Stratospheric microbiology at 20 km over the Pacific Ocean

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Abstract An aerobiology sampling flight at 20 km
was conducted on 28 April 2008 over the Pacific
Ocean (36.5° N, 118–149° W), a period of time that
coincided with the movement of Asian dust across the
ocean. The aim of this study was to confirm the
presence of viable bacteria and fungi within a trans-
oceanic, atmospheric bridge and to improve the
resolution of flight hardware processing techniques.
Isolates of the microbial strains recovered were
analyzed with ribosomal ribonucleic acid (rRNA)
sequencing to identify bacterial species *Bacillus* sp.,
Bacillus subtilis, Bacillus endophyticus, and the fungal
genus *Penicillium*. Satellite imagery and ground-based
radiosonde observations were used to measure dust
movement and characterize the high-altitude environ-
ment at the time of collection. Considering the
atmospheric residency time (7–10 days), the extreme
temperature regime of the environment ($-75°C$), and
the absence of a mechanism that could sustain
particulates at high altitude, it is unlikely that our
samples indicate a permanent, stratospheric ecosys-
tem. However, the presence of viable fungi and
bacteria in transoceanic stratosphere remains relevant
to understanding the distribution and extent of micro-
bial life on Earth.

1 Introduction

Microbial diversity in Earth's upper atmosphere
remains largely unexplored, despite its relevance to a
number of scientific disciplines. Bacteria and fungi are
injected into the atmosphere primarily by dust storms
over arid regions of the planet, but hurricanes,
volcanoes, fire, and anthropogenic sources also con-
tribute to the total amount of biological material in the
air. Meteorological factors control the altitude, distri-
bution, and residency time of particles aloft (Deshler
et al. 1993; Griffin et al. 2001; Bauman et al. 2003;
Kellogg and Griffin 2006; Wainwright et al. 2006;
Griffin 2007). Lower atmosphere (altitude 0–10 km)
collections have documented how microorganisms
can affect local ecology and climate. Global patterns
of atmospheric dust and long-range dispersion can
influence the spread of plant and animal pathogens

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(Griffin et al. 2001; Brown and Hovmøller 2002; Griffin 2007). Microbial cells contribute to precipitation as cloud condensation nuclei (CCN) or ice condensation nuclei (ICN), as discussed by Schnell and Vali (1976), Maki and Willoughby (1978), Szyrmer and Zawadzki (1997), Hamilton and Lenton (1998), Sun and Ariya (2006), Pratt et al. (2009), and Prenni et al. (2009). While most airborne microbes remain dormant during atmospheric transport, Sattler et al. (2001) documented growth of bacteria in supercooled cloud droplets (at or below 0°C) from samples collected at 3 km.

Clearly, bacteria and fungi are abundant in the troposphere, but what about even farther above the Earth's surface? The biologically challenging environment in the stratosphere (10–50 km) would suggest that there would be lower viable biomass as altitude increases. For instance, the stratosphere has greater extremes of ultraviolet (UV) irradiation, desiccation, cold temperatures, and nutrient deprivation than the troposphere (Brasseur and Solomon 1986; Lysenko and Demina 1992; Nicholson et al. 2000). Yet, several studies conducted in the stratosphere have reported viable, airborne microbes (Imshenetsky et al. 1978; Lysenko 1980; Harris et al. 2002; Wainwright et al. 2002; Narlikar et al. 2003; Griffin 2004, 2008), including novel species (Shivaji et al. 2006). Missions to sample stratospheric biota have been historically infrequent, making it difficult to answer even basic ecological questions. For example, what is the diversity and distribution of microbial species at high altitudes? Is there a limit to their dispersion (vertical and horizontal)? What influences cell viability at high altitudes? Finally, and perhaps most interestingly, can growth and/or reproduction occur in situ at altitude? Investigating these topics with subsequent stratosphere collections is critical to understanding the true boundaries of the terrestrial biosphere.

The environmental factor most relevant to each question above is residency time—defined here as the total time that microbes remain aloft in the upper atmosphere. To date, no study has been able to determine whether residency time is on the order of days, months, or possibly even longer. Although satellites and ground instruments can track dust storms, neither can remotely detect airborne microbes. Our group recognized that sampling an air mass above the open ocean would be an effective way to assign a residency time by (1) eliminating the possibility of local dust injections and (2) comparing satellite-imaged dust trajectories with DNA sequence information to infer place of origin. In Asia, dust generated by the Gobi, Takla Makan, and Badain Jaran deserts blows seasonally across the Pacific Ocean from approximately March to May (Duce et al. 1980; Kar and Takeuchi 2004), eventually reaching the west coast of North America. Chemical measurements (Jaffe et al. 2003) and satellite imagery (Griffin 2007) have been used to estimate that Asian dust typically takes 7–10 days to cross the Pacific. Along a transoceanic route, some fraction of microbes will be removed from the air by precipitation or gravitational settling (Griffin et al. 2006). Collecting dust deposited in North America would subject microbes to heavy dilution and contamination from local sources. To avoid this problem, our group sampled an air mass that was still in transit over the Pacific Ocean using a high-altitude aircraft that offered precise instrumental control and a large collection range. We hypothesized that most microbial species recovered from the transoceanic air samples would trace back to Asian isolates, several thousand kilometers from the desert source, due to the timing and location of our collection.

2 Materials and methods

2.1 Flight hardware

A modified impactor plate from the NASA Cosmic Dust Group at Johnson Space Center was designed to interface with a pressure-sealed, metal housing (serial number U2-144) that mounted onto the wingtip of a high altitude ER-2 aircraft (Lockheed Martin Corp. at NASA Dryden Flight Research Center) and was deployed by pilot command (Fig. 1). Details of the flight hardware have been published elsewhere (Griffin 2004, 2008). An exact replicate of the acrylic Cosmic Dust Group impactor plate (hereafter referred to as a 'flag' for its likeness in shape), measuring roughly 5.6 × 5.8 cm, was machined out of lightweight aluminum in order to make the system compatible with autoclaving. The aluminum flag was then textured on its leading-edge side with a sandblaster to increase its surface area and create additional attachment troughs for impacting atmosphere particles.

Fig. 1 Flight hardware.
a The flight hardware system (flag in housing) was loaded into the wingtip payload bay of the Lockheed Martin ER-2 high-altitude research aircraft at NASA Dryden Flight Research Center. **b** Upon pilot command, the hardware was deployed from the wingtip, extending the flag into the stratosphere. **c** View of the flag face if separated from housing to demonstrate individual system components. **d** Microscopic view of flag face surface after sandblasting treatment designed to increase adhesion of impacting atmosphere particles
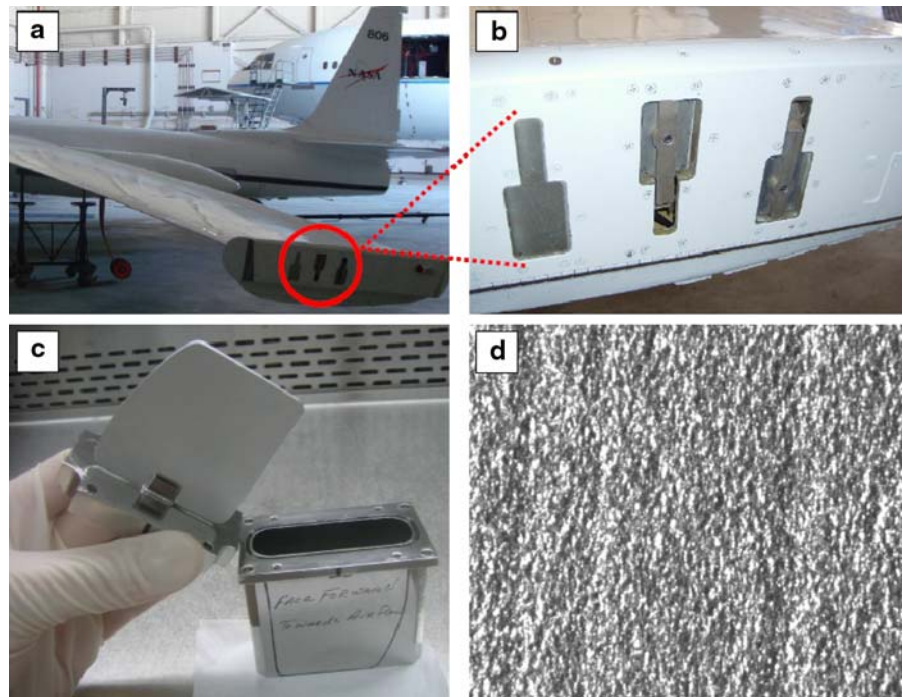
Surface topography was confirmed with a high-resolution video microscope (model VH-7000, Keyence Corp. of America, Woodcliff Lake, NJ, USA).

2.2 Microbiology procedures, hardware preparation, and sampling flight

Difco^{TM} R2A (18.2 g added to 1 l of deionized water) and potato dextrose agar (PDA) (39 g added to 1 l of deionized water; Difco media, Fisher Scientific, Pittsburg, PA) were used for solid growth of bacteria and fungi, respectively. A 10% dilution (3 g added to 1 l of deionized water) of tryptic soy broth (TSB) (Becton Dickinson and Company Sparks, MD) in 10 ml tubes was used for liquid growth of both bacteria and fungi.

Before flying, a series of hardware assembly/disassembly tests were conducted in the Space Life Sciences Laboratory at NASA Kennedy Space Center (FL, USA) to verify the sterility of autoclaved equipment, the sealed housing, and the hardware processing environment. All procedures were conducted under aseptic conditions, using a laminar flow hood (NuAire Inc., model NU-602-400, Plymouth, MN, USA) equipped with a UV irradiation light source. Prior to assembly, the flag and the metal housing were washed with 70%

isopropanol, mated, placed in a tape-sealed sterilization pouch (Tower Dual Peel® Sterilization Pouch, Baxter Healthcare Corporation, Deerfield, IL, USA) to reduce moisture, and double autoclaved at 121°C for 20 min along with other tools required for the procedure; notably, razor blades and Allen wrenches required for the housing screw mounts. The unit was then disassembled so that a pre-sterilized 15 μl aliquot of glycerol could be applied to the surface of the flag, using a micropipette and razor blade for uniform spreading. Once re-assembled and autoclaved, the unit was placed in the laminar flow hood for 48 h to simulate pre-flight transportation/idle time. Using sterile techniques, the flag was then removed from the housing and left exposed in the laminar flow hood for 8 h to mimic a typical flight-sampling period. After exposure, the flag was placed face-down (see below for detail) on R2A media for a 2-week incubation period in a 30°C growth chamber (Innova 4230, New Brunswick Scientific, Edison, NJ, USA), and no growth was observed.

The assembly method (described above) was repeated for the hardware intended for flight. The pre-sterilized glycerol aliquot spread onto the flag was also applied onto R2A plates for negative control testing and no growth occurred over a 4-week incubation period. The hardware was shipped to NASA Dryden Flight

Research Center where the complete system was mounted onto the wing payload bay of the ER-2 aircraft. On 28 April 2008, the aircraft departed (sortie # 08-6017) from Edwards Air Force Base (CA, USA) and deployed the flag at a sustained altitude of 20 km while maintaining an east-to-west transect (36.5° N, 118–149° W) over the Pacific Ocean (Fig. 2). The exposure to the stratosphere lasted for 7.5 h (opened 1416 Zulu and closed 2154 Zulu). The Pacific Ocean jet stream along the flight path was analyzed through infrared satellite images provided by the National Oceanic and Atmospheric Administration (NOAA) (Fig. 3). Environmental conditions at the sampling altitude were modeled using radiosonde observations and geopotential height contour maps provided by the Naval Research Laboratory and the California Regional Weather Server (D. Westphal and D. Dempsey, personal communication, June 2008) from the nearest land-based station, KOAK (WMO identification # 72493), located at 37.73° N, 122.22° W (Fig. 4).

2.3 Post-flight processing

Upon receiving the flight hardware, the assembly was unpackaged in a UV disinfected (12 h) laminar flow hood. A novel swabbing procedure was employed on the flight hardware which (1) increased the number of samples compared to historical assays (Griffin 2004, 2008), (2) used several growth media (liquid and solid) to encourage diverse cell recovery, and (3) would provide a useful comparison between the flag and the internal/external surfaces of the flight housing, divided into various sampling sections: F (flag), EH (external housing), and IH (internal housing) (Fig. 5). With the housing still closed, EH samples were taken with autoclaved 10-cm cotton applicators that were moistened in sterile, deionized H_2O. Each swab consisted of a surface rub of the cotton tip over the application spot. The contact side of the applicator was swabbed onto R2A media, then placed into 10 ml test tubes containing liquid TSB for a second growth opportunity. Negative controls with cotton applicators were performed periodically ($N = 10$) and there was no growth in liquid or solid media. Swabs were taken along the flag aluminum perimeter and backside before it was placed face-down on R2A. Finally, IH locations were swabbed, plated, and also introduced into liquid media. Test tubes and plates (parafilmed to prevent drying) were stored in a dark incubator at 30°C and monitored for growth. The face-down flag was transferred onto new R2A plates after time-steps of 1, 2, 4, 7, and 56 days.

2.4 Genetic identification

Bacterial cultures were isolated and archived at −20°C for genetic analysis. Subsets to be identified were grouped according to growth rate, medium preference,

Fig. 2 Sampling flight path. On 28 April 2008, the ER-2 aircraft (sortie # 08-6017) departed from Edwards Air Force Base (CA, USA) and traveled over the Pacific Ocean (36.5° N, 118–149° W) at an altitude of 20 km
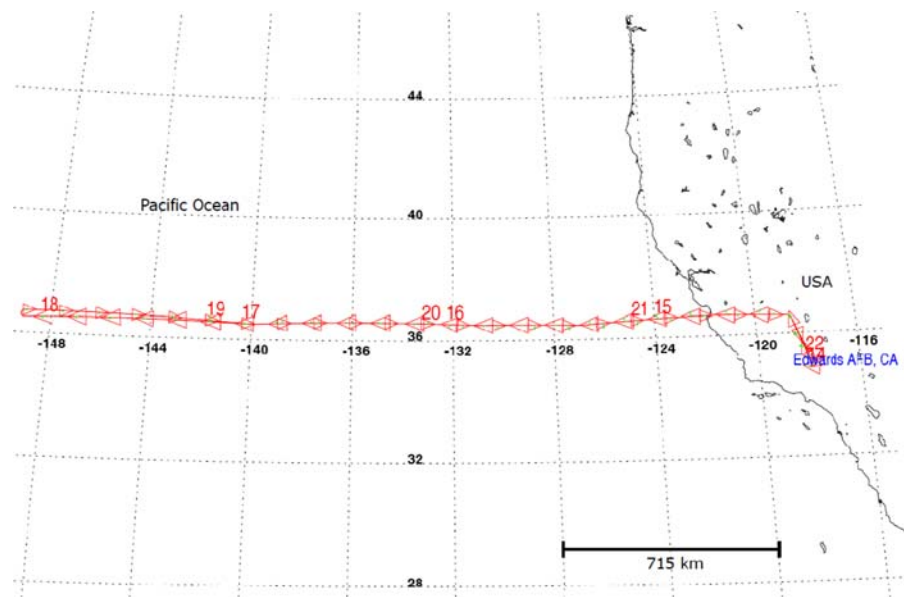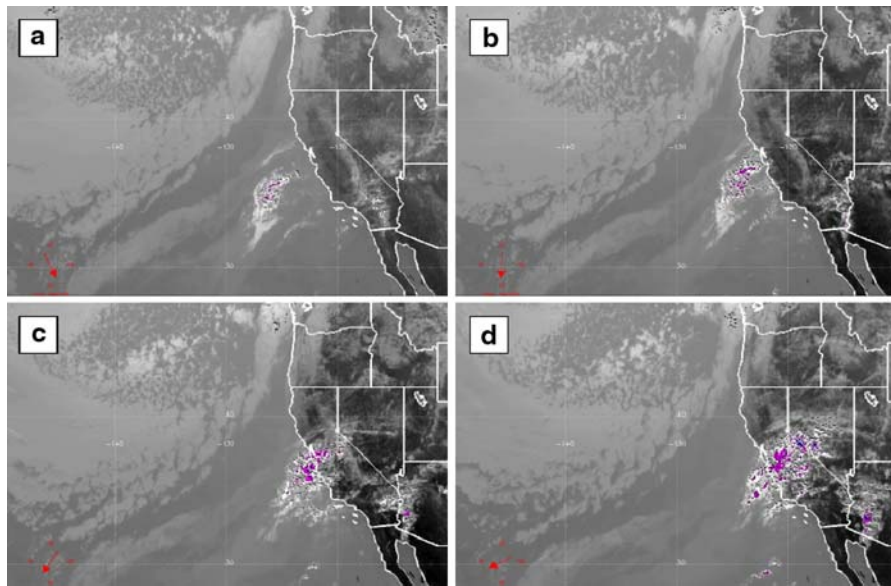
Fig. 3 Jet stream trajectory. Satellite images of atmosphere at 20 km on 28 April 2008, taken at IR wavelength by METEOSAT-5 and provided courtesy of NOAA/NASA. The west-to-east movement of the air mass can be observed by following the water content (see highlight) over time **a** 1615 Zulu, **b** 1815 Zulu, **c** 2015 Zulu, and **d** 2215 Zulu

Fig. 4 Environmental data at stratosphere sampling location. Radiosonde observations from station KOAK (37.73° N, 122.22° W) depict the temperature and dew point changes with altitude gain. At the 20 km sampling altitude (dotted line) the atmospheric pressure was estimated at 50 mb, with temperature and dew point at −75°C and −64°C, respectively. Wind was on a northeast heading at 4–9 m/s, blowing towards the coast of North America
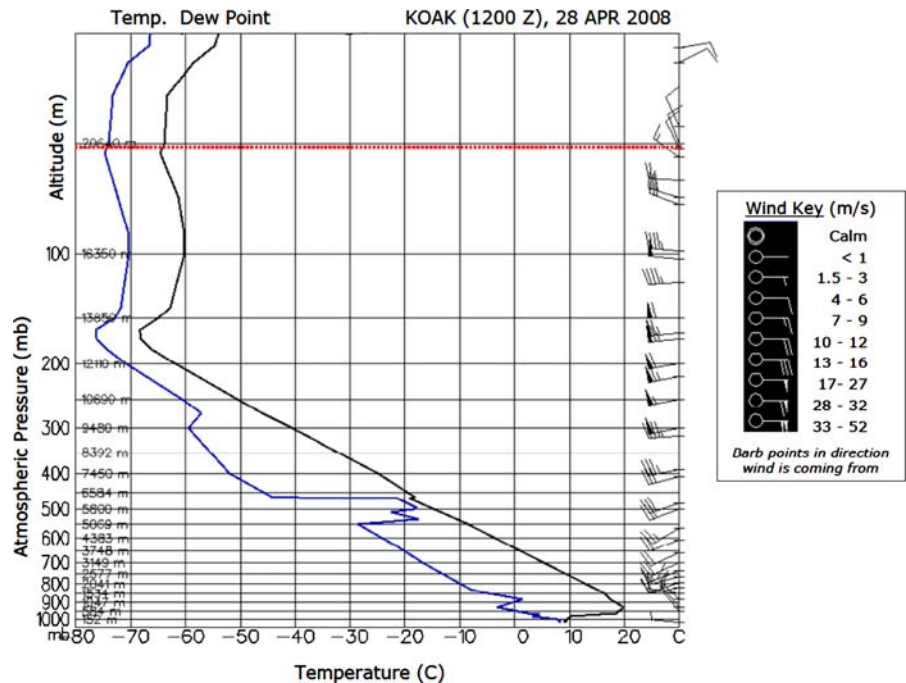
colony size, colony shape, pigmentation, and location on the sampling hardware. Deoxyribonucleic acid (DNA) extraction was performed, followed by the polymerase chain reaction (PCR) amplification of 16S ribosomal ribonucleic acid (rRNA), according to previously described protocols (Griffin 2004). Universal primers listed in Grasby et al. (2003) were used for eight bacterial isolates, generating 1538 bp

Fig. 5 Post-flight processing sequence and hardware nomenclature. (*Note*: numbers in brackets correspond to equivalent location on opposite side of hardware, not pictured.) **a** External housing (EH) was sampled immediately after removing the flight hardware from the shipping container. **b** Once the housing was opened, the leeward side of the flag (F) was processed. **c** Next, the perimeter of the flag and internal housing (IH) lid were sampled and then the flag was detached from the housing and placed face-down on R2A growth medium. **d** The remaining IH sites were processed to complete the processing procedure
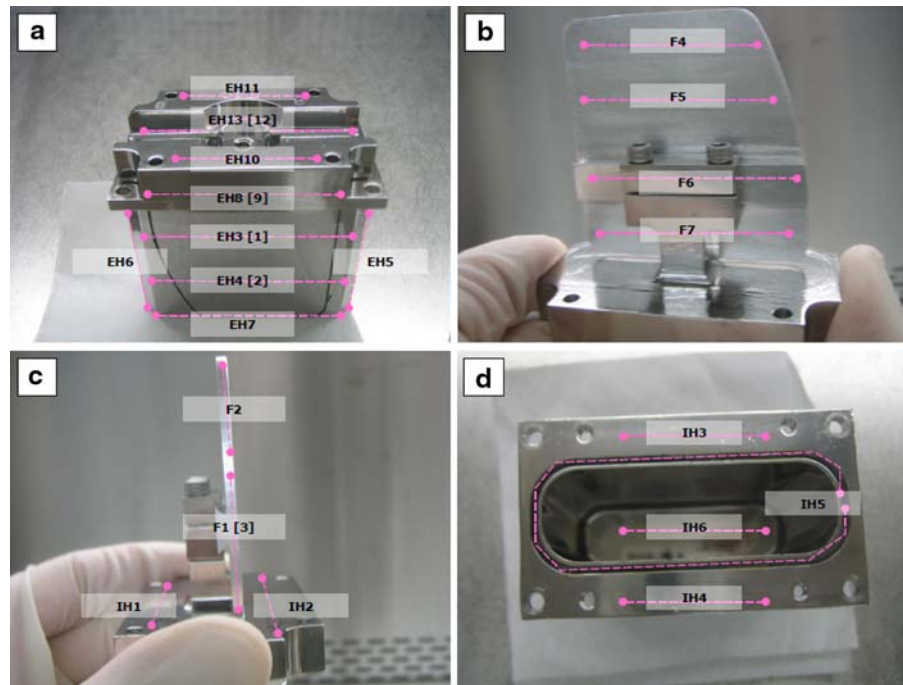
amplicons that were directly sequenced by North-woods DNA, Inc. (Becida, MN). GenBank Blast (http://www.ncbi.nlm.nih.gov/BLAST/) was utilized for sequence/isolate identification. Furthermore, three bacteria isolates were identified with a 466 bp 16S rRNA primer set described by Nadkarni et al. (2002) due to repeated sequencing errors noted with the larger primer set from Grasby et al. (2003). Table 1 provides the primer sets used for each bacterial isolate.

Fungal growth in liquid or solid media was immediately transferred to PDA and archived at 4°C for identification, noting growth rate and morphology for hyphae and fruiting bodies. After trying several primer sets in PCR, 18S rRNA for all fungal isolates were successfully amplified using primers NS3 and NS4 from White et al. (1990). Amplicons were directly sequenced and identified as described above with GenBank Blast.

3 Results

Bacteria were recovered from 6 of 27 possible locations on the flight hardware. The positive growth was from 4 F-sites and 2 EH-sites. Signs of growth in liquid media ranged from 24 to 96 h. Subsequently plated colonies

were small and medium sized, non-pigmented, and had either a smooth/round or sharp/jagged appearance. Analysis by 16S rRNA for F-bacteria showed strong homology with *Bacillus* sp. at the genus level, with most species matched to *Bacillus subtilis*. The EH cultures were recovered from the lid (EH11) and base (EH7) of the housing, on solid media from the swabbed locations. The EH7 colonies appeared to share morphological features with F-isolates, but sequence analysis revealed a relationship to *Bacillus endophyticus*. More surprising was the EH11 bacterium, which took nearly 3 weeks to recover on R2A and formed small colonies with red pigmentation. However, sequencing could only match the isolate to the genus-level of *Bacillus* sp. Interestingly, at least one (and, in some cases, many) of the closest GenBank neighbors were previously identified in China, Japan, Korea, or India (see Table 1).

Fungi were recovered from 8 of the 27 locations on the flight hardware: four isolates came from F-sites, while EH and IH-sites each yielded two isolates. When the flag was placed face down on R2A, it produced two adjacent fungal colonies near its perimeter after 72 h, which were subsequently removed to allow for additional microbe recovery. However, no additional growth was observed. Nor

Table 1 Bacterial growth

Site[a]	Recovery media	Time (h)	Colony description	Primers	% Homology (seq. length)	GenBank closest neighbor	Accession number	Other GenBank information
F1	TSB, R2A	24	Small, white, round	Grasby et al. (2003)	100 (560/560)	*Bacillus* sp. fj427970.1	FJ649341	Multiple *Bacillus* hits, primarily *subtilis*
	TSB, R2A	24	Small, white, jagged	Grasby et al. (2003)	99 (797/800)	*Bacillus* sp. eu168188.1	FJ649340	Matched isolate found in Chinese oil field
F3	TSB, R2A	72	Small, white, jagged	Grasby et al. (2003)	99 (559/560)	*Bacillus* sp. fj427970.1	FJ649337	Multiple *Bacillus* hits, primarily *subtilis*
F6	TSB, R2A	72	Medium, white, round	Grasby et al. (2003)	99 (560/561)	*Bacillus* sp. fj427970.1	FJ649339	Multiple *Bacillus* hits, primarily *subtilis*
	TSB, R2A	72	Small, white, round	Grasby et al. (2003)	99 (797/800)	*B. subtilis* dq846632.1	FJ649342	Matched isolate found in Korea
	TSB, R2A	72	Small, white, jagged	Nadkarni et al. (2002)	99 (407/408)	*B. subtilis* eu532192.1	FJ649343	Matched Indian isolate associated w/ jute plant
F7	TSB, R2A	72	Medium, white, round	Grasby et al. (2003)	99 (518/520)	*Bacillus* sp. FJ393325.1	FJ649338	Multiple *Bacillus* hits, primarily *subtilis*
	TSB, R2A	96	Medium, white, round	Nadkarni et al. (2002)	99 (420/423)	*Bacillus* sp. fj434652.1	FJ649344	Multiple *Bacillus* hits, primarily *subtilis*
	TSB, R2A	96	Small, white, jagged	Grasby et al. (2003)	99 (679/680)	*Bacillus* sp. FJ393325.1	FJ649345	Multiple *Bacillus* hits, primarily *subtilis*
EH7	R2A	72	Medium, white, round	Grasby et al. (2003)	98 (588/560)	*B. endophyticus* dq485415.1	FJ649336	Four same score hits with Chinese isolates
EH11	R2A	552	Very small, red, round	Nadkarni et al. (2002)	99 (407/409)	*Bacillus* sp. ab242649.1	FJ649346	Matched Chinese and Japanese isolates

[a] Cells not recovered from the following sites: flag face, F2, F4, F5, EH1, EH2, EH3, EH4, EH5, EH6, EH8, EH9, EH10, EH12, EH13, IH1, IH2, IH3, IH4, IH5, IH6

Table 2 Fungal growth

Site[a]	Recovery media	Time (h)	Growth description	Primers	% Homology (seq. length)	GenBank closest neighbor	Accession number	Other GenBank information
Flag face	R2A, PDA	72	Blue/green fruiting bodies, white mycelium	White et al. (1990)	99 (537/539)	*Penicillium* sp. EU827607.1	FJ649347	Closest 3 hits matched Chinese and Japanese isolates
F4	TSB, R2A, PDA	96	Blue/green fruiting bodies, white mycelium	White et al. (1990)	100 (200/200)	*Eurotiomycetes* sp. FJ458446.1	FJ649350	Matched numerous *Eurotiomycetes* sp.
F5	TSB, R2A, PDA	96	Blue/green fruiting bodies, white mycelium	White et al. (1990)	100 (489/489)	*Penicillium* sp. EU827607.1	FJ649353	Closest 3 hits matched Chinese and Japanese isolates
F6	R2A, PDA	552	Blue/green fruiting bodies, white mycelium	White et al. (1990)	100 (200/200)	*Eurotiomycetes* sp. FJ458446.1	FJ649348	Matched numerous *Eurotiomycetes* sp.
EH3	TSB, R2A, PDA	96	Blue/green fruiting bodies, white mycelium	White et al. (1990)	99 (497/500)	*Penicillium* sp. EU827607.1	FJ649352	Closest 3 hits matched Chinese and Japanese isolates
EH11	TSB, R2A, PDA	120	Blue/green fruiting bodies, white mycelium	White et al. (1990)	100 (486/486)	*Penicillium* sp. EU827607.1	FJ649354	Closest 3 hits matched Chinese and Japanese isolates
IH3	TSB, R2A, PDA	96	Blue/green fruiting bodies, white mycelium	White et al. (1990)	96 (185/192)	*Penicillium* sp. EU827607.1	FJ649351	Closest 3 hits matched Chinese and Japanese isolates
IH4	TSB, R2A, PDA	120	Blue/green fruiting bodies, white mycelium	White et al. (1990)	99 (537/538)	*Penicillium* sp. EU827607.1	FJ649349	Closest 3 hits matched Chinese and Japanese isolates

[a] Cells not recovered from the following sites: F1, F2, F3, F7, EH1, EH2, EH4, EH5, EH6, EH7, EH8, EH9, EH10, EH12, EH13

did growth occur when the flag was transferred onto fresh R2A plates at 2, 4, 7, and 56 day time-steps. Swab-based cultures recovered in liquid media showed first signs of growth between 96 and 120 h, while cells on solid media generated a wider range of growth time, 72–552 h. On both R2A and PDA media, fungi grew rapidly and were characterized by blue/green fruiting bodies and white mycelia. Sequences submitted to GenBank identified isolates F4 and F6 (located on the leeward side of the flag) to class Eurotiomycetes, using 200 bp sequence reads. The remaining six fungal isolates were matched to the same species of *Penicillium* sp., whose closest neighbors in GenBank originated from China or Japan (see Table 2). The positive growth at IH locations was close to the attachment point of the flag on the housing lid, but neither IH1 nor IH2 yielded fungi. Only one non-flag associated site, EH11, produced both bacterial and fungal cultures.

4 Discussion

Our study confirmed the presence of viable microbes in the stratosphere, supporting previous reports by Imshenetsky et al. (1978), Lysenko (1980), Harris et al. (2002), Wainwright et al. (2002), Narlikar et al. (2003), Griffin (2004, 2008), and Shivaji et al. (2006). However, our samples are the first documentation of microbiota recovered from a high-altitude location directly over the Pacific Ocean. Infrared satellite imagery from METEOSAT-5 showed air with an eastward movement across the ocean during the sampling time. Independently, the closest neighbors of many bacterial and fungal samples submitted to GenBank were Chinese, Japanese, Korean, and Indian-derived isolates; primarily *Bacillus* sp. and *B. subtilis* for bacterial species and *Penicillium* sp. and class Eurotiomycetes for fungi. The bacterial isolates reported in this study are all spore forming species and have been collected in prior stratospheric assays, at genus-level specificity (Imshenetsky et al. 1978; Wainwright et al. 2002; Narlikar et al. 2003; Griffin 2004). Although non-spore forming eubacterial species have been recovered from the stratosphere (Griffin 2008), sporulation likely enhances resistance to high-altitude stress (Riesenman and Nicholson 2000; Nicholson 2002).

The abundance of cells recovered in this study (12 out of 27 possible flight hardware sites) was surprising based on the previously described dilution effect on cells in the stratosphere (Griffin 2007). Either our extended horizontal range and modifications to the surface of the flag actually improved the collection efficiency, or the impactor collected spores that subsequently germinated and propagated inside the housing prior to recovery back at the laboratory. The spatial correlation of positive growth areas on the hardware would suggest the possibility of post-flight growth. While the recovery of bacteria and fungi was most abundant on flag-associated regions, the presence of *B. endophyticus*, *Bacillus* sp. and *Penicillium* sp. on the external housing must also be considered. This area was exposed to the atmosphere when attached to the aircraft wing, possibly allowing low-altitude contamination of the surface during (1) hardware installation/removal or (2) while the aircraft was on the ground or en route to 20 km altitude. It is interesting that the major bacterial outliers reported in this study—the sole representative of *B. endophyticus* and the only red-pigmented species of *Bacillus* sp.—both came from EH locations. Therefore, the highest confidence of stratosphere-sampled isolates should be reserved strictly for microbes recovered from the flag and IH locations (*Bacillus* sp., *B. subtilis*, and *Penicillium* sp.) since these areas were pressure-sealed inside the housing during the brief contamination windows discussed above. Nevertheless, ground contamination cannot explain the strong DNA sequence homology of microbes recovered in this study with previously described Asian isolates located on the other side of the Pacific Ocean. Moreover, if ground contamination was a factor one might expect an even greater diversity of species recovered, including human associated microbes.

While this study has provided evidence for a long-distance, stratospheric transport of microbes across the open ocean, it is important to emphasize that it does not indicate an independent, airborne microbial ecosystem. Rather, the viable bacteria and fungi reported herein were most likely associated with temporary, trans-Pacific dust events which last 7–10 days before most particles fall out of the atmosphere. While Narlikar et al. (2003), Shivaji et al. (2006) and Wainwright et al. (2006) use the discovery of non-culturable particulates and novel species as evidence of a distinct stratosphere ecology, no mechanism has

been presented to explain how microbes could remain aloft, growing or replicating. The possibility of a permanent, upper-atmosphere ecosystem seems unlikely based on several lines of evidence. First, the very low temperature of the sampling environment ($-75°C$) in this study was far below $-20°C$, the minimum temperature for microbial growth reported to date (Price and Sowers 2004; Junge et al. 2006). Although there is a temperature inversion with increasing altitude in the stratosphere (Ramaswamy et al. 2001), the extra warmth is gained due to absorption of solar radiation, including potentially biocidal UV wavelengths. Second, without a means of controlling altitude, airborne species should eventually (<5 years) fall out of the atmosphere, if aerosol extinction rates from the volcanic eruption of Mt. Pinatubo are used as a proxy (Bauman et al. 2003). Upper and lower atmosphere layers can mix according to Deshler et al. (1993), therefore how could microbes reported by Narlikar et al. (2003), Shivaji et al. (2006), and Wainwright et al. (2006) remain completely independent of terrestrial samples? The simplest way of explaining the unique stratospheric assemblages described by Narlikar et al. (2003), Shivaji et al. (2006), and Wainwright et al. (2006) is that these populations exist in terrestrial or aquatic ecosystems but have not yet been identified. However, in this study and others similar to it (Imshenetsky et al. 1978; Lysenko 1980; Harris et al. 2002; Narlikar et al. 2003; Griffin 2004, 2008), there was a strong genetic relationship between microorganisms recovered at high altitudes with those common in terrestrial or aquatic environments.

Significant improvements to the efficiency and accessibility of upper atmosphere sampling will be necessary to advance the study of high-altitude aerobiology. Hard surface impactors deployed by aircraft have demonstrated utility, but there is clearly a limit to their use. Assuming that a more robust collecting device can be engineered, special emphasis should be placed on culture-independent processing (Griffin et al. 2001; Griffin 2007) and measuring UV-induced DNA mutations (Wainwright et al. 2006). The ability to study high altitudes (up to 33 km) with sophisticated, long-duration science payloads may be available soon through ultra-long duration (up to 100 days) balloons developed by the United States Antarctic Program and NASA (Smith 2004; Gregory and Stepp 2004; National Science Foundation 2008). Measuring cells in situ

(Pratt et al. 2009) for weeks, or possibly longer, could resolve the residency time question addressed throughout this study. Ultimately, such information would strengthen our notion of the biosphere—the broad range of microorganisms within it and how the upper atmosphere may have influenced the diversity and distribution of life on Earth.

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