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Mini Review

Microbes in the Upper Atmosphere and Unique Opportunities for Astrobiology Research

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Abstract

Microbial taxa from every major biological lineage have been detected in Earth's upper atmosphere. The goal of this review is to communicate (1) relevant astrobiology questions that can be addressed with upper atmosphere microbiology studies and (2) available sampling methods for collecting microbes at extreme altitudes. Precipitation, mountain stations, airplanes, balloons, rockets, and satellites are all feasible routes for conducting aerobiology research. However, more efficient air samplers are needed, and contamination is also a pervasive problem in the field. Measuring microbial signatures without false positives in the upper atmosphere might contribute to sterilization and bioburden reduction methods for proposed astrobiology missions. Intriguingly, environmental conditions in the upper atmosphere resemble the surface conditions of Mars (extreme cold, hypobaria, desiccation, and irradiation). Whether terrestrial microbes are active in the upper atmosphere is an area of intense research interest. If, in fact, microbial metabolism, growth, or replication is achievable independent of Earth's surface, then the search for habitable zones on other worlds should be broadened to include atmospheres (*e.g.,* the high-altitude clouds of Venus). Furthermore, viable cells in the heavily irradiated upper atmosphere of Earth could help identify microbial genes or enzymes that bestow radiation resistance. Compelling astrobiology questions on the origin of life (if the atmosphere synthesized organic aerosols), evolution (if airborne transport influenced microbial mutation rates and speciation), and panspermia (outbound or inbound) are also testable in Earth's upper atmosphere. Key Words: Microbe—Aerobiology—Upper atmosphere—Stratosphere—Sampling. Astrobiology 13, 981–990.

1. Introduction: Airborne Microbes

Most of Earth's atmosphere is concentrated within the *boundary layer,* generally 0–2 km above the surface. Air is heavily mixed with the surface throughout the boundary layer and *troposphere* [spanning up to about 18 km above sea level (ASL)] because of weather systems and winds. The result of this convection is the lofting of common aerosols such as dust, smog, and smoke. Biological aerosols (*bioaerosols*) can also be swept up into the atmosphere, including individual and clumped microscopic cells (*e.g.,* bacteria and viruses) or reproductive components of multicellular life (*e.g.,* fungi and pollen). Most airborne biomass emanates from arid topsoil or marine sea spray (Kellogg and Griffin, 2006; Burrows *et al.,* 2009a; Frohlich-Nowoisky *et al.,* 2009; Womack *et al.,* 2010). Consequently, a rich variety of microbial archaea, bacteria, and eukaryotes have been isolated from the troposphere (Smith *et al.,* 2012, 2013). Landfills, wastewater treatment facilities, and farming also contribute significant quantities of human-generated bioaerosols (Burrows *et al.,* 2009b). Research combining the once disparate fields of climate science and microbial ecology has revealed that microbes actively influence cloud chemistry by serving as cloud and ice condensation nuclei (Bauer *et al.,* 2002; Vaïtilingom *et al.,* 2010, 2013; Després *et al.,* 2012; Šantl-Temkiv *et al.,* 2013a). Dispersal-specific adaptations and the viability of pathogens in the atmosphere are other topics of special interest in the recently reinvigorated field of *aerobiology* (Brown and Hovmøller, 2002; Griffin, 2007a; Toepfer *et al.,* 2011; Frazer, 2012; Polymenakou, 2012; Smith *et al.,* 2012; Ballester *et al.,* 2013; Smith *et al.,* 2013).

Before molecular methods in microbiology emerged, aerobiology studies relied upon culture-based analyses that presumably neglected the majority of microbes from air samples (Burrows *et al.,* 2009b; Womack *et al.,* 2010; Gandolfi *et al.,* 2013). The problems with culturing have been well documented from other environments and ecosystems, but the limitations are even more pronounced with air samples since microbes can be damaged or inactivated by desiccation and irradiation during atmospheric transport (Smith *et al.,*

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2011). Acquiring culture-independent microbiology data with atmospheric samples is not simple because the density of bioaerosols decreases with altitude (Fulton, 1966; Lighthart, 1997; Burrows *et al.,* 2009b; Yang *et al.,* 2009). Hospodsky *et al.* (2010) discussed the molecular detection limits for common bioaerosols. By processing large volumes of air, enough biomass can be collected to employ molecular assays, but air sampling systems must also be designed to prevent contamination. These experimental design and engineering challenges may explain why the upper atmosphere is among the least explored biological environments on Earth, rivaling deep oceanic and subsurface settings. The purpose of this review is to address the prevailing problems, propose solutions, and encourage additional microbiological exploration in Earth's upper atmosphere. First, sampling techniques enabling aseptic control and molecular methods are discussed in Section 2. Later, relevant astrobiology questions that can be uniquely examined in the upper atmosphere are outlined in Section 3.

2. How to Sample the Upper Atmosphere

Sampling methods for lower-altitude aerobiology investigations have been described elsewhere (Burrows *et al.,* 2009b; Yang *et al.,* 2009; Griffin *et al.,* 2010; Womack *et al.,* 2010; Gandolfi *et al.,* 2013). Therefore, this review focuses on techniques for collecting bioaerosols above the atmospheric boundary layer. While historically challenging, potential investigators should realize that a number of feasible options are available for conducting upper atmosphere microbiology studies.

2.1. Precipitation

Airborne microbes, viable or dead, can be efficient cloud and ice nuclei. Some bacteria have cell membrane proteins that raise the freezing point of water vapor in clouds, resulting in the selective growth of snow, hail, and graupel particles over other types of ice condensation nuclei (Govindarajan and Lindow, 1988; Šantl-Temkiv *et al.,* 2013a). Airborne microorganisms can also be passively entrapped when precipitation particles circulate inside a cloud, expand in size, and ultimately descend through the atmosphere. Thus, a simple and effective upper atmosphere sampling technique is gathering microbes returned to the surface by large hailstones (Christner *et al.,* 2008; Temkiv *et al.,* 2012; Šantl-Temkiv *et al.,* 2013a). The collection method is cheap, and there is practically no limit on sample size. But special precautions must be taken to minimize ground contamination. For instance, exterior layers of hailstones must be discarded due to contamination with the surface (Šantl-Temkiv *et al.,* 2013a), as well as any particles picked up during the descent through an entire column of lower tropospheric air. At most equatorial and subtropical locations, snow and hail have to be collected quickly before liquid melting can penetrate the samples. To reduce or eliminate the need for rapid collections, cores drilled through annual deposition layers in glaciers (Zhang *et al.,* 2007) and ice shelves (Priscu *et al.,* 2006) should contain a record of atmospheric microbes deposited by snowfall. However, surface conditions at the time of snowfall (*e.g.,* wind redistribution, erosion, sublimation, and melting) and subsequent metamorphism from burial could make it difficult to interpret aerobiology archived in ice.

2.2. Summit stations

Pioneering microbiologist Louis Pasteur (Pasteur, 1861) was the first to climb mountains to get above the boundary layer, and the approach is still useful today for microbial monitoring at high altitudes (Bowers *et al.,* 2009; Wiedinmyer *et al.,* 2009; Smith *et al.,* 2012, 2013; Vaïtilingom *et al.,* 2012). But the logistics of protecting and powering air sampling instrumentation at remote alpine settings can be difficult and dangerous, particularly in the wintertime. Thus, partnering with preexisting facilities at field stations like the Storm Peak Observatory, which is equipped to sample air quality (Bowers *et al.,* 2009), or the Mauna Kea Observatories that house telescopes (Mims and Mims, 2004) offer many obvious advantages. Air sampling infrastructure can be modified for aseptic collection and maintained by staff on-site. Moreover, pumps entrapping bioaerosols in filter membranes or liquid impingers can run continuously (Griffin *et al.,* 2010; Smith *et al.,* 2012), circumventing the problem of limited biomass that has traditionally prevented aerobiology studies from employing molecular methods. Furthermore, some field stations (*e.g.,* the Mt. Bachelor Observatory) already have atmospheric chemistry instruments that are useful for measuring meteorology and other aerosol species that can help explain the transport history of sampled microbes (Smith *et al.,* 2013). However, fixed field stations have limited spatial coverage, and certain sites may be vulnerable to the influence of adjacent topography or human activity, depending on the season and weather conditions (Weiss-Penzias *et al.,* 2006).

2.3. Aircraft

Researchers must leave the ground to target higher altitudes or specific air masses of interest. Unfortunately, aerosol collection instruments have not changed substantially since the earliest aerobiology investigations in which aircraft were used (see Timmons *et al.,* 1966; Yang *et al.,* 2008a). The fundamental problem is that instruments need to process high volumes of air without disrupting vehicle aerodynamics. A related issue is that if the air sampling device is located downwind of the fuselage or other major aircraft components, collected cells could be sloughed off from component surfaces instead of being native to the atmosphere. Previous studies have utilized wingtip-mounted air samplers to reduce the possibility of in-flight contamination (Griffin, 2004, 2007b; Yang *et al.,* 2008a; Smith *et al.,* 2010). If air is pumped into the aircraft fuselage, additional measures must be taken to clean sampling inlets and demonstrate that samples have been collected *in situ,* as argued by Smith and Griffin (2013) in response to results reported by Deleon-Rodriguez *et al.* (2013). Provided that robust sterilization protocols can be developed, establishing partnerships with the commercial airline industry may be a cost-effective way of collecting bioaerosol samples from the upper troposphere and lower stratosphere. Civil Aircraft for the Regular Investigation of the atmosphere Based on an Instrument Container (CARIBIC) and Lufthansa already have a robust atmospheric aerosol program in place (Feder, 2008). Unmanned aerial vehicles offer additional possibilities for atmospheric sampling (*e.g.,* Schmale *et al.,* 2012), particularly as lightweight, solar-powered aircraft enable extended spatial coverage and time aloft.

2.4. Balloons

Scientific balloons are made of a thin polyethylene film inflated with helium and can carry atmospheric sampling instruments on a gondola suspended underneath the balloon that eventually is returned to the surface on a parachute. For stratospheric flights between 30 and 40 km ASL, balloons typically reach the float altitude 2–3 h after launch and travel in the direction of the prevailing winds. Sampling time aloft depends on the total flight system weight, target altitude, launch site, and weather conditions. Previous stratospheric microbiology balloon missions have sampled air with battery-powered pumps connected to filter membranes (Yang *et al.*, 2008b) or by using cryogenic fluids to move air through a manifold of hollow steel tubing (Narlikar *et al.*, 2003; Wainwright *et al.*, 2003; Shivaji *et al.*, 2006, 2009; Rauf *et al.*, 2010). Each method is inefficient due to the limited volume of air that can be screened, highlighting the problem every aerobiology balloon mission must address: how to process large volumes of thin atmosphere without consuming large amounts of power or fuel. Even if a highly efficient pump could be engineered, the heat generated by the sampling system must be dissipated before affecting other flight hardware systems. Interestingly, advances in ultra-long-duration ballooning allowed a recent mission to remain aloft over 55 days at 39 km ASL (Ward, 2013). So while it may never be possible to produce an efficient pump for stratospheric balloon operations, flying an ordinary, low-volume pump on an ultra-long-duration mission could still collect unprecedented amounts of upper atmospheric biomass.

2.5. Rockets

Unlike other sampling options, rockets can reach any desired altitude in the atmosphere. Collection devices located in the upper stage can be separated from the rocket and capture air samples during ascent or descent. Only one team has ever reported collecting airborne microbes with rocketry, and the results are ambiguous (discussed in Section 3.1). In brief, the sampling system flown by Imshenetsky *et al.* (1978) consisted of a sticky polyethyleneteriphtolate film ejected from the rocket nose cone after reaching the mesosphere. Particles impacted the sticky side of the film, which was wound around a rotating roll that was sealed before descending on a parachute. A modern rocket sampler might use aerogel or some other semisolid matrix for entrapping microbes. As the commercial spaceflight industry matures, suborbital flight opportunities and access to the mesosphere should become easier and cheaper than they have been in the past (Stern, 2013). Nevertheless, rocket flights are short-lived (meaning less time for sampling air); and unless sampling devices are designed with separate chambers, it will be difficult to determine at what height particles were collected.

2.6. Remote sensing

Satellite observations feeding into a global circulation model (*e.g.*, Yu *et al.*, 2012) would be a comprehensive way to measure airborne life if bioaerosols were in dense enough concentrations to give a sharp spectral signature (Simmer and Volz, 1993). Bioaerosol distribution has been modeled by using ground and air measurements (Burrows *et al.*, 2009a), but no direct measurements of airborne microbial assemblages have been made from orbit. Remote sensing may be feasible by proxy observations since microbes are typically co-transported with other, more densely concentrated aerosols (Creamean *et al.*, 2013; Smith *et al.*, 2013). Thus, future aerobiology studies could examine existing ground/airborne datasets from a variety of field sites to look for regional correlations between microbes and co-transported aerosols in order to establish the most reliable proxy to measure from orbit. Candidate aerosols might include dust, sulfate, sea spray, and smoke, but more work is needed in this undeveloped topic in aerobiology.

3. Aerobiology Questions with Astrobiological Relevance

The biosphere boundary, habitable atmospheres, extremophiles, rapid evolution, intra- and interplanetary dispersal, contamination control, and planetary protection are just some of the broader astrobiology topics that can be examined at high altitudes. Each subsection below is meant to introduce guiding astrobiology questions and motivate future microbiology investigations in Earth's upper atmosphere.

3.1. Boundary of Earth's biosphere

Viable microorganisms have been found 2.7 km underground in Earth's mantle, and the extent of subsurface life may be even deeper still (Onstott *et al.*, 2009). Just like the search for life below the surface, the exploration of the upper atmosphere can help constrain the search for potentially habitable environments on other worlds. Microbes reach the upper atmosphere by strong upward winds (Wainwright *et al.*, 2006; Smith *et al.*, 2011); thunderstorms (Dehel *et al.*, 2008), hurricanes (Deleon-Rodriguez *et al.*, 2013), monsoons (Randel *et al.*, 2010), deep convection in the tropics (Holton *et al.*, 1995), electrostatic levitation (Rohatschek, 1996), and volcanic eruptions (Van Eaton *et al.*, 2013) are some of the mechanisms for pushing microbes to high altitudes (Fig. 1). Rocket and balloon launch activities also contribute to the number of microorganisms at extreme heights, but the amount may be insignificant compared to what global airplane traffic deposits. The US airline industry, alone, sends about 800,000 domestic and international commercial flights into the upper atmosphere every month (BTS, 2013), with each airplane shedding innumerable microbes. Similarly, aerosol exchange in the tropopause is increasing due to climate change (Randel and Jensen, 2013), suggesting a greater number of microorganisms will cross between the troposphere and stratosphere in the coming years.

Both the natural boundary of the biosphere and the artificial boundary generated by human activities are open questions in aerobiology because missions to the stratosphere (~18 to 50 km ASL) and mesosphere (~50 to 85 km ASL) are so infrequent (Table 1). The traditionally cited high mark for aerobiology is 77 km ASL (*e.g.*, Horneck *et al.*, 2010) based on a single rocket flight to the mesosphere by Imshenetsky *et al.* (1978). However, Imshenetsky *et al.* (1978) did not describe in any detail how their system was sterilized or prevented contamination beyond the unmeasured assertion that heat of friction during ascent should have sterilized portions of the nose cone. A subsequent mission was never

FIG. 1. Upper atmospheric, transatlantic transport of microorganisms in African desert dust plumes, visible as purple streaks by refracting sunlight on IODP Expedition 336 over the mid-Atlantic ridge at ~22°N, 46°W (Griffin, 2012). Thunderstorm convection is visible on the horizon, one of the many mechanisms responsible for lofting dust and terrestrial microbes to high altitudes. After reaching high altitudes, microbes can disperse globally and provide unique opportunities for astrobiology investigations (photo credit: D.W. Griffin, October 2011). Color images available online at www.liebertonline.com/ast

flown, and the simplest explanation for the spores collected in the mesosphere is that the microbes originated from the rocket itself or from soil contamination after the system impacted the desert regions of the Kazakhstan Republic. In fact, no mission to the stratosphere or above has flown consecutive flights (Yang *et al.*, 2009). Based on contamination control issues and the absence of reproducible data, results from these high-altitude missions must be conservatively interpreted. Even if previous extreme-altitude missions did collect microbes at altitude, the very small volumes of air sampled and reliance upon culture-based recovery methods would have provided an incomplete picture of actual microbial abundance and richness.

Detecting microbial signatures without false positives is a critical feature shared by upper atmosphere aerobiology and upcoming astrobiology missions to Mars. In either context, an unacceptable outcome is the inability to discriminate between *in situ* microorganisms and terrestrial contaminants hitchhiking on flight hardware. The possibility that microbes collected from the upper atmosphere are derived from sampling platforms rather than native, free-floating cells can only be resolved if stricter control measures are implemented. For instance, as a positive control fluorescent beads could be sputter-coated onto all flight systems to trace and identify contamination pathways on flight hardware (Juanes-Vallejo *et al.*, 2011). Identical sampling systems that are flown but not exposed to the upper atmosphere would serve as a convincing negative control. Future missions to the upper atmosphere could also employ multiple layers of containment along with in-flight sterilization measures to reduce bioburden (*e.g.*, dry heat microbial reduction, vapor phase hydrogen peroxide, or UV irradiation). All these approaches could potentially contribute to new methods for preventing the forward contamination of pristine worlds by spacecraft. Contamination control and life-detection technology readiness can be advanced with stratospheric microbiology missions where the rarified upper atmosphere imposes similar operational parameters on flight hardware that has to function autonomously. Other commonly used

Table 1. Overview of Stratospheric Microbiology Missions

Reference	*Top altitude (km)*	*Platform*	*Country*
Rogers and Meier, 1936	21	Balloon	USA
Soffen, 1965	41	Balloon	USA
Greene *et al.*, 1965	27	Balloon	USA
Imshenetsky *et al.*, 1978	77	Rocket	USSR
Narlikar *et al.*, 2003	41	Balloon	UK
Wainwright *et al.*, 2003	41	Balloon	UK
Griffin, 2004	20	Aircraft	USA
Shivaji *et al.*, 2006	41	Balloon	India
Griffin, 2007b	20	Aircraft	USA
Yang *et al.*, 2008b	35	Balloon	Japan
Shivaji *et al.*, 2009	41	Balloon	India
Smith *et al.*, 2010	20	Aircraft	USA
Rauf *et al.*, 2010	41	Balloon	UK

Mars analogues such as the Antarctic Dry Valleys reduce the need for instrument autonomy and, more critically, lack defining features of the martian environment, including hypobaria and extreme UV irradiation (*e.g.,* Smith *et al.,* 2011).

3.2. The atmosphere as an ecosystem

Comparisons between microbes in Earth's atmosphere (*aeroplankton*) and microbes in Earth's oceans (*phytoplankton*) are appealing. Chemically, both environments have an abundance of energy, carbon, and water; physically, both have prevailing currents and eddies that respond to location, topography, and season. Recent publications in aerobiology allude to the atmosphere as an ecosystem or habitat, but the debate is far from settled, and *in situ* measurements of microbial activity are needed (Womack *et al.,* 2010; Clarke *et al.,* 2013; Deleon-Rodriguez *et al.,* 2013; Diehl, 2013; Šantl-Temkiv *et al.,* 2013b; Smith *et al.,* 2013; Vaïtilingom *et al.,* 2013). The astrobiology connection follows simple logic: If the sky is more than just a transient environment, that is, microbes are actively metabolizing, growing, or reproducing at altitude, then the search for habitats beyond Earth must be broadened to include planetary atmospheres (see Section 3.3).

Inevitable fallout and environmental extremes are two major barriers to classifying the atmosphere as an ecosystem. Atmospheric aerosol lifetimes are referred to as *residence time.* Residence time is usually on the order of days for particles >1 μm but can be substantially longer for submicron particles at higher altitudes (Deshler *et al.,* 1993). However, longer time aloft increases the impact of environmental stress on airborne species, including cellular degradation from desiccation and irradiation. Accordingly, one hypothesis is that aeroplankton have evolved adaptations for returning quickly to the surface, particularly non-spore-forming species less resistant to harsh environmental conditions. It seems reasonable that cell membranes that promote cloud and ice condensation (leading to faster fallout) would be selected for after billions of years of terrestrial and marine microbes periodically drifting through the deadly atmosphere. A related, but alternative, hypothesis is that some aeroplankton have evolved cloud condensation adaptations not for accelerating deposition but for scavenging water and trace nutrients to metabolize while aloft. The profusion of cloud-borne microbial species capable of utilizing organic aerosols may be the first evidence of this lifestyle (Polymenakou, 2012; Deleon-Rodriguez *et al.,* 2013; Šantl-Temkiv *et al.,* 2013b; Vaïtilingom *et al.,* 2013). However, the alternative hypothesis does not account for how intracellular ice formation and high UV irradiation would severely constrain microbial activity in clouds. While some clouds can remain within the temperature realm of some psychrophilic bacteria (Clarke *et al.,* 2013), and it is possible that cloud opacity buffers biocidal irradiation, cellular metabolism, growth, or division rates would not make meaningful progression before most clouds dissipate. In fact, the average lifetime of convective systems is only about 10 h, and the average lifetime of cirrus clouds is about 18–24 h (Machado *et al.,* 1998). It is therefore difficult to imagine how clouds might be microbial oases for metabolically slow bacteria, considering some psychrophilic taxa take months per cell division (Mykytczuk *et al.,* 2013). Brief spurts of cellular activity may be plausible in warmer, long-lived clouds at lower altitudes, and a few teams have inferred airborne activity by measuring short-lived transcripts or metabolic by-products using ground simulation chambers (Amato *et al.,* 2007; Šantl-Temkiv *et al.,* 2013b; Vaïtilingom *et al.,* 2013). Ultimately, *in situ* measurements will be needed to help settle the debate about airborne microbial activity and whether the atmosphere is an ecosystem.

Submicron microbes pushed past the upper troposphere (as discussed in Section 3.1) can circumvent the residence time problem since stratospheric aerosols sometimes remain aloft for years (Deshler *et al.,* 1993). However, the stratosphere is extremely dry, irradiated, and depending on the location temperatures can drop down to about -70°C (Dao *et al.,* 1995; Smith *et al.,* 2011), which would be far too low for potential cellular growth or metabolism (Clarke *et al.,* 2013; Mykytczuk *et al.,* 2013). Moreover, O_3 generated by the photolysis of atmospheric O_2 might inhibit microbial activity in the stratosphere (Komanapalli and Lau, 1998).

3.3. Intra- and interplanetary dispersal

One of the simplest ways of explaining the cosmopolitan distribution of many microbial species on Earth is atmospheric transport (O'Malley, 2008). While it was previously understood that dust and particles larger than microbes could be transported vast distances in the atmosphere (*e.g.,* Betzer *et al.,* 1988), Brown and Hovmøller (2002) and Griffin (2002) were among the first to suggest that trade winds could carry viable microorganisms intercontinental distances. Recently, the hypothesis has been validated at independent sampling locations (Gorbushina *et al.,* 2007; Toepfer *et al.,* 2011; Favet *et al.,* 2012; Griffin, 2012; Hara and Zhang, 2012; Smith *et al.,* 2012; Yamaguchi *et al.,* 2012; Creamean *et al.,* 2013; Smith *et al.,* 2013). Atmospheric bridges also might help explain how life on Earth quickly "reboots" and recolonizes after major impact events, volcanic eruptions, or Snowball Earth episodes.

Panspermia is a controversial topic in astrobiology because the theory has been limited to ground collections and simulation studies (Nicholson, 2009), but life dispersing between planets is actually testable at the outer edge of Earth's atmosphere (Yang *et al.,* 2009). The topic has been supported by JAXA through a mission called Tanpopo that used an aerogel collector mounted on the exterior of the International Space Station orbiting at about 370 km to capture any outbound terrestrial microbes traveling through the thermosphere (Yamagishi *et al.,* 2008). However, to date, no detectable microorganisms have been captured by the mission (Tabata *et al.,* 2011). The most likely reason is that airborne cells cannot reach escape velocities necessary for breaking free of Earth's gravitational influence and that only impact events and spacecraft are capable of transporting terrestrial microbes outward into space (Wells, 2003; Nicholson, 2009; Nicholson *et al.,* 2009).

Inbound panspermia is another intriguing, testable topic in the upper atmosphere. If microbes were transported to Earth from other worlds or solar systems, the flux should be ongoing and measurable. Although there is still no convincing explanation for how microbes could endure extended transport time and lethal cosmic radiation levels in deep space, the roughly 40,000 tons of cosmic dust and micrometeoroids deposited into the upper atmosphere each year provide enough sample material to help settle the

debate (Brownlee, 2001). Cosmic dust and micrometeoroids arrive in various sizes and at various speeds and angles, but in general, particles $< 5\ \mu$m tend to survive entry and remain aloft in the stratosphere where they can be collected with stratospheric balloons or aircraft (Brownlee *et al.,* 1973; Brownlee, 2001). Wainwright *et al.* (2006) and Rauf *et al.* (2010) already claimed evidence of lithopanspermia through stratospheric cell-like structures analyzed by electron microscopy and UV spectroscopy. However, rudimentary microbiology methods were neglected in these studies, and morphology is a meager way of inferring the biological nature of samples. Simple molecular stains would have resolved any uncertainty about the nature of the microstructures. Even if the features observed by Wainwright *et al.* (2006) and Rauf *et al.* (2010) were in fact biological, there was no way of deciphering whether the cells were terrestrial contaminants from ground processing, flight instruments, or communication with the surface (as discussed in Section 3.1). Follow-up aerobiology investigations that collect and analyze cosmic dust for signatures of life will need to incorporate more sophisticated molecular assays, better contamination control measures, and focus on pristine particle interiors or samples collected prior to atmospheric entry in order to discriminate between native and alien cells (*e.g.,* Tabata *et al.,* 2011).

3.4. Life in other planetary atmospheres

After the origin of life on Earth, it is conceivable that terrestrial microorganisms were deposited by meteoritic panspermia into other planetary atmospheres and persisted. The atmosphere of Venus, for example, contains trace amounts of H_2O vapor and a variety of ingredients for theoretically powering redox reactions (Schulze-Makuch and Irwin, 2002; Konesky, 2009; Irwin and Schulze-Makuch, 2011). Along vertical gradients, temperatures and pressures are well within the known tolerances of terrestrial life (Clarke *et al.,* 2013; Harrison *et al.,* 2013), and the substantial density of the atmosphere would also enable longer particle residence times (James *et al.,* 1997). Schulze-Makuch *et al.* (2004) hypothesized that acidophilic microbes in venusian clouds (roughly 48 to 65 km above the surface) could use sulfur allotropes as photoprotective pigments for photosynthesis. Interestingly, an unknown UV absorber was observed in the lower cloud layer of Venus, but molecules indicative of life were not detected in previous flyby missions (Plummer, 1969). Still, Schulze-Makuch *et al.* (2004) argued that aeroplankton in the atmosphere would incorporate elemental sulfur and therefore mask hydrocarbon signatures. New information about terrestrial aeroplankton and unresolved atmospheric observations are compelling reasons for returning to explore Earth's nearest neighbor (Dorrington, 2010) or at least reconsider its planetary protection categorization. Venus is currently classified at the lowest level of planetary protection, designated as a Category 1 uninhabitable target by COSPAR (Nicholson *et al.,* 2009).

In the atmospheres of Titan and the distant ice giants Uranus and Neptune, extreme cold and inadequate photosynthetically active radiation make the propagation of terrestrial microbes deposited by meteors seem unlikely. Similarly, strong atmospheric convection in the gas giants Jupiter and Saturn would eventually destroy terrestrial microbes cycled between layers of temperature extremes (Vasavada and Showman, 2005). Sagan and Salpeter (1976) suggested that larger life-forms might control buoyancy but multicellularity and complexity could never evolve if ancestral microbes were continuously destroyed by atmospheric cycling processes. Although the gas and ice giants seem poor candidates for hosting atmospheric life, perhaps some terrestrial exoplanets have atmospheres so densely concentrated with aeroplankton that a direct biosignature spectrum would be detectable during host star transits.

3.5. Environment for rapid evolution of molecules and microbes

Due to extreme environmental conditions in the upper atmosphere (Horneck *et al.,* 2010; Smith *et al.,* 2011), the evolutionary history of life on Earth may have been influenced by airborne transport. Dobson *et al.* (2000) hypothesized that organic aerosols may have been essential prebiotic chemical reactors on early Earth. Once microbial life appeared, time aloft in the atmosphere could have accelerated natural selection and speciation by imposing a unique combination of stresses on microbes (*e.g.,* ultralow pressure, ultrahigh irradiation, desiccation, and severe oxidation) that was outside the range of conditions normally encountered on Earth's surface. By using extant species, it is worth investigating whether the independent or combined effect of these stressors can provoke novel types of cellular damage, response, or mutation, and if so measuring the ecological consequences when transformed cells return to the surface and germinate. Even cells killed by atmospheric transport might still affect distant gene pools by depositing viruses (*e.g.,* prophage) in distant environments (Gonzalez-Martin *et al.,* 2012). To assess the genomic and transcriptomic response of cells transported at high altitude and potential impacts on microbial evolution, future aerobiology studies could be modeled after exposure experiments recently conducted outside the International Space Station (Horneck *et al.,* 2012; Moeller *et al.,* 2012; Nicholson *et al.,* 2012).

Rapid selection in the upper atmosphere, due mostly to UV irradiation, might impose a bottleneck on airborne populations and play a substantial role in the distribution and composition of common microbial species (Smith *et al.,* 2011). Cell pigmentation, UV protection, DNA repair pathways, and the ability to form spores are just a few examples of what might be considered atmospheric specialization uncovered by recent surveys documenting taxa capable of surviving the rigors of high-altitude transport (Smith *et al.,* 2013). Understanding the identity and survival mechanisms of hardy microbes in the upper atmosphere might enhance our search for life in the Solar System and also contribute to planetary protection policies for missions, since post-landing UV may not kill all terrestrial microbes on the exterior of spacecraft (Schuerger *et al.,* 2013). The upper atmosphere is one of the most UV-intense environments on Earth and may also be a valuable location for gene mining and the isolation of enzymes that guard or restore radiation-damaged biomolecules (Ferrer *et al.,* 2007; Tiquia and Mormile, 2010). When the boiling hot springs of Yellowstone National Park were explored, a thermostable enzyme was isolated from *Thermus aquaticus,* which led to the prolific use of the polymerase chain reaction (PCR) across many biological sciences.

Similarly, efforts to recover novel, viable species from the upper atmosphere might yield immeasurable benefits for radiation biology and translational medical research fields.

4. Conclusions

Maybe the sky is *not* the limit, after all. Recent studies have indicated that the upper atmosphere is teeming with microorganisms, and a vast region of the sky is awaiting further biological exploration. It is not difficult to imagine astrobiologists partnering and sharing resources with other stakeholders interested in examining the effects of bioaerosols on global weather, climate, and disease. Astrobiology experiments in space can be expensive and infrequent, but many of the fundamental questions about life in the universe can be tested right here in our own atmosphere, through a variety of sampling platforms. Engineering challenges have long been the barrier to upper atmosphere microbiology research. The hope is that new air sampling technologies will soon facilitate *in situ* analyses and improved contamination countermeasures. While it may be impractical or impossible to fly contaminant-free hardware, some of the containment systems and controls developed for exploring Earth's upper atmosphere could inform Solar System astrobiology missions in the century ahead. At minimum, studying life in the upper atmosphere promises to challenge the traditional concept of the biosphere boundary and provide new insight about the diversity, distribution, and evolution of life on our planet.

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Abbreviation

ASL, above sea level.

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