GRAM-NEGATIVE, OXIDASE-POSITIVE BACTERIA IN RAINWATER AND WIND SAMPLES

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ABSTRACT

Gram–negative, rod–shaped, oxidase–positive bacteria, such as *Aeromonas* and *Pseduomonas*, are widespread in the environment. *Aeromonas* are emerging human pathogens associated with extraintestinal and opportunistic infections. Though there are various biological particles known in the atmosphere, these microbial communities are poorly characterized. Bacteria have the ability to remain suspended in the air for prolonged periods of time and can be transmitted through both airborne and droplet means. This study aimed to isolate *Aeromonas* and other similar bacteria from samples from the troposphere in order to learn more about the distribution of these organisms. Eleven precipitation and wind samples were aseptically collected in Abilene, Texas, and plated onto *Aeromonas* Blue Medium plates with and without ampicillin. The 16S rDNA sequences were amplified from 28 Gram–negative, oxidase–positive isolates. The analyzed sequences showed that none of the isolates belonged to the genus *Aeromonas*, but did include *Pseudomonas*, *Sphingomonas*, *Massilia*, *Naxibacter*, *Paracoccus*, *Novosphingobium*, and *Mesorhizobium*, giving clues to the distribution of these organisms. Furthermore, six isolates appeared to be novel species of bacteria, and several more were uncultured before this study.

INTRODUCTION

Aerobiology is the study of the occurrence, dispersion, and passive transport of airborne biological particles, which could be viable or nonviable, and are distributed in and throughout the air (11). A seasonal difference has been found among the amount of culturable bacteria from air samples; the highest concentrations were shown during the summer months (29). Microbial presence in air is an important factor that contributes to the transmission of infectious diseases, as evidence from previous studies has illustrated that pathogenic bacteria in the air have the ability to travel over long distances and come into contact (through inhalation and ingestion) with individuals who had no previous contact with the source of the infectious disease (8). Although hundreds of thousands of individual microbial cells can exist in a cubic meter of air, the diversity, interactions, and distribution of these organisms are poorly understood (4).

There are constant human–microbe interactions, both pathogenic as well as beneficial (17). Various bacteria have the ability to remain suspended in the air for prolonged periods of time and can be

transmitted through both aerosolized or airborne and droplet means (8). Aerosols in the atmosphere have the ability to influence cloud formation and precipitation development, impacting the earth's climate, water cycle, and atmospheric reactions (22,26). There is evidence suggesting that bacteria may be a major factor within the biological aerosols found in the atmosphere (33). The atmosphere has been described as "one of the last frontiers of biological exploration on Earth," which further supports the importance to investigate its impact on microbial life (34).

The water cycle describes the continuous movement of water around and through the earth as its physical state is altered between liquid, gas, and ice. Three percent of the earth's water is found in the atmosphere (22). The lowest part of earth's atmosphere, known as the troposphere, contains microbial communities that are poorly characterized at high elevations and in the air masses above the ocean (6). The poor characterization of these microbes is due to the difficulty in acquiring samples of adequate biomass, which leads to further challenges of finding sufficient DNA to be used for analysis (6).

Rainwater harvesting is becoming an increasingly popular drinking water source. However, the consumption of rainwater could be dangerous due to the potential health risks of chemical and microbiological contamination (1). Though there have been countless studies performed concerning chemical contamination of rainwater, microbial contamination continues to be somewhat unknown due to the difficulty involved in detecting pathogens (16). However, a small number of recent studies have emerged that do confirm the presence of waterborne pathogens in rainwater and air samples, but there is still much to learn (3,16,20).

Little is known about the occurrence and movement of particular genera of bacteria, such as *Aeromonas*, in the troposphere. *Aeromonas* cells are Gram-negative, oxidase-positive, non-spore forming, rod-shaped bacteria that are found in various aquatic habitats. This genus is a pathogen found in fish and other poikilothermic organisms, and is also regarded as an emerging pathogen in humans causing bacteremia, gastroenteritis, cellulitis, and sepsis (21). Our first hypothesis was that we would isolate *Aeromonas* from wind, rainwater, and hail samples because of its ubiquity in aquatic and other environments. However, *Aeromonas* was not found, so our second hypothesis was that we would isolate other unique bacteria with characteristics similar to *Aeromonas*. The second hypothesis was supported in the successful isolation of other Gram-negative, oxidase-positive bacteria such as *Pseudomonas*, *Aurantimonas*, and *Paracoccus*, which could potentially hold more importance than *Aeromonas* and give insight into the microbial communities of the troposphere.

MATERIALS AND METHODS

SAMPLING, MEDIA, AND GROWTH CONDITIONS

Rainwater, wind, and hail samples were collected in Abilene, Texas, from May to June of 2014 and from January to March of 2015. The rainwater and hail were collected using

sterile sampling jars that were set outside away from runoff of roofs and trees. Hail was allowed to melt in the lab at room temperature before processing. The wind was collected using plates of *Aeromonas* Blue Medium (ABM) (14) by standing outside in an open field, without buildings in the way to ensure as pure of a collection as possible, with the plate open against the direction of the wind for five minutes. The temperature, time, wind direction, wind speed, humidity, and dew point were recorded for each day of wind collection. ABM was made since it is not available commercially, and consisted of 4.0 g of soluble starch (Mallinckrodt, St. Louis, MO), 0.25 g of NH^4Cl (Mallinckrodt, Paris, KY), 1.0 g of tryptone (Sigma–Aldrich, St. Louis, MO), 0.5 g of yeast extract (Sigma–Aldrich, St. Louis, MO), 0.04 g of bromothymol blue (Ward's Natural Science, Rochester, NY) 15.0 g of agar powder (Alfa Aesar, Ward Hill, MA), 1 L of distilled water and was adjusted to a pH of 8.0 using 1M KOH. After autoclaving and cooling, 0.1 g of sodium desoxycholate (Sigma Chemical Company, St. Louis, MO), 5.0 mL of 0.41% L–tryptophan, and 5.0 mL of 0.99% L–phenylalanine were added and mixed. ABM was made with and without 32 µg/ml ampicillin (GBiosciences, St. Louis, MO). Ampicillin was added to the plates in later collection dates in order to select for *Aeromonas* and against *Pseudomonas* since many *Aeromonas* strains have chromosomally encoded β–lactamases and pseudomonads typically do not (14).

After collection of wind–generated samples, the ABM plates were incubated aerobically at 30˚C for 24 to 72 hours for colony growth. The rainwater and melted hail were diluted in 0.85% NaCl to obtain countable colonies and spread onto ABM plates with and without ampicillin and also incubated at 30˚C for 24 to 72 hours, depending on how quickly colony growth occurred.

After growth on the ABM plates, individual colonies were chosen and subcultured onto 0.2X Tryptic Soy Agar (TSA) plates and incubated at 30˚C for 24 to 48 hours. 0.2X TSA plates consisted of 8.0 g of TSA (Carolina, Burlington, NC) and an additional 12.0 g agar per liter of medium. Cultures were subcultured until pure cultures were obtained.

SCREENING AND AMPLIFICATION OF 16S RDNA FROM SELECTED ISOLATES.

Each presumptive aeromonad colony was tested for a positive oxidase reaction using OxiStrips (Hardy Diagnostics, Santa Monica, CA). Each colony that was oxidase–positive was kept for further testing, since *Aeromonas* species have cytochrome c oxidase in their electron transport chains. Then, they were Gram stained. Gram–negative, oxidase–positive rods were selected for amplification by colony PCR and sequencing of the 16S rRNA gene. The protocol is as follows: 5.0 µl of 10X Standard *Taq* Reaction Buffer (New England BioLabs Inc.), 1.0 µl of 10 mM dNTPs, 1.0 µl of 10 µM 27Fm Forward Primer 5'–AGAGTTTGATYMTGGCTCAG–3' (9) (Invitrogen, Carlsbad, CA), 1.0 µl of 10 µM 1492R Reverse Primer, 5'–TACCTTGTTACGACTT–3' (9) (Invitrogen, Carlsbad, CA), 0.25 µl of NEB *Taq* DNA Polymerase (New England BioLabs Inc.), 41.75 µl of Nuclease–free water (Invitrogen; Grand Island, New York) and template in the form of a colony, which totaled to 50 µl per reaction. Several of the genes from isolates could not be amplified using the colony PCR protocol, so pure DNA was extracted as previously described (30). The thermocycling conditions are as follows: initial denaturation: 95˚C for 5 minutes,

30 cycles of 95˚C for 30 seconds, 55˚C for 30 seconds, 68˚C for 1 minute 30 seconds followed by a final extension of 68˚C for 5 minutes. The products were visualized on a 0.8% agarose (Invitrogen; Carlsbad, CA) gel containing ethidium bromide. Products of 1.5 kb were purified using DNA Clean & Concentrator (Zymo Research Corporation, Irvine, CA) and sent to DNA Analysis Facility on Science Hill at Yale University (New Haven, CT) for sequencing.

DNA SEQUENCE ANALYSIS.

The National Center for Biotechnology Information (NCBI) BLASTn tool and Ribosomal Database Project (RDP) SeqMatch tool were used to search nucleotide databases and to analyze and interpret the results of each gene that was sequenced. Two databases were used in order to maximize the chance of finding accurate species identifications. Of the NCBI BLASTn tools, the following information was recorded for each sequenced sample: genus, species, strength (e-value), and identification number (2). Of the RDP SeqMatch tool, the following information was also recorded for each sequenced sample: genus, species and the strength (S_ab score) (19).

RESULTS

Table 1. Weather parameters at the time of rainwater sample collections. A "/" between NCBI and RDP genus names is shown when the two databases gave conflicting identifications.

Date	Genera Isolated	Low Temperature	High Temperature	Total Rainfall
2014-05-13	*Pseudomonas*	17.2ºC	8.9ºC	0.05 cm
2014-05-23	*Paracoccus, Pseudomonas*	28.3ºC	18.9ºC	0.61 cm
2014-05-25	*Massilia/Naxibacter, Pseudomonas*	23.3ºC	17.2ºC	2.39 cm
2015-01-21	None	0.6ºC	9.4ºC	7.20 cm
2015-01-31	*Agrobacterium/Aurantimonas, Mesorhizobium, Pseudomonas*	6.7ºC	12.2ºC	3.60 cm

The rainfall on the days rainwater was collected varied from 0.05 cm to 7.20 cm, with temperatures ranging from 0.6 ºC to 28.3 ºC (Table 1). Wind samples were collected only in May in winds ranging from 4.8 kph to 22.5 kph in temperatures from 22.8 ºC to 28.3 ºC. Wind direction, humidity, and dew point were also recorded and shown in Table 2.

After isolating, subculturing, testing for oxidase, and Gram staining, 28 DNA sequences from presumptive aeromonads were analyzed. None of these isolates were obtained from the hail sample, as the sample did not yield any bacteria with the phenotypes of interest. As shown in Table 3, of those 28 sequences, no isolates belong to the genus *Aeromonas*. However,

Table 2. Weather parameters at the time of wind sample collections. A "/" between NCBI and RDP genus names is shown when the two databases gave conflicting identifications.

Date	Genera Isolated	Time	Temperature	Wind Direction	Wind Speed	Humidity	Dew Point
2014-05-20	*Novosphingobium*/ *Sphingomonas*, *Pseudomonas*	11:00	26.1ºC	South	22.5 kph	48%	14.4ºC
2014-05-22	none	11:00	26.7ºC	South	19.3 kph	56%	16.1ºC
2014-05-23	*Novosphingobium*/ *Sphingomonas*	11:00	22.8ºC	South	20.9 kph	68%	16.7ºC
2014-05-26	none	16:00	28.3ºC	South-Southwest	4.8 kph	44%	15.0ºC
2014-05-27	none	11:00	23.3ºC	North	4.8 kph	68%	17.2ºC
2014-05-28	none	11:00	24.4ºC	North	22.5 kph	58%	16.1ºC

the NCBI BLAST database resulted in the following number and identities of bacteria: 14 *Pseudomonas*, 6 various bacteria that were previously uncultured and only identified through environmental sequencing methods, and 8 other bacteria: *Massilia varians*, 2 strains; *Paracoccus* 'Mali 27', 1 strain; *Novosphingobium* clone SeqSEEZ199, 1 strain; *Mesorhizobium opportunistum*, 2 strains; *Agrobacterium* H13-3, 1 strain; and *Paracoccus* QUEBA07, 1 strain. As a comparison, analysis of the sample using the RDP database resulted in the following number and identities of bacteria: 16 *Pseudomonas*, 4 various previously uncultured bacteria, and 8 other bacteria: *Naxibacter* 6981, 2 strains; *Sphingomonas* 44/40, 3 strains; *Aurantimonas ureilytica*, 2 strains; and *Rhizobium* Gls-4, 1 strain. The highest S_ab score for the collection of sequences was 1.00 while the lowest was 0.520 (for Isolate 6 and Isolate 23). The S_ab signifies that the sequences in this study had unique 7-base oligomers shared between the sample sequence and a given RDP sequence (which was then divided by the lowest number of unique oligonucleotides in either of the two sequences).

Data were analyzed based on date of sampling, source of isolation, and species identification (Fig. 1). *Pseudomonas* species were found both in rainwater and wind and both in May and January. Paracocuss and *Massilia/Naxibacter* and *Novosphingobium/ Sphingomonas* were only isolated in May, while *Agrobacterium/Rhizobium* and *Mesorhizobium/Aurantimonas* were isolated in January.

Table 3 (opposite page). NCBI BLAST Database and RDP Database Comparison for Sequence Samples. Isolates KEP1-KEP13 were isolated on *Aeromonas* blue medium without ampicillin and isolates KEP24-KEP28 were isolated on *Aeromonas* blue medium supplemented with 32 µg/ml ampicillin. All strength (E-value) scores were ≤ 2 × 10-165. Isolates with a star are those thought to be novel species. Uncultured refers to strains that were previously identified through environmental sequencing methods only.

	NCBI BLAST Database		RDP Database	
Isolate	Genus and Species	Identity	Genus and Species	Strength (S_abscore)
KEP1: Wind	*Pseudomonas putida*	99%	*Pseudomonas putida*	0.969
KEP2: Wind	Uncultured *Novosphingobium* clone *SeqSEEZ199*	99%	*Sphingomonas* 44/40	0.949
KEP3: Wind	Uncultured *Novosphingobium* clone *SeqSEEZ199*	99%	*Sphingomonas* 44/40	0.942
KEP4: Wind	Uncultured *Novosphingobium* clone *SeqSEEZ199*	99%	Uncultured *Sphingomonadaceae*	0.942
KEP5: Rain	Uncultured *Pseudomonas*	100%	*Pseudomonas fulva*	0.98
KEP6: Rain*	*Pseudomonas plecoglossicida*	89%	*Pseudomonas* CPA30	0.520
KEP7: Rain	*Pseudomonas* MC83	99%	*Pseudomonas* MC83	0.974
KEP8: Rain	*Massilia varians*	97%	*Naxibacter* 6981	0.870
KEP9: Wind	*Pseudomonas* JSM 2215099	100%	*Pseudomonas fulva*	0.997
KEP10: Rain	*Pseudomonas fulva*	100%	*Pseudomonas* 471-1	0.997
KEP11: Rain	*Pseudomonas* 9DLP	99%	*Pseudomonas* PSB1	0.992
KEP12: Rain	*Paracoccus* QUEBA07	99%	Uncultured Proteobacterium	0.974
KEP13: Rain	*Paracoccus* 'Mali 27'	99%	Uncultured Proteobacterium	0.962
KEP14: Wind	*Novosphingobium* clone SeqSEEZ199	99%	*Sphingomonas* 40/40	0.949
KEP15: Wind	*Pseudomonas putida*	99%	*Pseudomonas putida*	0.969
KEP16: Wind	Uncultured *Novosphingobium* clone SeqSEEZ199	99%	Uncultured *Sphingomonadaceae*	0.942
KEP17: Rain	*Pseudomonas* MC83	99%	*Pseudomonas* MC83	0.974
KEP18: Rain	*Massilia varians*	97%	*Naxibacter* 6981	0.870
KEP19: Rain	*Pseudomonas* JSM 2215099	100%	*Pseudomonas fulva*	0.997
KEP20: Rain	*Pseudomonas fulva*	100%	*Pseudomonas* U471-1	0.997
KEP21: Rain	*Pseudomonas* 9DLP	99%	*Pseudomonas* PSB1	0.992
KEP22: Rain	Uncultured *Pseudomonas*	100%	*Pseudomonas fulva*	0.983
KEP23: Rain*	*Pseudomonas plecoglossicida*	89%	*Pseudomonas* CPA30	0.520
KEP24: Rain*	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.911
KEP25: Rain*	*Mesorhizobium opportunistum*	94%	*Aurantimonas ureilytica*	0.916
KEP26: Rain	*Agrobacterium* H13-3	99%	*Rhizobium* Gls-4	0.972
KEP27: Rain*	*Pseudomonas syringae*	96%	*Pseudomonas fluorescens*	0.876
KEP28: Rain*	*Pseudomonas syringae*	93%	*Pseudomonas* S1(2011b)	0.552

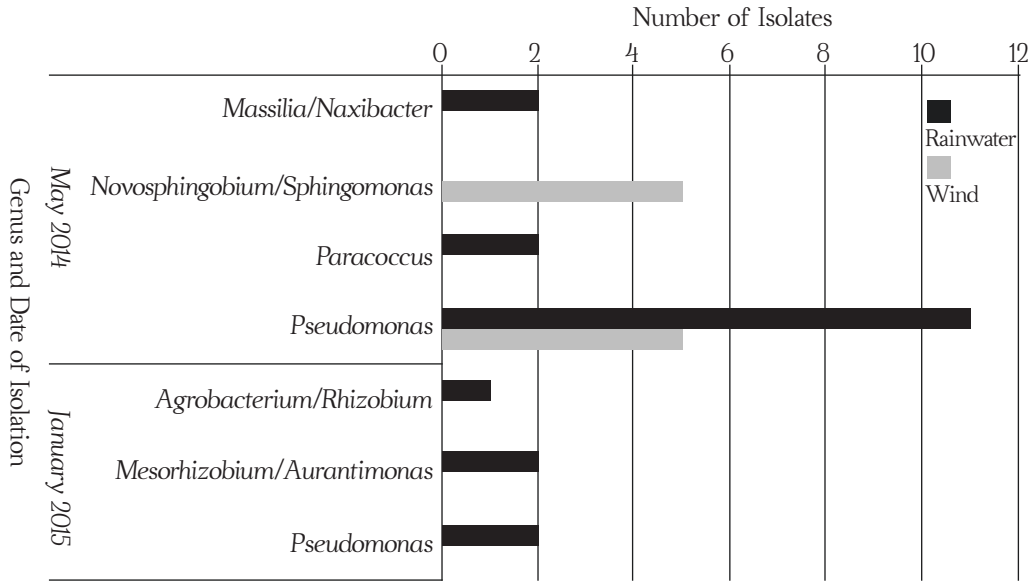

Figure 1. Number of isolates with regard to date of sampling, source of isolation, and identification. Only isolates with the phenotypes of interest that were further characterized are included in this graph. A "/" between NCBI and RDP genus names is shown when the two databases gave conflicting identifications.

Both databases resulted in identification of previously uncultured bacteria. Thus, this study resulted in successfully culturing those bacteria. For the NCBI BLAST database identifications, three of the six total previously uncultured bacteria were *Novosphingobium* (Table 3).

Of the samples that were sequenced, the identities of six of the samples appear to be new species (or at least type strains) of bacteria. According to the NCBI BLAST Database, these presumptive new species ranged from 89% to 96% identity to known species (Table 3). These isolates could be representatives of previously unknown species since their species identities are less than 97% identical to known species (9).

DISCUSSION & CONCLUSIONS

There are many reasons why *Aeromonas* may not have been 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 finally, 4) the microbe may not be present at all in precipitation.

Even though we did not find *Aeromonas* as we had hypothesized we would, we did find other interesting strains with a

diversity of implications. The majority of the isolates found were from the genus *Pseudomonas*, including *P. putida* (Isolate 1), *P. plecoglossicida* (Isolate 6, Isolate 23), *P. fulva* (Isolate 10, Isolate 19, Isolate 20, Isolate 22), *P. syringae* (Isolate 27, Isolate 28), and *P. fluorescens* (Isolate 27) (Table 3). Members of the genus *Pseudomonas* are often found in water and soil ecosystems, so their abundance among the samples collected in this study is not surprising (23,24). However, since members of this genus have also been associated with the transmission of diseases in humans, animals, and plants, the possibility of coming into contact with pathogens through rainwater or wind exists. (7).

Another significant characteristic of pseudomonads is that they are thought to play an important role in the atmosphere. *P. syringae* is associated with bioprecipitation. Bioprecipitation is a feedback cycle in which land plants produce airborne particles or aerosols containing microorganisms that impact the formation of clouds by their ice nucleation ability. This feedback cycle leads to rainfall that benefits both plant and microbial growth (25). The microorganisms that are encapsulated within these airborne particles are found in the center of snow and rain on five continents, with many of these microbes appearing in agricultural regions that are increasingly farmed. It is thought that the bacterium *P. syringae* is "the most prolific ice nucleator" because it can act within a set of warm atmospheric temperatures and may also cause other nucleators to lose their abilities (5). Furthermore, as a plant pathogen, *P. syringae* damages certain agricultural crops through the utilization of its ice making capabilities (5).

As stated before, pseudomonads are found in both soil and water. Since wind speeds ranged from 4.8 kph to 22.2 kph when the wind samples were collected (Table 2), it is possible for particulates from the ground to be swept up and included in the samples. Many of the sequenced isolates include genera often found in soil environments. Some of these isolates were identified as genera previously known to fix nitrogen, such as a *Pseudomonas* (12). Another bacterial isolate found that is also important in the nitrogen cycle was *Paracoccus*, which has previously been reported to reduce nitrate (13). As mentioned before, there were several bacteria whose closest sequence matches were previously identified through environmental sequencing but were not cultured until this study. Interestingly, two of these strains have previously been reported to fix nitrogen, a member of the order Rhizobiales and *Novosphingobium* (15,28). *Sphingomonas* was also found and is a genus known for its diversity of nitrogen-fixing capabilities as well as its association with plants and their roots (27).

Other soil bacteria of the genus *Naxibacter* and *Massilia* were also found among the isolates (35). Members of the *Massilia* genus are not only closely related to members of the *Naxibacter* genus, but both genera have been isolated from air samples in another study (32).

It was also no surprise that *Aurantimonas* was also found, since species within this genus have been isolated from air samples collected in the Republic of Korea (31). Finding *Aurantimonas*, *Naxibacter*, and *Massilia* suggests that there may be an important ecological role of these genera in air. Other isolates not usually isolated from air that were found in our study belong to the genus *Sphingomonas*. Members of this genus form biofilms within rainwater harvesting tanks that act as a bio-control agent and natural filter by removing

contaminants and bacteria from rainwater (18). These explanations illustrate that the isolates discovered in this study have unique capabilities and can survive amongst various environmental pressures.

Expanding both frequencies of collection and diversity of seasons will improve future research in this area. Wind samples were only taken in the summer. With samples taken throughout the year, this could increase the diversity of microbes found. As there are different forms of precipitation such as ice, snow, hail, sleet, and fog, collecting these forms of water will improve future research and allow for comparison between the microbes found in each state of water.

This study revealed the presence of interesting bacterial strains present in the troposphere. We found organisms important in bioprecipitation as well as those that have the potential to be important in the nitrogen cycle that are typically found in soil. In this study, we easily isolated previously uncultured bacterial strains as well as up to six novel species. This indicates that there is much to discover about the troposphere and how microbes are moved from environment to environment via transpiration and through updrafts of particulate matter from the terrestrial environment.

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REFERENCES

1. Ahmed W, Huygens F, Goonetilleke A, Gardner T. 2008. Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in southeast Queensland, Australia. *Appl. Environ. Microbiol.* 74:5490–96.

2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–10.

3. An HR, Mainelis G, White L. 2006. Development and calibration of real-time PCR for quantification of airborne microorganisms in air samples. *Atmos. Environ.* 40:7924–39.

4. Burrows SM, Elbert W, Lawrence MG, Pöschl U. 2009. Bacteria in the global atmosphere – Part 1: review and synthesis of literature data for different ecosystems. *Atmospheric Chem. Phyics.* 9:9263–80.

5. Cohen JM. 2012. Are we killing the rain? Meditations on the water cycle and, more particularly, on bioprecipitation. *ID 2458027*, Social Science Research Network, Rochester, NY

6. DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, et al. 2013. Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. *Proc. Natl. Acad. Sci.*

U. S. A. 110:2575–80.

7. Dobrowsky PH, Kwaadsteniet MD, Cloete TE, Khan W. 2014. Distribution of indigenous bacterial pathogens and potential pathogens associated with roof-harvested rainwater. *Appl. Environ. Microbiol.* 80:2307–16.

8. Fernstrom A, Goldblatt M. 2013. Aerobiology and its role in the transmission of infectious diseases. *J. Pathog.* 2013:

9. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl. Environ. Microbiol. 74:2461–70.

10. Gevers D, Cohan FM, Lawrence JG, Spratt BG, Coenye T, et al. 2005. Re-evaluating prokaryotic species. *Nat. Rev. Microbiol.* 3:733–39.

11. Gregory PH. 1973. *The Microbiology of The Atmosphere*. L. Hill. 418

12. Hatayama K, Kawai S, Shoun H, Ueda Y, Nakamura A. 2005. Pseudomonas azotifigens sp. nov., a novel nitrogen-fixing bacterium isolated from a compost pile. *Int. J. Syst. Evol. Microbiol.* 55:1539–44.

13. Hino S, Wilson PW. 1958. Nitrogen fixation by a facultative bacillus. *J. Bacteriol.* 75:403–8.

14. Huddleston JR, Zak JC, Jeter RM. 2007. Sampling bias created by ampicillin in isolation media for *Aeromonas*. *Can. J. Microbiol.* 53:39–44.

15. Im W-T, Kim S-H, Kim MK, Ten LN, Lee S-T. 2006. *Pleomorphomonas koreensis* sp. nov., a nitrogen-fixing species in the order Rhizobiales. *Int. J. Syst. Evol. Microbiol.* 56:1663–66.

16. Kaushik R, Balasubramanian R, de la Cruz AA. 2012. Influence of air quality on the composition of microbial pathogens in fresh rainwater. *Appl. Environ. Microbiol.* 78:2813–18.

17. Kellogg CA, Griffin DW. 2006. Aerobiology and the global transport of desert dust. *Trends Ecol. Evol.* 21:638–44.

18. Kim M, Han M. 2011. Composition and distribution of bacteria in an operating rainwater harvesting tank. *Water Sci. Technol. J. Int. Assoc. Water Pollut. Res.* 63:1524–30.

19. Larsen N, Olsen GJ, Maidak BL, McCaughey MJ, Overbeek R, et al. 1993. The ribosomal database project. *Nucleic Acids Res.* 21:3021–23.

20. Lee JY, Yang J-S, Han M, Choi J. 2010. Comparison of the microbiological and chemical characterization of harvested rainwater and reservoir water as alternative water resources. *Sci. Total Environ.* 408:896–905.

21. Martin-Carnahan A, Joseph SW. 2005. Genus i. Aeromonas stainier 1943, 213al. In *Bergey's Manual of Systematic Bacteriology, Vol. 2: The Proteobacteria, Part C*, ed G Garrity, JT Staley, DJ Brenner, NR Krieg, DR Boone, et al., pp. 557–78. New York: Springer. 2nd edition ed.

22. Möhler O, DeMott PJ, Vali G, Levin Z. 2007. Microbiology and atmospheric processes: the role of biological particles in cloud physics. *Biogeosciences.* 4:1059–71.

23. Palleroni NJ. 1992. Present situation of the taxonomy of aerobic pseudomonads. In *Pseudomonas: Molecular Biology and Biotechnology*, ed E Galli, S Silver, B Witholt, pp. 105–15. Washington, D.C.: ASM Press.

24. Rothschild LJ, Mancinelli RL. 2001. Life in extreme environments. *Nature.* 409:1092–1101.

25. Sands DC, Langhans VE, Scharen AI, De Smet G. 1982. The association between bacteria and rain and possible resultant meteorological implications. *J. Hung. Meteorol. Serv.* 86:148–52.

26. Solomon S, Qin D, Manning Z, Chen M, Marquis KB, et al., eds. 2007. *Contribution of working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge, UK.

27. Subashkumar R, Thayumanavan T, Vivekanandhan G, Lakshmanaperumalsamy P. 2006. Occurrence of *Aeromonas hydrophila in acute gasteroenteritis among children. Indian J. Med. Res.* 123:61–66.

28. Tiirola MA, Männistö MK, Puhakka JA, Kulomaa MS. 2002. Isolation and characterization of *Novosphingobium* sp. strain mt1, a dominant polychlorophenol-degrading strain in a groundwater bioremediation system. *Appl. Environ. Microbiol.* 68:173–80.

29. Tong Y, Lighthart B. 2000. The annual bacterial particle concentration and size distribution in the ambient atmosphere in a rural area of the Willamette Valley, Oregon. *Aerosol Sci. Technol.* 32:393–403.

30. Ulrich RL., Hughes TA. 2001. A rapid procedure for isolating chromosomal DNA from *Lactobacillus* species and other Gram-positive bacteria. *Lett. Appl. Microbiol.* 32:52–56.

31. Weon H-Y, Kim B-Y, Yoo S-H, Joa J-H, Lee KH, et al. 2007. *Aurantimonas ureilytica* sp. nov., isolated from an air sample. *Int. J. Syst. Evol. Microbiol.* 57:1717–20.

32. Weon H-Y, Yoo S-H, Kim S-J, Kim Y-S, Anandham R, Kwon S-W. 2010. *Massilia jejuensis* sp. nov. and *Naxibacter suwonensis* sp. nov., isolated from air samples. *Int. J. Syst. Evol. Microbiol.* 60:1938–43.

33. Wiedinmyer C, Bowers RM, Fierer N, Horanyi E, Hannigan M, et al. 2009. The contribution of biological particles to observed particulate organic carbon at a remote high altitude site. *Atmos. Environ.* 43:4278–82.

34. Womack AM, Bohannan BJM, Green JL. 2010. Biodiversity and biogeography of the atmosphere. *Philos. Trans. R. Soc. B Biol. Sci.* 365:3645–53.

35. Xu P. 2005. *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family "Oxalobacteraceae" isolated from China. *Int. J. Syst. Evol. Microbiol.* 55:1149–53.