Biogeosciences Discuss., 7, 6725–6747, 2010
www.biogeosciences-discuss.net/7/6725/2010/
doi:10.5194/bgd-7-6725-2010

Biogeosciences Discussions

Transport and characterization of ambient biological aerosol near Laurel, MD

J. L. Santarpia[1,2], D. Cunningham[1], J. Gilberry[1], S. Kim[1], E. E. Smith[1], S. Ratnesar-Shumate[1,2], and J. Quizon[1]

[1]National Security Technology Department, The Johns Hopkins University Applied Physics Laboratory, Laurel, MD, USA
[2]Department of Civil and Environmental Engineering, University of Maryland, Baltimore County, Baltimore, MD, USA

Received: 12 July 2010 – Accepted: 30 August 2010 – Published: 3 September 2010

Correspondence to: J. L. Santarpia (joshua.santarpia@jhuapl.edu)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Bacterial aerosol have been observed and studied in the ambient environment since the mid nineteenth century. These studies have sought to provide a better understanding of the diversity, variability and factors that control the biological aerosol population. In this study, we show comparisons between diversity of culturable bacteria and fungi, using culture and clinical biochemical tests, and 16S rRNA diversity using Affymetrix PhyloChips. Comparing the culturable fraction and surveying the total 16S rRNA of each sample provides a comprehensive look at the bacterial population studied and allows comparison with previous studies. Thirty-six hour back-trajectories of the air parcels sampled, over the two day period beginning 4 November 2008, provide information on the sources of aerosol sampled on the campus of Johns Hopkins University Applied Physics Laboratory in Laurel, MD. This study indicates that back-trajectory modeling of air parcels may provide insights into the observed diversity of biological aerosol.

1 Introduction

Biological aerosol particles are thought to have a wide variety of biological sources, including viruses, bacteria, fungi, plants (e.g. pollen grains) and human and other animal cells or cell fragments (Matthias-Maser and Jaenicke, 1995). Studies of these ambient bacterial aerosols have sought to provide a better understanding of the diversity, variability and factors that control the biological aerosol population. In principal, all past studies fall into one of three primary categories: studies of viable/culturable bacteria and fungi (Shaffer et al., 1997), studies of phylogenetic diversity using molecular methods such as clone sequencing (Hua et al., 2007; Neheme et al., 2008) or microarrays (Brodie et al., 2007), or spectroscopic characterization of biological aerosol (e.g. Pinnick et al., 1995; Reyes et al., 1999; Lee et al., 2004; Sivaprakasam et al., 2004; Pan et al., 2007; Steele et al., 2008). Several common observations have arisen from

BGD

7, 6725–6747, 2010

these studies. Bacteria can be transported to geographically distant locations from their sources (Hua et al., 2007). Meteorological conditions can affect both the observable biological aerosol concentration (Lighthart and Shafferm, 1995) and its diversity (Brodie et al., 2007; Fierer et al., 2008). Diverse bacteria and/or their genomic fingerprints have been found in air samples (Brodie et al., 2007). *Actinobacteria, Firmicutes* and *Proteobacteria* are ubiquitous in aerosol samples collected from diverse locations (Shaffer et al., 1997; Brodie et al., 2007; Fierer et al., 2008; Mancinella and Shulls, 1978). Gram-positive species dominate the culturable fraction of bioaerosols, while gram-negative species are the dominant fraction of the nucleic acid burden, probably because gram-negative organisms tend to be more fragile and are less likely to survive aerosol collection or even existence as an aerosol.

This study develops a method for understanding and comparing the molecular and culturable prokaryotic diversity in the ambient aerosol background. Samples were collected for microbiological analysis over a two day period from 4 November through 5 November. After collection, aerosol samples were characterized for bacterial diversity using Affymetrix PhyloChips (DeSantis et al., 2003). Using traditional culture and common biochemical tests, the diversity of culturable fungi and bacteria was examined. Comparing the culturable fraction and surveying the total 16S rRNA of each sample provides a comprehensive look at the bacterial population studied and allows comparison with the broad range of previous studies.

2 Methods

Air samples were collected over a two day period using an OMNI 3000 wetted-wall cyclone (Evogen, Inc., Kansas City, MO) programmed to collect samples at approximately 277 Lpm into approximately 11 mL of phosphate buffered saline (PBS). Four 4-h samples were collected during the sampling period, two from 05:30 to 09:30 EST and two from 13:30 to 17:30 EST on 4 and 5 November 2008. Typical environmental samples used in PhyloChip analysis are collected over at least a 24 h period (Brodie

BGD
7, 6725–6747, 2010

et al., 2007). Since the OMNI has a high sample collection rate and reportedly high collection efficiency values (Kesevan and Schepers, 2006), samples were collected over shorter periods to examine any short-term temporal variation in bioaerosol quantity or diversity. Due to changing weather conditions, this also allowed some anecdotal observations of the effects of precipitation. Collected aerosol samples were analyzed by dividing the 11 mL sample for cultivation and biochemical tests and analysis using Affymetrix PhyloChips (Fig. 1).

Immediately after recovery from the OMNI, 200 uL of the aerosol sample was removed and 50 uL aliquots were plated on each of Tryptic Soy Agar (TSA), Chocolate Agar (CA), Sheep's Blood Agar (SBA), and MacConkey Agar (MAC). The remaining 10.8 mL of sample was then concentrated via centrifugation at 4500 RPM for 10 min. To ensure the free nucleic acid present in the samples was not lost in this concentration process, 1 mL of the decanted supernatant was used to resuspend the sample pellets. Part of the concentrated sample was analyzed using the PhyloChip GeneChip (Affymetrix, Santa Clara, CA), and another part was plated the same way as the pre-concentrated sample, which allowed for the comparison of growth before and after the concentration process, serving as a concentration control and helping to further evaluate the viability of cultured organisms. Any remaining sample was labeled and retained. The OMNI was decontaminated with RNase Away (Molecular BioProducts, San Diego, CA) between sample collections. Negative control experiments were run using a laboratory PBS solution, the PBS that was used to collect the samples and the water used to replenish the fluid level in the OMNI during collection.

All plates were examined for growth after an incubation period of 24 h at 37 °C. The resulting colony forming units (CFUs) were grouped and categorized as unique isolates based on differential characteristics such as colony morphology, initial gram stain reaction, and results of basic biochemical tests. Isolates were then individually numbered and re-plated on TSA, CA, SBA, MAC, and Sabouraud Dextrose Agar (SDA). All plates, other than SDA, were incubated at 37 °C for 24 h. SDA plates were separately incubated at 30 °C and examined for growth every 24 h for up to 7 days.

BGD
7, 6725–6747, 2010

After the re-plating and 24 h incubation period described above, isolates were further characterized using additional biochemical tests and selective media. Simmons Citrate Agar, MAC, motility test medium, Triple Sugar Iron (TSI) and 6.5% NaCl Agar were used to examine the ability of the isolates to utilize citrate and ammonia, screen for gram negative organisms and characterize the ability to ferment lactose, assess motility, assess the ability to ferment carbohydrates, and assess the ability to grow in high salt conditions, respectively. The ability of the bacteria to produce catalase, indole and leucine amino peptidase (LAP) was determined by additional separate tests. The production of gas was determined using Durham tubes. The results of these tests were then compiled along with the gram stain reaction, colony morphology, and the presence or absence of growth on several differential media types, and the isolates were set aside to be stored in glycerol at −80 °C.

The range of media and protocols/procedures used in this study were limited by both time and logistics. There are a wide range of incubation conditions and specialized media types available, and if the full spectrum of these could be employed, this study may have yielded additional isolates; however, due to the study limitations, it was necessary to limit both the protocols implemented and the media selected for use to a small but broad group in order to satisfy the overall goals without exceeding the capacity of the study. This same approach was applied to the selection of basic and specialized biochemical tests used in this study to help characterize isolates.

The PhyloChip microarray (DeSantis et al., 2003) used in this project takes advantage of the 16S rRNA gene that all bacteria possess to identify bacterial components found in an environmental sample. There are greater than 500 000 probes arrayed on the chip. Operational taxonomical units (OTU) represent groupings of similar species within the 842 subfamilies identified by DeSantis et al., 2003. Each OTU corresponds to at least 11 probes (24 probes on average) thought to be prevalent in that OTU but dissimilar to sequences outside the OTU. In this way, unknown environmental bacteria can be phylogenetically classified based on their similarity to known categories of organisms.

BGD
7, 6725–6747, 2010

Once the environmental samples were concentrated, the DNA was extracted using an Ultraclean Microbial DNA Isolation kit (MO Bio Laboratories Inc., Carlsbad, CA). Polymerase chain reaction (PCR) amplification was then performed on the DNA extractions. The PCR products were cleaned and concentrated using Amicon Microcon YM-100 filters and then quantified on a UV spectrophotometer. The procedures for preparing the collected samples for analysis on the PhyloChip were adapted from Brodie et al., 2007.

Once each sample was processed with a PhyloChip, the resulting data was analyzed to retrieve the 16S-rRNA considered to be present in the sample. An OTU was considered present if at least 92% of its assigned probe-pairs for its corresponding probe set were positive. A probe-pair is considered positive if its signal value is above the mean noise threshold (MT). The MT is set according to: $MT = 13\ N^2$ where N was the noise value generated for each PhyloChip during processing (Brodie et al., 2007). Due to potential competitive amplification processes this relative abundance may not be representative of the original sample. This is an unfortunate necessity in most environmental samples since the quantity of unamplified DNA is relatively small and would not produce robust results.

Several controls were implemented to identify any false positive matches on the PhyloChips and to characterize background levels of 16S rRNA present in the collection and sample processing fluids. As part these controls, the PBS that was used as the collection fluid in the OMNI and the water aliquots used to replenish water lost due to evaporation during sample collection were screened using independent PhyloChips. Both the PBS and replacement water aliquots were processed using the same methods as previously described in the sample processing procedures with the addition of a nucleic acid concentration step using an Amicon Microcon YM-100 filter prior to sample extraction. After processing, the PBS controls showed no sign of positive OTU matches. The replacement water however, displayed a variety of positive matches on the array. Because the replacement water was prepackaged and sealed in unique bladder packs arriving directly from the manufacturer, steps to eliminate this background

BGD
7, 6725–6747, 2010

signal were not easily implemented. Because of this, all positive OTU matches found in the refill water were subtracted out as a background signal from all the processed samples. A phylogenetic tree of the identified bacterial orders was created and displayed using the Interactive Tree of Life (Letunic and Bork, 2007).

The National Oceanic and Atmospheric Association (NOAA) Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model (e.g. Draxler and Rolph, 2003; Rolph, 2003) was used to generate 36 h back-trajectories, using the Eta Data Assimilation System (EDAS) 40 km meteorological dataset, to indicate where the parcels of air collected during these experiments travelled prior to collection. A latitude and longitude of 39.1665, −76.897 was used as the collection point on the JHU/APL campus. The runs were initiated at 03:00 UTC for the collections between 05:30 and 09:30 and at 11:00 UTC for the collections between 13:30 and 17:30. The height used to initiate the model was 10 m, to represent the height of the mezzanine where samples were collected.

3 Results

Phylogenetic data from all four aerosol collection periods on the 4th and 5th (Fig. 2, Table 1) indicate that the biological diversity is increasing over the course of the 4 collection periods. One can see the increase not only in the number of positive OTUs with each consecutive collection but also with the number of phyla represented. Although collections one and two are similar to collections three and four in location and time, the differences may have been due to changing weather conditions affecting the environment. The effects of meteorology on bacterial aerosol have been noted in previous studies (Brodie et al., 2007; Lighthart and Shaffer, 1995). Observations by study personnel indicate that precipitation was observed at the collection site during the first aerosol sample collection (05:30 to 09:30 EST on 4 November 2008). National Weather Service NEXRAD data (not shown) indicated scattered showers across the state of Maryland throughout the day on 4 November 2008, and official National

BGD
7, 6725–6747, 2010

Weather Service observations from Baltimore/Washington International Airport indicate light precipitation periodically beginning between 10:18 and 10:54 EST and continuing at least until 22:00 EST. This precipitation may have resulted in the loss of airborne microorganisms through collection of these organisms by falling droplets, by their incorporation into the cloud and subsequently precipitation as cloud condensation nuclei (CCN) or simply by suppressing the source of these particles. The difference in diversity between samples collected 4 and 5 November (Fig. 2 and Table 1) may indicate that some organisms, or the aerosol particles associated with specific types of bacteria, are more suitable as CCN than others.

Although the diversity, measured by the microarray analysis, appears to increase over the course of the four sample periods, there are a few phyla that represent the bulk of 16S rRNA in the samples. Throughout all the samples Proteobacteria are found to represent 47.6 to 63.8% of all bacterial 16S rRNA found in the samples. The orders within this phylum that dominate the sample vary considerably from sample to sample, but Burkholderiales is found consistently to represent greater than 11% of 16S rRNA within this phylum. Firmicutes is the second most dominant phylum in these samples representing anywhere from 8.6 to 24.2% of the mitochondrial RNA in the samples. Not surprisingly, Bacilliales, Lactobacilliales and Chlostridiales represent greater than 70% of the 16S rRNA signatures identified in this phylum.

Initial isolate counts from the cultured samples yielded 41 unique isolates. Of the 41 isolates, 9 exhibited growth on SDA, along with macroscopic and microscopic morphology consistent with fungal isolates, and were therefore classified as fungal and not further characterized. Additionally, several isolates found within each sample that were initially thought to be separate organisms, were found to display similar gram stain and biochemical tests results after further isolation. These isolates were then re-categorized as the same organism. After the exclusion of the fungal isolates and the re-categorization of like isolates within samples, a total of 28 viable isolates with unique characteristics were found in this study (Fig. 3, Tables 2 and 3).

BGD
7, 6725–6747, 2010

Many of the isolates cultured initially appeared to be of low culturable concentrations as only 1 CFU of each isolate appeared after the initial incubation on the various media types. Additionally, there were several instances in which an isolate only appeared on a single media type as 1 CFU after the initial culture, but after replating displayed growth on all non-selective media. These indications of potential low concentrations of culturable organisms may be the result of more fragile organisms not surviving aerosol collection by the OMNI 3000. Because of this, it is possible that there were organisms present in the environment that would have been culturable, but were rendered non-viable by the collection system and therefore not isolated in this study.

Despite the limitations, it is clear that there is a highly diverse culturable microbiological load present in the characterized samples. Over all four samples taken, 24.3% of the isolates recovered were found to be conclusively fungal, 64.9% were conclusively bacterial and 10.8% were indeterminate but likely bacterial in nature. Of the 64.9% of the isolates that were known to be bacterial, 42% were found to be gram-negative rods, 33% gram positive rods, 21% gram positive cocci and 4%, despite controls, displayed consistently variable gram stains (Fig. 3a, Table 2). The 10.8% of the colonies that were considered indeterminate were unable to be removed from the media, which precluded further testing. Over the 4 collections, the fungal percent varied between 14.3% and 30% and the bacterial percent varied between 53.8% and 85.7%. The percent of the bacteria that were gram negative rods varied between 28.6% and 66.7%, gram positive rods between 20% and 42.9% and gram positive cocci between 0 and 40%. Three of the four indeterminate organisms were found during the second collection from 13:30 to 17:30 ET on 4 November (Table 2). The largest number of distinct colonies, thirteen, were isolated during that collection period.

The biochemical tests and selective media utilized in this study provided adequate differentiation of basic isolate characteristics (Fig. 3b, Table 3). Of the bacterial isolates subjected to these tests, 73.9% were catalase positive, 8.7% were indole positive, 43.5% were LAP positive, 17.4% grew on citrate agar, 65.2% displayed motility, 30.4% grew under 6.5% NaCl conditions, 5.9% produced gas, 21.7% were lactose

BGD
7, 6725–6747, 2010

fermenters, 78.2% were non-lactose fermenters. These basic morphological and biochemical characteristics were also useful for identifying common isolates, even between samples collected during different time periods. An example of this can be seen when looking at the results recorded for isolates 2–4 and 3–6 (Tables 2 and 3). Both isolates displayed the same growth characteristics, colony morphology, gram stain, and biochemical reactions indicating that they may be isolates of the same organism cultured from separate samples collected on different days. It should be noted, that due to several of the limitations discussed below, additional common isolates may have been present but not cultured in this study. Additionally, this study only consisted of two sets of two 4 h sample collections taken back to back. It is possible that a higher number of sample collections taken over a broader time period would yield a greater amount of common isolates, and potentially reveal trends associated with their prevalence such as environmental conditions or times of collection.

4 Summary and conclusions

Qualitatively, the comparisons between the bacteria identified by ribosomal RNA and the properties of the culturable bacterial aerosol agree with previous studies. Studies that have examined culturable bioaerosols (e.g. Lighthart and Shaffer, 1995; Mancinella and Shulls, 1978) find a preponderance of gram-positive bacteria, while molecular studies (e.g. Brodie et al., 2007) tend to indicate that the more fragile gram-negative bacteria dominate the nucleic acid. The present study indicates that 42% were gram-negative, 54% gram-positive and 4% gram variable. The PhyloChip results suggest that 68% to 85% of the bacterial phyla found are typically gram-negative, while 15% to 32% are gram-positive (assuming only Firmicutes and Actinobacteria are gram-positive). If the contribution from Proteobacteria is removed from the analysis, since its members are generally considered to be functional anaerobes and no attempt was made to cultivate these bacteria, the results are virtually identical to that of the cultured organisms. The mean across the four samples indicates that 46% of the bacterial load was

BGD
7, 6725–6747, 2010

gram-negative and 54% gram-positive (based on the assumptions above). Independently, the results of the molecular analysis and culture analysis generally agree with previous studies of the same type. Taken together these results present an interesting contrast that has been difficult to rectify by comparing disparate studies.

Proteobacteria and Firmicutes represent the dominant two phyla throughout all the samples (Fig. 2, Table 1); however, several other phyla are represented in the samples that may be important to consider. For instance, Acidobacteria and Actinobacteria are both common soil bacteria and are both found in all four air samples. This may indicate that at least some fraction of the aerosol collected in these samples was generated from soil. Although a small fraction of each sample, the presence of the phyla Planctomycetes and Cyanobacteria indicates that some of the aerosol in these samples had an open water source, possibly oceanic. The back-trajectory analysis indicate that all parcels had an oceanic origin east to north-east of New Jersey and traveled across both the Atlantic ocean and the land mass at heights close to the ground before being collected (Fig. 4). This is consistent with 16S rRNA found in the samples, which show evidence of both soil and oceanic origins.

Initially, the broad diversity of the organisms found in the aerosol samples was alarming. After comparison with the culturable fraction of the microorganisms, some confidence was gained in the results; however, this did not explain the presence of many of the phyla that should have had oceanic origins. The back-trajectory analysis provides a potential explanation of the source of the ribosomal signatures of these phyla. One exciting implication of these findings is that all air samples will carry a biological record of their history. It is well known from past studies that extreme events such as large desert sandstorms may carry bacteria to distant locations (Hua et al., 2007) and that hazardous bacteria may be transported locally over 100 s of meters (Cronholm, 1980). This study indicates that even under normal weather conditions, genetic evidence of the microbial communities within the path of the sampled air mass may exist. Since the urban environment is likely to have a number of unique microbial communities, this information may be used to help identify the source location of pollutants and

BGD
7, 6725–6747, 2010

provide additional evidence to support attribution of pollutants or hazardous emissions to specific locations. Further study of urban bacterial communities, especially in extreme environments such as factory smoke stacks and sewage treatment plants, using detailed genomic profiling may provide useful forensic information for pollution control and other source attribution applications.

Acknowledgements. We would like to acknowledge Bernard Collins and Michael Wagner for their efforts and support, Dorothy Paterno for critical review of the early findings, and Daniel Portwood for his review of the written work. We would also like to thank Anne E. Jedlicka and the other staff of the Gene Array Core Facility at the Malaria Research Institute of The Johns Hopkins University Bloomberg School of Public Health.

References

Brodie, E. L., DeSantis, T. Z., Moberg Parker, J. P., Zubietta, I. X., Piceno, Y. M., and Andersen, G. L.: Urban aerosols harbor diverse and dynamic bacterial populations, P. Natl. Acad. Sci., 104, 299–304, 2007.

Cronholm, L. S.: Potential health hazards from microbial aerosols in densely populated urban regions, Appl. Environ. Microbiol., 39, 6–12, 1980.

DeSantis, T. Z., Dubosarskiy, I., Murray, S. R., and Andersen, G. L.: Comprehensive aligned sequence construction for automated design of effective probes (CASCADE-P) using 16S rDNA, Bioinformatics, 19, 1461–1468, 2003.

DeSantis, T. Z., Stone, C. E., Murray, S. R., Moberg, J. P., and Andersen, G. L.: Rapid quantification and taxonomic classification of environmental DNA from both prokaryotic and eukaryotic origins using a microarray, FEMS Microbiol Lett., 245, 271–278, 2005.

Draxler, R. R. and Rolph, G. D.: HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) Model access via NOAA ARL READY Website (http://www.arl.noaa.gov/ready/hysplit4.html), NOAA Air Resources Laboratory, Silver Spring, MD, 2003.

Fierer, N., Liu, Z., Rodriguez-Hernandez, M., Knight, R., Henn, M., and Hernandez, M. T.: Short-term temporal variability in airborne bacterial and fungal populations, Appl. Environ. Microbiol., 74, 200–207, 2008.

Hua, N.-P., Kobayashi, F., Iwasaka, Y., Shi, G.-Y., and Naganuma, T.: Detailed identification of

BGD

7, 6725–6747, 2010

desert-oriented bacteria carried by Asian dust storms to Japan, Aerobiologia, 23, 291–298, 2007.
Kesavan, J. and Schepers, D.: Characteristics and Sampling Efficiencies of OMNI 3000 Aerosol Samplers, DTIC ADA457464, 2006.
Lee, W. B., Wu, J. Y., Lee, Y. I., and Sneddon, J.: Recent applications of laser-induced breakdown spectrometry: A review of material approaches, Appl. Spectrosc. Rev., 39, 27–97, 2004.
Letunic, I. and Bork, P.: Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation, Bioinformatics, 23, 127–128, 2007.
Lighthart, B and B. T. Shaffer: Airborne Bacteria in the Atmospheric Surface Layer: Temporal Distribution above a Grass Seed Field, Appl. Environ. Microbiol., 61, 1492–1496, 1995.
Mancinelli, R. L. and Shulls, W. A.: Airborne bacteria in an urban environment. Appl. Environ. Microbiol., 35, 1095–1101, 1978.
Matthias-Maser, R. and Jaenicke, S.: The size distribution of primary biological aerosol particles with radii > 0.2 mm in an urban/rural influenced region, Atmos. Res., 39, 279–286, 1995.
Neheme, B., Letourneau. V., Forster, R. J., Veillette, M., and Duchaine, C.: Culture-independent approach of the bacterial bioaerosol diversity in the standard swine confinement buildings, and assessment of the seasonal effect, Environ. Microbiol., 10, 665–675, 2008.
Pan, Y. L., Pinnick, R. G., Hill, S. C., Rosen, J. M., and Chang, R. K.: Single-Particle Laser-Induced-Fluorescence Spectra of Biological and Other Organic-Carbon Aerosols in the Atmosphere: Measurements at New Haven, Connecticut, and Las Cruces, New Mexico, J. Geophys. Res., 112, D24S19, doi:10.1029/2007JD008741, 2007.
Pinnick, R. G., Hill, S. C., Nachman, P., Pendleton, J. D., Fernandez, G. L., Mayo, M. W., and Bruno, J. G.: Fluorescence Particle Counter for Detecting Airborne Bacteria and other Biological Particles, Aerosol Sci. Technol., 23, 653–664, 1995.
Pinnick, R. G., Hill, S. C., Pan, Y. L., and Chang, R. K.: Fluorescence Spectra of Atmospheric Aerosol at Adelphi, Maryland, USA: Measurement and Classifcation of Single Particles Containing Organic Carbon, Atmos. Environ., 38, 1657–1672, 2004.
Reyes, F. L., Jeys, T. H., Newbury, N. R., Primmerman, C. A., Rowe, G. S., and Sanches, A.: Bio-aerosol Fluorescence Sensor, Field Anal. Chem. Technol., 3, 240–248, 1999.
Rolph, G. D.: Real-time Environmental Applications and Display sYstem (READY) Website (http://www.arl.noaa.gov/ready/hysplit4.html), NOAA Air Resources Laboratory, Silver Spring, MD, 2003.

BGD
7, 6725–6747, 2010

Sivaprakasam, V., Huston, A. L., Scotto, C., and Eversole, J. D.: Multiple UV Wavelength Excitation and Fluorescence of Bioaerosols, Optics Express, 12, 4457–4466, 2004.

Shaffer, B. T. and Lighthart, B.: Survey of Culturable Airborne Bacteria at Four Diverse Locations in Oregon: Urban, Rural, Forest and Coastal, Microbial Ecol., 34, 167–177, 1997.

Steele, P. T., Farquar, G. R., Martin, A. N., Coffee, K. R., Riot, V. J., Martin, S. I., Fergenson, D. P., Gard, E. E., and Frank, M.: Autonomous, broad-spectrum detection of hazardous aerosols in seconds, Anal. Chem., 80, 4583–4589, 2008.

BGD

7, 6725–6747, 2010

Table 1. Taxonomic distribution of OTUs identified in the four collected aerosol samples.

Phylum	Phylum % of OTU				Class	Order	Order % of Phylum			
	Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM			Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM
Acidobacteria	0.67	5.57	2.75	5.50	Acidobacteria	Acidobacteriales	100.00	55.00	77.78	53.49
					Acidobacteria-10	Unclassified	0.00	5.00	0.00	2.33
					Acidobacteria-4	Ellin6075/11-25	0.00	5.00	0.00	9.30
					Acidobacteria-6	Unclassified	0.00	10.00	5.56	20.93
					Acidobacteria-7	Unclassified	0.00	0.00	0.00	2.33
					Solibacteres	Unclassified	0.00	15.00	5.56	6.98
					Unclassified	Unclassified	0.00	10.00	11.11	4.65
Actinobacteria	1.34	6.41	10.08	8.06	Actinobacteria	Acidimicrobiales	0.00	0.00	6.06	7.94
						Actinomycetales	0.00	82.61	84.85	68.25
						Bifidobacteriales	0.00	0.00	1.52	9.52
						Coriobacteriales	0.00	0.00	1.52	1.59
						Rubrobacterales	50.00	8.70	4.55	3.17
						Unclassified	0.00	4.35	0.00	7.94
					BD2-10 group	Unclassified	50.00	4.35	1.52	1.59
AD3	0.67	0.28	0.15	0.13	Unclassified	Unclassified	100.00	100.00	100.00	100.00
Aquificae				0.13	Aquificae	Aquificales	0.00	0.00	0.00	100.00
Bacteroidetes	6.04	1.95	4.12	3.96	Bacteroidetes	Bacteroidales	11.11	0.00	11.11	16.13
					Flavobacteria	Flavobacteriales	11.11	0.00	11.11	6.45
					Sphingobacteria	Sphingobacteriales	66.67	85.71	66.67	67.74
					Unclassified	Unclassified	11.11	14.29	11.11	9.68
BRC1	0.67	0.56	0.31	0.13	Unclassified	Unclassified	100.00	100.00	100.00	100.00
Calditrix	0.00	0.00	0.15	0.13	Unclassified	Calditrichales	0.00	0.00	100.00	100.00
Chlamydiae	0.00	0.00	0.15	0.13	Chlamydiae	Chlamydiales	0.00	0.00	100.00	100.00
Chlorobi	1.34	0.28	0.31	0.64	Chlorobia	Chlorobiales	50.00	100.00	50.00	60.00
					Unclassified	Unclassified	50.00	0.00	50.00	40.00
Chloroflexi	2.68	3.06	1.83	2.17	Anaerolineae	Chloroflexi-1a	0.00	36.36	25.00	5.88
						Chloroflexi-1b	0.00	0.00	8.33	5.88
						Chloroflexi-1f	0.00	0.00	0.00	5.88
						Unclassified	100.00	0.00	25.00	23.53
					Chloroflexi-3	Roseiflexales	0.00	0.00	0.00	5.88
					Chloroflexi-4	Unclassified	0.00	9.09	8.33	11.76
					Dehalococcoidetes	Unclassified	0.00	27.27	16.67	17.65
					Thermomicrobia	Unclassified	0.00	0.00	0.00	5.88
					Unclassified	Unclassified	0.00	27.27	16.67	17.65
Coprothermobacteria	0.00	0.00	0.15	0.13	Unclassified	Unclassified	0.00	0.00	100.00	100.00
Cyanobacteria	0.67	0.56	0.46	1.66	Cyanobacteria	Chloroplasts	0.00	100.00	33.30	46.15
						Chroococcales	0.00	0.00	0.00	7.69
						Nostocales	0.00	0.00	0.00	7.69
						Plectonema	100.00	0.00	33.30	7.69
						Prochlorales	0.00	0.00	0.00	7.69
					Unclassified	Unclassified	0.00	0.00	33.30	23.08
Deinococcus-Thermus	0.00	0.56	0.31	0.38	Unclassified	Unclassified	0.00	100.00	100.00	100.00
DSS1	0.00	0.00	0.00	0.13	Unclassified	Unclassified	0.00	0.00	0.00	100.00
Firmicutes	24.16	8.64	19.69	23.53	Bacilli	Bacillales	47.22	32.26	38.76	32.89
						Lactobacillales	27.78	12.90	20.93	19.08
					Clostridia	Clostridiales	13.89	22.58	31.78	38.16
						Unclassified	2.78	3.23	0.78	1.97
					Desulfotomaculum	Unclassified	0.00	9.68	2.33	2.63
					Mollicutes	Acholeplasmatales	0.00	0.00	1.55	0.66
						Anaeroplasmatales	5.56	6.45	0.00	1.32
						Mycoplasmatales	0.00	0.00	0.00	0.66
					Symbiobacteria	Symbiobacterales	2.78	6.45	1.55	1.32
					Unclassified	Unclassified	0.00	6.45	2.33	1.32
Gemmatimonadetes	0.00	0.56	0.61	0.77	Unclassified	Unclassified	0.00	100.00	100.00	100.00
Lentisphaerae	0.00	0.28	0.15	0.13	Unclassified	Unclassified	0.00	100.00	100.00	100.00
Natronoanaerobium	0.67	0.56	0.61	0.64	Unclassified	Unclassified	100.00	100.00	100.00	100.00

BGD
7, 6725–6747, 2010

Table 1. Continued.

Phylum	Phylum % of OTU				Class	Order	Order % of Phylum			
	Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM			Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM
NC10	0.00	0.28	0.15	0.26	NC10-1	Unclassified	0.00	100.00	100.00	100.00
Nitrospira	0.00	0.28	0.31	0.51	Nitrospira	Nitrospirales	0.00	100.00	100.00	100.00
OP10	0.67	0.28	0.46	0.38	CH21 cluster	Unclassified	0.00	100.00	100.00	33.33
					Unclassified	Unclassified	100.00	0.00	0.00	66.67
OP3	0.00	0.28	0.15	0.13	Unclassified	Unclassified	0.00	100.00	100.00	100.00
OP8	0.00	0.28	0.15	0.00	Unclassified	Unclassified	0.00	100.00	100.00	0.00
OP9/JS1	0.00	0.56	0.31	0.26	OP9	Unclassified	0.00	100.00	100.00	100.00
Planctomycetes	1.34	0.84	0.31	0.64	Planctomycetacia	Planctomycetales	100.00	100.00	100.00	100.00
Proteobacteria	53.69	63.79	50.08	47.57	Alphaproteobacteria	Acetobacterales	0.00	1.32	0.61	1.06
						Azospirillales	0.00	1.76	1.52	1.06
						Bradyrhizobiales	0.00	4.41	3.94	3.72
						Caulobacterales	4.94	1.76	1.21	1.60
						Consistiales	0.00	1.32	0.91	1.60
						Ellin314/wr0007	0.00	0.88	0.61	0.53
						Ellin329/Riz1046	0.00	1.32	0.61	0.53
						Rhizobiales	0.00	6.17	7.27	10.90
						Rhodobacterales	1.23	3.52	2.42	3.72
						Rickettsiales	2.47	3.08	1.52	2.39
						Sphingomonadales	8.64	7.49	5.76	3.99
						Unclassified	1.23	6.17	4.55	3.72
					Betaproteobacteria	Burkholderiales	11.11	15.42	14.55	16.76
						Ellin6095/SC-I-39	0.00	0.44	0.30	0.27
						Methylophilales	1.23	0.44	0.30	0.27
						MND1 clone group	0.00	0.00	0.61	1.06
						Neisseriales	0.00	0.00	0.00	0.27
						Nitrosomonadales	0.00	1.76	1.21	1.86
						Rhodocyclales	1.23	1.76	1.82	1.86
						Unclassified	0.00	0.44	0.30	0.27
					Deltaproteobacteria	AMD clone group	0.00	0.00	1.52	1.33
						Bdellovibrionales	0.00	0.00	0.00	0.27
						dechlorinating clone group	0.00	0.44	0.30	0.27
						Desulfobacterales	0.00	5.73	4.55	3.99
						Desulfovibrionales	1.23	0.00	3.03	3.19
						Desulfuromonadales	0.00	0.88	0.61	0.80
						EB1021 group	0.00	0.00	0.00	0.53
						Myxococcales	1.23	1.32	1.21	2.66
						Syntrophobacterales	0.00	2.20	1.52	1.60
						Unclassified	1.23	1.76	1.52	1.06
					Epsilonproteobacteria	Campylobacterales	12.35	7.93	6.36	6.12
					Gammaproteobacteria	Acidithiobacillales	0.00	0.00	0.91	0.27
						Aeromonadales	1.23	0.00	0.30	1.06
						Alteromonadales	3.70	2.64	4.85	1.86
						aquatic clone group	0.00	0.00	0.00	0.80
						Chromatiales	1.23	1.32	0.91	1.60
						Ellin307/WD2124	0.00	0.00	0.00	0.27
						Enterobacteriales	34.57	3.96	10.61	2.39
						Legionellales	3.70	3.08	2.12	1.86
						Methylococcales	0.00	0.44	0.61	1.06
						Oceanospirillales	0.00	1.32	1.52	0.53
						Pseudomonadales	4.94	0.44	2.12	1.33
						Shewanella	0.00	0.00	0.30	0.00
						SUP05	1.23	0.00	0.61	0.53
						Symbionts	0.00	0.44	0.61	0.53
						Thiotrichales	0.00	0.88	1.21	1.86
						Unclassified	1.23	1.76	1.21	1.60
						uranium waste clones	0.00	0.00	0.00	0.27
						Xanthomonadales	0.00	2.64	0.61	1.86

BGD
7, 6725–6747, 2010

Table 1. Continued.

Phylum	Phylum % of OTU				Class	Order	Order % of Phylum			
	Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM			Nov 4 AM	Nov 4 PM	Nov 5 AM	Nov 5 PM
					Unclassified	Unclassified	0.00	1.32	0.91	1.06
SPAM	0.00	0.28	0.31	0.26	Unclassified	Unclassified	0.00	100.00	100.00	100.00
Spirochaetes	0.00	0.00	0.46	1.66	Spirochaetes	Spirochaetales	0.00	0.00	100.00	100.00
Synergistes	0.67	0.28	0.61	0.77	Unclassified	Unclassified	100.00	100.00	100.00	100.00
Thermodesulfobacteria	0.00	0.00	0.00	0.13	Thermodesulfobacteria	Thermodesulfobacteriales	0.00	0.00	0.00	100.00
TM6	0.00	0.00	0.00	0.13	Unclassified	Unclassified	0.00	0.00	0.00	100.00
TM7	0.00	0.28	0.15	0.38	TM7-3	Unclassified	0.00	100.00	0.00	33.33
					Unclassified	Unclassified	0.00	0.00	100.00	66.67
Unclassified	1.34	1.95	1.22	1.41	Unclassified	Unclassified	100.00	100.00	100.00	100.00
Verrucomicrobia	2.68	1.95	0.76	0.64	Unclassified	Unclassified	25.00	28.57	20.00	40.00
					Verrucomicrobiae	Verrucomicrobiales	75.00	71.43	80.00	60.00
WS3	0.00	0.56	0.31	0.13	Unclassified	Unclassified	0.00	100.00	100.00	100.00
WS5	0.00	0.00	0.00	0.13	Unclassified	Unclassified	0.00	0.00	0.00	100.00

BGD
7, 6725–6747, 2010

Table 2. Morphological and gram-stain properties of cultivated isolates from the four collected aerosol samples.

Isolate Number	Colony Morphology						Growth Conditions		Gram Stain	
	Size	Color	Shape	Edge	Surface	Elevation	Media	Time (hr)	Stain	Shape
1–1, 2	small	white	filamentous	filamentous	rough	raised	TSA, SDA	24		N/R
1–3	large	opaque	circular	filamentous	rough	raised	TSA, SDA	48		N/R
1–4	large	orange	circular	entire	rough	raised	TSA, SDA	48		N/R
1–5	medium	off-white	circular	entire	glistening	raised	Chocolate	24	GN	rods
1-6	medium	yellow	circular	entire	glistening	raised	SBA	24	GN	rods/cocci
1–7	medium	gray/white	irregular	entire	smooth	raised	SBA	24	GP	rods
1–8	pinpoint	clear	circular	entire	smooth	raised	SBA	24	GN	rods
1–9	small	white	filamentous	filamentous	rough	raised	SBA	24		unable to pick
1–10, 11, 13	pinpoint	clear	circular	entire	smooth	raised	TSA	48	GP	rods
1–12	small	white	circular	entire	smooth	raised	SBA	48	GN	small rods
2–1	small	white	filamentous	filamentous	smooth	raised	TSA	24		unable to pick
2–2	medium	orange	circular	entire	smooth	raised	TSA, Chocolate	24	GP	rods
2–3	large	opaque	irregular	entire	smooth	convex	TSA	24	GP	rods
2–4	medium	gray/white	circular	entire	glistening	raised	SBA	24	GP	cocci clusters
2–5	medium	yellow/gray	irregular	entire	glistening	raised	SBA	24	GP	rods
2–6	small	white	circular	entire	smooth	raised	SBA	24	GP	cocci clusters
2–7	large	gray	circular	entire	rough	raised	Chocolate, SDA	24		N/R
2–8	medium	gray	circular	entire	glistening	raised	Chocolate, SDA	48		N/R
2–9	large	off-white	circular	entire	dry	raised	Chocolate	24		N/R
2–10, 13	small	white	filamentous	filamentous	rough	raised	Chocolate	24	GN	rods
2–11	medium	white	filamentous	filamentous	rough	raised	TSA	48		unable to pick
2–12	small	opaque	circular	entire	glistening	raised	TSA	48	GN	rods
2–14	large	orange	circular	entire	rough edges, smooth center	convex	Chocolate, SDA	48		N/R
3–1	medium	white	filamentous	filamentous	rough	raised	TSA	24	GP	rods
3–2	medium	white	filamentous	filamentous	rough	raised	TSA, Chocolate, SBA, SDA	24		N/R
3–3	small	white	circular	entire	smooth	raised	TSA, Chocolate	24	GP	cocci clusters
3–4	medium	white	circular	entire	glistening	raised	TSA	24	GN	rods
3–5	large	yellow	circular	entire	glistening	raised	Chocolate	24	GN	small rods
3–6	large	white	circular	entire	smooth	raised	SBA	24	GP	cocci clusters
3–7	large	yellow, darker center	circular	entire	glistening	raised	SBA, SDA	24		N/R
4–1	very large	white	circular	entire	rough edges, smooth center	raised	TSA, SDA	24		N/R
4–2	large	yellow	circular	entire	rough edges, smooth center	raised	TSA	48	GP	rods
4–3	medium	bright orange	circular	entire	smooth	raised	TSA	48	GP	rods
4–4	small	white	circular	entire	smooth	raised	TSA	48	GN	rods
4–5	pinpoint	opaque	circular	entire	smooth	raised	TSA	48	GP	cocci clusters
4–6	small	opaque	circular	entire	glistening	raised	Chocolate	48		Variable
4–7	small	yellow	circular	entire	glistening	raised	Chocolate	48	GN	rods

BGD
7, 6725–6747, 2010

Table 3. Biochemical properties of cultivated isolates from the four collected aerosol samples.

Isolate Number	Catalase	Indole	LAP	Citrate	Motility	TSI	NaCl	Gas	MacConkey
1–5	+	–	–	–	–	K/K	–	–	LF
1–6	+	+	+	–	+	N/G	–	–	LF
1–7	–	–	–	–	+	A/A	–	–	NG
1–8	+	–	–	–	–	NC	–	–	NG
1–12	–	–	+	–	–	NC	–	–	LF
2–2	+	–	–	–	+	K/A	+	–	NG
2–3	+	–	–	+	+	K/A	+	+	NG
2–4	+	–	–	–	–	A/A	+	–	NG
2–5	+	–	–	+	+	A/A	+	–	NLF
2–6	+	–	–	–	+	K/A	+	–	NG
2–12	+	–	+	–	+	NC gas	–	–	LF
3–1	–	–	–	–	–	NC gas	–	–	NG
3–3	–	–	–	–	+	K/A	+	–	NG
3–4	+	+	+	–	+	A/A	-	-	NLF
3–5	+	–	+	+	–	K/A	–	–	NLF
3–6	+	–	–	–	–	A/A	+	–	NG
4–2	+	–	–	–	+	K/K	-	N/R	NG
4-3	+	–	–	+	+	K/K gas	N/R	N/R	NG
4–4	+	–	+	–	+	NC gas	N/R	N/R	NG
4–5	+	–	+	–	+	A/A	N/R	N/R	NG
4–7	+	–	+	–	+	NC gas	N/R	N/R	NG
1–10, 11, 13	–	–	+	–	–	NC gas	–	–	NG
2–10, 13	+	–	+	–	+	NC	–	–	LF

BGD
7, 6725–6747, 2010

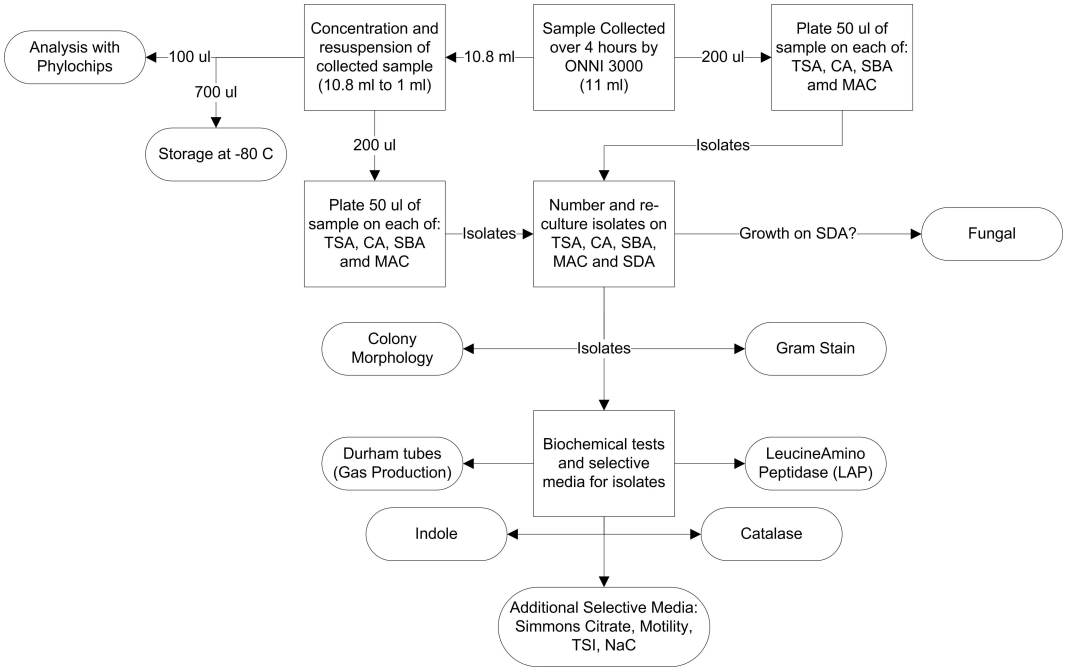

Fig. 1. Flow chart for processing collected aerosol samples.

BGD
7, 6725–6747, 2010

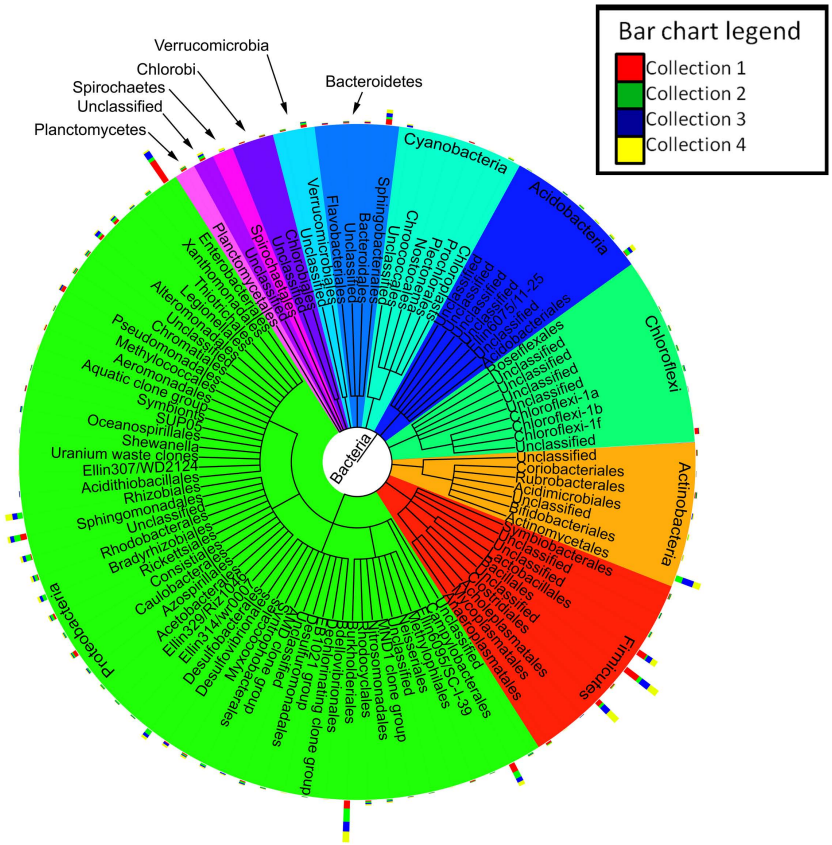

Fig. 2. Phylogenetic distribution of bacterial orders with OTUs that represent greater than 1% of the OTUs found in the four collected aerosol samples. The outer stacked bar chart indicates the relative abundance of distinct OTUs from each order in each sample that is also shown in Table 1.

BGD
7, 6725–6747, 2010

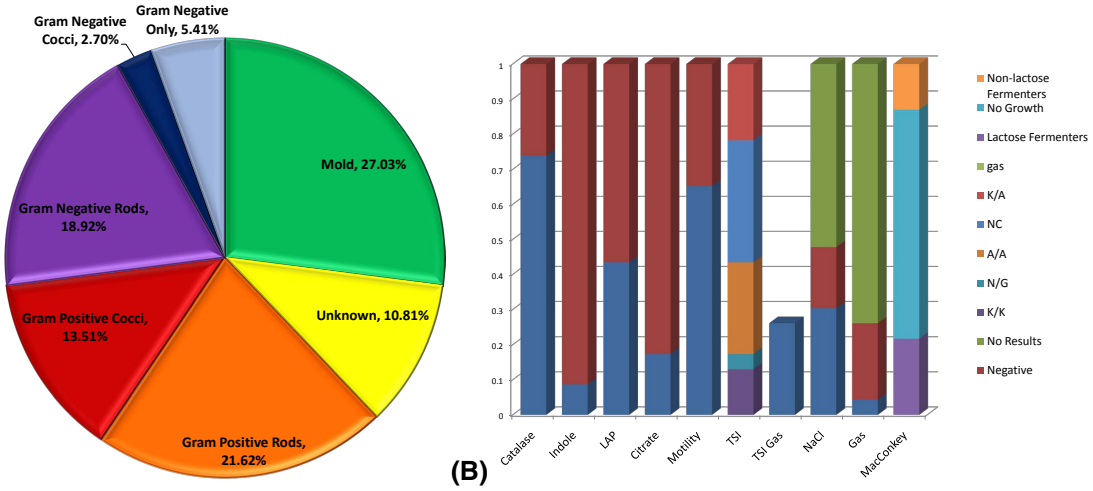

Fig. 3. **(A)** Morphological distribution of cultivated microbial isolates from the four collected aerosol samples. **(B)** Summary of the tested biochemical properties of all bacterial isolates from the four collected aerosol samples.

BGD
7, 6725–6747, 2010

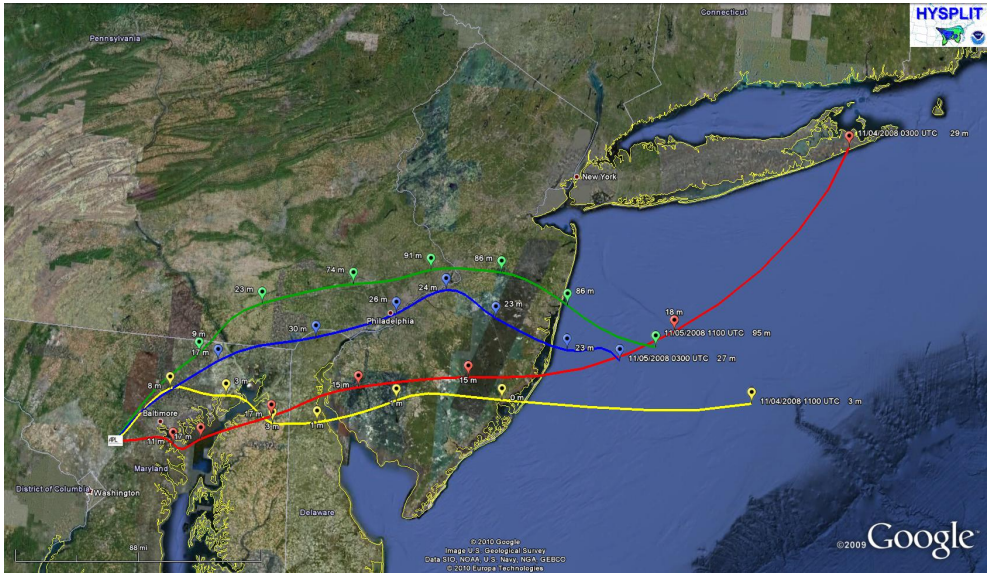

Fig. 4. Thirty-six hour back trajectories from each of the four collected aerosol samples. The altitude is given adjacent to each point denoted on the trajectories. The trajectory for each sample indicates that all samples travelled at relatively low altitudes over both ocean and land. Mapping and satellite imagery generated using Google Earth.

BGD
7, 6725–6747, 2010