Isolating *Aeromonas* from Precipitation and the Atmosphere

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Abstract

Aeromonas is a ubiquitous, Gram-negative, rod-shaped, oxidase-positive anaerobe. Aeromonads are emerging human pathogens associated with extra-intestinal infections after coming into contact with or consuming contaminated water or food. Though there are various biological particles that are known to be in the atmosphere, microbial communities are poorly characterized at high altitudes and in air masses. Bacteria have the ability to remain suspended in the air for prolonged periods of time and transmit diseases through both aerosolized "airborne" and "droplet" means. Various precipitationand wind samples were taken in Abilene, TX with a sterile sampling jar and plated onto *Aeromonas* blue medium plates with and without ampicillin. Colonies were subcultured onto plates and then tested for the presence of oxidase. 16S rRNA DNA sequences were amplified from oxidase-positive, Gram-negative rods and then sequenced. After receiving results, sequences were analyzed using two online databases: NCBI BLAST and RDP. No isolates appeared to belong to the genus *Aeromonas*. There were various other bacteria found. There are many reasons why *Aeromonas* was not found in the samples: 1) the lack of a proximal lake or stream as an initial source of *Aeromonas* prevents detection of these types of cells, 2) *Aeromonas* may not be able to survive in the atmospheric or wind conditions of the study, 3) *Aeromonas* may be present in the precipitation but the methods were not sensitive enough to detect them, and 4) the genus may not be present at all in precipitation. Though *Aeromonas* was not found, many other genera of rod-shaped bacteria were. Future research for this study include expanding the collection site to sites near aquatic environments and other areas outside of Texas, collecting different forms of precipitation and at different seasons of the year.

Introduction

Members of the genus *Aeromonas* are ubiquitous aquatic bacteria found in virtually every environmental niche where bacterial ecosystems exist (3). This bacteria maintains the ability to grow over a wide variety of temperatures, pH's and turbidities (3). As the infection of *Aeromonas* often occurs after coming into contact with or consuming contaminated water or food, the potential health hazards that microorganisms present in water are heavily illustrated in humans as well as in animals (1, 2). Aerobiology is the study of the movement and effects of microorganisms in the atmosphere. The atmosphere has been described as "one of the last frontiers of biological exploration on Earth" (8). The lowest part of earth's atmosphere known as the troposphere contains microbial communities, which are poorly characterized (5). Recent studies confirm the presence of waterborne pathogens in rainwater samples (4). The purpose of this study was to determine the presence of *Aeromonas* in rainwater and wind samples and investigate how these bacteria move throughout the environment and the atmosphere.

Citations

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Materials and Methods

Sampling. The rainwater was collected using sterilized sampling jars and the wind was collected using *Aeromonas* blue medium plates from May to June of 2014 and January to March of 2015 in Abilene, TX.

Media and growth conditions. The bacterial cultures were plated and grown onto *Aeromonas* blue medium with and without ampicillin using the spread plate technique and incubated at 30° C for 24 to 72 hours. **Isolating and subculturing of pure colonies.** Individual colonies of interest were subcultured onto tryptic soy agar plates (TSA) and isolated until arriving at pure cultures. **Presumptive identification as aeromonads.** The pure colonies were evaluated by using the oxidase test. A positive test indicates cytochrome oxidase activity in the bacteria. The oxidase-positive colonies were Gram-strained, and those that appeared to be Gram-negative rods were designated as presumptive aeromonad colonies and further pursued. **Colony PCR and gel electrophoresis.** Colony PCR for 16S rRNA DNA was performed on the fifteen individual presumptive aeromonad colonies using the standard 27Fm Forward Primer and 1492r Reverse Primer. **DNA extraction.** The samples underwent DNA extraction using a DNA clean and concentrator process to isolate the chromosomal DNA. The samples were then purified using a Zymo Research DNA purification kit before being DNA sequenced.

	NCBI		RDP	
Isolate: source	**Identification**	**% Identity****	**Identification**	**Strength (S_abscore)**
1: wind	*Novosphingobium* clone SeqSEEZ199	99%	*Sphingomonas* 44/40	0.949
2: wind	*Pseudomonas putida*	99%	*Pseudomonas putida*	0.969
3: wind	Uncultured *Novosphingobium* clone SeqSEEZ199	99%	Uncultured Sphingomonadaceae bacterium	0.942
4: rain	*Pseudomonas* MC83	99%	*Pseudomonas* MC83	0.974
5: rain	*Massilia varians*	97%	*Naxibacter* 6981	0.870
6: rain	*Pseudomonas* JSM 2215099	100%	*Pseudomonas fulva*	0.997
7: rain	*Pseudomonas fulva*	100%	*Pseudomonas* 471-1	0.997
8: rain	*Pseudomonas* 9DLP	99%	*Pseudomonas* PSB1	0.992
9: rain	Uncultured *Pseudomonas*	100%	*Pseudomonas fulva*	0.983
10: rain	*Pseudomonas plecoglossicida*	89%	*Pseudomonas* CPA30	0.520
*11: rain	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.911
*12: rain	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.916
*13: rain	*Agrobacterium* H13-3	99%	*Rhizobium* Gls-4	0.972
*14: rain	*Pseudomonas syringae*	96%	*Pseudomonas fluorescens*	0.876
*15: rain	*Pseudomonas syringae*	93%	*Pseudomonas* S1(2011b)	0.552

**All strength values (E-value) were $p \leq 2\times10^{-165}$
*Ampicillin was used in the isolation medium of these strains

Table 1. NCBI BLAST Database and RDP Database Comparison for Sequence Samples

Results and Discussion

Having used various testing techniques that resulted in presumptive samples belonging to the genus *Aeromonas,* the DNA sequences of fifteen samples were analyzed using two comparative databases: NCBI BLAST and RDP. Table 1 displays the results of the analysis of the samples using the two comparative databases. The results of the analyzed sequences illustrate that no samples belong to the genus *Aeromonas*. Analyzing the sample sequences using the NCBI BLAST database resulted in various identifications of organisms. The highest sequence similarity was 100%, including three samples identified from the genus *Pseudomonas*. Of the remaining sample sequence similarities, five of them were less than 97%, which could indicate them as representatives of previously unknown species as their species identities are less than 97% identity to known species. Using the RDP database, the highest S_ab score for the collection of sequences was 0.997 while the lowest was 0.520. Organisms that make up the genus *Pseudomonas* play a vital role in the atmosphere as they are involved in the carbon cycle of nature and are abundantly found in environmental water samples (10). Many members of this genus are causative agents in nosocomial infections of those who have compromised immune systems (7). Members of *Naxibacter* bacteria have been isolated from soil samples (15). *Massilia* bacteria members, are not only closely related to members of the *Naxibacter* genus, but both genera have been isolated from air samples in another study (Weon *et al.,* 2010). Organisms of the genus *Aurantimonas* are strictly anaerobic and oxidase positive as is *Aeromonas*. Species within this genus have been isolated from air samples collected in the Republic of Korea (12). After analysis, there were various bacteria that were identified as being uncultured. Members of the genus *Novosphingobium,* have been known to have the ability of nitrogen fixation among other similar capabilities (9). One of the samples was a close match to a previously uncultured *Sphingomonas* bacterial strain. This genus of bacteria is known for its diversity of nitrogen-fixing capabilities as well as its association with plants and their roots (11, 14).

Conclusions

Though no *Aeromonas* was found among samples sequenced, there were a variety of other bacteria that were found. There are a number of reasons as to why *Aeromonas* was not found among the samples. With a lack of a closely located body of water where *Aeromonas* may reside, this prevented any detection or collection of aeromonads. As the study of the presence of *Aeromonas* in the atmosphere is currently on its frontier, members of this genus may not have the ability to survive in atmospheric conditions used in this study. Though *Aeromonas* may be ubiquitous in aquatic environments, the methods of experimentation used throughout this study may have not been sensitive enough to detect its presence. As knowledge of the presence of *Aeromonas* in the atmosphere is been limited, it may not be present.

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