonas* species has boomed. This genus is essentially ubiquitous in every aquatic environmental niche (Janda 2010). This bacteria is a disease-causing pathogen, giving rise to opportunistic wound infections in humans, and when ingested, gastroenteritis. *Aeromonas* is capable of growth over a wide range of temperatures, pHs, and turbidities (Janda 2010). *Aeromonas* is able to grow readily on *Aeromonas* Blue Media for laboratory identification.

Aerobiology is the study of microorganism movement in the atmosphere. Many bacteria, including *Aeromonas*, are capable of remaining suspended in the air for long periods of time (Fernstrom 2013). This factor could contribute to the transmission of *Aeromonas* via rainwater. The purpose of this project was to determine the presence of an *Aeromonas* species in rainwater samples.

References

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Table 1. 16S rRNA sequence results (F- forward and R- reverse) from 5 rainwater colonies

Sample	Source	Identity	% Identity
1F	1/23- rainwater	P. rhodesiae	98%
1R	1/23- rainwater	P. rhodesiae	99%
2F	2/18- rainwater	B. species	97%
2R	2/18- rainwater	B. species	97%
3F	2/3- rainwater	P. rhodesiae	98%
3R	2/3- rainwater	P. species	99%
4F	1/23- rainwater	P. rhodesiae	94%
4R	1/23- rainwater	P. rhodesiae	99%
5F	1/23- rainwater	P. rhodesiae	99%
5R	1/23- rainwater	P. rhodesiae	99%

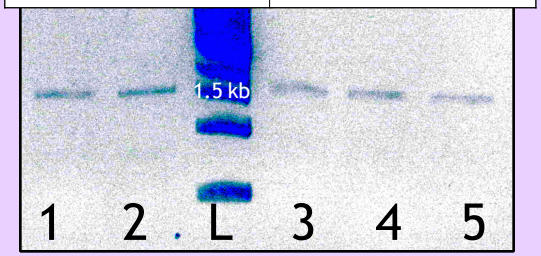

Figure 1. Amplification of 16S rRNA genes from 5 colonies isolated from rainwater.

Methods

The rainwater, snow, and ice samples were collected using sterilized jars. The bacterial cultures were grown on *Aeromonas* Blue Medium using the spread plate technique, and incubated at 30°C for 48 hours. Colonies of interest were evaluated using the oxidase test and subcultured if visibly positive. A positive test indicates cytochrom oxidase activity in the bacteria. Colony PCR for 16S rRNA was performed on five individual colonies that were suspected *Aeromonas*. The samples underwent a DNA Clean and Concentrator process before being DNA sequenced. Table 1 shows the results of nanodrop spectroscopy and sequencing.

Results

Although preliminary results showed that *Aeromonas* is present in rainwater, it was not found to be in this study. There is a possibility that the bacterium was present in the rainwater samples, but did not appear suspicious when plated on *Aeromonas* Blue Medium. From the six precipitation samples that were collected, suspicious colonies were only present when the precipitation fell as rainwater. Figure 1 shows the results of amplification of the 16S rRNA gene. All five DNA fragments were 1.5 kb. The results of DNA sequencing are found in Table 1.

The genus *Pseudomonas* plays an important role in the carbon cycle and are abundantly present in environmental water samples (Sazakli 2005). In conjuncture with *Aeromonas*, this bacteria is a gram-negative rod that typically gives a positive result to oxidase testing. *P. rhodesiae* had not previously been isolated from precipitation samples.

Bordetella is a bacterium that is common to hospital environments. Many species of *Bordetella* are pathogenic to humans, causing a range of respiratory diseases (Mooi 2010). Some species of *Bordetella* are oxidase positive and gram-negative rods, similar to *Aeromonas*. For sample #2, *B. petrii* was the closest named species at 97% identity. This species is commonly isolated from river sediment and has an unknown pathogenic significance (Wintzingerode 2001).

Conclusions

Bordetella and *Pseudomonas* species were found in the precipitation samples, but no *Aeromonas*. This has implications for the ecology and natural reservoirs of these organisms, in addition to its transmission to different environments. Neither species had previously been isolated from rainwater samples. Rainwater samples will continue to be analyzed for the presence of *Aeromonas* in order to determine if this is a possible mode of transfer to other natural environments.