

Assessing Panspermia Hypothesis by Microorganisms Collected from The High Altitude Atmosphere

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Abstract

Microbiology at the high altitude atmosphere is important for assessing the chances and limits of microbial transfer from the earth to extraterrestrial bodies. Among the microorganisms isolated from the high-atmospheric samples, spore formers and vegetative *Deinococci* were highly resistant against harsh environment at high altitude. From limited knowledge available to date, it is suggested that terrestrial microorganisms may have had chances to be ejected and transferred to outer space. Survival of these organisms during their space travel and proliferation on other planets might be also feasible. Directed Panspermia from Earth to extraterrestrial bodies is discussed on the basis of findings reported in literatures. ©2009 Jpn. Soc. Biol. Sci. Space; Article ID: 092303017

Introduction

Great attentions have been paid to the questions 'How did life on Earth originate?' and 'Is life on Earth alone in the universe?'. Many efforts have been made to answer to these questions. Abiogenesis (also known as spontaneous generation) hypotheses such as the Miller-Urey Experiment (Miller, 1953), protenoid (Fox, 1973), and the RNA World (Gilbert, 1986), have been proposed and widely discussed with many findings and observations. The panspermia hypothesis is another hypothesis for the origin of the life on Earth. The panspermia hypothesis was initially proposed by Arrhenius (1903). It argues that terrestrial life was seeded by the extraterrestrial life forms after traveling by radiation pressure (Weber and Greenberg, 1985) or meteorites (Melosh, 1988). Directed panspermia could be either from outer space to Earth (Crick and Orgel, 1973; Hoyle and Wickramasinghe, 1979) or from Earth to seed extraterrestrial bodies (Mautner and Matloff, 1979).

Verification of the panspermia hypothesis had been difficult due to the limit of human ability to investigate the extraterrestrial space during the earlier years. However, in the past several decades, space technology has allowed us to deploy probes into outer space beyond Mars. Recent exploration of the polar region of Mars (Titus *et al.*, 2003; Bandfield, 2007; Kerr, 2008) indicates that at least water, an essential requirement for life, existed on Mars. It should be noted that space probes may carry terrestrial microbes even strict sterilization procedures will be taken, and that some microbes may survive on extraterrestrial bodies through spacecraft crash on them (La Duc *et al.*, 2004; McLean *et al.*, 2006). There is a risk of spreading terrestrial microbes to the extraterrestrial world at conducting human activities. It is very likely that directed panspermia from Earth to other planets has been going on since the beginning of our space exploration.

Another means for terrestrial microbes to travel outer space are by natural carriers such as meteorites (Mileikowsky *et al.*, 2000), dust, or even microbes alone. Such natural transfer could have been made since billions of years ago when microbes were widely distributed on Earth. Microbes could have reached other bodies in our solar system and even extrasolar systems. It is also possible that some of the transferred microbes remain alive or dormant on extraterrestrial bodies under favorable conditions for their preservation (Yang *et al.*, 2009c).

Terrestrial microbes resulting from such transfer would pass through Earth's atmosphere before reaching outer space. The gravitational force decreases at higher altitude. Presumably, the higher the altitude that microbes can reach, the higher the possibility they escape into outer space. Except the input by human activities and rare events of large meteorite impact, a possible mechanism to steadily eject microbes from high altitude to outer space has been suggested. The forces of Earth's electric fields on charged microbes at high altitudes are at times strong enough to counteract the gravitational force on the microbes and uplift them into near-earth space (Dehel, 2006). Also, the high altitude atmosphere (HAA) resembles outer space in some physical conditions (desiccation, low pressure, low temperature and high levels of radiation). Therefore, information on microbes in the HAA is important for assessing the chances and limits of directed panspermia of terrestrial life. Here we review microbiological studies in the HAA for providing valuable information on the panspermia hypothesis. Reviews on the microbes in the HAA associated with disease spread and cloud nuclei formation could be found elsewhere (Lundheim, 2002; Kellogg and Griffin, 2006; Griffin, 2007).

Microbes in the HAA

There is no common definition of high altitude. High altitude is defined to begin at 1.5 km above sea level by the International Society of Mountain Medicine. Altitude illness, however, rarely occurs below 2.5 km. In this article, we define high altitude to begin at 2.5 km above

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sea level, except as specifically clarified. The upper boundary of the earth's atmosphere is where outer space starts, but there is no clear definition of outer space either. The most common definition is that outer space starts at the Kármán line, which is 100 km above the surface of Earth (Becerra Ortiz, 2008). 99.99997 % of the atmosphere by mass is below 100 km. By such definitions, the HAA starts from the upper troposphere, extends to the stratosphere and the mesosphere, and fades into space from the thermosphere (Fig. 1).

Transport mechanisms of microbes to the HAA

It is certain that there are microbes in the HAA. Earth's surface atmosphere contains various microbiota such as viruses, bacteria, algae, fungi and protozoa from a variety of sources (Brown *et al.*, 1964; McGovern *et al.*, 1965; Schlichting, 1969; Broady, 1979; Pósfai *et al.*, 2003). The microbiota in the low altitude atmosphere, within troposphere, can get upward transportation via vertical atmospheric convection. Radar studies showed that some birds such as passerines fly at heights up to 3 km and wader up to 7 km during long migration flights (Bruderer, 1997). It is very likely that birds traveling at high altitude carry microbes and release them at that height. Since the late 1800's and early 1900's, human activities started to contribute the distribution of microbes into the HAA by balloons, aircrafts and rockets. Human skydivers might have also brought microbes up to the middle stratosphere (McCrary, 2004). Other mechanisms of transporting microbes into the HAA include wind storms (Kellogg and Griffin, 2006), volcanic ejection (Robock, 2002; Antuña *et al.*, 2003; Oman *et al.*, 2005) and meteorite impacts (Alvarez *et al.*, 1980). Simulation study showed that, electric fields found during thunderstorms could accelerate charged micro-sized particles rapidly up to the upper stratosphere against the forces of drag of neutral atmosphere and gravity (Dehel *et al.*, 2008).

Microbes in the HAA

The presence of microbes in the HAA has been known for more than a century. However, the HAA remains a poorly explored area of microbiology. There is only fragmentary information about microbial populations in the HAA. A few samples have been collected in the HAA and the microbes in these samples were studied (Table 1). Fungi and pigmented endospore-forming bacteria from the high atmosphere were first reported in Europe (Cristiani, 1893; Harz, 1904). Later studies in the USA, Soviet Union, and India reported the recovery of fungi, and endospore-forming bacteria such as *Bacillus* (Rogers and Meier, 1936; Soffen, 1965; Bruch, 1967; Wainwright *et al.*, 2003; Griffin, 2005), and non-spore-forming *Micrococci* and *Mycobacterium* (Fulton, 1966; Bruch, 1967; Imshenetsky *et al.*, 1978; Griffin, 2008). We isolated *Deinococci* and endospore-forming bacteria from samples collected in the upper troposphere and stratosphere in Japan (Yang *et al.*, 2008a, 2008b). Algae, viruses and protozoans have not been reported to date. Spore-forming bacteria and fungi were the predominant species of reported HAA isolates. The predominant non-spore-forming isolates reported were *Micrococci*, a group

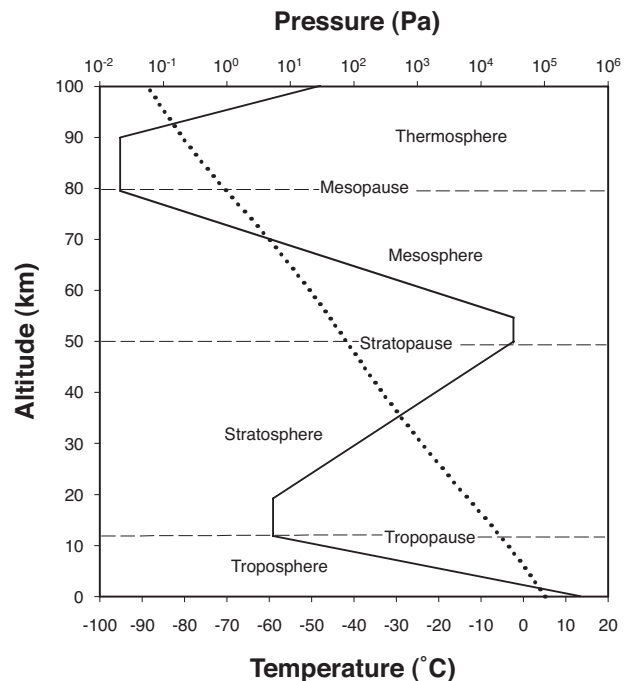


Fig. 1. Layers of the Earth's atmosphere. Changes in the average global atmospheric temperature (solid lines, simplified) and pressure (dotted line) with altitude are indicated.

to which the *Deinococci* previously belonged (Murray, 1992).

Due to their high resistance to desiccation and radiation, *Bacillus* spores and *Deinococcus radiodurans* cells have been frequently used in ground-based space research (Dose and Gill, 1995; Dose *et al.*, 1996, 2001; Dose and Klein, 1996; Mancinelli and Klovstad, 2000; Horneck *et al.*, 2001; Saffary *et al.*, 2002; Fajardo-Cavazos *et al.*, 2005; Nicholson and Schuerger, 2005; Diaz and Schulze-Makuch, 2006; Tauscher *et al.*, 2006; de la Vega *et al.*, 2007; Stöffler *et al.*, 2007; Osman *et al.*, 2008) or exposure experiments in near-earth orbits (Horneck *et al.*, 1995; Reitz *et al.*, 1995; Brack *et al.*, 2001; Rettberg *et al.*, 2002a; Rabbow *et al.*, 2003; Sancho *et al.*, 2007). Since the microbes isolated from the HAA had high chance to escape from the earth and survive under outer space environment, they can be representatives of terrestrial microbes for panspermia. In particular, *Bacillus* and *Deinococcus* isolates from the HAA that exhibited high levels of radiation- and desiccation resistance (Shivaji *et al.*, 2006; Yang *et al.*, 2008b, 2009a, 2009b) would be good candidates for future tests of interplanetary transfer of life.

Microbial concentrations in the HAA

It is generally thought that population density of microbiota decreases with increasing altitude in the troposphere. However, the height dependence of microbial population density has not well been studied. From the available data on captured microbes (Table 1), we estimated the microbial density by the altitude at ambient temperature and pressure (ATP). The microbial

Table 1 Microbiological studies in the high atmosphere.

References	Mission	Sampling method	Altitude (km)	Microbial collection (cultivated bacteria and fungi)	Total colony number	Sampled air volume ^{ATP} (m ³)	Sampled air volume ^{STP} (m ³)	Density ^{ATP} (microorganisms per m ³)	Density ^{STP} (microorganisms per m ³)
Rogers and Meier, 1936	Balloon	Sterile cylindrical sampling device descending by parachute	11-21	<i>Bacillus</i> sp., <i>Microsporium</i> sp., <i>Rhizopus</i> sp., <i>Penicillium</i> sp. and <i>Aspergillus</i> sp.	10	66	8.45	0.152	1.17
Soffen, 1965	Balloon	Drawing air through filter papers by a motor blower	38-41	<i>Penicillium</i> sp.	14	56.6	0.182	0.247	76.92
Greene <i>et al.</i> , 1964; Bruch, 1967	Balloon	Drawing large volumes of air by a fan through polyurethane foam filter	3-9 9-18 18-27	(Predominant isolates) <i>Micrococci</i> and spore-forming rods, <i>Aspergillus</i> sp., <i>Alternaria</i> sp., <i>Penicillium</i> sp. and <i>Cladosporium</i> sp.	847 678 304	2095 8014 14611	1070 1522 665	0.404 0.085 0.021	0.78 0.44 0.2
Fulton, 1966	Aircraft	Air pass through a gelatin foam filter in sampling probe in front of bombardier's compartment	0.69 1.6 3.1	(Predominant isolates) <i>Bacilli</i> , <i>Micrococci</i> , <i>Aspergillus</i> sp., <i>Alternaria</i> sp. and <i>Horrodendron</i> sp.	not given not given not given	not given not given not given	not given not given not given	128 ^a 48 ^a 26 ^a	142 ^a 58 ^a 37 ^a
Imshenetuky <i>et al.</i> , 1976	Rocket	Sterile medium exposed on the forepart of the rocket during ascent	48-77	<i>Mycobacterium</i> sp., <i>Micrococcus</i> sp., <i>Circinella</i> sp., <i>Aspergillus</i> sp., <i>Papulaspora</i> sp. and <i>Penicillium</i> sp.	31	unknown	unknown	unknown	unknown
Yang <i>et al.</i> , 2008a	Aircraft	Drawing air through membrane filters by pumps	0.8-12	<i>Deinococcus</i> sp., <i>Streptomyces</i> sp., <i>Bacillus</i> sp. <i>Paenibacillus</i> sp., and unidentified fungi	8	5.51	2.69	1.47	2.97
Wainwright <i>et al.</i> , 2003	Balloon	Cryosampler comprised of sterile cylindrical probes	41	<i>Bacillus</i> sp., <i>Staphylococcus</i> sp., <i>Engyotontium</i> sp.	7	6.69	0.017	1.05	406.98
Griffin, 2005	Aircraft	Sterile impacter plates exposed on the underside of aircraft	20	<i>Bacillus</i> sp., <i>Penicillium</i> sp., <i>Micrococcaceae</i> and <i>Microbacteriaceae</i> , <i>Staphylococcus</i> and <i>Brevibacterium</i>	592	unknown	unknown	unknown	unknown
Yang <i>et al.</i> , 2008b	Balloon	Drawing air through membrane filters by pumps	12-35	<i>Bacillus</i> sp., <i>Paenibacillus</i> sp.	4	9	0.35	0.44	11.31

Air density at the respective mean sampling altitude was used for conversion between ATP (ambient temperature and pressure) and STP (standard temperature and pressure (0 °C and 100 kPa)). a), Approximate mean density values.

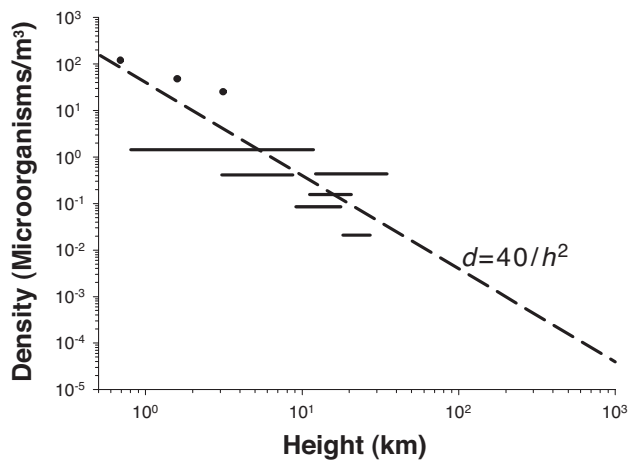


Fig. 2. Microbial density depending on altitude. Horizontal lines or filled circles were drawn based on the data at ambient temperature and pressure (ATP) in Table 1. Because some microbial samplings were done in certain height ranges, the horizontal lines show the respective height ranges. The dashed regression line ($d = 40/h^2$) indicates the estimated microbial density (d) versus height (h).

density data above 35 km in Table 1 was not used in the estimation, due to the small volumes of air collected (less than 0.2 m³, STP). The estimated result was close to the regression line $d = 40/h^2$ (Fig. 2), where d is the microbial density and h is the altitude in km. It suggests that microbial density generally decreases at higher altitudes over the troposphere. At the top of the atmosphere (100 km altitude), the expected microbial density is at an order of 10⁻²~10⁻³/m³. The altitude-dependence of microbial density in Fig. 2 must be taken to be a rough estimate, however, since microbial density data at specific altitudes from independent investigations may have ambiguity due to various factors such as variable atmospheric conditions, sampling methods, and even the isolation media used. More data, particularly in the atmosphere higher than 25 km, is needed to obtain a better understanding of the microbial distribution in the HAA. It is worth emphasizing that the data in Fig. 2 is limited to cultivated microbes, although generally less than 1 % of the microbes in the natural environment can be cultivated. Wainwright *et al.* (2004) found non-cultivable bacterial clumps in HAA samples using viable fluorescent staining. The actual density of microbes at various altitudes might be two orders of magnitude higher than that shown in Fig. 2.

Microbial Survival under HAA Conditions

The physical environment in the HAA is harsher than the low altitude atmosphere, not only in temperature, humidity, density and pressure, but also in the levels of various types of radiation. Micro-sized particles such as bacteria and spores drifting over the tropopause may take 1-2 years to sediment on Earth's surface, while the residence time in the upper troposphere is typically weeks (Kirkby and Carslaw, 2006). Although the atmospheric environment generally does not support microbial growth,

some microbes, particularly those isolated from the HAA, are presumably able to survive in the harsh environment for at least weeks to years.

Survival under HAA thermal environment

Air temperature profile in the atmosphere is a major criterion for the definition of atmospheric layers (Fig. 1). The temperature in the troposphere and the mesosphere decreases as altitude increases. The temperature increases as altitude increases in the stratosphere and the thermosphere. At high altitude, the atmospheric temperature is usually lower than the freezing point of water. However, the situation is not simple for the particles at higher altitude due to dramatically decreasing air density. Ambient temperature of dilute air or charged particles does not determine the temperature of the particles *in situ*. Thermal environment for microbe in the HAA is dominated by radiative heating made by solar light and long wave emission from Earth surface and radiative cooling of microbe to deep space (3 K). Since the heat input has daily cycle, the temperature of particles is high during day time and low at night, thus the heat cycle will affect the microbial survival in the HAA. *Escherichia coli*, a bacterium widely distributed on Earth's surface but never isolated from the HAA to date, is susceptible to subzero temperatures and is severely damaged, probably due to the presence of fragile cell components such as the cytoplasmic membrane (Moussa *et al.*, 2008). Some microbes may exhibit metabolic activity below 0 °C and even grow in clouds (Carpenter *et al.*, 2000; Sattler *et al.*, 2001; Hansen *et al.*, 2007). *Bacillus* spores and *Deinococci*, the species frequently found in the HAA, are rarely inactivated by subzero temperatures (Diaz and Schulze-Makuch, 2006; Yang *et al.*, 2009c); rather, they are better preserved at -70 °C (Yang *et al.*, 2009c). However, the effect of the daily temperature cycle on microbes has not been assessed.

Survival under HAA desiccation conditions

Water is a crucial element for terrestrial life. Water vapor content in the troposphere usually varies with weather and location. Its partial pressure decreases rapidly with altitude, varying from several thousand Pa near the surface to several hundred milli Pa in the lower stratosphere (Seidel, 2002). Above the tropopause, the atmosphere becomes almost completely dry. Atmospheric dryness at high altitude is caused not only by the scarcity of water, but also the low atmospheric pressure (Fig. 1). Drying of microbes proceeds when they are heated by solar radiation in vacuum. In solar system, a snow line (2.7 of Earth revolution radius) is defined whether water stays as ice or not. It depends on the temperature of the body under the radiation heat balance. Under 150 K or so, water stays in ice form. Around Earth or even Mars, volatile chemical species sublime by solar radiation, while sublimation of water does not occur beyond the snow line.

Microbes exhibit very different levels of resistance to desiccation. Some (e.g. *Bacillus* spores) may survive up to a million years but some die within several hours under desiccation (Atlas and Bartha, 1997). Presumably,

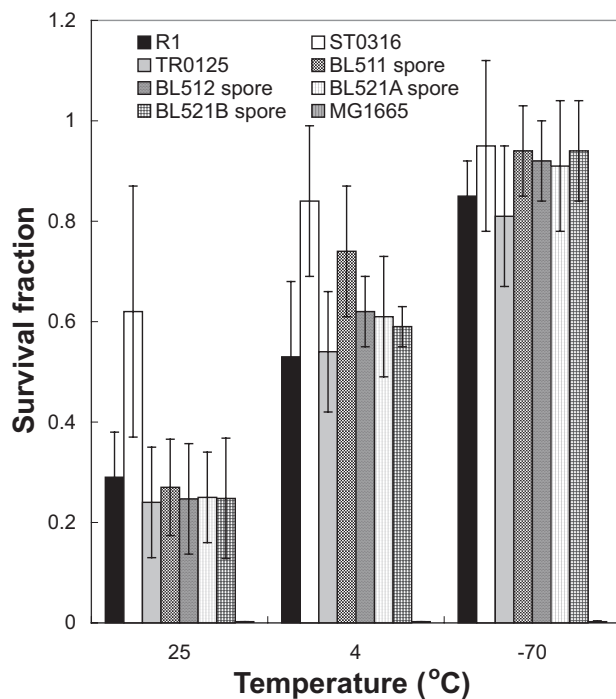


Fig. 3. Bacterial survival after 6-weeks of desiccation. Materials and methods were described previously (Yang *et al.*, 2009c). Strain R1 (*D. radiodurans*) is a ground isolate well known for desiccation tolerance. *Deinococcus* strains ST0316 and TR0125, *Bacillus* strains BL511, BL512, BL521A, and BL521B, are the isolates we obtained from the HAA (Yang *et al.*, 2008a, 2008b). Strain MG1665 (*E. coli*) is an Earth surface isolate, which is sensitive to desiccation, and its survival fraction was quite low as indicated in this figure. Data for each strain was from at least three independent experiments.

high resistance to desiccation is needed for microbial survival in the HAA. Desiccation-sensitive microbes would not be able to survive in the HAA for long periods. However, little is known about the desiccation resistance of HAA microbes, as the desiccation resistance of HAA isolates has not been evaluated in previous studies. To gain knowledge regarding the desiccation resistance of HAA microbes, the isolates we obtained from the HAA were exposed to desiccation conditions for six weeks. All of the tested HAA isolates showed greater desiccation resistance than the earth's surface isolate *E. coli* (Fig. 3). In particular, the *Deinococci* readily survived desiccation conditions (Yang *et al.*, 2009c) and may persist in the HAA for extended periods. *Deinococci* are well known for radiation resistance, which largely results from their extraordinary ability to repair DNA (Cox and Battista, 2005). High intracellular manganese and low iron concentrations in *Deinococci* were suggested to facilitate cellular protection from protein oxidation during dehydration and radiation (Daly *et al.*, 2004; Fredrickson *et al.*, 2008). A correlation between desiccation and radiation resistance of *Deinococci* has been suggested by several studies (Mattimore and Battista, 1996; Tanaka *et al.*, 2004; Rainey *et al.*, 2005; Chanal *et al.*, 2006). A major reason for radiation and desiccation resistance of bacterial endospores is likely the protection of spore DNA

by α/β -type small acid-soluble proteins (Nicholson *et al.*, 2000).

Survival in ozone-containing HAA

Up to the upper boundary of the atmosphere, there is almost no change in the relative composition of dry air (78 % nitrogen, 21 % oxygen, 1 % argon, and trace gases) except for ozone, whose composition is high in the stratosphere. The average concentration of ozone in the stratosphere is about 10 ppm compared to approximately 0.04 ppm in the troposphere. Many microbes may be killed within minutes by similar level of ozone as in the stratosphere (Li and Wang, 2003; Young and Setlow, 2004; Tseng and Li, 2006). However, the toxicity of ozone is greatly affected by environmental factors such as relative humidity and temperature. Lower temperature may decrease ozone effectiveness on the microbes (Wickramanayake and Sproul, 1988). Microbes were killed more readily by ozone in an atmosphere having a high rather than low relative humidity (Ewell, 1946). At relative humidity < 45 %, the germicidal power of ozone on airborne microbes was negligible (Elford and van de Eude, 1942). It seems that moisture in a cell is needed to be inactivated by ozone. Not only that desiccated microbes are more resistant than hydrated cells to ozone, but once desiccated, some cells are difficult to rehydrate sufficiently to be susceptible to ozone sterilization (Guérin, 1963). Considering the subzero temperature and extremely low water vapor content in the stratosphere, it is not likely that stratospheric ozone directly inactivates microbes.

Survival at HAA pressure

Atmospheric pressure decreases with increasing altitude (Fig. 1). About 2.5 kPa (corresponding to the atmospheric pressure at about 25 km altitude) may be the lower limit for bacterial growth (Thomas *et al.*, 2006). The associated desiccation at low atmospheric pressure may also affect microbial viability and therefore may play a role in selecting for survivors in the HAA. It was reported that increasing levels of desiccation during vacuum caused DNA damage (Dose *et al.*, 1991; Dose and Gill, 1995; Wehner and Horneck, 1995). Desiccation stress induces reactive oxygen species, which can cause irreversible damage on membrane systems, proteins and DNA (Mattimore and Battista, 1996; Adhikari *et al.*, 2009). Under extremely low pressures (high vacuum), survival rates of microbes can be very different depending on the species and strains (Imshenetsky *et al.*, 1977; Horneck, 1981; Dose *et al.*, 1996; Miyamoto-Shinohara *et al.*, 2000; Saffary *et al.*, 2002). Many bacteria can turn into dormancy (a reversible state of very low metabolic activity) to survive subzero temperature and high vacuum (Mileikowsky *et al.*, 2000; Bar *et al.*, 2002; Price and Sowers, 2004). Bacterial DNA is hardly damaged by the stable low temperature and stable dry state. Freezing down to the temperature of liquid nitrogen and vacuum are often used to store bacteria for long periods. Much higher bacterial survival in response to low pressures occurs at subzero temperature compared to temperatures above zero, and in *Bacillus* spores and *Deinococci*

compared to *E. coli* (Dose and Klein, 1996; Diaz and Schulze-Makuch, 2006).

Survival under radiation in the HAA

Another serious challenge for microbial survival in the HAA is thought to be radiation. Cosmic ray in the HAA originates from the sun and galactic system. The composition and fluence of cosmic ray vary throughout the atmosphere, partially depending on the altitude (Eisenbud, 1987; Bouville and Lowder, 1988). The level of cosmic radiation in the HAA is generally too low to be a threat for microbes. At spacecraft altitude, the dose of cosmic radiation averages about 0.2 mGy per day (Goossens *et al.*, 2006), while about 100 Gy is necessary to inactivate 37 % of *E. coli* (Quint *et al.*, 2002), a bacterium that is very radiation-sensitive compared to bacterial isolates from the HAA (Yang *et al.*, 2009b). Cosmic rays are thought to be much less effective in damaging terrestrial life than UV radiation (Galante and Horvath, 2007).

The ultraviolet section (< 400 nm) of the solar radiation spectrum accounts for about 7 % of total solar radiation. With every 1 km increase in altitude, UV levels generally increase by 10 to 20 %, depending on latitude, weather, and time of day and year (Blumthaler *et al.*, 1997; Seckmeyer *et al.*, 1997; Schmucki and Philipona, 2002; World Health Organization, 2002). The microbes that survive in the HAA are presumably resistant to ambient UV radiation. Considering the increase of biologically effective irradiation at high altitudes (Nybakken *et al.*, 2004), and the higher susceptibility of some microbes to the combined effects of radiation and other factors such as vacuum (Saffary *et al.*, 2002; Diaz and Schulze-Makuch, 2006), a lethal radiation dose for some microbes could be reached at altitudes in the HAA. However, data on UV resistance is available for few isolates from the HAA. The microbes isolated by Imshenetsky *et al.* (1976) were found to be very resistant to UV radiation, with the exception of the unpigmented *Micrococcus albus* (Imshenetsky *et al.*, 1977). The four *Bacillus* strains isolated from the HAA by Shivaji *et al.* (2006) were also claimed to be very UV-resistant. Shivaji *et al.* also related the UV resistance of their isolates with more intense UV radiation in the HAA than on the ground. The bacterial isolates we obtained from the HAA by aircraft exhibited higher UV resistance than the ground isolates from campus and the airport (Yang *et al.*, 2008a). Besides containing pigments, the *Deinococcus* isolates from the HAA (Yang *et al.*, 2008a) tended to form cell clumps or aggregates, which might help their survival under UV radiation. The presence of viable cell clumps has been reported from the samples collected in the upper stratosphere (Harris *et al.*, 2001). Also, spores of the isolates we obtained from the HAA by balloon generally exhibited greater UV resistance compared to *E. coli* (Yang *et al.*, 2008b). Therefore, it is likely that UV radiation plays a crucial role in selecting microbial species able to survive in the HAA.

Overall, typical HAA conditions such as low temperature, desiccation, low pressure or radiation, alone or in some combination, may affect the survival of microbes to varying degrees. This is consistent with the isolation of vial spore-formers and *Deinococci* from the HAA, as these organisms are generally much more resistant to these conditions than *E. coli*. It is worth noting that the actual conditions in the HAA result from the combination of all individual HAA conditions. Survival of microbes in the HAA is determined by a synergistic effect of these conditions, rather than on a single condition, and the synergistic effect is not simply the additive effect of the single conditions.

Microbial Survival from the HAA to Outer Space

Most of the physical conditions of outer space resemble those of the upper atmosphere. The radiation of outer space, which is different from that of the HAA in spectra and density, is considered to be a most serious threat for microbial survival during interplanetary transfer. Extraterrestrial UV radiation can kill unprotected *B. subtilis* spores within seconds (Horneck and Brack, 1992). Since unprotected microbes rarely survive a long trip in space, lithopanspermia (interplanetary transfer of life inside meteorites) is thought to be more likely (Clark *et al.*, 1999; Mileikowsky *et al.*, 2000) and will be tested by the Living Interplanetary Flight Experiment (Warmflash *et al.*, 2007). However, some microbes might form cell clusters or carried by airborne or cosmic dusts or meteorites as their inclusion. The dusts or outer layers of cell clusters might provide some protection from extraterrestrial radiation to the cells inside. Bacterial spores, if protected by dead spores or a dust layer of just a few microns to centimeters thick, could survive solar UV exposure for a significantly longer period (Mancinelli and Klovstad, 2000; Rettberg *et al.*, 2002b). Microbes therefore might not require meteorites for their carrier to survive during space travel. Thermal environment determined by intensity of solar light could be another serious challenge for unprotected microbes during interplanetary transfer. The heat-resistant bacteria would have more chances to survive the thermal environment. Some *Deinococci* can grow at about 55 °C (Ferreira *et al.*, 1997). Their lethal temperatures have not been reported. Endospores of many bacterial species can sustain heat treatment higher than 80 °C. *E. coli* in desiccated state exposed to short heating pulses under vacuum conditions survived up to 250 °C, while the same heating under atmospheric pressure led to complete sterilization (Pavlov *et al.*, 2007). It is unknown that up to which high temperature desiccated *Deinococci* and spores can tolerate under vacuum condition. Microbes traveling opposite to the sun would suffer less solar heat. If microbes are ejected from Earth and travel at the high speeds as suggested by Dehel (2006), the traveling time to Mars or Titan could be less than 1 year.

Landing of HAA Microbes onto Extraterrestrial Bodies

The panspermia process may be divided into three phases, i.e. 1) ejection from a donor planet, 2) transit through interplanetary space, and 3) entry and landing onto a recipient planet (Mileikowsky *et al.*, 2000; Nicholson, 2004). Terrestrial microbes, particularly spores and *Deinococci*, can be transported into the HAA and viably ejected from Earth for space travel as discussed above. The gravitational field of another planet may increase chance of capturing panspermian carrier. Aerodynamical heat or shock suffered during their ascent or descent is a major factor contributing to killing of microbes (Fajardo-Cavazos *et al.*, 2005; Meyer *et al.*, 2006). For extraterrestrial bodies to capture small microbe-associated particles (SMP), however, their speed relative to the target body might be more important than the additional speed gained by gravitational potential. Survival during atmospheric entry is largely dependent on the size and entry speed of the particles, and thickness of the atmosphere. Generally, the smaller and slower particles survive intact. For particles entering the earth's atmosphere at speeds < 20 km/s the survival rate is essentially 100 % for diameter < 5 µm and is less than 50 % for diameter > 100 µm (Brownlee, 2001). In the case of extraterrestrial bodies with thick atmospheres (e.g. Mars and Titan), entry of SMP can be largely buffered, provided adequate injection angle, thus their viable landing on the bodies is likely. In the case of collision with bodies with thin atmospheres (e.g. Mercury and the Moon), SMP would likely experience accelerated impact on the surface. Survivability of microbes after high impact collision has been tested (Horneck *et al.*, 2001; Mastrapa *et al.*, 2001; Benardini *et al.*, 2003; Willis *et al.*, 2006). Large fractions of spores or *Deinococci* survived impact with hard surfaces at the impact speeds less than 1 km/s. Survivability of *E. coli* after exposure to shock wave was much lower than *Bacillus* spores and *Deinococcus*.

Microbes could have been launched with our space probes or ejected by meteorite impacts into outer space at high speeds. A sustained 'outflow' of microbes into near-earth space propelled by electromagnetic fields and

further into deep space via magnetospheric plasmoids that leave the Earth at 700 km/s or more have been suggested (Håland *et al.*, 1999; Dehel, 2006), although the modeling study has not been done above the stratosphere (Dehel *et al.*, 2008). Ejected microbes may travel interplanetary space freely after leaving the Earth gravitational potential well under gravitational force of Sun until leaving its gravitational potential well. Depending on size and composition of the ejected microbes, their interplanetary travel may also be propelled outward from Sun by radiation pressure force (Wilck and Mann, 1996). Therefore, microbes might be widely present in deep space and some may have reached other planets.

The density of terrestrial microbes dispersed to distant space would be inverse square proportional to the distance from Earth. Since the regression line of microbial density at the vicinity of Earth happens to show inverse square dependence on the height from Earth surface, same regression line ($d = 40/h^2$, replacing h with distance) was applied to estimate density of terrestrial microbes hitting the extraterrestrial bodies such as Moon, Mars and Titan listed in Table 2. We further estimated the amount of microbes that could encounter these bodies. The total number of microbes that annually encounter a body was estimated by the multiplication of microbial density at the body's altitude (microorganisms/km³), moving speed (km/s) of the body in our Solar system, and the largest cross section (km²) of the body. The Moon could trap 7.5×10^{13} microbes per year. If microbial decay were avoided on some Moon areas, microbial density on the Moon would be high after accumulation for billions of years. The craters in permanent shadow near the lunar poles can be of astrobiological interest. Mars and Titan could trap fewer amounts of terrestrial microbes per year. If hospitable conditions (e.g. water or methane) for microbial growth existed or still exist on Mars and Titan, even the amount of seed microbes might be small, but flourished in some regions of the bodies once viable transfer was achieved.

Challenges

Sampler carriers

Microbial sampling in the HAA requires sampler carriers such as aircraft, balloon or rocket. These

Table 2 Estimated microorganisms in outer space and yearly deposition on some extraterrestrial objects.

Object	Mean distance from Earth's surface (km)	Extrapolated microbial density at the distance (microorganisms/km ³)	Average moving speed of the object (km/s)	Target cross section ^{a)} of the object (km ²)	Expected encounter (microorganisms/year)
ISS	3.5×10^2	3.3×10^5	7.7 ^{b)}	—	—
Moon	4.0×10^5	2.5×10^{-1}	1.0 ^{b)}	9.5×10^6	7.5×10^{13}
Mars	2.3×10^8	7.6×10^{-7}	24 ^{c)}	3.6×10^7	2.1×10^{10}
Titan	1.4×10^9	2.0×10^{-8}	9.7 ^{d)}	2.1×10^7	1.3×10^8

a) Target cross section is assumed to be $\pi \times r^2$ (r , mean radius of the object).

b) Orbital speed around the Earth.

c) Orbital speed around the sun.

d) Saturn's orbital speed around the sun.

requirements have greatly limited sampling opportunities. Cooperation with carrier administrators and operators is necessary for conducting such flight experiment. The cost of a flight is usually much higher than that of the biological investigation. Among them, the cost for aircraft is the least, while the cost for rocket is the most.

There is always concern that the carrier may introduce contamination. Preventing contamination by carriers remains a difficult task. Although the sampler may be thoroughly sterilized before launch and kept clean before sampling in the HAA, it is not feasible to completely sterilize the carrier. Strictly speaking, it is impossible to absolutely avoid potential contamination from the carrier, as long as the sampler is sent with the carrier from the ground and operated close to the carrier in the HAA. For samplings facilitated by aircraft (Fulton, 1966; Griffin, 2005; Yang *et al.*, 2008a) or rocket (Imshenetsky *et al.*, 1978), the probability of such contamination was low, since the air collectors were positioned at the forward (ram) direction of the high-speed carrier. Furthermore, sterilization of the rocket was reached by the heating (up to 600 °C) during its high speed take-off through dense air (Imshenetsky *et al.*, 1978). However, the probability of contamination from balloons is relatively high, since the air collectors are usually hung below the slowly ascending balloons (Rogers and Meier, 1936; Greene *et al.*, 1964; Soffen, 1965; Wainwright *et al.*, 2003; Yang *et al.*, 2008b). To evaluate sampling reliability using a balloon, we recommend that future investigations include control sampling from the balloon surface immediately before launch, and an air sample before reaching the designated sampling altitude. Sampling without contamination by the carrier might be achieved by dropping a sampling device after being carried to the HAA, but sampling time and therefore the air volume would be very limited in this case.

Microbial collection and detection methods

Standard methodology has not yet established for microbial investigation in the HAA. Various samplers have been designed and used in past studies. Some included air filter papers or impact media, while some collected only air for later processing in the laboratory. Collecting efficiency could depend on the types of filter papers or impact media used. In addition, the volumes of air impacted with media were unknown. The methods for recovering microbes from the samples (culture media and conditions) were also different. Therefore, it is difficult to compare the microbiology data among the various studies. There is a need to standardize or develop adequate types of filter papers (pore size and material) for studying the HAA microbes. If multiple parallel sampling can be conducted in future studies, several types of culture media and conditions should be used for microbe isolation.

Determination of the overall microbial diversity in the HAA is essential for our full understanding of which microbes may spread around the earth via the HAA and possess the potential for traveling to outer space. As discussed above, many species of microbes have

been isolated in past investigations. However, the vast majority of microbial diversity might have been limited by cultivation-based methods. Little has been done to determine the diversity of non-cultivable microbes in HAA samples. The presence of viruses, which are the smallest microbes, has rarely been investigated in the HAA, although they may stay in the HAA for longer periods of time than larger microbes. Culture-independent techniques such as 16S rRNA gene sequence analysis and metagenomic sequencing can provide a less biased perspective on HAA microbes. One challenge is the low concentration of HAA microbes. Large numbers of microbes might be collected on filter membranes by filtering huge volumes of air with deployment of powerful sampling devices and extended sampling times; however, this strategy would be more costly. In the case of few microbes in the sample, micromanipulation and single cell techniques (Brehm-Stecher and Johnson, 2004), which have been used to facilitate the isolation of pure eukaryotic cells or prokaryotic cultures, may be necessary.

Perspective

The stress resistance of the spore formers and vegetative *Deinococci* explains their prevalence in the harsh HAA environment. Applying culture-independent techniques might result in the discovery of more microbial populations in the HAA. Exposing laboratory-prepared microbes to the HAA may test the synergistic effects of atmospheric conditions on their survival. Sampling by using a rocket (Imshenetsky *et al.*, 1978) has been the sole method for investigation at altitudes higher than 50 km. Development of balloons capable of flying at altitudes higher than 50 km (Yamagami *et al.*, 2004) makes it possible to conduct more investigations at the top of the earth's atmosphere. For collection of HAA microbes facilitated by high-speed aircraft or rockets, it is worth testing another medium (Aerogel), which has been used to capture micrometeoroids and debris in outer space (Hörz *et al.*, 2000), and simulate aero-capture in a planetary atmosphere (Burchell *et al.*, 2001). Detection of microbes that may have escaped from Earth and testing of microbial survival at International Space Station (ISS) altitude for multi-year periods constitute goals of the TANPOPO project (Yamagishi *et al.*, 2007). Invaluable information on the 'Panspermia' of terrestrial life might be obtained from the project.

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