



African desert dust in the Caribbean atmosphere: Microbiology and public health

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Abstract

Air samples collected on St. John in the U.S. Virgin Islands were screened for the presence of viable bacteria and fungi to determine if the number of cultivatable microbes in the atmosphere differed between “clear atmospheric conditions” and “African dust-events.” Results indicate that during “African dust-events,” the numbers of cultivatable airborne microorganisms can be 2 to 3 times that found during “clear atmospheric conditions.” Direct microbial counts of air samples using an epifluorescent microscopy assay demonstrated that during an “African dust-event,” bacteria-like and virus-like particle counts were approximately one log greater than during “clear atmospheric conditions.” Bacteria-like particles exhibiting autofluorescence, a trait of phototrophs, were only detected during an “African dust-event.”

1. Introduction

It has long been recognized that dust clouds originating from African storms regularly impact the Atlantic and Caribbean (Prospero, 1970). In the mid 1800's while sailing in the Atlantic aboard the *Beagle*, Darwin noted dust in the atmosphere and concluded the source was Africa (Darwin, 1845).

“The wind had been for twenty-four hours previously E.N.E., and hence, from the position of the ship, the dust probably came from the coast of Africa. The atmosphere was so hazy that the visible horizon was only one mile distant.”

Charles Darwin, 1845

Modern oceanographic research indicates that African dust significantly impacts water quality in the Sargasso Sea and its light scattering capability may play an important role in climate-forcing throughout the tropical and subtropical North Atlantic Ocean (Jickells, 1999; Li, 1996). Dust flux from Africa to the atmosphere has been estimated to be as high as 1 billion

tons per year (Moulin, 1997). Reported estimates of African dust impacting the North Eastern Amazon Basin were 280 thousand tons per event, adding up to 13 million tons per year, with maximum westward dust flux occurring between the months of February and April (Swap, 1992, 1996). Maximum transport to the Caribbean, Central and North America occurs between the months of June through October and is concentrated between latitudes 15° and 25° North (Graham, 1979). Research on Barbados conducted by the University of Miami since the late 1960's has reported an increase in dust flux over the last twenty-five years coinciding with the onset of the current North African drought (Prospero, 1968, 1986, 1999). The same research group, monitoring African dust transport at a site in south Florida over the last 23 years, reported deposition rates between 10 to 100 $\mu\text{g}/\text{m}^2/\text{day}/\text{dust-event}$. Large dust-events have been reported to impact approximately 30% of the landmass of the continental United States, with Florida receiving approximately 50% of the

total annual deposition reaching the U.S. (Perry, 1997).

African dust has been identified using element ratios (aluminum/calcium, aluminum/titanium, titanium/zirconium, etc.) as the most important parent material in Caribbean island soils and provided the clay minerals needed for production of pre-Columbian Indian pottery on San Salvador island in the Bahamas (Mann, 1986; Muhs, 1990). African dust has been implicated as a significant source of primary nutrients such as iron and calcium to the oceans and it has been estimated that approximately 50% of the phosphorus transported to the oceans via the atmosphere is due to African dust deposition (Graham, 1979; Henriksson, 2000; Savoie, 1980). Long-range transport of nutrient laden desert dusts from the Gobi and Takla Makan deserts has been identified as sustaining Hawaiian rainforests growing in highly weathered soils (Chadwick, 1999). Dust clouds originating from these Asian deserts have recently been observed impacting Oregon and Washington State in images obtained from the National Aeronautics and Space Administrations (NASA) Earth Probe-Total Ozone Mapping Spectrometer (TOMS) satellite (Arimoto, 1996).

Sediment core studies conducted in the Atlantic Ocean west of Africa reported the presence of freshwater diatoms and phytoliths in sediment cores and attributed their presence to African dust deposition (Maynard, 1976; Parmenter, 1974). Atmospheric transport of macro-scale organisms such as the desert locust *Schistocerca gregaria* from Africa to the Caribbean islands of Barbados and Dominica has also been documented (Ritchie, 1989; Rosenberg, 1999).

African dust has been implicated in various disease outbreaks in coral reef environments throughout the Caribbean, and the fungus *Aspergillus sydowii*, causative agent of the current outbreak of Caribbean seafan disease, has been identified in atmospheric samples collected in the Caribbean during African dust events (Shinn, 2000; Smith, 1996; Weir, 2000). Exposure to desert dust has been identified as the source of a number of outbreaks of terrestrial diseases including: (1) Aspergillosis in desert locusts (Venkatesh, 1975), (2) Coccidioidomycosis in humans (MMWR, 2000; Williams, 1979), (3) Al Eskon disease in humans (Korenyi-Both, 1992) and (4) desert lung syndrome in humans (Nouh, 1989). The National Institute of Health's National Institute of Allergy and Infectious Diseases identifies airborne dust as the primary source of allergic stress worldwide

(www.niaid.nih.gov/publications/allergens/intro.htm). A good example of long range atmospheric transport of microbial pathogens is the "*Puccinia* Pathway", where the fungal wheat pathogen *Puccinia graminis* is transported from southern Texas and northern Mexico to the Northern U.S and Canada in the spring, and back during the summer and fall (Pedgley, 1986). A review of plant rust pathogens reported the possibility of transoceanic transport of the same pathogen from Australia to South Africa due to similarities in species isolated and regional wind patterns (Nagarajan, 1990). These authors also reported the possibility of transatlantic wind transport of a coffee rust pathogen in 1966 from Angola to Brazil (Nagarajan, 1990).

The identification of viable pathogenic fungal spores in Caribbean air samples (Smit, 1999), and cited evidence of long range microbial transport, resulted in our current effort to determine if there is an increase in the numbers of microbes in the atmosphere when the region is being impacted by African dust.

2. Material and methods

2.1 Sample site

Atmospheric samples for cultivatable microbiological analysis were collected at Lind Point on St. John in the U.S. Virgin Islands during both normal atmospheric conditions and African dust-events. For direct count analysis the dust-event sample was collected at Mamey Peak (St. John) and the clear condition sample collected offshore (southeast of St. John). Atmospheric conditions at the time of sampling were determined visually, with clear skies indicating non-dust conditions, and reduced visibility indicating dust-events. TOMS ultraviolet satellite images, which enhance resolution of atmospheric conditions (<http://toms.gsfc.nasa.gov>), show the daily distribution of dust and other aerosols for the entire globe from 1979 to present (Chiapello, 1999).

2.2 Air samples for isolation of microbes

Presterilized filter housings containing 47 mm diameter analytical test filters with a pore size of 0.2 μm were obtained from Fisher Scientific (catalog #09-74030G, Atlanta, GA). To take the air sample, the filters were removed from their respective sterile bags, placed on an analytical filter manifold, lids removed and vacuum applied using a vacuum pump for a set

period of time. Airflow rates through the filters were 9.3 liters/minute for 15 to 29 minutes per sampling. To control for handling contamination, an additional filter was removed from its bag, the filter placed on the manifold and allowed to sit without removing the lid. Both filters were then removed from the manifold, lids sealed with parafilm, replaced in their respective bags, sealed with tape and refrigerated until shipment. Once the filters were received at the United States Geological Survey (USGS) microbiological laboratory in St. Petersburg, Florida, they were refrigerated until analysis. All analysis was conducted within a horizontal laminar airflow cabinet using sterile technique. R2A agar (Fisher Scientific, Atlanta, GA) was utilized for microbial analysis. One quarter to one half of each filter was cut using sterile scissors and placed on R2A agar sample side up. Filters were incubated in the dark at room temperature and monitored for growth over a 2-week period. Fungi and bacterial colonies were isolated from each other by isolation streaking on fresh plates of R2A. Once isolated, colonies were grown overnight in Tryptic Soy Broth (Fisher Scientific, Atlanta, GA) and the following day 1 ml of culture was transferred to a sterile cryogenic storage tube containing 200 μ l of sterile glycerol. These isolated colonies were then stored at -70°C for cataloging.

2.3 Air samples for direct counts

Pall Gelman 25 mm air filter holders (Fisher Scientific, Atlanta, GA) were loaded with an 8.0 μ m pore size support filter (cat. #AAWP02500, Fisher Scientific, Atlanta, GA) and a 0.02 μ m pore size glass Whatman Anodisc filter (Fisher Scientific, Atlanta, GA). Filters/holders were individually wrapped in parafilm, placed in a plastic bag and shipped to sample site. For sampling, three of the filter holders were unwrapped and placed on a vacuum manifold. Using a vacuum pump, air was pumped for a set period of time (range of 10 to 15 minutes at a rate of 14.65 to 29.98 L/minute). The filters were then removed from the manifold, wrapped in parafilm and refrigerated until shipment to the USGS St. Petersburg, Florida, laboratory. Upon arrival samples were refrigerated until processing. For processing the filters were placed sample-side up on top of a drop of diluted SYBR Gold nucleic acid stain (Molecular Probes, Eugene, OR: 97.5 μ l of 0.02 μ m filtered H_2O + 2.5 μ l of a 1/10 dilution of SYBR Gold) and incubated at room temperature in the dark for $\sim$ 10–15 minutes. The filters were then removed from the drop of diluted

SYBR Gold, excess stain was removed by blotting the back of the filter on tissue paper, and placed on a glass slide. Twenty-seven microliters of antifade solution (990.0 μ l 50% 1X PBS/50% Glycerin + 10.0 μ l 10% P-phenylene diamine) was placed on a coverslip, and the coverslip placed over the filter. The coverslip was lightly pressed to expel any trapped air and the slide was then refrigerated in the dark until counted by epifluorescent microscopy. Ten fields per slide were counted. The average numbers of microbe-like particles were obtained by averaging both field and sample counts.

2.4 Genetic identification of microbial isolates

The polymerase chain reaction (PCR) was used for 16S and 18S rDNA amplification using universal prokaryote and fungal primer sets [EF3 and EF4 for fungi] respectively (Shah, 1997; Smit, 1999). For bacterial DNA extraction, bacterial isolates were touched with a sterile pipette tip, and the tip was then used to inoculate 180 μ l of lysis buffer recommended for extraction of DNA from gram positive bacteria in a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA). The DNeasy Tissue Kit protocol was followed and purified DNA was eluted in 100 μ l of the kit elution buffer. For fungal DNA extraction, approximately 5 mg of fungal isolate tissue was placed into a 1.5 ml microfuge tube. A modified freeze-thaw protocol was then followed (Griffin, 2001) after suspending the tissue in DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) buffer AP1 (400 μ l) and RNase A as outlined in the kit protocol. After the freeze-thaw assay was completed the DNeasy Kit protocol was followed (starting at step three with the addition of 130 μ l of buffer AP2) and purified DNA was eluted in 100 μ l of the kit elution buffer. For both the bacteria and fungi, 5 μ l of purified eluate was used for PCR. The PCR master mix recipe per reaction was: 10 μ l of GeneAmp 10X PCR buffer (Applied Biosystems, Foster City, CA), 12 μ l of 25 mM MgCl_2 (Applied Biosystems, Foster City, CA), 2 μ l of 10 mM dNTP mix (Promega, Madison, WI), 0.5 μ l of 5 units/ μ l Taq polymerase (Promega, Madison, WI), 1 μ l each of 10 nM upstream and downstream primer (synthesized by Operon Technologies, Inc. Alameda, CA) and 69.0 μ l of 0.02 μ m filter sterilized autoclaved H_2O . PCR amplification profile used for both 16 S and 18 S rDNA amplification was: one cycle for 2 minutes at 94°C , 40 cycles of [30 seconds at 94°C , 30 seconds at 45°C , 2 minutes at 72°C], one cycle of 10 minutes at 72°C and hold at

Table 1. Microbiological data of air samples collected on St John, United States Virgin Islands between 18 July 2000 and 28 July 2000

Date of sample	Wind speed (m/s)/wind direction (degrees)	Dust-event Visual/TOMS	Number of colonies isolated. Bacteria/fungi	Equivalent numbers of colonies per liter of air filtered. Total (Bacteria/Fungi)
18 July 2000	2.5/64°	No/No	7/4	0.16 (0.10/0.06)
23 July 2000	3.9/83°	Yes/Yes	14/5	0.21 (0.16/0.06)
26 July 2000	3.1/119°	Yes/Yes	36/5	0.40 (0.35/0.05)
27 July 2000	4.2/77°	Yes/Yes	7/2	0.12 (0.09/0.03)
28 July 2000	4.2/103°	No/Yes	28/7	0.26 (0.21/0.05)
Total			92/23	

Dust-Event – Visual = determined by site at the time of sampling.

Dust-Event – TOMS = determined using the NASA satellite Earth Probe – Total Ozone Mapping Spectrometer (TOMS).

4 °C. After PCR, amplicon was cleaned and eluted to 30 µl in 0.02 µm-filtered water using a QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA). Pure amplicon was then cloned into a plasmid vector using a TOPO TA Cloning Kit (Invitrogen Corp., Carlsbad, CA). After the plasmid was amplified overnight in the TOPO TA Cloning Kit host/clone (*E.coli*), concentrated plasmid was purified using a Wizard® Plus SV Miniprep Kit (Promega, Madison, WI). Clones were verified using EcoR I digestion (Promega, Madison, WI, following manufacturers directions) and electrophoresis. Plasmid insert (PCR amplicon) was sequenced (single strand, one reaction, ~750 bases) by the University of Florida DNA Sequencing Core Laboratory (Gainesville, FL). GenBank Blast search (<http://www.ncbi.nlm.nih.gov/BLAST/>) was used for amplicon/isolate identification.

3. Results

Table 1 lists the results of the 5 samples taken between the 18th and 28th of July 2000. A total of 115 microbes were cultured from approximately 469 liters of air, which included samples taken during both clear conditions and dust-events. Figure 1 is a panel showing TOMS images of atmospheric aerosols moving towards or through the Caribbean from

Africa with the image dates corresponding to the sample dates in Table 1. On 18 July 2000, no dust was noted visually or with TOMS and 11 microbes (7 bacteria and 4 fungi) were cultured from 69.75 liters of air (0.16 microbes/liter). On 23 July 2000, dust was noted visually and with TOMS and 19 microbes (14 bacteria and 4 fungi) were cultured from 88.35 liters (0.21 microbes/liter). On 26 July 2000, dust was noted visually and with TOMS and 41 microbes (36 bacteria and 5 fungi) were cultured from 102.3 liters (0.40 microbes/liter). On 27 July 2000, dust was noted visually and with TOMS and 9 microbes (7 bacteria and 2 fungi) were cultured from 74.4 liters (0.12 microbes/liter). On 28 July 2000, dust was only noted by TOMS and 35 microbes were cultured from 134.85 liters (0.26 microbes/liter). No growth occurred on any of the negative control filters.

Table 2a lists the genera and/or species of bacterial and fungal isolates that were identified on 18 July 2000 using ribosomal sequences. Of the 7 bacteria identified, 3 were pigmented gram positives (*Curtobacterium citreum* isolates and *Bacillus megaterium*), 2 were non-pigmented gram positives (both *Arthrobacter globiformis* isolates), 1 was a pigmented gram negative (*Sphingomonas* sp.) and 1 was a pigmented unknown. The four fungal isolates were *Coccodinium bartschii*, *Cladosporium cladosporioides*, *Pleospora rudis* and *Gibberella puli-*

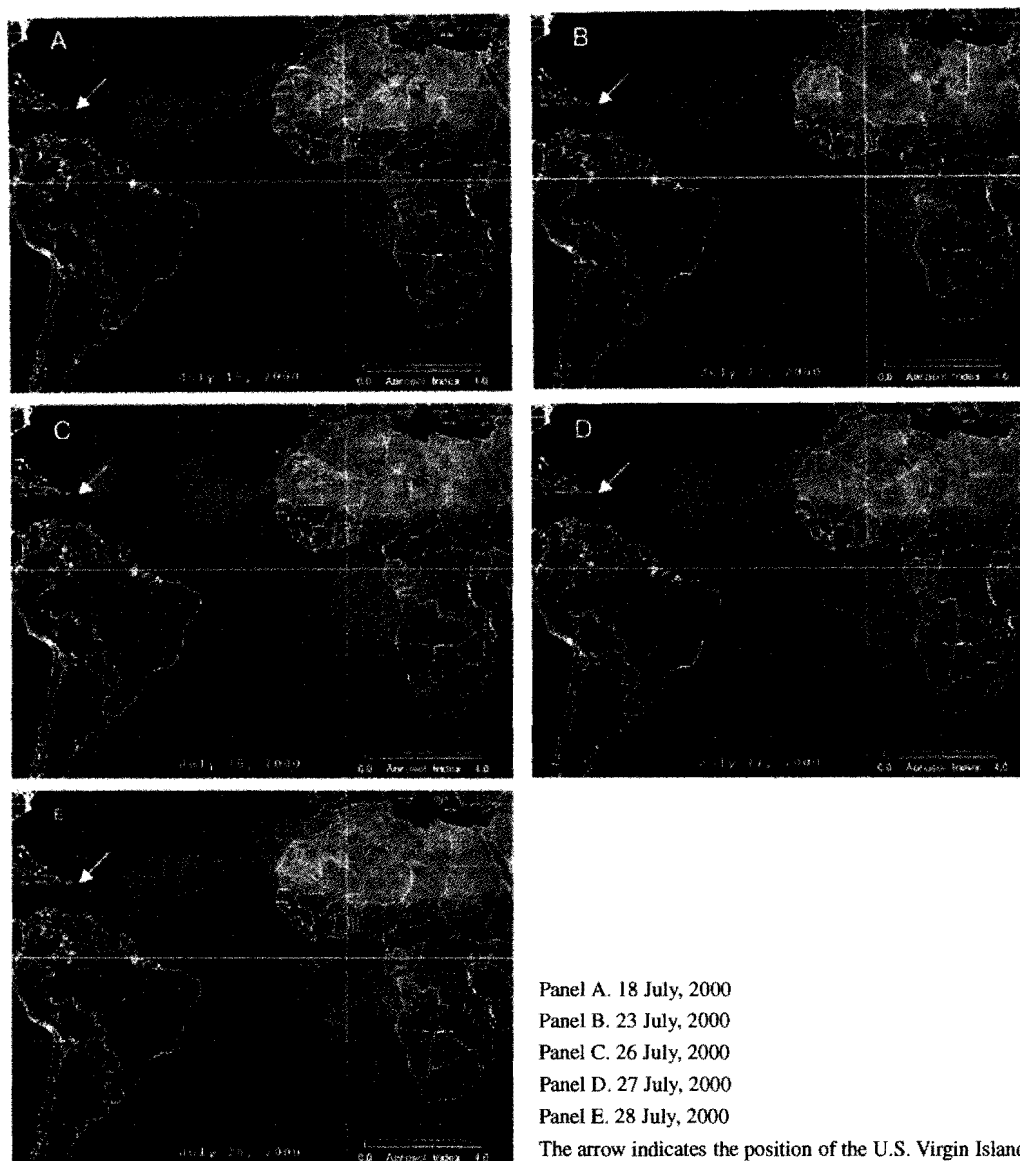


Figure 1. Polar orbiting Earth Probe TOMS Aerosol images for the dates 18, 23, 26, 27 and 28 July 2000. Images were captured at approximately 11:15am local solar time each day and are compliments of the Laboratory for Atmospheres, Goddard Space Flight Center, Greenbelt, MD. <http://jwocky.gsfc.nasa.gov/>.

caris. Non-pigmented bacteria represented 28% of the total isolates. Thirty-six percent of the isolates were plant or human pathogens.

Table 2b lists the genera and/or species of bacteria and fungi that were identified on 23 July 2000. All 12 original bacterial isolates were pigmented. Of the original 12 isolates only 11 could be recovered from storage. Five of the eleven were gram negatives, 5 were gram positive and one showed sequence simil-

arity to a purple non-sulfur bacteria (626/655 bp, 95%). Two of the bacteria, *Bacillus megaterium* and *Curtobacterium citreum* have been identified as plant pathogens. Three of the four fungal isolates were identified as *Cladosporium cladosporioides*, and the remaining isolate as *Coccodinium bartschii*.

Table 2c lists the genera and/or species of bacteria and fungi that were identified on 26 July 2000. Of the 32 bacteria identified all 23 gram positives

Table 2a. Identified bacteria and fungi using 16S and 18S rDNA sequences and GenBank blast. Clear atmospheric conditions, 18 July 2000

Genus/species	Type	Number of Isolates (% homology)	Number of matched bases	Information
<i>Arthrobacter globiformis</i>	Bacteria/Gram +	2	673/686 (98%), 743/745 (99%)	Common non-pathogenic environmental isolate
<i>Bacillus megaterium</i>	Bacteria/Gram +	1	622/625 (99%)	Elm pathogen
<i>Curtobacterium citreum</i>	Bacteria/Gram +	2	734/735 (99%), 723/726 (99%)	Bean pathogen
<i>Sphingomonas</i> sp.	Bacteria/Gram –	1	656/667 (98%)	Plant/marine/soil isolate
Unknown	Bacteria/	1	648/667 (97%)	Arizona soil isolate
<i>Cladosporium cladosporioides</i>	Fungi	1	687/688 (99%)	Common environmental isolate. Human and plant pathogen
<i>Coccodinium bartschii</i>	Fungi	1	643/651 (98%)	aka – sooty mould
<i>Gibberella pulicaris</i>	Fungi	1	688/691 (99%)	Causes potato dry rot (storage disease)
<i>Pleospora rudis</i>	Fungi	1	636/644 (98%)	Plant isolate

Table 2b. Identified bacteria and fungi using 16S and 18S rDNA sequences and GenBank blast. Dust event, 23 July 2000

Genus/species	Type	Number of Isolates (% homology)	Number of matched bases	Information
<i>Bacillus megaterium</i>	Bacteria/Gram +	1	718/722 (99%)	Elm pathogen
<i>Curtobacterium albidum</i>	Bacteria/ Gram +	1	678/681 (99%)	Rice isolate
<i>Curtobacterium citreum</i>	Bacteria/Gram +	1	680/683 (99%)	Bean pathogens
<i>Curtobacterium luteum</i>	Bacteria/Gram +	1	687/687 (100%)	Soil Bacteria
<i>Microbacterium</i> sp.	Bacteria/Gram +	2	689/702 (98%), 689/703 (98%)	Soil bacteria
<i>Pseudomonas riboflavina</i>	Bacteria/Gram –	1	668/699 (95%)	Soil isolate
<i>Sinorhizobium</i> sp.	Bacteria/Gram –	1	673/708 (95%)	Plant symbiont
<i>Sphingomonas trueperi</i>	Bacteria/Gram –	1	719/739 (97%)	Soil isolate
<i>Sphingomonas</i> sp.	Bacteria/Gram –	2	661/680 (97%), 505/512 (98%)	Plant/marine/soil isolate
Unidentified proteobacterium	Purple non-sulfur bacteria	1	626/655 (95%)	Grouped with <i>Zoogloea</i> sp. Marine isolate
<i>Cladosporium cladosporioides</i>	Fungi	3	767/768 (99%), 795/797 (99%), 772/774 (99%)	Common environmental isolate. Human and plant pathogen
<i>Coccodinium bartschii</i>	Fungi	1	773/789 (97%)	aka – sooty mould

were pigmented, 6 were pigmented gram negatives (*Sphingomonas* sp. isolates, *Pseudomonas alcalophila*, *Pseudomonas oleovorans* and the unidentified) and 3 were non-pigmented gram negatives (both *Sphingomonas pruni* isolates and *Paracoccus aminovorans*). Of the 5 fungal isolates, 2 were *Coccodinium bartschii*, 2 were *Pleospora rudis* and one was *Cochliobolus sativus*. Non-pigmented bacteria represented

9% of the total isolates. Three of the original 35 bacteria isolates could not be recovered from storage. Twenty-eight percent of the total identified isolates were plant pathogens. No human pathogens were identified at the species level. *Escherichia coli* was used as a control strain to verify accurate 16S rDNA isolation and identification.

Table 2c. Identified bacteria and fungi using 16S and 18S rDNA sequences and GenBank Blast. Dust event, 26 July 2000

Genus/species	Type	Number of Isolates (% homology)	Number of matched bases	Information
<i>Arthrobacter sp.</i>	Bacteria/Gram +	3	724/731 (99%), 716/730 (98%), 734/754 (97%)	PCB degrading soil bacteria and human pathogens
<i>Bacillus pumilus</i>	Bacteria/Gram +	1	706/707 (99%)	Most frequently recovered bacilli in Ethiopian spices
<i>Curtobacterium citreum</i>	Bacteria/Gram +	4	705/707 (99%), 685/688 (99%), 725/727 (99%), 595/598 (99%)	Bean pathogens
<i>Curtobacterium luteum</i>	Bacteria/Gram +	3	742/743 (99%), 678/679 (99%), 576/619 (93%)	Soil bacteria
<i>Kocuria erythromyxa</i>	Bacteria/Gram +	1	737/741 (99%)	Marine isolate
<i>Microbacterium arborescens</i>	Bacteria/Gram +	3	687/687 (100%), 734/735 (99%), 722/724 (99%)	Soil bacteria
<i>Microbacterium testaceum</i>	Bacteria/Gram +	6	645/653 (98%), 642/645 (98%), 645/656 (98%), 680/690 (98%), 703/711 (98%), 712/723 (98%)	Soil bacteria
<i>Paracoccus aminivorans</i>	Bacteria/Gram -	1	587/603 (97%)	Activated sludge isolate. Thiocyanate-utilizing facultative chemolithotroph
<i>Paracoccus sp.</i>	Bacteria/Gram -	1	640/652 (98%)	Marine isolate
<i>Pseudomonas alcalophila</i>	Bacteria/Gram -	1	684/688 (99%)	Marine isolate
<i>Pseudomonas oleovorans</i>	Bacteria/Gram -	1	697/705 (98%)	Cooking oil waste isolate
<i>Sphingomonas pruni</i>	Bacteria/Gram -	2	733/751 (97%), 677/687 (98%)	Plant pathogen
<i>Sphingomonas sp.</i>	Bacteria/Gram -	2	641/652 (98%), 703/721 (97%)	Plant/marine/deep-soil isolate
Unidentified	Bacteria/Gram +	1	645/649 (99%)	Rape rhizosphere isolate. 98% homology (656/654) with <i>Curtobacterium sp.</i>
Unidentified	Bacteria/Gram +	1	691/696 (99%)	Causes red stripes in rice and is 98% homologous (685/694) to <i>M. testaceum</i>
Unidentified	Bacteria/Gram -	1	630/632 (99%)	Trickling filter isolate. 97% homology (641/659) to <i>Sphingomonas sp.</i> (marine isolate)
<i>Coccodinium bartschii</i>	Fungi	2	672/680 (98%), 665/672 (98%)	?
<i>Cochliobolus sativus</i>	Fungi	1	672/674 (99%)	Grass pathogen
<i>Pleospora rudis</i>	Fungi	2	638/644 (99%), 637/644 (98%)	Plant isolates

Table 3. Microbiological data of air samples collected on St John, United States Virgin Islands between 1 October 2000 and 17 March 2001

Date of sample	Wind speed (m/s)/wind direction (degrees)	Dust-event Visual/TOMS	Number of colonies isolated. Bacteria/fungi	Equivalent numbers of colonies per liter of air filtered. Total (Bacteria/Fungi)
1 Oct. 2000	3.4/97°	Yes/No	0/2	0.02 (0.00/0.02)
8 Oct. 2000	3.7/125°	No/No	0/2	0.02 (0.00/0.02)
19 Nov. 2000	5.4/66°	No/No	0/0	0.00
17 Feb. 2001	3.0/127°	No/No	0/2	0.01 (0.00/0.01)
3 Mar. 2001	2.8/126°	No/No	0/0	0.00
9 Mar. 2001	6.2/147°	No/No	0/2	0.01 (0.00/0.01)
17 Mar. 2001	3.6/105°	No/No	0/0	0.00

Table 4. Microbial direct counts

Type	Date	Atmospheric condition	Counts/liter of air
Bacteria-like	1 October 2000	Dust	157.8
Virus-like particles	1 October 2000	Dust	213.0
Orange autofluorescence	1 October 2000	Dust	201.5
Bacteria-like	19 November 2000	Clear	18.1
Virus-like particles	19 November 2000	Clear	18.1
Orange autofluorescence	19 November 2000	Clear	0.0

Bacteria-like = rod shaped bacteria and large cocci/spore shaped fluorescent particles. Did not include any autofluorescent particles.

Virus-like particles = very small to fine cocci shaped fluorescing particles.

Orange autofluorescence = all orange autofluorescent particles. Orange autofluorescence is typically caused by phototrophs.

Table 3 lists the results of 7 samples taken during the end of the St. John dust season, in months during which clouds of African desert dusts do not typically impact the region. No bacterial growth was noted on any of these sample filters. The first of October 2000 was the only sample visually classified as a dust event. TOMS images for this date do not show any significant dust cloud activity. Two fungal colonies were noted after 48 hours of incubation on R2A. Microbial direct count results for this date can be found in Table 4. Of the remaining six sample dates all were classified as clear atmospheric conditions both visually and by TOMS. Microbial direct count information for the 19 November 2000 sample can be found in

Table 4. No growth was noted on the samples taken on 19 November 2000, 3 March 2001 and 17 March 2001. Two fungal colonies were isolated from each of the other sample dates, 8 October 2000, 17 February 2001 and 9 March 2001.

Table 4 lists the microbial direct counts obtained during dust and clear conditions (1 October 2000 and 19 November 2000 respectively). Counts obtained from the dust condition sample were 157.8 bacteria-like particles, 213.0 virus-like particles and 201.5 autofluorescent bacteria-like particles per liter of air filtered. The counts obtained from the clear condition sample were 18.1 bacteria-like particles, 18.1 virus-like particles and 0.0 autofluorescent bacteria-like

particles per liter of air filtered. Negative control filters were stained for both samples to account for microbial contamination of staining reagents and handling. Control counts were averaged and subtracted from the sample counts in calculating the above reported counts.

4. Discussion

The numbers of bacteria listed in Table 1 by date demonstrates a flux in the number of viable airborne microbes in the U.S. Virgin Islands as the region is being impacted by African dust. The panels in Figure 1 which match the respective sample dates show what appears to be clear atmospheric conditions on the 18 of July 2000 and African dust clouds of various sizes impacting the region between the dates of 23 and 28 July 2000. The data in Table 1 indicates that higher numbers of cultivatable microbes can be recovered from air samples when dust is impacting the region. It should be stated that when the dust is confined to the boundary layer below 1 km, the standard Aerosol Index dust images (which are the TOMS aerosol images used in Figure 1), may not show the presence of dust over a region. Most of the time, dust plumes are approximately 5 km from the sea surface, and are easily detected by TOMS. A physical property that may affect the number of detectable airborne microbes both before and after dust impacts the region is wind shear. Wind shear may cause dust particles near the ground to linger in light wind conditions. This results in heavy particles quickly falling out of the atmosphere leaving light particles such as very fine dust and microbes behind as the main dust plume moves on. This may have been the case for the microbes collected on 18 July 2000 when no dust was noted in the region visually or by TOMS. TOMS images on the 15th and 16th of July show dust clouds in the region.

A majority of the isolates listed in Tables 2a, 2b and 2c are commonly found in soils or in association with plants. Of the eleven 18 July 2000 isolates, four (36%) were identified at the species level as potential plant pathogens (*C. citreum*, *B. megaterium* and *C. cladosporioides* isolates). The fungus isolate *Cladosporium cladosporioides*, has been identified as both a plant and human pathogen and is one of the most common species of fungi recovered from air samples (Drabick, 1990; Gugnani, 1978). Of the twelve 23 July 2000 isolates, five (42%) have been identified as potential plant pathogens. Of the

thirty-five 26 July 2000 isolates, seven (20%) were identified at the species level as potential plant or grass pathogens (*S. pruni*, *C. citreum* and *C. sativus* isolates). Three of the 26 July 2000 isolates have previously been identified in marine environments (*P. alcalophila*, *Paracoccus* sp. and *K. erythromyxa*). Microbial movement from marine environments to the atmosphere has been demonstrated via physical surface activity that results in the formation of sea-foams/sprays (Maynard, 1968a,b). It is possible that dust clouds traversing marine environments serve as a vehicle for long-range transport of aerosolized marine microorganisms. Of the three dates in which the isolates have been identified at least 10% were *Sphingomonas* sp., a genera which has been previously shown to be the closest genetic match to the unidentified bacteria that causes white plague (a coral disease) throughout the Caribbean. The direct count data in Table 4 shows an increase in all categories of Microbes when dust is impacting the region. Bacteria-like particles were 8.7 times higher during the dust event than during clear atmospheric conditions. Virus-like particles were over a log higher and autofluorescing bacteria-like particles were only detected during dust conditions. As the cultivatable counts versus the direct counts indicate (Tables 3 and 4 respectively), a majority of the microbes present in air samples may not be cultivatable due to a variety of factors such as viability, media selection, incubation temperature and aerobic versus anaerobic incubation. Microbial ecology studies have shown that less than 1 percent of microbes are cultivatable from environmental samples (Torsvik, 1990; Eilers, 2000).

It is also interesting to note the differences in cultivatable organisms during the dust season (Table 1) versus the non-dust season (Table 3). Of the five samples collected during the dust season, the concentration of cultivatable microbes averaged 0.23/liter of air sampled. Of the 7 non-dust season samples, the concentration of cultivatable microbes averaged 0.01/liter of air sampled. Over one log difference despite the fact that more samples were taken in the non-dust season and typically more air was sampled per sample date (average, 94.27 liters/sample versus 130.06 liters/sample, dust season verses non-dust season respectively).

This preliminary data indicates that microbes are being transported from Africa via dust flux to the Caribbean and the Americas. It was originally believed that few viable microbes would be isolated from each sample due to inactivation from ultravi-

olet (UV) light exposure as they traversed the Atlantic in the clouds of dust. We now believe that as the dust plumes traverse the Atlantic, the dust particles at higher altitudes attenuate ultraviolet light, which provides sufficient UV shielding at lower altitudes to allow microbial survival during the estimated 5 to 7 day journeys. Within large dust plumes UV light may be attenuated by more than 50% (Herman, 1999). Additionally, microbes may attach to and survive within cracks and crevasses of inorganic dust particles, which could shield them from UV light.

The high concentration of dust impacting the Caribbean may pose a significant public health threat, particularly as it pertains to respiratory disease. It is known that exposure to desert silica can result in development of silicosis (Patial, 1999). Once sensitized to fungi, exposure to small concentrations of airborne fungal spores can trigger allergic responses (Gravesen, 1979). There has been a seventeen-fold increase in asthma prevalence in Barbados between 1973 and 1996, and acute asthma attacks accounted for 22.3% of Queen Elizabeth Hospital emergency room visits in 1999 (Howitt, 1998 2000). This increase corresponds to the observed increase in African dust flux impacting Barbados (Prospero, 1999). An additional public health concern is cistern contamination from pathogenic microbes transported in African dust as many of the Caribbean Islands utilize cisterns to collect drinking water.

This and other research demonstrates that dust clouds may serve not only as a periodic source of nutrients for terrestrial plants and primary producers in nutrient depleted oceanic waters, but may also serve as a medium for the global transport of microorganisms. Pathogenic microbes associated with dust clouds may pose a risk to ecosystem and human health as the clouds traverse regions. Research is ongoing and will focus on isolate identification, total microbial counts using nucleic acid stains to address the question of percent cultivatable/viable, anaerobe and archeobacteria populations, the presence of viruses and factors impacting both ecosystem and public health.

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