Microbial Distributions and Survival in the Troposphere and Stratosphere

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MICROBIAL DISTRIBUTION AND SURVIVAL IN THE TROPOSPHERE AND STRATOSPHERE

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Biological Sciences

by

Noelle Celeste Bryan
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LIST OF ABBREVIATIONS

6-4PP: 6-4 photoproduct
AoA: age of air
ASL: above sea level
ATP: adenosine triphosphate
BDC: Brewer-Dobson circulation
CBL: convective boundary layer
CCN: cloud condensation nuclei
CFU: colony forming unit
CPD: cyclobutane pyrimidine dimer
cT: continental tropical
DNA: deoxyribonucleic acid
DSB: double strand breaks
EM: eddy mixing
FOV: field of view
GPS: global positioning system
HASP: high altitude student platform
HEPA: high efficiency particulate air
HR: homologous recombination
HYSPLIT: HYbrid single particle lagrangian integrated trajectory
IN: ice nucleation
IR: ionizing radiation
LD₉₀: lethal dose require to reduce 90% of the microbial population
mT: maritime tropical
NER: nucleotide excision repair
ND: not determined
NH: northern hemisphere
QBO: quasi-biennial oscillation
RC: residual circulation
RH: relative humidity
ROS: reactive oxygen species
rRNA: ribosomal ribonucleic acid
SASP: small, acid soluble spore proteins
SE: standard error
SEM: scanning electron microscopy
SH: southern hemisphere
SP: spore photoproduct
UV: ultraviolet radiation
UVER: UV damage endonuclease excision repair
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ABSTRACT

Although bioaerosols in the lower altitudes of the atmosphere have been extensively studied, little is known about the distribution and nature of microorganisms at altitudes above the Earth’s surface. To examine the vertical distribution of microorganisms in the atmosphere, a helium balloon payload system was developed to sample bioaerosols at stratospheric altitudes of up to 38 km, where temperature, air pressure, relative humidity, and ultraviolet-C (UV-C) radiation conditions are similar to the surface of Mars. Bioaerosol concentrations between 3 and 29 km were similar to each other, ~three-fold lower than those in the convective boundary layer (CBL; 0-3 km; 1 x 10^6 cells m\(^{-3}\)), and decreased to 8 x 10^4 cells m\(^{-3}\) between 35 and 38 km. From these data we estimate that Earth’s atmosphere contains at least 100-fold more microorganisms (~10^24 cells) than previously thought (Burrows et al., 2009a), ~40% of which may exist of bioaerosols in the stratosphere. Isolates recovered from 6 to 29 km were evaluated for the tolerance to desiccation and UV-C in order to better constrain their ability to survive transport in the stratosphere. Based on the survival data, UV-C was determined to be the limiting factor for microbial survival in the atmosphere. Populations of L6-1, the most UV-C tolerant isolate examined, at the low end of stratospheric cell concentrations (3.8 x 10^5 cells m\(^{-3}\)) would be inactivated in 43 days at 20 km, a value that decrease to ~2 hours at 30 km, above the shielding effects of the ozone layer. Based on these data, we hypothesize the ozone layer may represent the upper limit of the biosphere.
CHAPTER 1. Introduction

1.0 Importance of bioaerosols

Bioaerosols are defined as intact aerosolized viruses, bacteria, fungi, and pollen, or other particulate biological material (Després et al., 2007; O’Sullivan et al., 2015; Šanti-Temkiv, et al., 2015), and are ubiquitous in the air just above the surface of the Earth, where they comprise a year-round significant portion of the total aerosol population, ranging from 5 to 50% at a semi-urban site and 20 to 30% at a rural location (Jaenicke, R., 2005). Bioaerosols are aerosolized from soil, the phyllosphere, or aquatic environments by passive transport mechanisms, including turbulence, convection, raindrops (Joung et al., 2017), and wave breaking (Aller et al., 2005). Additionally, some microorganisms have adapted specialized structures for active (i.e., acospores of Gibberella zeae) or passive (i.e., conidiophores of Penicillium sp.) release into the atmosphere (Wyatt et al., 2013). The study of bioaerosols has relevance for understanding the dispersal of human (Amato et al., 2006; Griffin et al., 2003; Kellogg et al., 2004; Li et al., 2017) and plant pathogens (Amato et al., 2005; Amato et al., 2006; Griffin et al., 2001; Griffin et al., 2007; Kellogg et al., 2004; Monteil et al., 2014; Smith et al., 2012; Vaitilingom et al., 2012), mitigating bioterrorism (Davis and Johnson-Winegar, 2000; King et al., 2011; Schmid and Kaufmann, 2002), examining their role in global atmospheric processes (Morris et al., 2008; Monteil et al., 2014), and use as astrobiological analogues (Khodadad et al., 2017).

Recent reports highlight the risk of exposure to pathogenic bioaerosols downwind of wastewater treatment facilities (Li et al, 2016) and manure application sites (Jahne et al., 2015). Bioaerosols generated in the confines of commercial farms may also pose
threats to human health. Between 40 to 56% of employees in a swine confinement building tested positive for human pathogenic bacteria and the *tet* gene, responsible for resistance to the antibiotic tetracycline, regardless of whether or not they wore protective face masks for five days (Létourneau et al., 2010). The presence of pathogens in the nasal swabs of those workers who wore the facemasks suggests long-term colonization (>5 days) in the workers, who may serve as asymptomatic carriers and potentially spread disease (Létourneau et al., 2010). Li et al., (2017) documented significant increases in urban viable bioaerosol concentrations during haze episodes (as opposed to sunny, cloudy, or rainy days) and found more than 60% of the bioaerosols were <5 µm, the size range of respirable particles. The concentration of viable bioaerosols in this semi-arid city with a population of 8.5 million people ranged from 0.60 to 1.8 x 10³ CFU m⁻³, exceeding local health risk guidelines (Li et al., 2017). Recently, van Leuken et al., (2016) modeled the range of an airborne *Coxiella burnetii* outbreak, the causative agent of Q fever, in response to increasing global temperatures. Not surprisingly the meteorological factors that most greatly influenced horizontal dispersal (wind speed) and wet deposition (precipitation), resulted in the most significant changes in the model (van Leuken et al., 2016). Precipitation increased in each of the modeled scenarios of increased global temperatures, however wind speed increased only when the model conditions also allowed for large-scale changes in circulation patterns (van Leuken et al., 2016). However, the study failed to account for the decreased viability of *C. burnetii* during aerial transport, a critical component for predicting disease occurrence (van Leuken et al., 2016).
The intentional aerosolization and release of human pathogens is one of the most dangerous types of bioterrorism (King et al., 2011). A lethal attack using *Bacillus anthracis* spores was carried out using mail delivery in 2001 (Anonymous, 2001). The average size of *B. anthracis* spores is 0.8 by 1.2 µm (Schmid and Kaufmann, 2002) and naturally occurs in the respirable size range (Davis and Johnson-Winegar, 2000). However, the weaponization of the spores includes milling techniques to prevent clumping, thereby aiding in aerial dispersal (Davis and Johnson-Winegar, 2000). The spore preparation employed during the 2001 attack was equivalent to those produced by the U. S. military biowarfare program and resulted in 5 fatalities (Schmid and Kaufmann, 2002). The ability of the spores to maintain viability during aerial dispersal, coupled with the high rate of lethality, make *B. anthracis* spores the Department of Defense’s top biowarfare threat to the military (Davis and Johnson-Winegar, 2000; Schmid and Kaufmann, 2002).

In addition to the dispersal of potential human pathogens, bioaerosols that impact plant health may also reach new habitats after transport through the atmosphere. The potential for aerial dispersal of plant pathogens was recently reviewed by Schmale et al., (2015), and highlights the need for a better understanding of the potential scale of disease transmission to better guide plant disease management practices. The plant pathogen, *Pseudomonas syringae*, is commonly isolated from clouds (Amato et al., 2007; Vaïtilingom et al., 2012), and may influence precipitation formation due to its ice nucleating capacity (Morris et al., 2008; Monteil et al., 2014). Plant growth promoting bacteria, from 13 different families, capable of phosphate solubilization, siderophore and indolacetic acid production (reviewed by Compant et al., 2010) have been also isolated.
from atmospheric samples, demonstrating a potentially beneficial role to plant health in the dispersal of bioaerosols (Strulik et al., 2016).

2.0 Environmental stress encountered during aerial transport

Cells lose water upon aerosolization, and the efflux of the water from a cell when exposed to a gas with a water activity lower than the cell itself is termed desiccation (Potts, M., 1994). Water activity can be defined as the ratio of the water vapor pressure in a substance to the water vapor pressure of pure water at the same temperature (Labuza, T., 1980). Alpert et al., (2006) estimates the drying of cellular water content to 10% would be equilibrated to air with 50% RH at 20 °C. Shrinkages in Serratia marcescens and Mycobacterium bovis BCG (a surrogate for multidrug-resistant M. tuberculosis) bioaerosol size correlated to decreased RH (Ko et al., 2000). Desiccation is a prevalent environmental stress that terrestrial bacteria must overcome, especially in arid environments (Barnard et al., 2013; Cytryn et al., 2007; van Hamm et al., 2016), and results in a wide range of damaging effects in the cell (Lebre et al., 2017). However, low temperatures (4 °C) significantly improved desiccation survival in a variety of food borne pathogens (Gruzdev et al., 2012a; Gruzdev et al., 2012b; Koseki et al., 2015). Survival while desiccated increased at 4 °C (17%), relative to 25 °C (0.6%; Gruzdev et al., 2012a), and S. enterica serovar Typhimurium SL1344 survived ~2 y at 4 °C with only a 5 log reduction in viability (Gruzdev et al., 2012b). While microorganisms are capable of synthesizing proteins and DNA at temperatures as low as -15 ºC (Amato et al., 2010; Christner et al., 2002), the decreased rates of metabolism may indicate that cellular activity is targeting repair of cellular components rather than growth and cell division (Amato et al., 2010). Although generally RH decreases with altitude, the reduced
temperatures in the upper atmosphere may offer microorganisms an advantage over surface level temperatures while being exposed to low RH (Ch. 3).

Exposure to desiccating conditions is a common phenomenon, yet only a limited number of studies have investigated the specific mechanisms used by microorganisms to combat this stress (Finn 2013; Gruzdev et al., 2012; Hingston et al, 2015). The majority of these studies have focused on the persistence of food borne pathogens under low water activity storage conditions. Desiccation results in reduced cell membrane integrity, and *Escherichia coli* increase the fraction of saturated fatty acids in the membrane, resulting in a more tightly packed, gel phase lipid bilayer (Scherber et al., 2009). *Listeria monocytogenes*, with a mutation in the branched-chain α-keto acid dehydrogenase complex, have increased levels of unsaturated fatty acids and demonstrated extreme desiccation tolerance when compared to the wild-type (Hingston et al., 2015). Other bacteria respond by increasing membrane fluidity, such as *Salmonella enterica*, who induces the gene *ddg* (Gruzdev et al., 2012b) responsible for the substitution of the saturated lipid A acyl chain laurate with the unsaturated acyl chain palmitoleate (Carty et al., 1999). Desiccation also results in protein denaturation, and the accumulation of compatible solutes may help reduce this process by potentially interacting with the hydrophilic domains or preferentially excluding water molecules and increasing the compact nature of the protein (Jain and Roy 2009; Leslie et al., 1995), as well as helping to maintain turgor pressure (Billi and Potts, 2002; Potts, M. 1994). Compatible solutes include amino acids (i.e., glutamate, glutamine, and proline), polysaccharides (i.e. trehalose, sucrose), glycine betaine, and potassium ions (Lebre et al., 2017; Potts, M. 1994) and they are either synthesized by the cells (Cytryn et al., 2007), taken up from the
environment (Gruzdev et al., 2012b), or both (Finn et al., 2013). However, the ability to adapt to the stressors of desiccation is ultimately dependent upon the rate of water lost (Gruzdev et al., 2012a).

Oxidative stress may be one of the most detrimental effects of desiccation (Franca et al., 2007). Reactive oxygen species (ROS), such as superoxide anions (\(\bullet O_2^-\)), hydrogen peroxide (\(H_2O_2\)), and the hydroxyl radical (\(\bullet OH\)), are formed during aerobic respiration (Franca et al., 2007, Friedburg et al., 1995). However, desiccation induced protein cross-links and denaturation are predicted to interrupt normal metabolic pathways and membrane permeability, thereby resulting in a dysfunctional electron transport chain and only the partial reduction of oxygen (Billi and Potts, 2002; Franca et al., 2007; Friedburg et al., 1995; Lebre et al., 2017). ROS can have detrimental interactions with any macromolecule they interact with and are considered to be the primary source of DNA damage encountered during desiccation (Franca, et al., 2007; Potts, M., 1994; Lebre et al., 2017). Antioxidants such as catalase, superoxide dismutase, and peroxidase convert ROS to nonlethal products (Franca et al., 2007; Friedberg et al., 1995). Disruption of the glutathione peroxidase gene in *L. monocytogenes* resulted in a desiccation sensitive mutant (Hingston et al., 2015), and *B. japonicum* was shown to significantly induce genes encoding peroxidases and superoxide dismutases while desiccated (Cytryn et al., 2007).

The DNA double strand breaks (DSBs) produced by ROS during desiccation must be efficiently repaired to maintain viability upon rehydration (Mattimore and Battista, 1996). The accumulation of DSBs after 42 days was similar in both the desiccation sensitive *E. coli* K-12 strain A1157 and the desiccation tolerant strain *Deinococcus radiodurans* R1, implying *D. radiodurans* is highly efficient at DNA repair and not
immune to DNA damage (Mattimore and Battista, 1996). Although there was no visible
evidence for DSBs after 2 days of desiccation, the number of surviving \textit{E. coli} decreased
1000-fold (Mattimore and Battista, 1996), indicating the loss of viability in \textit{E. coli} is
likely due to a combination of the cellular damages described above. The DNA DSBs
incurred by \textit{D. radiodurans} R1 during 6 weeks of desiccation (equivalent to a minimum
of 60 DSBs) was similar to that produced by $5.2 \times 10^3$ Gy of ionizing radiation
(Mattimore and Battista, 1996). Because both desiccation and ionizing radiation produce
similar forms of damage, selection for desiccation tolerance has proven to be a successful
method for identifying ionizing radiation resistant strains (Rainey et al., 2005; Sanders
and Maxcy, 1979).

Variations in transcriptional responses to desiccation are highly dependent upon
the species, RH, time exposed to desiccating conditions, media, and temperature (Cytryn
et al., 2007; Deng et al., 2012; Finn et al., 2013; Gruzdev et al., 2012b). Levels of
desiccation tolerance varies significantly among 5 different \textit{S. enterica} serovar
Typhimurium strains and 3 \textit{S. enterica} serotypes, indicating survival to desiccation is also
highly strain specific (Gruzdev et al., 2012b). Desiccation at 30% RH resulted in a
significant decrease in metabolic activity of \textit{S. enterica}, with <5% of the total genome
was transcribed (Deng et al., 2012). During desiccation, microorganism may upregulate
various genes including those involved in ribosome biogenesis, the transport and
metabolisms of amino acids, lipids, and carbohydrates, stress responses, the synthesis and
uptake of compatible solutes, and DNA repair (Cytryn et al., 2007; Finn et al., 2013,
Gruzdev et al., 2012a). Even though global metabolism may be drastically reduced
beyond the level of reproductive growth, and altered transcriptional responses measured
over time (4 to 528 h) demonstrate that microorganisms are actively mitigating the stress of desiccation (Cytryn et al., 2007; Deng et al., 2012). Desiccation tolerant, symbiotic nitrogen fixing bacteria has also been investigated (Cytryn et al., 2007; van Hamm et al., 2016). Differential gene expression was observed after desiccating *Bradyrhizobium japonicum* strain USDA 110 under 27% RH, and the transcriptional responses varied with time indicating a dynamic response to desiccation (Cytryn et al., 2007). By having a better understanding of the specific mechanisms of desiccation tolerance, the ability to isolate legume symbionts, such as *Rhizobium leguminosarum*, capable of surviving on the seed and successfully colonize the emerging plant will be greatly improved (van Ham et al., 2016). The identification of desiccation tolerant symbionts of pasture legumes represents a pivotal advancement in the development of successful seed inoculum, especially for drought tolerant soybeans, clover, and other legumes (Cytryn et al., 2007; van Hamm et al., 2016).

As bioaerosols are transported to higher altitudes they will be exposed to increase UV fluence rates (Blumthaler and Ellinger, 1997). UV can be divided into three categories: UVA (320-400 nm), UVB (280-320 nm), and UV-C (190-280 nm; Coohill and Sagripanti, 2008; Santos et al., 2013). DNA most efficiently absorbs UV-C at 254 nm (Brash, D., 1988; Friedberg et al., 1995), causing the formation of cyclobutane pyrimidine dimers (CPD), which are covalent bonds formed between neighboring pyrimidine bases, and most commonly involves two thymines (Brash, D., 1988; Friedberg et al., 1995). Based on the *D. radiodurans* genome size and GC content, a dose of 0.5 kJ m\(^{-2}\) could induce as many as 5.0 x 10\(^3\) thymidine based CPD per genome, yet because these lesions are efficiently repaired, still not result in the loss of viability (Earl
et al., 2002). The CPD alters the conformation of the DNA helix, which may result in decreased protein binding affinity and interrupted DNA replication (Brash, D., 1988; Friedberg et al., 1995). Although less frequent than CPD, the cross-linked pyrimidine-pyrimidone (6-4PP) photoproduct is created from UV-C exposure and also disrupts the shape of DNA (Brash, D., 1988; Friedberg et al., 1995). Battista (1995) and Tanaka et al., (2005) describe DNA repair and their representative *D. radiodurans* genes: nucleotide excision repair (NER; *uvrA*), UV damage endonuclease excision repair (UVER; *uvsE*), and homologous recombination repair (HR; *recA*). The construction of *D. radiodurans* mutants revealed HR played the most significant role in response to UV-C damage during exponential phase, however the contributions of UVER and NER were increased during stationary phase (Tanaka et al., 2005). NER efficiently removes the 6-4PP, and UVER eliminates both 6-4PP and CPD (Tanaka et al., 2005). More recently, a fourth method to mitigate DNA damage was proposed for the combined interactions of the novel *D. radiodurans* proteins, PprA, DdrA, and DdrD (Selvam et al., 2013). While not directly involved in the removal of CPD or 6-4PP, these proteins are hypothesized to have a stabilizing effect on the damaged DNA, facilitating its repair (Selvam et al., 2013). Although UV-C emitted from the sun is attenuated by the absorption of ozone (Ch. 4), microorganisms surviving transport in the stratosphere must have the capacity to repair UV-C induced DNA damage. Because UV-C radiation is so deleterious to DNA, it is widely employed for decontamination, a better understanding the types of microorganisms that display high levels of UV-C tolerances will impact many aspects of current microbial inactivation protocols (Cooper et al., 2016; King et al., 2011; Koutchma et al., 2009; Rose et al., 2009) and planetary protection (NASA, 2005).
In response to stressors such as desiccation and UV-C, some bacteria (i.e., *Bacillus* and *Clostridia*) can form metabolically dormant structures called endospores (hereafter referred to as spores), and the major differences between *Bacillus* and *Clostridia* have been reviewed by Paredes-Sabja et al., (2014). The physiological structure of the spore is distinct from the vegetative cells and provides a protective advantage against harsh environmental conditions (reviewed by Legget et al., 2012; Setlow, P. 2006). Within the spore core are α/β small, acid soluble spore proteins (SASPs), which when bound to DNA, result in the A-helix conformation (reviewed by Nicholson et al., 2000). This altered conformation is less prone to the formation of CPD, and favors the formation of the formation of the efficiently repaired DNA lesion, the spore photoproduct (SP; Nicholson et al., 2000; Setlow and Li, 2015). During desiccation, the DNA bound SASPs protect against single strand breaks (Fairhead et al., 1994), however the exact mechanism remains unknown (Nicholson et al., 2000). An increased concentration of dipicolinic acid in the spore core results in decreased water content (28 to 50%, as compare to 75 to 80% of the wet weight of the vegetative cell) and decreased damage from ROS (Nicholson et al., 2000; Setlow, P., 1995). Because the spore is metabolically inert, the survival of the spore is dependent upon decreased levels of DNA damage incurred as a spore and the capability to repair the damage upon germination (Nicholson et al., 2000). The activation of the spore specific SP lyase during the first minutes of germination efficiently repairs the accumulated SP (Nicholson et al., 2000; Setlow et al., 2006; Setlow and Li, 2015). These highly resistant, specialized structures with small (<1.0 µm) aerodynamic diameters may explain the prevalence of
spore-forming isolates from atmospheric samples (Griffin et al., 2001; Griffin, D., 2004; Shavji et al., 2006; Smith et al., 2010).

3.0 Physical characteristics of the atmosphere

3.1 The convective boundary layer (CBL)

The lowest part of the atmosphere, the CBL, is the portion that directly interacts, both mechanically and thermally, with the Earth’s surface (Cushman-Roisin, B. 2014). Solar energy warms the Earth’s surface and this heat is transmitted into the atmosphere (Stull, R., 2006). Convective air creates turbulence, resulting in a well-mixed CBL of uniform moisture and potential temperature with altitude (Cushman-Roisin, B. 2014; Stull, R., 2006; Wang et al., 2016b). At the top of the homogenous CBL is the entrainment zone, where temperature sharply decreases, resulting in a “knee” in the temperature profile (Stull, R., 2006). The height of the CBL varies with geographic location, season, and with precipitation and dust events (Ouwersloot et al., 2012; Wang et al., 2016a; Wang et al., 2016b).

The vast majority of bioaerosol concentration data have been obtained in the CBL, and Burrows et al., (2009a) reviewed the factors thought to contribute to their spatial and temporal variation. Bioaerosol emissions from various ecosystems have been used to estimate global bioaerosols concentrations, implying that there are roughly $10^{22}$ cells in Earth’s atmosphere (Burrows et al., 2009b). Depending on the ability of the microorganism to serve as a cloud condensation nuclei (CCN) or an ice nucleating (IN) particle, the average CBL residency time of a 1.0-µm bacteria is predicted to range from 3.4 to 7.5 days (Burrows et al., 2009). Based on an estimate derived from a single culture-based study (Lightheart and Shaffer, 1994), deserts were predicted to not be a
significant source of bioaerosols (Burrows et al., 2009a). However, due to the increased likelihood of convective transport into the free troposphere and decreased precipitation, 1.0-um desert bioaerosols have the longest predicted residency times (9.6 to 14.4 days; Burrows et al., 2009b). Although the estimates of Burrows et al., (2009a) are solely based on data from the surface and CBL, models of global microbial scale flux, transport, and contributions to atmospheric processes (e.g., Burrows et al., 2013; Hoose et al., 2010; Sesartic et al., 2012) have been forced to use them in the absence of actual data from the free troposphere.

3.2 The free troposphere

The majority of convective turbulence is limited to the CBL, and the free troposphere is the atmospheric layer directly above the entrainment zone, or knee, where temperature, pressure, and relative humidity (RH) decrease with altitude (Ouwersloot et al., 2012; Seinfeld and Pandis, 1998; Stull, R., 2006). The majority of water vapor exists in the troposphere, containing 80% of the total water mass in the atmosphere (Seinfeld and Pandis, 1998). Clouds and precipitation form in the free troposphere, and freezing can be catalyzed by IN microorganisms or other aerosols (Creamean et al., 2016; Stopelli et al., 2016). Intercontinental transport of dust and microorganisms can occur in the free troposphere (Creamean et al., 2013; Griffin et al., 2006; Smith et al., 2012) and occurs on the order of 5 to 10 days (Griffin et al., 2006; Smith et al., 2013).

3.3 The tropopause

The tropopause is the boundary between the free troposphere and stratosphere, and can be defined by temperature lapse rates or variations in potential vorticity (Seidel et al., 2001). The cold point tropopause is the altitude with the lowest temperature value
(Seidel et al., 2001) and will be the definition of the tropopause employed in this study (Ch. 3). The height of the tropopause varies with geographic location and season, with an average altitude and temperature at the equator were 16.9 km above the surface and -81 °C, respectively (Seidel et al., 2001). The height of the tropopause decreases as latitude increases, to approximately 8 km over the poles (Seinfeld and Pandis, 1998). Temperature also increases with distance from the equator with average values of ~70° at latitudes of 25°N and 25°S (Seidel et al., 2001).

Convection causes moist tropospheric air in the tropics (30°N to 30°S) to rise, and the water condenses as it encounters low temperatures of the tropical tropopause (Mote et al., 1994). Therefore, air entering the stratosphere will be “marked” by low water vapor entry concentrations (between 3 and 4.5 ppmv), and these values will increases with height as a result of the oxidation of methane in the stratosphere (Mote et al., 1996). By monitoring the water vapor profiles, the ascent rate of a parcel of air from the tropical tropopause into the overlying stratosphere can be determined (Mote et al., 1996; Mote et al., 1998; Niwano et al., 2003). The ascent rate varies biannually with the quasi-biennial oscillation of stratospheric winds and with altitude, latitude, and season (Mote et al., 1998; Niwano et al., 2003). Maximum ascent rates (0.26 to 0.36 mm s\(^{-1}\)) occur during the months of December to February, and minimum rates (~0.2 mm s\(^{-1}\)) are typical during June to August (Niwano et al., 2003). Based on tropical ascent rates of 0.4 mm s\(^{-1}\) from 16 to 18 km, 0.2 mm s\(^{-1}\) from 19 to 23 km, and an increased rate of 0.4 mm s\(^{-1}\) due to Rossby wave propagation above 23 km (Mote et al., 1998), it would take a parcel of air 2 years to ascent vertically from the tropical tropopause to 36 km in the stratosphere.
3.4 The stratosphere

The stratosphere is defined as the layer of air above the tropopause, where temperature increases with altitude, and was originally divided into two separate air masses: the overworld (at midlatitude altitudes above 15 km) and the lowermost stratosphere (between the local tropopause at ~14 km and 15 km at midlatitudes), based on different water vapor mixing ratio observations (Dessler et al., 1995). The overworld, more commonly referred to as the middle to upper stratosphere, corresponds to the classical description of the stratosphere, with air that does not physically interact with the local tropopause at (Dessler et al., 1995; Gettelman, et al., 2011). Middle to upper stratospheric air originates from the tropical tropopause where low temperatures reduce the water vapor mixing ratios (<7 ppmv, Dessler et al., 1995). The lowermost stratosphere contains a mixture of diabatically descending overworld air and air that has entered through the extratropical tropopause (>30°, Dessler et al., 1995; Mote et al., 1996). Because temperatures in the extratropical tropopause are warmer than those in the tropical tropopause, the resulting water vapor mixing ratios are also increased to tens of ppmv (Dessler et al., 1995).

As altitude increases above the tropopause and into the stratosphere, so does the concentration of ozone (O₃; Gettelman et al., 2011) with maximum values between 20 and 30 km (Jacobs, D., 1999). O₃ is formed by the photolysis of molecular oxygen (O₂) by UV-C wavelengths below 242 nm, generating single oxygen atoms (O) that react with other O₂ molecules to form O₃ (Seinfeld and Pandis, 1998). O₃ strongly absorbs UV light from 240 to 320 nm, the most biologically harmful portion of the UV spectrum, and will dissociate back into O₂ and O producing heat (Seinfeld and Pandis, 1998). This reaction
takes place in shorter time scales in the lowermost stratosphere, where the presence of O$_3$ dominates, resulting in the accumulation of ozone (Seinfeld and Pandis, 1998). The ratio of [O]/[O$_3$] increases as altitude increases up to 30 km, and pressure and reaction rates decrease (Jacobs, D., 1999; Seinfeld and Pandis, 1998).

Figure 1 illustrates the Brewer-Dobson circulation (BDC), where air enters the stratosphere after ascension through the tropical tropopause (red dashed line, Fig. 1) and is transported poleward through the where it descends into the high latitude troposphere (Butchart, 2014; Plumb, R. A., 1996, 2002). This residual circulation (RC) pattern was independently proposed by Brewer (1949) and Dobson (1929, 1956) to describe observations of stratospheric water vapor and ozone, respectively. Once in the stratosphere, air is transported along two major pathways of the BDC (Fig 1). The slower, deep branch (blue arrows, Fig. 1) of BDC moves air in the middle and upper stratosphere (Butchart et al., 2014; Gettelman, et al., 2011; Konopka et al., 2015). The breaking of planetary-scale Rossby waves formed as the Earth rotates, drives the poleward motion of stratospheric air, and the strongest effects of this are seen during NH winter (Plumb R. A., 2002, 2007). Downward advection of the deep branch transports air to the lowermost stratosphere during transport toward the poles (Ploeger et al., 2015). Strong horizontal mixing occurs in the midlatitude stratosphere due to the dissipation of Rossby waves, which creates the highly mixed surf zone between ~20 to 75°N (Plumb, R. A., 1996). Transport barriers form at the edges of the surf zone, and these prevent large-scale, horizontal mixing of stratospheric air into either the polar or tropic zones (Ploeger et al., 2015; Plumb R. A., 1996, 2002). Air in the faster, shallow branch (~20 km) of the BDC
moves by advection from the tropics to higher latitudes (Gettelman et al., 2011; Konopka et al., 2015; Plumb R. A., 2002).

A parcel of air is comprised of aerosols that have traveled a variety of paths, and each aerosol has a unique transit time (Butchart et al., 2014; Konopka et al., 2015). The mean age of air (AoA) in the stratosphere is defined as a statistical distribution of all these transit times from the tropopause into the stratosphere (Gettelman, 2011; Konopka, et al., 2015; Plumb, R. A., 2002). The transit times of atmospheric tracer molecules are

![Diagram](image_url)

Fig. 1: Northern hemisphere transport of air from the equator across the tropopause (dashed red line) to the stratosphere and the poles. The green arrows represent the time to ascend (calculated from Mote et al., 1998) to the shallow (red arrows) and deep (blue arrows) branches of the BDC. Sampling latitude for is indicated with the star and dashed grey line (Ch. 3). The mean AoA at the sampling latitude are labeled in black boxes (Haenel et al., 2015). Purple arrows indicate the descent of stratospheric air to the surface. The shaded blue area represents the upper troposphere and the shaded grey area represents the lowermost stratosphere, where a mixture of tropospheric stratospheric air occurs. Adapted from Gettelman, et al., 2011; Haenel et al., 2015; Konopka et al., 2015; Plumb, R. A., 1996, 2002, 2007; Stiller et al., 2012.
used to compare concentrations at a given location in the stratosphere to its relative concentration in the tropopause. Suitable tracers, such as carbon dioxide (CO$_2$) and sulfur hexafluoride (SF$_6$), should have linear tropospheric mixing ratios and no significant sources nor sinks in the stratosphere (Plumb, R. A., 2002, Stiller et al., 2012). The mean AoA can be used to infer the speed, or strength, of the particular branch of the BDC. Stiller et al., (2012) and Haenel et al., (2015) used a modified definition of the mean AoA as determined by SF$_6$ (concentrations in stratosphere were compared to the well-mixed troposphere to account for the prolonged ascent time through the tropical tropopause) to uncover different decadal trends in the AoA of the deep and shallow branches of BDC from 2002-2012. The youngest air was consistently observed over the tropical tropopause, the point of entry into the stratosphere (Plumb, R. A., 1996), and the mean AoA increased with altitude and latitude towards the poles (Haenel et al., 2015; Fig. 1).

There are several factors, including season and latitude, that can influence the mean AoA of both branches of BDC, and they do not transport air at equal rates in both hemispheres (Gettelman et al., 2011; Haenel et al., 2015; Konopka et al., 2015; Stiller et al., 2012). An increase in the magnitude of upwelling through the tropical tropopause will decrease the AoA, and a significant linear decrease was detected in the shallow branch of the BDC in the southern hemisphere (SH) tropics and midlatitudes, indicative of elevated transport rates via BDC (Haenel et al., 2015; Ploeger et al., 2015; Stiller et al., 2012). Though not as pronounced as the decrease of SH AoA, the shallow branch of the NH midlatitude BDC decreased in AoA from 2002 to 2012, amplified during the spring and summer, with a “flushing” of younger air originating from low latitudes (Ploeger et al.,
This flushing implies that air masses were horizontally mixed from the tropical tropopause or lower stratosphere (Bönisch et al., 2009).

Because the age of air is relative to when it crossed the tropical tropopause, tropospheric air is considered older than stratospheric air (Haenel et al., 2015; Stiller et al., 2012). Quasi-horizontal mixing of older, tropospheric eddies was hypothesized as a cause for the increase of stratospheric AoA (Haenel et al., 2015; Stiller et al., 2012). Ploeger et al., (2015) extended the work of Haenel et al., (2015) and Stiller et al., (2012) in an attempt to model the contribution of tropospheric eddy mixing, relative to the RC of the BDC, to the observed regional and temporal changes of AoA. Additional simulations of the contribution of eddy mixing to AoA of the shallow branch of the BDC were always predicted to be stronger in the NH than in the Southern hemisphere (SH) resulting in midlatitude NH air having older AoA, with maximum values from July to November (Konopka et al., 2015).

Contrary to the hypothesis of Dessler et al., (1995), the transport of tropospheric air into the stratosphere is possible. Indeed, satellite observations of the SF6 concentrations along the deep branch of the BDC in the NH midlatitudes displayed an overall positive trend (Haenel et al., 2015), and agreed well with the +0.24 year decade\(^{-1}\) previously proposed for 30 to 50ºN at 24 to 35 km implying that the relative contribution of eddy mixing is increasing (Engel et al., 2009). Modeled contributions of tropospheric eddy mixing were most evident in the NH midlatitudes above 20 km, resulting in up to a 2-year increase in the mean AoA, as opposed to AoA variability in the tropics that is almost completely governed by RC (Ploeger et al., 2015). The quasi-biennial oscillation (QBO) describes the shift of stratospheric winds from easterly to westerly phases...
governed by the propagation of Rossby waves (Plumb and Bell, 1981), which influences RC and eddy mixing effects on AoA (Ploeger et al., 2015). During the QBO easterly phase, NH midlatitude AoA is at a minimum, but as local eddy mixing increases during the westerly phase, and AoA values are oldest just before returning to the easterly phase (Ploeger et al., 2015).

In addition to BDC and eddy mixing, episodic events can also transport tropospheric air into the stratosphere. Biomass burning (Glatthor et al., 2013), thunderstorms (Wang, P., 2003), and even relatively weak volcanic activity coupled with convective monsoon systems (Bourassa et al., 2012; Clarisse et al., 2014), have all been attributed to the tropospheric exchange of air into the stratosphere. A recent case study detected in situ cirrus cloud particles in the midlatitude stratosphere suggests that local, small-scale events may have important implications in stratospheric chemistry and radiative forcings (Müller et al., 2015).

Stratospheric parcels of air can be also transported downward into the local troposphere (Gettelman et al., 2011). Jurkat et al., (2014) measured ozone (O₃) and nitric acid concentrations to detect two way mixing of air across the tropopause over west Africa (25° to 32°N) during August and September 2012. The intrusion of stratospheric air containing high O₃ concentrations negatively affects human respiratory and pulmonary health, and in 2015 the U.S. Environmental Protection Agency strengthened its National Ambient Air Quality Standard by lowering the acceptable baseline O₃ concentrations from 75 to 70 ppbv (EPA, 2015). Stratospheric intrusions are of particular importance to high elevation (>1.4 km ASL) sites in the western United States, accounting for 43% of the variability in surface O₃ observation from 1990-2012 (Lin et
al., 2015a). Excluding years impacted by the large-scale stratospheric injection from Mt. Pinatubo (1992-2012), stratospheric intrusions contributed to 15 to 25 ppbv to the springtime surface O$_3$ concentrations over the western United States (Lin et al., 2015). These intrusions occurred most frequently from April to May during years following La Niña winters (Lin et al., 2015), when ocean surface temperatures are ≥0.4 °C cooler than the long term average temperatures for August through October (Ternberth, K. 1997). Although the intrusions do not frequently reach the CBL during El Niño, periods, stratospheric intrusion still significantly influences O$_3$ in the free troposphere (Lin et al., 2015). There is a multitude of evidence that frequent exchanges of air occur across the midlatitude tropopause, but because of the lack of bioaerosol concentrations above the free troposphere, we cannot predict how these events may impact the transport of bioaerosols.

4.0 Previous studies of bioaerosols above the CBL

Over the last decade, there have been several studies that focused on the survival and diversity of bioaerosols above the CBL. Viable bacteria, predominantly spore formers, were cultured from air samples collected in the western United States (U.S.) that were transported from Asia in the troposphere (Smith et al., 2012). DeLeon-Rodriguez et al., (2013) observed an average concentration of $1.5 \times 10^5$ cells m$^{-3}$ over the continental U.S. and Gulf of Mexico, and estimated 60 to 100% were viable based on stains that detect membrane integrity. The ability of the plant pathogen $P.$ syringae to survive aerial dispersal is a critical component for its ability to find new habitats (Morris et al., 2008). Additionally, clouds have been identified as low temperature (-15 to 10 °C; Amato et al., 2007), aquatic reservoirs (average 98% RH; Bowers et al., 2009) of bioaerosols, with
concentrations ranging from 0.06 to $1.2 \times 10^5$ cells m$^{-3}$ of cloud volume (Amato et al., 2005; Bauer et al., 2002; DeLeon-Rodriguez et al., 2013). The culturable fraction of the cloud population is dependent upon time and location of sampling and varies from 0.02 to 2.2% (Amato et al., 2005; Amato et al., 2007; Bauer et al., 2002). The extensive collection of isolates recovered from clouds has permitted laboratory investigations into their physiology and potential for aerial dispersal (Amato et al., 2005; Amato et al., 2007; Vaïtilingom et al., 2012). Many of these bacteria demonstrate the ability to metabolize organics commonly found in clouds (i.e., acetate, formate, succinate, lactate; Amato et al., 2005). Under simulated cloud conditions, the microbial rates of organic degradation rivaled those of photodegradation, suggesting they may play an important role in cloud chemistry (Vaïtilingom et al., 2011). While information of predicted survival of laboratory strains during aerial transport is widely available (Lin and Li, 2012; King et al., 2011; Ko et al., 2000), very little information exists for isolates originating from atmospheric samples. Cloud isolated strains of *P. syringae* and *P. fluorescens* (Vaïtilingom et al., 2012), were evaluated for their ability to survive aerial dispersal in a cloud simulation chamber (Amato et al., 2015). Based on these experiments, it was predicted that only 1 in $10^6$ cells would survive the desiccation encountered during the predicted atmospheric residency time of 3.4 days (Amato et al., 2015). Survival data such as this allows for more accurate predictions of dispersal patterns, and the potential for microorganisms to survive atmospheric transport and colonize new habitats.

In addition to potentially influencing cloud chemistry, bioaerosols may also contribute to the formation of clouds of and precipitation by serving as CCN and IN particles, respectively. Biological IN particles are able to trigger the formation of ice at
temperatures as warm as -2 °C (Christner et al., 2008) to -10 °C (Stopelli et al., 2015), whereas mineral particles are only have IN activity at temperatures below -10 °C (Lohmann, U., 2002). Bowers et al., (2009) found the average concentration of IN particles was significantly greater in clouds as opposed to clear skies. Total cell concentrations in clouds ranged from 0.06 to 71 IN m\(^{-3}\) active from -6 °C to -14 °C, with >99.3% of the IN activity at temperatures above -10 °C attributed to biological IN particles (Joly et al., 2014). Although the fraction of bacteria capable of IN activity may be as low as 1 in 10\(^5\) cells (Xia et al., 2013), as few as 10 IN particles m\(^{-3}\) are predicted to be sufficient to catalyze freezing Crawford et al., (2012). When compared to the total particle (Stopelli et al., 2015) and cell (Stopelli et al., 2017) concentrations in snow, biological IN particles are more efficiently removed by ice nucleation activity and wet scavenging during precipitation events. The preferential removal of biological IN particles, such as \(P.\ syringae\), may reduce atmospheric residency times, thereby increasing chances for survival during aerial dispersal (Stopelli et al., 2017).

Determining the vertical distribution of bioaerosols and the high altitude limit for microbial life represent outstanding questions for bioaerosols research (Ohno et al., 2017). The sampling of bioaerosols above the CBL poses the challenges of access to the sample as well as low concentrations of bioaerosols observed in the troposphere (Ch. 3). Table 1 describes the advantages and disadvantages of various sampling methodologies that have been used for bioaerosol sampling in the free troposphere and stratosphere. Mountain top research stations offer the advantage of being able to sample without the need of aircraft and the ability to use off the shelf aerosol technology. Observatories such as the Storm Peak Laboratory (Bowers et al., 2009; Wiedinmyer et
al., 2009), Mt. Bachelor Observatory (Smith et al., 2012; Smith et al., 2013), the Atmospheric Research Station at Puy de Dôme (Amato et al., 2007; Vaïtilingom et al., 2012) and the Research Station Jungfraujoch (Stopelli et al., 2015; Stopelli et al., 2016; Stopelli et al., 2017) have instrumentation to monitor atmospheric chemistry and meteorological conditions, allowing for the characterization of the air masses carrying bioaerosols. However, these mountain top observatories are limited to a single sampling location and altitude. Aircraft based measurements allow for bioaerosol sampling from specific atmospheric conditions, such as hurricanes (DeLeon-Rodriguez et al., 2013), and at lower altitudes in the stratosphere (Griffin D., 2004, Smith et al., 2010; Yang et al., 2008a). However, since the collection efficiency of the samplers (DeLeon-Rodriguez et al., 2013; Yang et al., 2008a) and volume collected (Griffin D., 2004; Smith et al., 2010) was not determined, it is not possible to accurately infer bioaerosol concentrations from these data. Balloon based platforms have also been used with a variety of sampling devices to sample microorganisms in high altitude air masses. The spatial resolution of sample collection is limited either to the vertical path of the balloon on the ascent, or its horizontal journey at a float altitude (Ch. 3). Similar to aircraft observations, data for collection efficiency and volumes sampled are sparse (Harris et al., 2002; Wainwright et al., 2003; Yang et al., 2008b). Although previous efforts have been successful in the recovery of viable microorganisms from stratospheric altitudes (Griffin, 2004; Griffin, 2008; Harris et al., 2002; Imshenetsky et al., 1978; Shivaji et al., 2006; Smith et al., 2010; Yang et al., 2008), there have been concerns about surface contamination and a lack of procedural controls to verify these data (Smith, D. J., 2013).
<table>
<thead>
<tr>
<th>High altitude sampling technologies</th>
<th>Collection substrate</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Collection efficiency</th>
<th>Other factors</th>
<th>Refs.</th>
</tr>
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<tbody>
<tr>
<td>Pump</td>
<td>Filter</td>
<td>Reduced pressures result in higher efficiency.</td>
<td>Loss of viability over the course of sampling.</td>
<td>Dependent upon inlet conditions.</td>
<td>Sample volume, efficiency.</td>
<td>Griffin D., 2004; Ohno et al., 2011; Griffin-Rodiguez et al., 2008a; Stahl et al., 2011; Chalmers et al., 2013; Del Carmen-Rodiguez et al., 2010.</td>
</tr>
<tr>
<td>Impaction</td>
<td>Liquid</td>
<td>Sample concentrated into small volume.</td>
<td>Sample is concentrated into small volume.</td>
<td>Collection efficiency often not determined.</td>
<td>Collection efficiency often not determined.</td>
<td>Griffin et al., 2011.</td>
</tr>
<tr>
<td>Impaction</td>
<td>Plates</td>
<td>Large surface area can lead to increased background contamination.</td>
<td>Large surface area can lead to increased background contamination.</td>
<td>Easy to determine volume sampled.</td>
<td>Easy to determine volume sampled.</td>
<td>Ohno et al., 2017; Smith et al., 2010.</td>
</tr>
<tr>
<td>Impaction</td>
<td>Rods</td>
<td>Highly efficient for wide range of particle sizes.</td>
<td>Highly efficient for wide range of particle sizes.</td>
<td>Loss of viability over the course of sampling.</td>
<td>Low collection efficiency for aerosols &lt; 10 µm.</td>
<td>Yang et al., 2008a; Griffin et al., 2011; Griffin-Rodiguez et al., 2010.</td>
</tr>
<tr>
<td>Impaction</td>
<td>This study (Ch. 3)</td>
<td>Small area resulting in reduced pressures result in small sample volume.</td>
<td>Small area resulting in reduced pressures result in small sample volume.</td>
<td>Efficiency dependent upon inlet conditions.</td>
<td>Efficiency dependent upon inlet conditions.</td>
<td>Yang et al., 2008a; Griffin et al., 2011; Griffin-Rodiguez et al., 2010.</td>
</tr>
</tbody>
</table>

Table 1: Currently available technologies for the collection of bioaerosols and their relevance to high altitude sample collection.
5.0 The stratosphere as a Mars analogue environment

Many terrestrial environments have been argued to be analogs for conditions on Mars; however, only the stratosphere provides the unique combinations of conditions (high UV fluence and decreased RH and pressure) present on the surface of Mars. The hyper-arid Atacama Desert, Chile, with low levels of organic molecules (Azua-Bustos et al., 2015; Navarro-González et al., 2003) and low mean RH values in the atmosphere (17%) and soil (14%), is often used to simulate the surface environment of Mars (Azua-Bustos et al., 2015). Likewise, the Antarctica Dry Valleys are also used in astrobiology studies because they are cold and arid locations with landforms and soil characteristics comparable to the Mars surface (Marchant and Head, 2007; Musilova et al., 2015). While such locations may recreate the soil characteristics and temperature environments similar to Mars, neither fully replicates the desiccating and UV radiation conditions present.

The Rover Environmental Monitoring Station (REMS) on the Curiosity rover in Gale Crater on Mars (~4.59°S, 137.44°E, -4.5 km elevation; Gómez-Elvira et al., 2014) reported pressure values that ranged from 7.0 to 9.5 mbar during the first 1160 sols (Martínez et al., 2016), and these data are highly similar to pressure values reported for 31 to 32 km (9.6 mbar, Khodadad et al., 2017; 8.4 mbar, AMA soundings Ch. 3). REMS also monitors temperate and RH, at sunrise, when temperatures are at the lowest (-76 °C), RH reaches between 45 to 49% (Saviji et al, 2015). As temperature increases during the sol, RH drops below 1%, only to rise again after sunset, reaching a maximum of 9 to 13% near midnight (Saviji et al, 2015). Data compiled from the first 1160 sols indicated cycles of annual variation, with RH values that peak near 70% in the winter, and drop below 10% in the late spring and early summer (Martínez et al., 2016). The total UVABC
environment (200-380 nm) of Gale Crater is also recorded by REMS, and data from sol 100 indicate total fluence peaks at values near 20 W m$^{-2}$, a value that decreased to 15 W m$^{-2}$ on sol 96 during a regional dust storm (Gómez-Elvira et al., 2014). More recently Martínez et al., (2016) have extended the UV data set to the first 1160 sol and calculated the total surface dose of UVABC at Gale Crater to range from 0.7 to 1.1 MJ m$^{-2}$ sol$^{-1}$.

Planetary protection is defined as the pursuit of knowledge of our solar system in a manner that does not introduce terrestrial microorganisms to extraterrestrial environments, safeguarding the pristine space environment for the future study of “life form, precursors, or remnants” (COSPAR, 2011; Rettberg et al., 2016). The policies of planetary protection must be dynamic, and should constantly evolve as new data become available through exploration (Rettberg et al., 2016). The stratosphere provides a unique UV environment that mimics both the wavelengths and diurnal variations encountered on the surface of Mars, conditions that are not yet possible to replicate under laboratory settings (Dartnell et al., 2012; Khodadad et al., 2017). The stratosphere is the best representation of a Mars analogue environment, and future investigations conducted here could help to construct updated policies and procedures. Such investigations may include extensions of the microbial inactivation study of Khodadad et al., (2017) to include different spacecraft materials and geometries (Schuerger et al., 2003), the presence of soils to provide shielding from UV (Hansen et al., 2009), or the inactivation of UV-C tolerant microorganisms, including those not capable of forming endospores (described in Ch. 4). Additionally, these inactivation experiments could assess total cell concentration levels. The current bioburden protocols are limited to heat resistant endospore concentrations (NASA, 2010), which may grossly underestimate the total concentration.
of microorganisms associated with spacecraft materials. With data collection capabilities from the stratosphere that occurs on the order of hours to days, analyses and interpretation of results do not have to be postponed for years to account for spacecraft travel to Mars.

In addition to the detection of intact microorganisms, certain biogenic molecules, referred to as biosignatures, can serve as evidence of past or present life (reviewed by Botta et al., 2008; Summons et al., 2011). Molecular biosignatures include, but are not limited to, enantiomers of amino acids, nucleic acids, proteins, lipids, carbohydrates, polycyclic aromatic hydrocarbons (PAHs), and ATP (Summons et al., 2014). Quantifying rates of biosignature degradation due to UV exposure in the stratosphere could be useful for guiding the design of future sampling life detection missions (Dartnell et al., 2012; Poch et al., 2014). Dartnell et al., (2012) predict a 10-hour window on Mars to sample for recently exposed subsurface PAHs before a significant reduction in fluorescence. Similar efforts have defined optimal sampling windows for glycine (Poch et al., 2014; ten Kate et al., 2006). After UV exposures equivalent to 80 sols on the surface of Mars, concentrations of DNA, amino acids, and microbial viability were significantly reduced even when shielded by 1.5 mm of permafrost, however there was no significant reduction of the total organic carbon (Hansen et al., 2009). Under simulated Mars conditions, the UV mediated rate of destruction of extracellular ATP would take 10 orders of magnitude longer than the rate of bacterial endospore inactivation, indicating biosignatures may remain well after the complete destruction of viable microorganisms (Schuerger et al., 2003). The search for extraterrestrial biosignatures may be hindered by the persistence of these contaminating molecules from earthly microbes on spacecraft surfaces, even after
their inactivation by UV-C (Schuerger et al., 2003). Without updated decontamination procedures to limit the both the viable microorganisms and the molecules, the detection of a signal above this background is a formidable challenge (Summons et al., 2014). Therefore a thorough understanding of how these molecules react under extraterrestrial, and in particular Mars-like conditions, could be used to constrain the risk of forward contamination and likelihood of returning a false positive life detection result.

6.0 Objectives of this study

Despite increased research on bioaerosols at altitudes above the CBL, there are no data to understand their vertical distributions in the troposphere and stratosphere. There are also outstanding questions concerning the potential of microbes to survive high altitude transport and disperse to new environments. To address this knowledge gap, this study developed a helium balloon payload that sampled bioaerosols at altitudes of up to 36 km. Decontamination protocols and quantitative procedures were developed to determine levels of residual contamination incurred prior to, during, and after flight in order to establish levels of detection and confident quantification of the signal above background. The collection of bioaerosol samples provide the first evidence of the microbial contribution to the total aerosol population above the free troposphere. The repeated recovery of culturable isolates from sampled collected between 6.1 to 26 km allowed investigations into the tolerance of these species to desiccation and UV-C. These data were used to examine the potential of such species to survive high altitude aerial dispersal in the stratosphere to assess if this region should be considered a portion of Earth’s biosphere.

1.0 Introduction

Microorganisms aerosolized from the Earth's surface are transported both vertically (Fulton, 1966) and horizontally (Burrows et al., 2009; Griffin et al., 2001) in the atmosphere. Cells with an aerodynamic diameter less than 10 µm are geographically disseminated over long distances and have been shown to retain their viability after intercontinental transport (Griffin et al., 2006; Hara and Zhang, 2012; Murata and Zhang, 2014; Smith et al., 2012; Smith et al., 2013). Certain microbes may even retain their metabolic function in the atmosphere (Sattler et al., 2001; Vaïtilingom et al., 2011) or have active roles in meteorological processes such as ice nucleation (Christner et al., 2008; Joly et al., 2014) or cloud formation (Bauer et al., 2002; Pöschl et al., 2010).

Sampling microorganisms in the troposphere and stratosphere is challenging, and consequently, there are very few data available on the concentration and nature of microbial life in the high atmosphere. There are reports of viable microorganisms isolated from samples collected from 20–77 km above sea level (ASL; hereafter, all altitudes referenced above sea level; Griffin, 2004; Griffin, 2008; Harris et al., 2002; Imshenetsky et al., 1978; Shivaji et al., 2006; Smith et al., 2010; Yang et al., 2008); however, there are quantitative data only for altitudes below 10 km (Amato et al., 2005; DeLeon-Rodriguez et al., 2013; Huffman et al., 2010; Huffman et al., 2012; Vaïtilingom et al., 2012).

“This chapter 2 previously appeared as [N.C. Bryan, M. Stewart, D. Granger, T.G. Guzik, B.C. Christner, A method for sampling microbial aerosols using high altitude balloons, 2014]. It is reprinted by permission of Noelle Bryan—see Appendix B.”
Conclusions based on observations of microbial growth from samples collected at altitudes at or above 41 km (e.g., Wainwright et al., 2003; Imshenetsky et al., 1978) have resulted in extraordinary claims for the tenacity of life in the stratosphere and mesosphere. However, the aforementioned studies have lacked rigorous measures to exclude the possibility of microbial contamination, and to date, these observations have not been verified.

Here we report on an autonomous balloon sonde system for sampling bioaerosols to altitudes in the stratosphere. Environmental conditions at high altitude make standard aeromicrobiological sampling approaches challenging, necessitating the use of technology that functions under low extremes of pressure and temperature. Since the concentration of bioaerosols is anticipated to decrease with altitude, high sensitivities are required to exceed the signal to noise ratio threshold. Therefore, reduction and assessment of microbial contamination associated with system components and sampling substrates are a very relevant aspect of these measurements. We discuss the application of our bioaerosol sampling approach for studies interested in examining the geographic boundaries of microbial dispersal via the atmosphere, defining the upper altitude limits for life in the biosphere, and assessing habitability in extraterrestrial atmospheres.

2.0 Materials and methods

2.1 Balloon vehicle

The vehicle used to carry the Life's Atmospheric Microbial Boundary (LAMB) bioaerosol sampling payload to stratospheric altitudes up to 38 km consisted of a 2.0-kg latex sounding balloon, a parachute, a flight termination unit, the primary radio beacon, and a video camera. The total weight suspended below the balloon is about 5.4 kg and all
balloons were inflated with helium to achieve an initial ascent rate of about 350 m per minute. The balloon carried all components up to a pre-programmed cut altitude, at which point the flight termination unit triggers. When triggered, the termination unit melts a nylon string connecting the balloon to the top of the parachute, releasing the balloon and allowing the payload components to descend by parachute for recovery.

The primary radio beacon includes a Trimble Copernicus II global positioning system (GPS) receiver and Byonics Micro-Trak RTG FA High Altitude Combo transmitter to report the real-time latitude, longitude, and altitude of the balloon vehicle throughout the flight. The beacon broadcasts these data using an Automatic Packet Reporting System (APRS) communication on frequency 144.390 MHz. This system allows the balloon vehicle to be continuously tracked over a very wide area from a fixed ground location or mobile station. During payload flight operations, two ground vehicles were outfitted with a radio transceiver and laptop tracking system capable of receiving the APRS packets and mapping the beacon location. This enabled the ground crew to follow the balloon vehicle and quickly reach the landing site for payload recovery.

Located above the LAMB payload is a Kodak Zx1 HD camera. This down-facing camera records high definition video of the payload throughout the flight. The video provides visual evidence to verify the successful opening and closing of the sampling chambers, as well as diagnostic information when malfunctions occurred.

2.2 The LAMB payload

The LAMB payload that was flown for all experimental campaigns is shown in Fig. 1. The following sections provide details about the construction and operation of this payload.
2.2.1 Mechanical system

The main structural support of the payload is centered on the electronics box (Fig. 1), an aluminum framed structure which houses the control electronics, flight power supply, data storage, linear actuators for opening and closing sample chamber doors, and a GPS receiver and radio beacon for real time transmission of position and altitude co-ordinates. The dimensions of the box are approximately 25 cm ×25 cm and 20 cm high. Each sampling chamber was constructed of milled aluminum and the inner cross-sectional area of the chamber is 45 cm$^2$ (Fig. 1). The chambers are mounted between a

Figure 1: A top and side view of the LAMB payload. The central electronics box includes the flight control, monitoring and power systems. Each payload includes two sampling chambers (one on the right and one on the left) and each chamber has two doors (top and bot- tom) that are operated by linear actuators. The top view shows the top door on the right hand side fully retracted exposing the sampling rods and rod holders. The third procedural control chamber is not pictured here. The scale bar is in cm.
pair of side rails, which are then connected to the electronics box. The upper and lower doors slide along a pair of grooves milled into the rails. The fixed ends of the actuators are connected to a center post in the electronics box and the moveable shafts of the actuators are connected to the chamber doors. Retracting the actuator shafts opens the chamber while extending them closes and seals the chamber. Teflon™ coating was applied to reduce the sliding friction between the doors and the side rails.

Each detachable sampling chamber holds forty Rotorods® (IMS Health, Inc.), hereafter referred to as rods, that are secured in place by a rod holder shown in the top view image in Fig. 1. Each rod holder (6.0 cm × 1.3 cm) secures twenty rods spaced 0.25 cm apart, and two rod holders are staggered in each chamber. The rod holder position is maintained by shallow grooves milled on either side of the inner-chamber walls and is fixed in place by screws. Each rod (22 mm × 1.6 mm) has an impact-sampling surface of 35 mm². O-rings placed in grooves milled on the top and bottom surfaces of the chamber walls provide a seal when the doors are closed. A threaded Luer lock fitting was attached to the side of each chamber to accommodate a 0.22 µm syringe filter, allowing pressure equalization between ambient and the chamber interior when the chamber is sealed. A third, structurally identical control chamber, was flown in addition to the sampling payload (Fig. 1) for each mission.

The LAMB payload was modified for use on the High Altitude Student Platform (HASP, http://laspace.lsu.edu/hasp), a zero-pressure, high altitude balloon, and sampled for 8.4 h at 38 km in 2013. Procedural controls from eight flights during 2013, including HASP, were analyzed according to the methods described below.
2.2.2 Electronics and power supply

The LAMB payload electronics consist of a control and power system. The control system is responsible for monitoring payload altitude, actuator shaft position, actuator temperature, external temperature, internal temperature, external relative humidity, as well as controlling the opening and closing of the chamber doors at pre-programmed altitudes. The control system is comprised of an Arduino MEGA 2560 microcontroller, a custom GPS receiver–micro secure digital (SD) storage board, and an actuator control board. The boards are stacked and communicate across a bus interface. Connected to the control system's GPS unit is the secondary beaconing system, which is embedded in the payload and provides real time transmission of position and altitude coordinates. Like the primary, it is also a Byonics Micro-Trak and is specifically used as a backup to the primary beaconing system should it fail. This secondary beaconing system is also used to enhance position resolution as the payload nears the ground upon descent.

The Arduino MEGA 2560 microcontroller executes all instructions, digitizes up to 16 analog signals, controls all actuator input/output (I/O) functionality, and communicates with the external serial devices. It is also responsible for executing instructions based upon input from the GPS receiver, temperature and relative humidity sensors, as well as actuator shaft position data. All flight sensor and diagnostic data managed by the Arduino is written to files on the microSD card for post-flight analysis.

The actuator control board controls the four linear actuators (one for each chamber door). Power to each actuator is provided through an h-bridge integrated circuit chip, allowing the actuator to be extended and retracted by reversing the polarity between
power and ground. A pair of I/O lines to the h-bridge allows control of the polarity for each actuator.

System power is provided by 10 lightweight Energizer Ultimate Lithium “AA” batteries wired in series to supply 15 to 18 VDC to power all payload components, including the electronics and actuators. During flight, the average current draw from the batteries is 130 mA, but can range from 120 mA to 1300 mA depending upon whether the radio transmitter and door actuators are active. The battery pack is specified to supply 3.0 Ah at 0 °C (Energizer®, designation: ANSI 15-LF, IEC- FR6), which taking into account the duty cycles of the various powered components, allows the payload to operate autonomously for about 20 h.

2.2.3 Flight software

The payload is controlled by an embedded system and is designed to operate autonomously, making operational decisions based upon the data collected by the control system. At the heart of the control system is the Arduino MEGA microcontroller. A logic flow chart describing the software that runs on the microcontroller is shown in Fig. 2. The primary functions of the flight software are to collect positional information from the GPS receiver, command the linear actuators to open or close the sampling chamber doors at programmed altitudes, and to ensure that the doors are closed during payload or balloon failure modes. Further, the software records data from the multiple temperature sensors and a relative humidity sensor, with archiving by the software occurring every 3 s. Errors are logged together with critical information as they occur, so that in-flight problems can be analyzed after the flight. The flight control system software continuously polls the GPS receiver for a position string that contains, among other information, the longitude, latitude and altitude of the payload. A new position string should be available to the control system once every 3 s. Once the string is received, it is
parsed, scanned for errors, and made sure to contain accurate positional data before it is logged and deemed usable. Only then is the position data used to determine the direction of motion of the payload, which requires several consecutive position strings that report motion in the same direction. The sample chamber doors are opened only when the direction of motion is determined to be ascending, the minimum sampling altitude has been exceeded, the maximum sampling altitude has not been exceeded, and at least one valid position packet has been received within the last minute. If the doors are already open, they will be closed if the direction of motion is determined to be descent or if a valid position string has not been received for over 1 min (Fig. 2). The system timestamps and logs ‘event’ records, status updates, and errors to the control system's on-board microSD card. The events are logged periodically while the errors and updates are logged as they occur. Timestamps are obtained from the GPS disciplined free-running system clock, which is zeroed upon reboot.

The time stamped archived data provides a complete and accurate timeline of the flight and provides information such as when doors are opened and closed, error diagnostics, and environmental parameters. The event record contains the following information: the last position packet data, each door position recorded in millimeters, all actuator motor temperatures, interior payload temperatures, exterior payload temperature, and exterior relative humidity. The errors that are logged during normal execution of the code include: “microSD card initialization failure”, “GPS position string buffer empty for over some time period”, “GPS position string buffer out-of-bounds error”, “position string parse error”, “position string incorrect checksum error”, “checksum calculation error”, “descent warning — closing all doors”, “unable to open/ close door to its proper
position”, and “too much time has passed since the receipt of the last good positional packet — closing all doors”. Some examples of status updates are: “door 1 was opened”, “a new file was created on the microSD card”, “a position string was received from the GPS receiver”, etc.

Figure 2: Flow chart describing the logic flow of the autonomous embedded flight control system software. The system relies on accurate GPS data in order to open and close sampling chamber doors at predetermined altitudes. Errors are handled through the normal logic flow of the software. For example, if too much time passes since the last good GPS packet was received, the sampling chamber doors are closed. Once GPS packets are received again, the logic dictates that the doors will be opened if the payload is ascending and is in sampling range.
2.2.4 Component and payload testing

To ensure that the pressure equalization of the sample chamber was through the syringe filter, each sampling chamber was sealed and a leak test was performed on all chambers. The chamber leak rate varied for each, but was always less than 0.05 kPa per min. The flow rate through the filter was determined to be approximately 5.0 kPa per min, two orders of magnitude greater than the leak rates measured for the sealed chambers.

Preflight system tests were also performed in a thermal vacuum chamber to simulate the low pressure and temperature conditions that the payload encounters during flight. During testing in the thermal vacuum chamber, the chambers were commanded opened and closed repeatedly and shown to operate correctly down to pressures of 1.1 kPa. In addition, a series of low temperature tests were conducted in a freezer to evaluate the effectiveness of the payload insulation to keep the payload electronics operational.

2.3 Microbiological analysis

2.3.1 Bacterial strains and culturing conditions

Cultures of *Bacillus atrophaeus* (ATCC 9372) were grown aerobically with shaking (250 rpm) overnight in 5.0 mL of tryptic soy broth (TSB, DifcoTM, cat. no.: DF0370-17-3) at 20 °C. An aliquot of *B. atrophaeus* was plated onto Schaeffer's sporulation media (Schaeffer et al., 1965) and incubated for five days at 20 °C. The presence of endospores was verified by phase contrast microscopy. The cell and endospore populations were harvested by centrifugation for 10 min. at 17,000 ×g and suspended in 1.0 mL of phosphate-buffered saline (PBS), followed by a second wash with 1.0 mL of PBS. *Deinococcus radiodurans* (ATCC 13939) was cultured aerobically
overnight in 5.0 mL of TSB with shaking at 20 °C; the cells were harvested and suspended as described above. Suspensions of each bacterial culture were applied to the surface of triplicate rods and allowed to air dry for 1 h within the laminar flow hood.

For the flight control rods, the presence of viable microorganisms was assessed by placing the silicone-coated portion of each rod into a culture tube containing the liquid media R2A (DifcoTM, cat. no.: 218262), 1% R2A, TSB, and 1% TSB amended with 100 µg mL\(^{-1}\) cycloheximide (Acros Organics, cat. no: AC35720010) to inhibit the growth of fungi (Ha et al., 1995). Each rod was placed in media within 12 h of recovery and incubated at 4 °C (\(n = 160\)) for 60 days. Enrichment cultures were then shifted to 20 °C and monitored for 21 days. Over the course of 81 days, aliquots of the enrichments cultures were plated onto solid media (R2A and TSA) and monitored for colony growth at 20 °C.

2.3.2 Silicone coating of the sampling rods

All sides of the sampling rods were exposed to \(1.3 \times 10^5 \mu\text{W cm}^{-2}\) of germicidal ultraviolet radiation (UV-C; 254-nm) for 20 min., soaked in 2000 ppm sodium hypochlorite for 20 min., rinsed with 70% (v/v) ethanol, and allowed to dry in a laminar flow hood (Labconco, cat. no.: 3612504) for 1 h. The rods were subsequently coated in silicone grease (IMS Health, Inc.) by the method of Frenz et al. (2001). After 24–48 h, the rods were exposed to ethylene oxide (450–650 mg L\(^{-1}\)) for 4 h at 55 °C and a relative humidity of 30–50% (Ecosterile, Inc.).

2.3.3 Decontamination of rods inoculated with bacteria

Three rods containing cells of each bacterial strain were placed into 1 mL of PBS and serially diluted to determine the number of cells that remained viable after air-drying.
This value was designated as the initial colony forming unit (CFU) concentration that each decontamination treatment was compared to. Ten-fold serial dilutions of each culture were spread plated onto tryptic soy agar (TSA, DifcoTM, cat. no.: 236590) and incubated at 20 °C. The total number of CFU was recorded after 72 h of growth. The average initial concentration of CFU for the individual treatments of ethylene oxide, UV-C, and sodium hypochlorite were $9.7 \times 10^5$ ($\pm 8.7 \times 10^4$, standard deviation, $n = 3$) and $1.7 \times 10^6$ ($\pm 6.0 \times 10^4$, $n = 3$) CFU rod$^{-1}$ for *B. atrophaeus* and *D. radiodurans*, respectively. For the combined treatment, the average initial concentrations were $3.0 \times 10^5$ ($\pm 4.4 \times 10^4$, $n = 3$) and $4.9 \times 10^7$ ($\pm 6.8 \times 10^6$, $n = 3$) CFU rod$^{-1}$ for *B. atrophaeus* and *D. radiodurans*, respectively. Additionally, inoculated rods were exposed to one of four treatments: ethylene oxide, UV-C, sodium hypochlorite, or a combined treatment of UV-C, sodium hypochlorite, and ethylene oxide. The samples were then placed into sterile Petri dishes and stored at 20 °C for 24 h.

After each treatment, cells on the rods were dislodged from the silicone grease by placing the rods into 1.0 mL of sterile PBS and vortexing the mixture for 10 min. The three replicates were ten-fold serially diluted and plated onto TSA, incubated at 20 °C, and the number of CFU was determined after 72 h. Assuming 100% removal of cells from the rods during vortexing and considering the number of CFU required for a standard dilution plate (i.e., between 30–300 CFU on a 100 mm diameter plate), the limit of quantification for our method was 300 CFU rod$^{-1}$. For samples that had <300 CFU, a value of 29 was substituted (Eaton et al., 1998), allowing calculation of the reduction in culturable cells for all treatments. The log reduction of CFU (or mol ATP; see below) after decontamination was calculated by subtracting the mean log density of the treated
samples from the mean log density of the untreated, air-dried rods.

2.3.4 External cleaning of the chambers after recovery

Once the payload was recovered, all chambers were inspected to ensure that the doors had maintained a seal. The chambers were subsequently removed from the payload base and placed in clean plastic bags for return to a clean area for processing. All chambers were manipulated and opened in a portable laminar flow hood (Fungi Perfecti, LLC, Item no.: E-ALFH1). Prior to opening the chambers, all exposed surfaces were wiped down with 70% (v/v) ethanol to remove surface dust and debris encountered during flight and landing. The rod holders were aseptically removed from the chambers and placed into sterile Petri dishes for immediate processing (ATP analysis and culturing) or stored at −20 °C for subsequent analysis.

2.3.5 Direct counting of cells via epifluorescence microscopy

The number of DNA-containing cells on each flight control rod was estimated by staining with equal volumes of 0.22 µm-filtered Tris–borate–EDTA (1×) buffered SYBR™ Gold (Molecular Probes, Inc., cat. no. S-11494) and an antifade solution (0.1% phenylenediamine in a 1:1 solution of PBS and glycerol). A rod stage adaptor (IMS Health Inc.) was used to hold the samples and a 10 µL aliquot of the stain: antifade solution was applied directly to the surface of the rods, followed by the application of a coverslip. The samples were stained for 20 min. in the dark. The cells were visualized with an Olympus bx51 epifluorescence microscope. For each rod, sixty random fields of view (FOV) were counted, with each FOV representing $2.8 \times 10^4 \mu m^2$. Fluorescing particles smaller than 0.7 µm were not counted because they were difficult to distinguish from autofluorescing particulates. The cell concentration per rod was calculated from the
average number of cells per field and extrapolated to the total sampling area. The cell densities on 21 flight control rods, obtained from the seven August 2013 sampling missions, were determined using this method.

### 2.3.6 Measurement of the cellular adenosine triphosphate (ATP) concentration

To measure the concentration of ATP in cells attached to the inoculated and flight control rods, samples were analyzed using an ATP Biomass Kit HS (Biothema, Inc. cat. no. 266–112). An ATP solution (100 nmol L$^{-1}$) supplied by the manufacturer was diluted into 0.22 µm filtered, autoclaved deionized water (DIW) to generate a standard curve. Free ATP was removed by placing the rod into a 1:1 solution of 0.22 µm filtered, autoclaved DIW and ATP eliminating reagent (Biothema, Inc.), followed by incubation for 17 min at 20 °C. Cellular ATP was then extracted according to the manufacturer's instructions, the luciferase enzyme solution was added to the sample, and luminescence was measured using a 20/20n luminometer (Turner Biosystems, cat. no.: E5331). The light produced was measured in relative light units (RLUs) and converted to ATP concentration using the standard curve. Based on the addition of $1.0 \times 10^3$ pmol of the ATP standard to each sample, there was no sign of inhibition of the reaction from the samples when compared to the addition of the standard to water. A total of 96 flight control rods from seven flights during August 2013 were quantified for cellular ATP within 12 h of sampling.

### 2.3.7 Total particle concentration and characterization

Six rods from the August 2013 flight controls were prepared for scanning electron microscopy by sputter coating with gold/palladium for 4 min to apply a layer that was approximately 1.0 Å thick. Particles embedded in the silicone grease were enumerated
using a JSM-6610LV scanning electron microscope under the high vacuum mode (−0.8 kPa). Sixty randomly chosen FOV (each FOV represents $1.3 \times 10^4 \mu m^2$) for each rod were examined at a voltage of 10–12 kV. A digital image of each FOV was captured and the particle boundaries were traced and analyzed using ImageJ software (Schneider et al., 2012). The area, perimeter, major and minor axis lengths, and circularity for each particle were measured. Circularty was calculated by the following formula (Royston-Bishop et al., 2005; Schneider et al., 2012):

$$\text{Circularity} = \frac{4\pi \cdot \text{area}}{\text{perimeter}^2} \quad (1)$$

### 2.4 Statistical analyses

Statistical analyses were performed using StatPlus:mac statistical analysis software (AnalystSoft, Inc.) and SAS® University Edition (SAS Institute Inc., copyright, 2014). Student’s t-test was used to compare the mean log density of samples to the mean log density of the controls, and when the variances were not equal, the heteroscedastic Student’s t-test was used. The one-way ANOVA, with post hoc Tukey–Kramer test, was utilized to compare the effectiveness of the decontamination treatments. All data were normalized to account for differences in the initial CFU concentrations and are reported as the mean (± the standard deviation) of the log-transformed data.

To determine the limits of detections for each assay, the data were first checked for the possibility of a nonparametric distribution. The data are reported as the mean (± the standard error of the mean) calculated by the SAS software using the PROC GLIMMIX statement with the negative binomial distribution and Kenward–Roger adjustments.
3. Results

3.1 Reduction of cellular viability and ATP by the decontamination methods

To examine the effectiveness of several decontamination methods on the reduction of microbial cells and their associated molecules from the sampling rods, a series of experiments were conducted. Fig. 3 shows the log reduction in CFU and mol of ATP after rod decontamination by UV-C, ethylene oxide, sodium hypochlorite, and a combination of all three treatments. In these experiments, $10^5$ to $10^7$ CFU mL$^{-1}$ of the type strains *B. atrophaeus* and *D. radiodurans* were applied to rod surfaces and the survival of the cells and persistence of cellular ATP was quantified.

In Fig. 3, sodium hypochlorite and the combination treatments were the most effective decontamination methods for reducing culturable cells of *B. atrophaeus* (3.4 ± 0.11 log units) and *D. radiodurans* (4.8 ± 1.0 log units), as well as cellular ATP from *D. radiodurans* (0.76 ± 0.21 log units). Statistical analysis revealed that ethylene oxide was as successful as sodium hypochlorite and the combined treatments in the reduction of *D. radiodurans* CFU (Fig. 3, indicated by the treatments labeled with a lower case “a”). Ethylene oxide was significantly less effective than sodium hypochlorite and the combined treatments in the reduction of *B. atrophaeus* CFU and *D. radiodurans* ATP (Fig. 3, indicated by the lower case “b”). UV-C was the least effective decontamination treatment for either analyte. Although the concentration of CFU rod$^{-1}$ for *B. atrophaeus* was significantly reduced by the UV-C treatment (1.2 ± 0.32 log units, $p = 0.02$), rods inoculated with *D. radiodurans* were not statistically different from the CFU rod$^{-1}$ or the concentration of ATP from control samples (Fig. 3, indicated by the asterisks). A single ATP data point from the decontamination experiment (specifically, the sodium...
hypochlorite treatment; \( n = 4 \)) was deemed to be an extreme outlier (greater than three deviations from the mean) and was subsequently removed from the analysis.

### 3.2 Balloon missions

One flight test was conducted in May 2013 at the NASA Columbia Scientific Balloon Facility (CSBF) balloon launch site near Palestine, TX and seven additional flights were completed in August 2013 at the NASA CSBF launch site near Fort Sumner, New Mexico. During these flights, the payload was shown to operate correctly up to an altitude of 38 km. Following recovery of the balloon payloads, the recorded diagnostic

![Fig. 3. Reduction of bacterial culturability and ATP on rods decontaminated by ethylene oxide, UV-C, sodium hypochlorite, and a combination of all three. Each value represents the mean and the error bars indicate the standard deviation (CFU, \( n = 3 \); ATP, \( n = 5 \)). The letters a–c rank the treatments for each sample and are based on one-way ANOVA with post hoc Tukey–Kramer analysis. Asterisks represent p values that were not statistically different from the controls (\( p \geq 0.05 \); heteroscedastic Student's t-test).](image-url)
data and flight video were examined to assess payload performance. The flight video verified that the chamber top doors always opened at the pre-programmed altitude. The chamber bottom doors, however, usually required several attempts before a successful opening was achieved.

Key diagnostic parameters for one test flight are shown in Fig. 4, plotted against Mission Elapsed Time (MET) in units of hours and minutes. In panel A, the altitude profile for the test flight (solid black line) shows a fairly consistent ascent up to about 30 km, where the flight was terminated at ~1:22 MET and the payload began its descent on parachute. The external temperature data (dark gray line) shows a profile typical of these altitudes with the coldest temperatures (~48 to ~51 °C) occurring when the vehicle passes through the tropopause at 0:39 MET on ascent and again at 1:30 MET on descent. The constant temperature indicated by the flat line occurring at 1:30 MET indicates that the payload experienced colder temperatures that were out of sensor range (<−51 °C) during the descent. The internal temperature (light gray line) remained above 0 °C, consistent with the temperature time constant seen during laboratory testing.

The temperature of the linear actuators dipped as low as −35 °C and continued to operate correctly, opening and sealing the chambers in extreme cold conditions. Panel B of Fig. 4 shows the chamber door positions recorded during the flight and the sequence of chamber door operation, which was verified with the flight video. The top doors on both chambers opened as expected upon command, but when sampling in the stratosphere, there was a delay in opening the bottom doors. This effect is presumably due to chilling of the chambers as the payload passed through the tropopause, increasing the door seal resistance.
Fig. 4. Key flight diagnostic parameters from the test flight, ACES-42. The x-axis is Mission Elapsed Time (MET) in units of hours and minutes starting with launch at 0:00. Panel A shows the payload's altitude (black line), the internal temperature (light gray line) and the external temperature (dark gray line). Panel B shows the opening and closing of the chamber doors. Chamber one's doors are represented by the dark gray lines while chamber two's are represented by the light gray lines. The solid lines represent the top chamber doors while the dashed lines represent the bottom doors. Panel C of the figure shows the occurrence(s) of unexpected errors of which there was only one during this flight.

Nevertheless, a few minutes of solar heating of the bottom door after the top door opened were sufficient to resolve the problem. The plot in panel C of Fig. 4 indicates the occurrence of an unexpected error during the flight from diagnostic information recorded on-board the payload. For this flight, a single GPS error was recorded,
indicating that a valid GPS string was not properly received or processed for at least 60 but not more than 63 seconds. Had the sample chamber doors been open at the time that this error occurred, they would have closed and reopened once a normal and valid GPS string receipt resumed. However, the error occurred after the set maximum sampling altitude was exceeded, and therefore, no action was taken.

### 3.3 Determination of detection limits for cell and molecular analysis

As a result of the May 2013 flight, it was determined the flight control rods did not receive microbial contamination during flight or landing that was significantly higher than the sampling rods that never left the confines of the laboratory. Using microscopic examination, it was determined that there was not a significant difference between the cell ($p = 0.77, n = 6$) and ATP ($p = 0.06, n = 17$) concentrations between the flight and laboratory controls.

The cell, ATP, and particle concentration data distributions were positively skewed and all had variances greater than their respective means, indicating negative binomial distributions. The number of cells per rod ranged from 260 to 750 and averaged 520 ($\pm 29, n = 21$) cells per rod. A total of 530 bacterial cells were counted from 21 independent rods, subsampled from 7 different flight controls. With a mode of zero, 95% of the FOV counted contained two or fewer cells. Only two FOV contained four cells, the highest number of cells counted per FOV. Based on limits of detection at 3-sigma and the epifluorescence microcopy data, our decontamination protocol allows the detection of as few as 87 cells per rod (Table 1). The average concentration of cellular ATP on the flight control rods was $7.5 \times 10^{-16}$ ($\pm 0.85 \times 10^{-17}$ mol rod$^{-1}$, $n = 96$) and an ATP concentration greater than $2.6 \times 10^{-16}$ mol rod$^{-1}$ provides a 3σ confidence level of
detection above the background. Since an entire rod can be placed into a single culture tube with liquid media, one colony-forming unit per rod is theoretically sufficient to detect growth. Of the 160-flight control rods placed into enrichment culture, growth was observed in only 1 of these cultures during the course of this study.

A total of 58 particles were counted from six flight control rods that flew on two separate flights. Particles on these rods ranged from 1.0 to 13 µm in diameter, with a mode of 3.9 µm. Of the particles measured, 95% were smaller than 11 µm in diameter. The number of particles counted in 60 FOV for each rod ranged from 1.0 to 29 and the average concentration of particles rod\(^{-1}\) was 480 (±2.0 × 10\(^2\)). The average particle area and major axis length (diameter) were 12 (±1.7) µm\(^2\) and 4.4 (±0.33) µm, respectively. In Eq. (1), a perfect circle has a value of 1.0. The average circularity of the particles analyzed on flight control rods was 0.74 (±0.11). In order to achieve a signal 3-sigma above the controls, a total of 6.0 × 10\(^2\) particles must be collected per rod (Table 1).

Table 1: Limits of detection for microbiological and particulate analyses conducted on decontaminated rod surfaces.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Method</th>
<th>3σ limit of detection above the flight control rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell concentration</td>
<td>SYBR Gold(^\text{TM}) enumeration</td>
<td>87 cells ((n=21))</td>
</tr>
<tr>
<td>Viable cells and biomass</td>
<td>ATP quantification</td>
<td>2.6 × 10(^{16}) mol ATP or 85 cells(^a)</td>
</tr>
<tr>
<td>Culturable cells</td>
<td>Liquid enrichment</td>
<td>1 colony forming unit (^b) ((n=160))</td>
</tr>
<tr>
<td>Total particle concentration</td>
<td>SEM enumeration</td>
<td>600 particles ((n=6))</td>
</tr>
</tbody>
</table>

\(^a\) Based on an estimated 3.0 x 10-18 mol ATP per bacterial cell (Franzen and Binkley, 1961)

\(^b\) Theoretical limit based on the ability of 1 CFU on each rod to enumerate in culture media.
4.0 Discussion

Microbial aerosol sampling at altitudes above the atmospheric boundary layer (~1 km above sea level) can be conducted from mountaintop observatories (Amato et al., 2005; Bowers et al., 2009; Smith et al., 2012, 2013; Xia et al., 2013), using aircraft (DeLeon-Rodriguez et al., 2013; Griffin, 2004; Smith et al., 2010), or high altitude balloon platforms (Harris et al., 2002; Rogers and Meier, 1936; Yang et al., 2008). Although a ground-based approach provides obvious advantages, including the ability to sample large volumes of air, the air masses may be influenced by surface level interactions (Xia et al., 2013) and sampling is restricted to altitudes in the troposphere. Aircraft based sampling for microorganisms has been accomplished up to altitudes of 20 km (DeLeon-Rodriguez et al., 2013; Griffin, 2004; Smith et al., 2010), providing the necessary flexibility to examine vertical profiles in the atmosphere and allowing large air volumes to be sampled. For example, DeLeon-Rodriguez et al. (2013) filtered 2.2 to 15 m$^3$ of air at altitudes of 3.0 to 10 km for their microbiological analysis. While aircraft-based platforms have distinct advantages for characterizing the spatial and temporal variability of microbial aerosols in the Earth–atmosphere system, they are also limited by the fact that sampling has been restricted to altitudes below ~20 km and an aircraft requires enormous financial and logistic support resources.

Balloon-based approaches to detect and describe microbes in the stratosphere were first attempted over 75 years ago (Rogers and Meier, 1936). To date, most of the high altitude studies have focused on detecting only culturable microorganisms, which is an approach well known to bias for a minor fraction of the total microbial diversity (Staley and Konopka, 1985; Stewart, 2012). In cloud water samples enriched at 5 °C and
17 °C, less than 0.28% of the total bacterial cells were found to be cultured using standard methods (Vaïtilingom et al., 2012). It is also important to note that strategies used previously in the sampling of stratospheric microbes have not provided data on the quantitative abundance of microorganisms or assessed the level of background contamination associated with flight operations and subsequent analysis. For example, Harris et al. (2002) used liquid neon-cooled cryopumps to sample at altitudes from 19–41 km and reported the presence of one to three microscopic cell “clumps” per sample. However, since the amount of air sampled during these missions is not known, relating the abundance of the bioaerosols to the source air mass is not possible. Further, Yang et al. (2008) report the recovery of two CFU from filters that concentrated 0.35 m$^3$ of air at 20–30 km, but in the absence of procedural controls and repeat measurements, it is difficult to prove that such results represent bona fide recoveries of stratospheric microbes rather than artifacts resulting from contamination.

Given the state of knowledge in the field of high altitude microbiology and the inherent challenges involved, our objective was to develop a quantitative sampling strategy that collected microbial aerosols from defined air mass volumes and sampled multiple altitude ranges per mission. Equally important for this effort was to establish protocols that assessed the cleanliness of the payload and sampling substrates, allowing the authenticity of results obtained from atmospheric sampling to be verified. To sample bioaerosols in the high atmosphere, we adapted the Rotorod impaction sampling method (Frenz et al., 1996) and developed a light weight device that could sample while ascending through the atmosphere or through rotation at a fixed altitude. The sampling chambers hold up to 40 individual rods, providing the opportunity for replication of
measurements and purposing the samples to various types of microbiological and molecular analyses. Since the payload samples at altitudes where the atmospheric pressure is 50 to 100 times lower than that at sea level, a complete seal on the chambers would create an undesirable situation where a partial vacuum would be created in the chamber interior when the payload returned to surface pressure. In this scenario, an uncontrolled leak could allow extraneous microbes to enter the chamber and contaminate the sample. To prevent this, we equipped each chamber with a syringe filter to establish a “controlled leak” that equalizes the chamber interior and exterior pressure while preventing all particles >0.22 µm from entering the sampling chamber.

The volume of air sampled by a single rod is the area of its upward facing side (35 mm²) multiplied by the change in altitude during its sampling flight path, with each rod passing through 0.035 m³ per km of altitude sampled. The efficiency at which aerosols are collected on a rod is a function of the ascent rate (5.8 m s⁻¹) and particle size. For example, the collection efficiency for 6.0 µm particles traveling at 5.0 m s⁻¹ was 56% (Frenz, 1999), but decreased to 0.5% for particles 0.5 to 1.0 µm in diameter (Magill et al., 1968). Hence, owing to the small volumes sampled, efficiency of the rod collection method, and the minimum thresholds required to overcome the signal to noise ratio (Table 1), the altitudinal resolution of our approach is low. Based on previous reports of cell concentrations between 1.5 and 10 km (10⁴ to 10⁵ cells m⁻³; Amato et al., 2005; DeLeon-Rodriguez et al., 2013; Xia et al., 2013), the minimum vertical resolution necessary to breach the detection limit for cells 6.0 µm in diameter (Table 1) by our method is 0.5 km in a tropospheric air mass.

The ability of the sampling chambers to keep samples pristine during balloon
missions was evaluated by flying sampling chambers that remained sealed, providing procedural control data for each mission. Background contamination on the procedural controls was determined to not be significantly different from identical rods that were not removed from the laboratory, indicating that the chambers were effective at excluding external microbial contamination during all phases of launch and recovery. The abundance of cellular and molecular contamination associated with our procedural controls allowed us to estimate the level of detection for various parameters important to microbial aerosol characterization (Table 1). Detection of viable microbial biomass in the atmosphere via the quantification of cellular ATP will require at least $2.6 \times 10^{-16}$ mol of ATP, which corresponds to ~ 85 viable bacterial cells per rod (based on $3.0 \times 10^{-18}$ mol of ATP per bacterial cell; Franzen and Binkley, 1961). This was nearly identical to the cell concentration values (87 DNA-containing cells per rod) required to exceed the background threshold for epifluorescent microscopy. Based on data for particles in the size range of 0.5 to 10.4 µm, a collection of 600 particles would be sufficient to achieve a signal above the controls. It is important to note that measurable quantities of cells and their molecules (i.e., ATP) remained detectable after decontamination procedures. Therefore, the parallel analysis of procedural controls is an important consideration when measuring microbial concentrations that approach the signal to noise ratio.

The levels of detection reported in Table 1 could presumably be lowered if the background level of cells and molecules were reduced by more effective decontamination methods. High temperature combustion or autoclaving were not possible with all the materials we used, specifically the rods. Therefore, we evaluated the effectiveness of sodium hypochlorite, UV-C irradiation, and ethylene oxide for microbial disinfection of
the materials. In these tests, we chose *B. atrophaeus* because its endospores have shown high levels of resistance to ethylene oxide treatment (Mosely et al., 2005) and *D. radiodurans*, which is extremely resistant to UV-C and desiccation (Bauermeister et al., 2011). Therefore, the decontamination data presented here should be considered a conservative estimate of microbial survival under these conditions.

A variety of biological molecules commonly used as proxies for microbial biomass (e.g., ATP; Vaïtilingom et al., 2012) and diversity (DNA; DeLeon-Rodriguez et al., 2013) can persist, even in materials that have been sterilized (Venkateswaran et al., 2003). Therefore, the ability to eliminate and assess the level of biochemical contamination was important. There was not a significant difference between the sodium hypochlorite and the combined treatments. The use of sodium hypochlorite alone would be a viable option if payload materials were not compatible with UV-C or ethylene oxide sterilization. Although ethylene oxide was less effective at decreasing the viability of endospores and concentration of cellular ATP than sodium hypochlorite, it is an effective decontamination method to consider in applications that are incompatible with sodium hypochlorite. Sodium hypochlorite offers several advantages for use as a chemical decontaminant; it is inexpensive, readily available, and easily adaptable for field-based protocols.

Environmental conditions recorded during balloon flights indicated that the payload functioned at pressures and temperatures as low as 0.7 kPa and −51 °C, respectively. The primary reason the payload configuration that we present here possesses only two sampling chambers is to remain compliant with weight restrictions specified by the United States Federal Aviation Administration (Code of Federal
Regulations, title 14, sec. 101.1). It is possible to fly larger weight payloads, at the expense of a much more complicated flight system and flight authorization process, making it possible to increase the sampling capacity and accommodate additional sample chambers per payload. In addition, the payload can be adapted for float sampling. We sampled with LAMB for ~8 h while at float altitude (38 km) by rotating the payload on a fixed axis, allowing the sampling of ~2.0 m$^3$ per rod. As such, the payload that we describe here can be adapted to sample microbial aerosols on any type of balloon platform.

In conclusion, we have developed a robust and reliable approach for the quantitative sampling of microbial aerosols at altitudes in the troposphere and stratosphere. Data from procedural controls flown on actual balloon missions allowed us to demarcate the limits of detection for microbial cells and their associated molecules. In addition to providing data on the upper limits of Earth's biosphere and geographical movement of microorganisms on a global scale, the integration of microbiology into high altitude balloon research also has relevance to astrobiology. The properties of microbes surviving extreme combinations of pressure, temperature, relative humidity, and UV irradiation are important for assessing the habitability of other planetary environments. Further, our research is also relevant to discussions regarding the prevention of forward contamination during future extraterrestrial missions, where sample integrity will be a fundamental concern (e.g., Christner et al., 2005; Eigenbrode et al., 2009; Venkateswaran et al., 2004).
Acknowledgments

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CHAPTER 3. Quantitative Detection and Characterization of Bioaerosols in the Troposphere and Stratosphere*

1.0 Introduction

Although microorganisms comprise a portion of the bioaerosols that are ubiquitous and transported in Earth’s atmosphere (Jaenicke et al., 2005; Smith et al., 2013; Griffin et al., 2006), little is known about the properties of species that disseminate in high altitude air masses. Furthermore, a dearth of bioaerosol concentration data have hampered meaningful assessments of microbial abundances in higher layers of the atmosphere (Fig. S1). Given the potential for microorganisms to disperse between continents as bioaerosols (Smith et al., 2013; Griffin et al., 2006), directly or indirectly affect climatic processes (Creamean et al., 2013), and remain metabolically active under certain atmospheric conditions (Griffin, D., 2004; Sattler et al., 2001; Vaïtillingom, et al., 2012), there is a critical need for new observations that improve understanding of their distribution in the atmosphere.

2.0 Materials and Methods

2.1 Balloon flights and sampling

A 2-kg latex helium sounding balloon was used for short duration (<3 h) missions at altitudes from 1.3 to 29 km. Data from the following nine sounding balloon flights are presented in this study (Table 1): two missions in Louisiana [June 24, 2010 near Church Point, LA (30.4047° N, 92.2169° W, elevation 0.01 km above sea level), November 21, 2010 near Denham Springs, LA (30.4797° N, 90.9542° W, elevation 0.01 km above sea level]); one mission in Texas [May 22, 2013 near Palestine, TX (31.7581° N, 95.6386° W, elevation 0.15 km above sea level)]; and six missions in New Mexico [between August 17 and September 4, 2013 in the vicinity of Ft. Sumner, NM (34.4731° N, 88.3791° W, elevation 0.05 km above sea level)].
104.242° W, elevation 1.2 km above sea level). Rates of ascension for the sounding balloon flights ranged between 6.5 and 7.9 m s\(^{-1}\), with an average of 7.1 m s\(^{-1}\). The project website provides more detailed information on flight trajectories, systems, and payloads for the sounding balloon missions ([http://laspace.lsu.edu/aces/flightinfo/flightinfo.php](http://laspace.lsu.edu/aces/flightinfo/flightinfo.php); ACES-17, 21, 36-42).

Aerosol sampling at designated altitudes was conducted using the autonomous payload system described by Bryan et al., (2014). To assess the level of background microbial and particle contamination, each flight included a procedural control chamber that was identical to and decontaminated with the sample chambers (Bryan et al., 2014). Particles and cells in the air masses were sampled by impaction during ascent on silicon-coated Rotorods® (IMS Health, Inc.; 40 rods per chamber) that were exposed by opening retractable doors on the top and bottom of the chambers with linear actuators. Sampling was terminated by commanding the linear actuators to extend, closing the doors and sealing the chamber for subsequent return to the surface. The volume of air sampled per rod (Table 1) was calculated by multiplying the altitude interval by the area of a rod (35 mm\(^2\)). While the density of air molecules decreases as pressure decreases, the physical volume sampled is calculated as an altitude independent value. At altitudes of approximately 30 km, the payload string was physically separated from the sounding balloon, facilitating a controlled descent to the surface via parachute. Upon payload recovery (typically <1 h after touchdown), the chambers were inspected to verify their seal during impact, and the doors were manually locked to prevent breach during transport. The chambers were returned to a field laboratory, opened in the laminar flow of
a microbiological safety cabinet with high efficiency particulate air (HEPA) filters, and the samples were processed within 6 h of recovery.

To sample at float altitude on the High Altitude Student Platform (HASP; Guzik et al., 2008), the payload was configured to rotate on a fixed axis, allowing the chambers to pass through the air at an average velocity of 2.1 m s^{-1} (Bryan et al., 2014). The Table 1: Flight data and results from air mass sampling in New Mexico, Louisiana, and Texas.

<table>
<thead>
<tr>
<th>Median sampling altitude (range) km</th>
<th>Date of flight</th>
<th>Volume sampled m³ rod⁻¹</th>
<th>Concentration† N m⁻³ x 10⁶ (±SE)</th>
<th>Mass µg m⁻³ (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cells 1.0-2.0 µm</td>
<td>Particles 1.0-6.0 µm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±SE)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±SE)</td>
<td>ND</td>
</tr>
<tr>
<td>2.2 (1.3-3.0)</td>
<td>8/17/13</td>
<td>0.062</td>
<td>1.2 (±0.061)</td>
<td>ND</td>
</tr>
<tr>
<td>2.3 (1.4-3.1)</td>
<td>8/18/13</td>
<td>0.056</td>
<td>1.4 (±0.085)</td>
<td>105 (±1.6)</td>
</tr>
<tr>
<td>6.1* (3.0-9.1)</td>
<td>6/24/10</td>
<td>0.21</td>
<td>0.22 (±0.012)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>11/21/10</td>
<td></td>
<td>0.30 (±0.023)</td>
<td>ND</td>
</tr>
<tr>
<td>8.1 (5.5-11)</td>
<td>8/17/13</td>
<td>0.18</td>
<td>0.40 (±0.021)**</td>
<td>0.12 (±0.0060)</td>
</tr>
<tr>
<td></td>
<td>8/18/13</td>
<td></td>
<td>&lt;0.36***</td>
<td>7.2 (±0.12)</td>
</tr>
<tr>
<td>21 (18-23)</td>
<td>8/21/13</td>
<td>0.16</td>
<td>&lt;0.40***</td>
<td>1.7 (±0.042)</td>
</tr>
<tr>
<td>24 (19-29)</td>
<td>9/1/13</td>
<td>0.35</td>
<td>0.53 (±0.017)</td>
<td>1.2 (±0.025)</td>
</tr>
<tr>
<td>26* (22-29)</td>
<td>5/22/13</td>
<td>0.25</td>
<td>0.42 (±0.012)</td>
<td>NG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(±SE)</td>
<td>ND</td>
</tr>
<tr>
<td>26 (24-29)</td>
<td>8/22/13</td>
<td>0.19</td>
<td>0.38 (±0.025)</td>
<td>1.8 (±0.042)</td>
</tr>
<tr>
<td></td>
<td>9/4/13</td>
<td>0.17</td>
<td>0.45 (±0.028)</td>
<td>2.0 (±0.046)</td>
</tr>
<tr>
<td>36 (35-38)</td>
<td>9/2/13</td>
<td>2.2</td>
<td>0.077 (±0.0051)</td>
<td>2.5 (±0.049)</td>
</tr>
</tbody>
</table>

†: Corrected values based on the particle collection efficiency and sampling velocity (Sect. 2; Fig. S3, Table S2).
*: All cells collected during these flights were assumed to be 1.0 µm for the efficiency correction. The 6.1 and 26 km flights were conducted in Louisiana and Texas, respectively.
**: Only cells in the 1 µm size bin were at concentrations significantly higher than the procedural controls (Sect. 2).
***: Data are not significantly greater than the controls and reported at the procedural limits of detection.
modified payload was integrated on the HASP and launched September 2, 2013 (14:57 UTC) from Ft. Sumner, NM. The HASP support vehicle was tethered to a 28,000 m³ polyethylene, zero pressure helium-filled balloon and provided power, uplink commanding, and downlink telemetry for payload science operations. Upon reaching float altitude (~36 km), the chambers were warmed opened. All operations were confirmed in the downlinked telemetry as well as in the real-time video on-board HASP. Sampling was conducted for 8.4 h, the payload was commanded to stop rotation, and the chambers were closed in preparation for flight termination. Based on a rotation rate of 92 rpm, the cross sectional area and number of rods, and the distance from the rotation axis to the center of the chamber, the total volume of air sampled at float altitudes of 35 to 38 km was estimated at 2.2 m³ rod⁻¹ and a total volume of 177 m³. Flight termination ensued when HASP was ~1000 km west of the launch site. Payload touchdown occurred at 33.96° N, 112.98° W on September 3, 2013 (14:10 UTC). HASP was accessible ~12 h after touchdown, the payload was transported in a cooler containing blue ice, and the samples were processed within 48 h. More detailed information on the 2013 HASP flight is available on the project website (http://laspace.lsu.edu/hasp/Flightinfo.php?py=2013).

2.2 Physical properties of the air masses

Temperature and relative humidity (RH) data collected by National Oceanic and Atmospheric Administration balloon sounding flights (http://weather.uwyo.edu/upperair/sounding.html) were examined from Amarillo, TX (35.2220° N, 101.8313° W, approximately 170 miles to the east of Fort Sumner, NM) and Albuquerque, NM (35.0853° N, 106.6056° W, approximately 160 miles to the west of Fort Sumner, NM). Temperature profiles and air mass origins for both locations (at
12:00 UTC) showed similar characteristics during each flight; therefore, only temperature data from Amarillo are shown in Figures 1A and S2. Temperature and RH in the free troposphere over Lake Charles, LA (30.2266° N, 93.2174° W) for the flight dates in 2010 showed similar characteristics during each flight; therefore, only temperature data from Amarillo are shown in Figures 1A and S2. Temperature and RH in the free troposphere over Lake Charles, LA (30.2266° N, 93.2174° W) for the flight dates in 2010 were very similar to those observed at Amarillo, TX during 2013 (Fig. 1A).

Unfortunately, no temperature or RH data are available for the stratospheric altitudes sampled in the vicinity of Palestine, TX on May 22, 2013. Based on the Amarillo temperature profiles during the flights in August and September 2013, the CBL (from the surface to approximately 3 km), free troposphere (3 to 15 km), tropopause (15 to 18), and stratosphere (altitudes above 18 km) were identified (Figs. 1A and S2). The CBL is well mixed, and as shown in Fig. S2, its upper boundary with the free troposphere is indicated by the region where temperature decreases linearly with increasing altitude, producing a “knee” in the temperature profile (Stull, R., 2006; Cushman-Roisin, B.,

![Image](image_url)

Fig. 1. Temperature, microbiological, and particle data with altitude. (A) Temperature profiles obtained at Amarillo, TX from 1.1 to 33 km above sea level (ASL). (B) Cell (grey circles) and particle (black circles) concentrations are plotted at the median sampling altitude. The values for “viable” cells (black triangles) were derived from the ATP data (Table S4). Open symbols at 8.1 and 21 km are cell data that were not significantly different from the controls; the values plotted are the procedural limits of detection (Table 1). (C) Mass of cell carbon (grey diamonds) and particles (black diamonds) with altitude. The standard errors for data in B and C are reported in Tables 1 or S4.

(Table 1) were very similar to those observed at Amarillo, TX during 2013 (Fig. 1A).

Unfortunately, no temperature or RH data are available for the stratospheric altitudes sampled in the vicinity of Palestine, TX on May 22, 2013. Based on the Amarillo temperature profiles during the flights in August and September 2013, the CBL (from the surface to approximately 3 km), free troposphere (3 to 15 km), tropopause (15 to 18), and stratosphere (altitudes above 18 km) were identified (Figs. 1A and S2). The CBL is well mixed, and as shown in Fig. S2, its upper boundary with the free troposphere is indicated by the region where temperature decreases linearly with increasing altitude, producing a “knee” in the temperature profile (Stull, R., 2006; Cushman-Roisin, B.,
2014; Ouwersloot et al., 2012). For flights targeting the lowest sampling altitudes (17 and 18 August 2013), the top of the CBL was at 2.1 km and 2.5 km, respectively (Fig. S2). The troposphere and stratosphere are separated by the tropopause, which was at a height of 15 km to 18 km during the 2013 flight campaign. The stratosphere is the layer of air immediately above the tropopause, where air temperature increased with altitude (Fig. 1A).

2.3 Air mass trajectory and classification

The trajectories of air masses traveling towards the launch site were forecast prior to each flight with the HYbrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model using data from the Global Data Assimilation System (Draxler et al., 2004). The model output was used to predict the boundary altitudes for the CBL, free troposphere, and stratosphere based on temperature. The predicted air mass trajectories were verified post-flight by generating 72-h backward trajectories with archived data using HYSPLIT. The trajectory history was used to categorize air masses as continental tropical (cT) or maritime tropical (mT) according to the method of Rauber et al., (2008).

2.4 Characterization of cells and particles

Particles and SYBR® Gold-stained cells on the sampling rods were microscopically enumerated as previously described (Bryan et al., 2014). Particles were sized using ImageJ software and cell dimensions were estimated using a calibrated ocular scale and epifluorescence microscopy; diameter was based on the major axis length. Particle data were binned in fifteen categories, with ten 0.1 mm bins for particles 1.0 to 1.9 µm in diameter and five bins for particles 2.0 to 6.4 µm (Table S2). The size distributions were used to estimate particle mass (Table 1) by assuming a particle density
of 1.0 g cm\(^{-3}\) (Huffman et al., 2012). The cell data were separated into 1-µm bins of 1.0 and 2.0 µm in diameter. Cellular carbon was calculated based on representative volumes for the 1.0 (0.52 µm\(^3\)) and 2.0 µm (4.2 µm\(^3\)) cell size bins, a dry cell biomass density of 1.1 g cm\(^{-3}\) (Bratbak and Dundas, 1984), and that carbon represented 50% of the cell dry weight (West, et al., 1986).

The mean number of particles or cells per field of view in the controls was subtracted from the mean number of particles or cells per field of view in each sample. Error was propagated by combining the standard errors of the control and sample following the method of Baker and Nissim, 1963. Data from the samples and procedural controls were scaled to the sampling rod area, corrected for efficiency (Fig. S3), and divided by the air volume sampled (Table 1). The procedural level of detection for each altitude interval sampled in Table 1 is based on cell abundance in the controls (460 cells rod\(^{-1}, n=21\)) and the volume of air sampled (range of 0.059 to 1.0 x 10\(^6\) cells m\(^{-3}\)). Cell abundance data that were not significantly different from the procedural controls (Fig. 1B open symbols, α > 0.05) are reported at limit of detection values (Eaton et al., 1998).

For global bioaerosol estimates, a rudimentary atmospheric structure was presumed to derive the volume of four concentric atmospheric layers: the CBL (0 to 3 km, volume of 1.5 x 10\(^9\) km\(^3\)), the free troposphere and tropopause (3 to 20 km, 8.7 x 10\(^9\) km\(^3\)), and two altitude ranges in the stratosphere [20 to 30 km (5.1 x 10\(^9\) km\(^3\)), and 30 to 40 km, (5.2 x 10\(^9\) km\(^3\))]. The minimum and maximum concentration values available at altitudes <30 km (Table 1) were used for the calculations (i.e., the CBL, 0 to 3 km, 1.2 to 1.4 x 10\(^6\) cells m\(^{-3}\); the free troposphere and tropopause, 3 to 20 km, 2.2 to 4.0 x 10\(^5\) cells m\(^{-3}\); the lower stratosphere, 20 to 30 km, 3.8 to 5.3 x 10\(^5\) cells m\(^{-3}\)). The single data point
at 36 km (7.7 x 10⁴ cells m⁻³) was used for the estimate at altitudes of 30 to 40 km. Similarly, the biomass and cell carbon data (Table 1) were also used to estimate their magnitude in the atmosphere (Table S3).

2.5 Aerosol collection efficiency

The aerosol collection efficiency was measured by rotating two sampling chambers at different velocities on the roof of a building at Louisiana State University (30.4500° N, 91.1400° W, elevation ~0.02 km). For each trial, the velocity of the payload sampling chambers was varied by adjusting the length of the rotational arm affixed to the sampling chambers. During these experiments, the velocity of chamber one ranged from 2.8 to 3.1 m s⁻¹, with an average velocity of 2.9 m s⁻¹. Based on the radial distance from center, each sampling rod traveled from 3.8 to 4.2 m per revolution, sampling between 0.35 and 0.39 m³ of air after one hour. The average sampling velocity of chamber two was 4.7 m s⁻¹ (ranging from 4.6 to 4.9 m s⁻¹), the distance traveled by the rods was 5.9 to 6.3 m per revolution, and the total volume of air sampled per rod during one hour ranged from 0.58 to 0.62 m³.

Simultaneously with rotational aerosol collection, a six-stage (cutpoint aerodynamic diameters of 10, 2.5, 1.4, 0.77, 0.44, and 0.25 µm) Model 131 MOUDI™ (MSP Corporation) cascade impactor was used to sample (100 L min⁻¹; total of 12 m³ air sampled in two hours) at the same height as the sampling chambers and within a horizontal distance of ~1 m. Prior to sampling, the foil substrates used as impaction surfaces were coated with silicone spray (CRC Industries) and allowed to dry for 15 minutes in a laminar flow hood. Particles impacted on the collection substrate (3.5 x 10⁹ µm² of sampling surface) from three stages of the MOUDI sampler were obtained: stage
1 (with a cutpoint diameter of 8.5 µm), stage 2 (cutpoint diameter 2.5 µm) and stage 3 (cutpoint diameter 1.4 µm). Based on the cell size distribution observed in the air masses sampled (Table S1), the data from stage 3 were selected for determination of the size and abundance of particles 0.95 to 6.4 µm (Table S2).

For each velocity test, the particles in 60 fields of view (total area of 7.7 x 10^5 µm² per rod) for each sample rod (n=3) were counted and measured. Particles on three replicate samples of the substrate from stage 3 were enumerated and sized by examining 30 fields of view per replicate (area of 9.6 x 10^4 µm² per replicate). Identical collection substrates were prepared and processed in parallel to serve as procedural controls. The particles in each field of view were manually traced, counted, and sized (Bryan et al., 2014), and the average number of particles per field of view for each size bin was determined (Table S2).

2.6 Quantification of cellular ATP

Free ATP was enzymatically eliminated and the concentration of cellular ATP was determined as described by Bryan et al. (2014). Procedural control rods from seven flights (~12 rods per flight) were analyzed to determine the level of background and analytical detection limit for cellular ATP. The level of background associated with the procedural controls averaged 7.5±0.85 x 10^2 amol ATP rod⁻¹ (n=96). The concentrations reported (Table S4) were adjusted for background using the same procedure described for the cell data. Measurements of C:ATP weight ratios in exponentially growing bacteria cells range from 28 to 500, with 250 commonly used as a mean value for environmental studies to account for the various taxa and growth rates of the microbial community (Karl, D., 1980). Because the majority of the bioaerosols collected are not predicted to
exponentially growing, the values of 25:1 and 250:1 were used to estimate cell carbon from the ATP data to represent a sample with low and mean C:ATP weight ratios, respectively. To make comparisons per volume of air sampled, cell carbon values derived from the ATP data were partitioning according to observed cell size (Table S1), converted to biovolume (West et al., 1986), and the number of 1 and 2 µm cells per rod was estimated (Table S4). The cell concentration inferred from the ATP data was corrected for efficiency (Table S2) and divided by the sampling volume (Fig. 1B; Table S4).

2.7 Enrichment culturing and isolation of bacteria

Within six hours of payload recovery (48 h for the HASP flight), the sealed sampling chambers were opened under class 100 conditions, and individual rods were aseptically removed and placed into culture tubes containing 5 mL of sterile media. For the samples collected in November 2010 and May 2013, ten sample and ten control rods were placed into R2A broth (Difco cat. no.: 218262) amended with 100 mg mL\(^{-1}\) cycloheximide to inhibit fungal growth. Each media used during the 2013 New Mexico campaign, [R2A broth, 1% (v/v) R2A broth, tryptic soy broth (Difco cat. no.: 236950), and 1% (v/v) tryptic soy broth] was amended with 100 mg mL\(^{-1}\) cycloheximide and individually inoculated with five sample and five control rods.

The cultures were stored at 4 °C in the dark until returned to Louisiana State University (< 3 weeks), where they were incubated aerobically at 22°C. Microbial growth in the liquid cultures was monitored by visual inspection and biweekly spread plating 100 µL of each onto agar-solidified media of identical composition. Individual colonies that
appeared on the agar media following aerobic incubation at 22°C were isolated into pure cultures using a standard three-phase streak technique.

2.8 Amplification and sequencing of 16S rRNA gene sequences

Liquid cultures of each isolate were incubated until turbid and the cells were harvested by centrifugation (17,000 × g; 10 min). The cells were lysed chemically (10% SDS w/v) and enzymatically (lysozyme, 10 mg mL⁻¹), and genomic DNA was extracted and purified using phenol-chloroform followed by ethanol precipitation (Sambrook and Russell, 2001). The DNA obtained was suspended in 0.22 µm filtered and autoclaved deionized water, and quantified by absorbance at 260 nm using a Nanodrop ND 1000 spectrophotometer (Thermo Scientific, Inc.).

A portion of the 16S ribosomal RNA gene (~1.4 kb) was amplified in a 50 µL polymerase chain reaction (PCR) containing 1.5 units of Taq DNA polymerase (5PRIME, cat. no.: 2200000), 1X TaqMaster PCR enhancer, 1X MasterTaq buffer, 200 µM deoxynucleotide triphosphates (dNTPs), 20 µM of each primer [27F, 5`-AGAGTTTGATCCTGGCTCAG-3` (Lane, D., 1991); and 1492R, 5`-GGTTACCTTGTACGACTT-3` (Turner et al., 1999)], and approximately 100 ng of genomic DNA. After 4.5 min. at 95°C, thirty cycles of the PCR were conducted with a 30 s denaturation at 95°C, 30 s of annealing at 50.8°C, and 1 minute of extension at 72°C, followed by a final 5 minute extension at 72°C. The predicted size of the amplicons was verified by electrophoresis through a 1.0% agarose gel, and subsequently, the DNA was purified using the UltraClean PCR Clean-up Kit (MO BIO Laboratories, Inc., cat. no.: 12500-50).
The purified PCR products were sequenced using BigDye® terminator cycle sequencing (Applied Biosystems™), an ABI 3130XL Genetic Analyzer (Applied Biosystems™), and the following primers: 27F, 515F [(5’-GTGCCAGCMGCGCGGTAA-3’ (Turner et al., 1999); and 907R, 5’-CCGTCAATTCTTTAGTTT-3’, Lane, D., 1991)], and 1492R. The DNA sequence electropherograms were manually checked for accuracy and ambiguous base calls. Individual sequences were aligned using BioEdit software (2013) to create a consensus sequence based on a minimum of 2X coverage for each position in the alignment (227 to 1257, E. coli numbering). The nearest neighbors to the queried 16S rRNA gene sequences were identified with the SINA aligner using the SEED database (Pruesse et al., 2012). Distance analyses were performed with MEGA7 (Kumar et al., 2016).

2.9 Statistical analysis

Statistical analysis was performed using SAS® University Edition (2014). Following tests for normality, the PROC GLIMMIX procedure for a negative binomial distribution, along with the LSMEANS statement and the DUNNETTU option, was used for significance testing. The limit of detection for measured parameters was determined using the PROC GLIMMIX procedure with 95% confidence intervals. Linear regressions were performed using StatPlus:mac statistical analysis software (AnalystSoft Inc.).

3.0 Results and Discussion

We investigated the size, concentration, and viability of microorganisms at select altitudes between 1.3 and 38 km above sea level (Fig. 1; Table 1) using helium balloon payloads specifically developed for high altitude aerosol sampling (Bryan et al., 2014). Twelve air masses were sampled during ten flights in Louisiana, Texas, and New Mexico.
between 2010 and 2013 (Sect. 2; Table 1). The CBL, free troposphere, and stratosphere were identified based on ambient temperature measurements (Sect. 2; Figs. 1A and S2). According to 72-h back trajectories, the sampled CBL air masses (2.2 and 2.3 km) were categorized as continental tropical, whereas the higher altitudes sampled in the free troposphere and stratosphere were maritime tropical air masses (Sect. 2). Air temperature in the CBL ranged between 10 °C and 20 °C (Fig. S2) with relative humidity (RH) values of 58% to 76%, which contrasted to higher altitudes in the troposphere and stratosphere that had low RH (20% to 30%) and a minimum temperature of -74 °C (Sect. 2; Fig. 1A).

DNA-containing cells (Fig. 2 A-F) and particles (Fig. 2H) impacted on the rods were characterized using epifluorescence and scanning electron microscopy, respectively. Based on comparison with data from rods in sampling chambers that remained sealed on each flight, only cells larger than 0.5 µm and less than 2.0 µm in diameter (Fig. 2 A-F) were at abundances statistically higher than those in the procedural controls (Sect. 2). Therefore, size bins of 1.0 and 2.0 µm were used to parse the cell concentration data (Table S1). Similarly, abundances of particles larger than 6.0 µm were statistically indistinguishable from those in the controls, and the data for particles 1.0 to 6.0 µm was binned according to Table S2. The efficiency at which the rods collected particles 1.0 to 6.0 µm was empirically determined (Fig. S3), corrected for the sampling velocity (average of 7.1 m s⁻¹ on ascent or 2.1 m s⁻¹ for rotational sampling), and the coefficients derived were used to estimate the concentration of each size bin per volume of air mass sampled (Table S2). Particle concentration and mass decreased exponentially with altitude (Fig. 1), and values from the free troposphere were 16- and 10-fold lower, respectively, than those observed in the CBL. The lowest particle abundances were 69
observed in samples from the stratosphere, which ranged in concentration from 1.2 to 2.5 x 10^6 particles m^{-3} (Table 1) and had highly similar size and mass distributions (Fig. S4).

In general, the concentration of microbial cells and associated cellular carbon decreased with increasing altitude from the CBL into the stratosphere (Fig. 1; Tables 1 and S1). Microbial abundances in the CBL (average of 1.3 x 10^6 cells m^{-3}) were at the high end of

---

**Fig. 2.** Cells and particles collected from air masses in the troposphere and stratosphere. Epifluorescence micrographs of SYBR® Gold-stained cells observed at 2.2 km (A), 26 km on 22 August 2013 (B,C), 26 km on 4 September 2013 (D,E), and at 36 km (F). A scanning electron microscopic comparison of particles observed in the procedural controls (G) and a sample from 36 km (H). The scale bars are 2.5 µm.
the three order-of-magnitude concentration range reported in the literature, and similar to values obtained at mountain atmospheric research observatories (Fig. S1).

Cell concentration estimates for air masses in the free troposphere were ~2-fold higher than those reported at 9 to 10 km above the Caribbean Sea before, during, and after tropical hurricanes (DeLeon-Rodriguez et al., 2013; Fig. S1). Slightly higher cell concentrations were observed in the lower portion of the stratosphere (19 to 29 km, 3.8 to 5.3 x 10^5 cells m^-3) when compared to values from the free troposphere (2.2 to 4.0 x 10^5 cells m^-3), but these differences were not statistically significant (n=7, p=0.11). Although rods from two of the flights (8.1 and 21 km) had cell numbers that on average were higher than the controls, the experimental data were not significantly different and are thus reported at the procedural detection limits (Sect. 2). The concentration of cells and cell carbon was lowest (7.7 x 10^4 cells m^-3 and 0.086 µg m^-3, respectively) at the highest altitude sampled (36 km).

Having cell and biomass concentration data from altitudes considerably higher than previously available (Figs. 1 and S1) allowed an estimate of the atmospheric reservoir of microbes and comparison with prior inventories (Burrows et al., 2009a; Whitman et al., 1998). Extrapolation of the CBL (0 to 3 km above the surface) data range (Table 1) to its global volume yields 1.8 to 2.1 x 10^{24} cells (Sect. 2; Table S3), which is more than five orders-of-magnitude higher than estimates based on culturable bacteria (5.0 x 10^{19} colony forming units; Whitman et al., 1998) and two orders-of-magnitude higher than parameterizations used in global bioaerosol transport models (1.3 to 1.7 x 10^{22} cells; Burrows et al., 2009b). Comparison of global cell and biomass estimates for 0-20 km (3.8 to 5.6 x 10^{24} cells; 2.0 to 2.7 Tg cell carbon) with 20-40 km (2.4 to 3.1 x 10^{24}
cells; 2.1 to 2.5 Tg cell carbon) suggests the stratosphere may contain ~40 to 50% of the microbial cells and biomass in the atmosphere (Table S3). Clearly, global estimates based on spatially- and temporally-limited data should be viewed as tentative and refined when new information is available. Nevertheless, our results support the hypothesis that the stratosphere is a significant reservoir of bioaerosols. Since ~80% of microorganisms in the stratosphere may exist at altitudes of 20 to 30 km (Fig. 1B; Table S3), periodic exchange with and dispersal in tropospheric air masses is possible, particularly over northern hemisphere midlatitudes above 20 km (Konopka et al., 2015; Ploeger et al., 2015).

There are a variety of stresses encountered by aerosolized microorganisms at high altitudes, but few data on the maintenance of cell viability during long-distance atmospheric transport. Statistically significant levels of cellular adenosine triphosphate (ATP), a proxy for potentially viable biomass, were measured in samples collected from 2.2, 24, and 36 km (Sect. 2; Table S4). Consistent with the cell data (Fig. 1B), the highest ATP concentrations were detected at 2.2 km (4.8 x 10^4 amol rod\(^{-1}\) m\(^{-3}\), n=4, p=0.0201) and lowest at 36 km (5.9 x 10^2 amol rod\(^{-1}\) m\(^{-3}\), n=60, p=<0.001). To estimate the number of cells per volume of air from the ATP data, the ATP mass per rod was converted to cell carbon (250:1 C:ATP, Karl, D., 1980), partitioned according to the observed cell size fractions, and the values were corrected for collection efficiency (Sect. 2; Table S4). The number of “viable” cells in the CBL inferred from the ATP data (3.8±0.10 x 10^5 cells m\(^{-3}\)) suggested ~30% of the DNA-containing cells were potentially viable (Fig. 1B). In contrast, the data from 24 and 36 km (1.1±0.062 x 10^5 cells m\(^{-3}\) and 7.6±0.25 x 10^3 cells m\(^{-3}\), respectively) implied that cell viability decreased (20% and ~10%, respectively) with
altitude in the stratosphere (Fig. 1B; Table S4). When using the conservative estimate of 25:1 C:ATP weight ratio (Karl, D., 1980) to account for the potential for decreased metabolism in high altitude bioaerosols would reduce the estimates for “viable” cells m$^{-3}$ by one order of magnitude.

To investigate if cultivable microorganisms were present in the bioaerosols sampled, liquid media were inoculated with sampling rods and the cultures monitored for evidence of growth (Sect. 2). Growth occurred in samples from the five flights examined (Table 2) after one to two weeks of incubation at 25$^\circ$C. Isolation of the cultured microorganisms followed by amplifying and sequencing a portion of their 16S rRNA gene revealed a cohort of 19 isolates that were comprised of eight different bacterial genera in the phyla Actinobacteria, Firmicutes, and Proteobacteria (Table 2). Approximately 80% (15 of 19) of the isolates belonged to genera observed at more than one sampling altitude, geographical location, and/or season, implying these taxa were prevalent in the atmosphere and tolerant to the multiple stresses associated with atmospheric transport.

Members of the genus *Curtobacterium* were the most frequently cultured isolates and recovered from three of the sampled stratospheric air masses (21 km and 26 km over New Mexico, and 26 km over Texas). An isolate recovered from samples collected at 21 km (L6-1) had 100% 16S rRNA gene identity to *C. flaccumfaciens pv. flaccumfaciens* LMG 3645 (Table 2), implicating a potential agriculturally-relevant plant pathogen (Haverson et al., 2015) capable of withstanding stratospheric RH and increased UV fluence rates. Previous reports have documented species of *Paenibacillus* and the closely related genus *Bacillus* at select altitudes between 20 and 41 km (Harris et al., 2002;
Rogers and Meirer, 1936; Yang et al., 2008a; Yang et al., 2008b; Griffin, D. 2004; Smith et al., 2010), and such species should be ideally suited for surviving the combination of extremes associated with atmospheric transport as environmentally-resistant endospores. However, most of the recovered isolates are not affiliated with bacterial taxa known to differentiate into metabolic dormant cysts or spores, and elucidation of the adaptations promoting atmospheric survival in these species warrants more detailed investigation. These microorganisms were recovered from a vegetative state and must display tolerance to the environmental conditions encountered in the stratosphere.

The tropopause is a semipermeable barrier that limits exchange between the troposphere and stratosphere, and aerosols are injected into the stratosphere in the tropics via Brewer-Dobson circulation (Haenel et al., 2015), and in various other regions due to biomass burning (Glatthor et al., 2013), convective clouds, volcanic activity, and midlatitude troposphere-stratosphere exchange due to quasi-horizontal eddy mixing above 20 km (Haenel et al., 2015; Konopka et al., 2015; Ploeger et al., 2015). Particle removal from the troposphere occurs by wet deposition and sedimentation (Burrows et al., 2008b), while sedimentation is the only mechanism relevant to the stratosphere (Benduhn and Lawrence, 2013). As such, predicted residency times for 1- and 2-µm particles at 25 km in the stratosphere are 1 to 2 years (Benduhn and Lawrence, 2013), compared with days to weeks in the troposphere (Burrows et al., 2008b). Further, gravito-photophoresis may negate the force of gravity on particles the size of cells (Cheremisin et al., 2011), providing an additional explanation for the relatively high cell and particle concentrations observed in the stratosphere (Fig. 1B).
Table 2: Identification of the isolated bacteria based on analysis of 1030 base pairs (227 to 1257, E. coli numbering) of the 16S rRNA gene.

<table>
<thead>
<tr>
<th>Isolate designation</th>
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<th>Nearest neighbor (Genbank accession number)</th>
<th>Percent sequence identity</th>
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<td>99.7</td>
<td>KY635892, KY635894, KY635895, KY635896</td>
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</tbody>
</table>

**: These isolates were cultured from separate rods in the same sampling mission and had 100% 16S rRNA sequence identity.

***: Isolates co-recovered from an enrichment culture that was inoculated with one sampling rod.

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**Genbank accession number,** **isolation, and location:**

- **ML2-1:** KY629944, New Mexico.
- **ML2-3:** KY635886, Louisiana.
- **L6-1:** ML2-4, ML2-3, ML2-1.
Based on these considerations, microorganisms surviving transport to the stratosphere are expected to have high tolerance to desiccation and UV radiation (Yang et al., 2008b). Future investigations defining the levels of tolerance to desiccation and UV will provide valuable insight to the potential for stratospheric bioaerosols to be disseminated along global circulation patterns (Haenel et al., 2015), or if their presence in the stratosphere is the result of local troposphere to stratosphere exchange (Konopka et al., 2015; Ploeger et al., 2015).

We developed a robust method to sample bioaerosols at high altitudes (Bryan et al., 2014) and used this approach to generate the first quantitative microbiological data above 10 km, extending the observations available to altitudes of 38 km. Future studies of the spatial, temporal, and altitudinal variation of microorganisms in the atmosphere would improve understanding of how atmospheric processes affect the quantity and dispersion of aerosolized microbes, as well as resolve the potential roles bioaerosols may play in meteorological processes and Earth’s radiative balance. A seminal result from this study is the relatively high concentrations of microorganisms that were observed in the stratosphere (Fig. 1B), with repeated recovery of phylogenetically diverse isolates (Table 2). Multiple studies have reported recovering culturable microbes from stratospheric air masses (Harris et al., 2002; Rogers and Meirer, 1936; Yang et al., 2008a; Yang et al., 2008b; Griffin, D., 2004; Smith et al., 2010), but it is uncertain whether or not the extreme conditions prevent all but the most tolerant of species (e.g., endospore-formers) from surviving the time frames necessary for reentry to the troposphere and return to surface habitats. The mean age of midlatitude stratospheric air ranges from 3 years at 20 km to 7 years at 30 km, values that double as air moves poleward where it will descend.
back into the troposphere (Haenel et al., 2015). Therefore, eddy mixing of tropospheric air into the stratosphere (Haenel et al., 2015; Konopka et al., 2015; Ploege et al., 2015) may provide a more plausible explanation for the presence of viable microorganisms, whose potential for long distance transport in the stratosphere remains unknown. Particle size is the most important parameter to consider for bioaerosol transport and deposition, and while the data support the presence of microbes the size of prokaryotic cells and viruses in the stratosphere, larger bioaerosols consisting of eukaryotic microorganisms could be present at abundances below our detection thresholds.

Acknowledgements

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CHAPTER 4. Desiccation and UV-C Tolerance of Bacteria Recovered From Altitudes of 6.1 to 26 km ASL: Potential for Survival and Dispersal in the Stratosphere

1.0 Introduction

Aerial dispersal of bioaerosols involves three steps: aerosolization and emission from the surface, atmospheric transport and survival, and deposition (Monteil et al., 2014). The flux of microbial bioaerosols in the convective boundary layer (CBL) can be estimated (Burrows et al., 2009a; 2009b; 2013). However, survival during long distance dispersal at high altitudes is poorly constrained. Numerous studies have documented the dispersal of viable bioaerosols during Asian (Hara and Zhang, 2012; Jeon et al., 2011; Maki et al., 2013; Smith et al., 2012; Smith et al., 2013; Yuan et al., 2016) and African (Griffin et al., 2001; Griffin et al., 2003; Griffin et al., 2006; Griffin, D., 2007) dust events, with cell densities positively correlating with the total coarse particle concentrations (Griffin et al., 2006; Griffin et al., 2007; Hara and Zhang, 2012; Jeon et al., 2011).

To survive aerial dispersal, cells must overcome two major sources of stress: desiccation and increased UV fluence. Desiccation is defined as the loss of cellular water due to exposure to a gas with a lower moisture level than the cell itself (Potts, M., 1994), and begins when cells are aerosolized from the surface, the first step of aerial transport. Desiccation will continue to be a challenge as bioaerosols are lofted to higher altitudes where RH decreases, and those that reach the stratosphere will be exposed to UV-C (190-280 nm) and increased fluence rates of UV-A (320-400 nm) and UV-B (280-320 nm; Coohill and Sagripanti, 2008; Ordonez, et al., 2009; Santos et al., 2013). Previous reports have documented the recovery of Bacillales from the stratosphere, microorganisms
capable of forming endospores (Griffin, D., 2004; Rogers and Meirer, 1936; Shivaji et al., 2006; Smith et al., 2010; Wainwright et al., 2003; Yang et al., 2008b), a metabolically dormant cell state that is well documented for its ability to survive harsh environmental conditions (i.e., heat, desiccation, UV and gamma irradiation, reviewed by Nicholson et al., 2000). Non-spor forming isolates have also been recovered from the stratosphere (Griffin, D., 2008; Wainwright et al., 2003), however only a handful of stratospheric isolates have been evaluated for their ability to withstand UV exposures (UV-B, Shivaji et al., 2006; UV-C, Yang et al., 2008a and Yang et al., 2008b).

Many genera of bacteria are capable of surviving long distance, tropospheric transport. Gram-positive, high GC Actinomycetes and low GC, endospore-forming Firmicutes dominate the viable fraction of bioaerosols associated with desert dust events (Griffin et al., 2006; Griffin et al., 2007; Hara and Zhang, 2012; Kellogg et al., 2004; Smith et al., 2012; Yuan et al., 2016). Cloud water droplets are also known to harbor a diverse assemblage of culturable bioaerosols (Amato et al., 2005; Amato et al., 2006) dominated by the Alphaproteobacteria Sphingomonas and the Gammaproteobacteria Pseudomonas (Vaïtilingom et al., 2012). Viable isolates with high 16S rRNA gene similarity to plant (Amato et al., 2005; Amato et al., 2006; Griffin et al., 2001; Griffin et al., 2007; Kellogg et al., 2004; Monteil et al., 2014; Smith et al., 2012; Vaïtilingom et al., 2012), animal (Griffin et al., 2001; Griffin et al., 2003; Griffin et al., 2007; Kellogg et al., 2004), and human (Amato et al., 2006; Griffin et al., 2003; Kellogg et al., 2004) pathogens have been detected in desert dust events, clouds, and precipitation, providing evidence that many different isolates closely related to pathogens are capable of surviving aerial dispersal.
Air is transported into the stratosphere by a variety of routes: convective upwelling in the tropics as part of the BDC (Brewer, A., 1949; Haenel et al., 2015), convective clouds, biomass burning (Glatthor et al., 2013), volcanic activity (Vernier et al., 2016), and quasi-horizontal eddy mixing at NH midlatitudes (Haenel et al., 2015; Konopka et al., 2015; Ploeger et al., 2015). Intercontinental transport of aerosols in the size range of bacteria (smaller than 5 µm) in the free troposphere is possible in 5 to 10 days (Griffin et al., 2006; Smith et al., 2013), and stratospheric transport from the tropics to the midlatitudes via the Brewer Dobson circulation measurements averaged 3 to 7 years for altitudes between 20 and 30 km (Haenel et al., 2015; Stiller et al., 2012). However, there are few data on the survival of atmospheric bacteria at low RH and high UV radiation flux. Understanding the limits for survival during aerial dispersal is critical for understanding the transport boundary of a given species, including those that may have the potential to cause disease (Kellogg et al., 2004; Smith et al., 2012; Monteil et al., 2014).

The recovery of bacteria from samples collected at 6.1 to 29 km indicates that a variety of species remain viable during transport in the stratosphere (Ch. 3). Here the effects of desiccation and UV-C (200-280 nm) exposure on microbial survival were evaluated to predict how long the isolates may remain viable under these conditions. In addition to providing insight on the potential for transport in the stratosphere, altitudes of ~30 km in Earth’s atmosphere have a similar temperature, pressure, RH, and UV-C fluence to the surface of Mars (Khodadad et al., 2017; Schuerger et al., 2003). Hence, the stratosphere provides the most suitable Earthly analog for examining microbial survival under conditions that mimic those on the surface of Mars.
2.0 Materials and Methods

2.1 Strains and culture conditions

Cultures of *D. radiodurans* R1 (ATCC 13939), *E. coli* MG1655, and the atmospheric isolates (Table 1) were grown aerobically with shaking (175 RPM) at 30 °C in liquid R2A broth. Optical density at 620 nm was quantified to measure culture turbidity, and growth of each isolate in the exponential and stationary phases was examined. Cells harvested from the stationary phase of growth were serially diluted in 10 mM MgSO₄, spread plated on R2A agar (Difco™, cat. no.: 218263), and incubated at 30 °C in the dark. After 3 to 7 days, the number of colony forming units (CFUs) recovered from 100 µL was used to determine the original concentration of cells mL⁻¹.

2.2 Desiccation Survival

Aliquots of the suspensions (10 µL) were spotted onto sterile glass coverslips. The samples were dried under a stream of 0.22 µm filtered, N₂ gas (~10 min) or placed in a desiccation chamber containing Drierite® (≥98% CaSO₄, W.A. Hammond Drierite Company, Ltd., cat. no.: 7778-18-9) until the liquid evaporated (~4 h). RH in the chamber was monitored using a dial hygrometer (Fisher Scientific™, cat. no.: 11-661-6B) with an accuracy of ±2.5%. The dried cells were removed from the coverslip by vigorous vortexing for 10 min in 1 mL of 10 mM MgSO₄ or by rinsing and suspending the material with a pipet into 1 mL of 10 mM MgSO₄. Three replicates of each sample were prepared. At the t=0 time point, the number of CFU recovered was designated as the starting population size for each isolate surviving drying on the coverslip.
Table 1: Identification of the isolated bacteria based on analysis of 1030 base pairs (227 to 1257, E. coli numbering) of the 16S rRNA gene.

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<td>26, 9/4/13, New Mexico</td>
<td>Modestobacter versicolor (JX841004)</td>
<td>98.7</td>
<td>KY629942</td>
</tr>
<tr>
<td>L9-7</td>
<td>26, 9/4/13, New Mexico</td>
<td>Paenibacillus barrengolzi (AY167184)</td>
<td>98.3</td>
<td>KY629943</td>
</tr>
<tr>
<td>L9-9A</td>
<td>26, 9/4/13, New Mexico</td>
<td>Paenibacillus barrengolzi (AY167184)</td>
<td>98.3</td>
<td>KY629943</td>
</tr>
<tr>
<td>L9-9A**</td>
<td>26, 9/4/13, New Mexico</td>
<td>Roseomonas vinacea (EF389888)</td>
<td>98.0</td>
<td>KY629946</td>
</tr>
<tr>
<td>L9-9A**</td>
<td>26, 9/4/13, New Mexico</td>
<td>Roseomonas vinacea (EF389888)</td>
<td>98.0</td>
<td>KY629946</td>
</tr>
</tbody>
</table>

**: These isolates were cultured from separate rods in the same sampling mission and had 100% 16S rRNA sequence identity.

*: These isolates were cultured from an enrichment culture that was inoculated with one sampling rod.

**: Isolates co-recovered from separate rods in the same sampling mission and had 100% 16S rRNA sequence identity.
The RH of the desiccation chambers was maintained between 15 and 25% RH by adding additional Drierite®. Samples were removed from the desiccation chamber after 1, 2, 4, 6, 8, 10, 12, and 14 days. Plates were monitored daily for growth, and reexamined 3 days after enumeration for any additional growth.

The surviving fraction of each strain was determined from the ratio of \( N/N_0 \), where \( N \) represents the number of CFU per mL at each time point and \( N_0 \) represents the number of CFU per experiment that survived after dehydration \((t=0, \text{ as described above})\). Each time point was evaluated when \( N \geq 30 \) CFU, the limit of detection for survival assays. Each experiment was performed in triplicate. To determine the average (± the standard deviation) parameters (y-intercept, slope, and \( R^2 \) values) of the desiccation survival curves, the exponential equation, \( N = N_0 e^{-kt} \), where \( k \) is the inactivation rate, and \( t \) is the time of exposure applied to each of the triplicate samples.

2.3 UV-C Survival

Desiccated bacteria were exposed to UV-C using a model 3UV-38 lamp (Part no.: 95-0341-01, UVP LLC.) set at the 254 nm configuration, which emits UV at wavelengths of 200 to 280 nm at a dose rate of 10.6 to 11.4 W m\(^{-2}\). In order to determine the concentration of cells required to achieve monolayers and avoid potential shading during UV exposure, aliquots of approximately \( 10^8 \) and \( 10^7 \) CFU \((n=3)\) were applied to coverslips, desiccated, and exposed to 0.65 kJ m\(^{-2}\). The log transformed average number of the \( N \) was compared to the average number \( N_0 \) to determine if there was a loss of viability.

The atmospheric isolates were initially screened by exposure to 0.65 kJ m\(^{-2}\) UV and spot plating 10 \( \mu \)L of serially diluted culture onto agar-solidified media. Populations
that exhibited no growth after this dose on the spot plates were subjected to a lower range of exposures (0.15 to 0.65 kJ m\(^{-2}\)). The isolates capable of forming colonies after exposure to 0.65 kJ m\(^{-2}\) were subjected to higher UV doses (0.65 to 2.8 kJ m\(^{-2}\)). Each experiment was conducted in triplicate. For the UV survival curves, the equation was \(N = N_0 e^{-kF}\), where \(F\) represents the UV fluence (kJ m\(^{-2}\); adapted from Lin and Li, 2002). The average (± the standard deviation) inactivation rates of the UV survival curves were calculated and compared for statistical significance.

2.4 Microscopic analysis of desiccated samples for UV-C exposure

The aliquots of \(10^8\) and \(10^7\) CFU dried on coverslips were stained with crystal violet and examined under 1000X magnification for the presence of monolayers using an Olympus bx51 epifluorescence microscope.

2.5 Statistical analysis

Student's t-test was used to compare survival data after a treatment was applied (drying method, cell removal technique, effect of growth phase, and CFU concentration) to that at \(t=0\), and when variances were not equal, the heteroscedastic Student's t-test was performed using StatPlus:mac statistical analysis software (AnalystSoft, Inc.). This procedure was used for determining significant differences for optimization of experimental procedures in Figures 1-3.

Each experimental time point was performed in triplicate, and the average (± standard deviation) \(k\), y intercept, and \(R^2\) values for each curve determined (Tables 3 and 4). These parameters were used to model the reduction in viability (Figs. 4 and 5) and the exposure that reduced the population by 90% (LD\(_{90}\); in days of desiccation or kJ m\(^{-2}\) UV-
When comparing the survival curves of individual isolates, statistical differences between \( k \) values were evaluated using a one-way ANOVA followed by post-hoc Tukey HSD analysis (StatPlus:mac statistical analysis software, AnalystSoft, Inc), with values of \( p<0.05 \) considered to be statistically significant.

### 2.6 Phylogenetic analysis of 16S rRNA genes

Extraction, purification, and amplification of 16S rRNA gene sequences from the isolates were performed as described in Chapter 3. A consensus was created for the 16S rRNA gene sequences as described in Ch. 3, based on a minimum of 2X coverage for each position in the alignment (227 to 1257, \textit{E. coli} numbering; BioEDit v. 7.2.5, 2013). The 16S rRNA gene sequences of the nearest type strains and environmental strains were identified with the SINA aligner using the SEED database (Pruesse et al., 2012). Phylogenetic analysis was conducted with MEGA7 (Kumar et al., 2016) using the maximum likelihood method, based on the Jukes-Cantor model with 1000 bootstrap replicates.

### 3.0 Results

#### 3.1 Optimization of Experiments

To determine if the drying rate effected viability, approximately \( 9.5 \times 10^5 \) CFU of exponentially growing \textit{D. radiodurans} were placed onto coverslips and rapidly dried under a stream of dry \( \text{N}_2 \) or were allowed to dry over \( \text{CaSO}_4 \) for 4 h. Although the surviving fraction from the 4 h drying treatment (4.1\( \pm \)1.2\%) was slightly higher than that from rapid drying with \( \text{N}_2 \) (2.4\( \pm \)2.0\%), the data were not significantly different \((p=0.19727)\). Based on these results, all of the isolates were desiccated in the chambers, with the viable count after 4 h designated as \( t=0 \) for the experiments. Vortexing and
washing by manually pipetting MgSO₄ onto the coverslip were evaluated for their effectiveness in removing cells from coverslips. The washing method was selected as it yielded statistically higher recovery when the starting concentration was less than 10⁸ CFU (Fig. 1). Growth on agar plates was monitored over the course of 7 days, depending on the individual growth rates of the isolates.

The effect of the growth phase on desiccation tolerance was evaluated in *D. radiodurans* by harvesting populations from exponential and stationary growth phase and examining survival to desiccation after 48 h (Fig. 2A). The effect of the growth phase on desiccation tolerance was evaluated in *D. radiodurans* by harvesting populations from exponential and stationary growth phase and examining survival to desiccation after 48 h (Fig. 2A).

Cells harvested from early exponential phase (2.7±0.45 x 10⁵ CFU) showed a significant decrease in viability after 48 h of desiccation (*p*=0.013, indicated with an asterisk), whereas the survival of populations harvested from the stationary phase was not significantly different from the initial population (*p*=0.069). Several of the atmospheric isolates (ML2-3, ML2-4, L3-6, L3-7, L7-3, L7-5, L7-7A) were evaluated for desiccation tolerance when harvested from exponential and stationary phase cultures (Fig. 2B), with the stationary phase populations (open symbols) consistently demonstrating significantly higher survival rates than those from exponentially phase (closed symbols). Based on these results, subsequent experiments were performed with cells harvested within the first 6 hours of entering stationary phase, as verified by measurements of optical density.
Figure 1: The relative effectiveness of removing desiccated cells by pipetting or vortexing. The data are plotted as the log transformed mean ± the standard deviation, (n=3). *Indicates data that are significantly different from the controls (p≥0.05).

Figure 2: The effect of growth phase of desiccation survival of (A) D. radiodurans after 48 h. The data are plotted as the log transformed mean ± the standard deviation (n=3). and (b) Atmospheric isolates were harvested in either stationary (open symbols) or exponential phase (closed symbols) and evaluated for desiccation tolerance for 14 days.
However, identical experiments using 1.0±0.15 × 10^7 D. radiodurans CFU had significantly reduced viability after the UV treatment (p=0.0053). Micrographs of the desiccated samples confirmed stacking of cells on coverslips containing 10^8 CFU, whereas a monolayer of cells was observed in the 10^7 CFU preparation, when the original culture was diluted 10-fold (Fig 3B). Therefore, all subsequent UV exposure experiments were conducted with populations of 10^5 to 10^7 CFU, based on the original cell populations during incubation, to mitigate the effect of cell stacking and shading.

3.2 Resistance to desiccation

Eighteen bacterial isolates that were recovered from samples collected at altitudes of 6.1 to 26 km (Table 1) were evaluated for desiccation tolerance (Fig. 4). The desiccation tolerance of E. coli and D. radiodurans were evaluated as examples of bacteria with low and high desiccation tolerance, respectively (Mattimore and Battista, 1996). The 20 strains could be organized into 5 categories based on their response to the experimental conditions.

Figure 3: (A) The effect of varying the starting concentrations of D. radiodurans survival after exposure to 0.65 kJ m⁻² of UV-C. The blue bars represent a 1:10 dilution of the original concentration (red bars) The data are plotted as the log transformed mean ± the standard deviation (n=3). *Indicates data that are significantly different from the controls (p≥0.05). (B) Monolayers of D. radiodurans were achieved when starting with ≤10^7 CFU.
desiccation. Three isolates (L3-4, L9-1, ML2-1) were classified as desiccation sensitive isolates, as no CFU were recovered when the samples were desiccated at $t=0$. The tropospheric isolate ML2-3 (6.1 km, LA; $p=0.3766$) and *D. radiodurans* ($p=0.86719$) were categorized as desiccation resistant, with no significant loss of viability after 14 days. Three tolerance categories (low, moderate, and high desiccation tolerance) were based on the similarity of survival curves obtained (Fig. 4).

Survival curves for each isolate were generated in triplicate and the average ($\pm$standard deviation) parameters derived from each experiment are detailed in Table 2. The data in Table 2 were used to generate decay models that described the reduction in microbial culturability (Figure 4), and were used to estimate the dehydration time, in days, necessary to reach the LD$_{90}$ (Table 2). The survival of each isolate while desiccated fit an exponential decay function well, with negative values of $k$ providing an estimated inactivation constant (Erkmen, O., 1995). Increasingly negative $k$ values indicate a reduced amount of time, in days, to reach the LD$_{90}$ (Table 2). Isolates were arbitrarily categorized as displaying low, moderate, and high tolerance to desiccation based on the $k$ values of the modeled survival curves (Table 2).

![Figure 4: Desiccation tolerance of atmospheric isolates over 14 days. The curves are plotted as the average parameters reported in Table 2.](image-url)
Highly desiccation tolerant strains were categorized by $k$ values from -0.075 to -0.18. A $k$ value difference of -0.09 separated the moderate group, whose $k$ values ranged from -0.27 to -0.50. The $k$ value difference between the moderate and low tolerance groups was more pronounced (0.15 decrease), and the $k$ values for the low desiccation tolerant strains ranged from -0.65 to -1.2 for *E. coli*. Culturable populations of *E. coli* were below the limit of quantification (<300 CFU mL$^{-1}$) after 8 days of desiccation, therefore the parameters of the survival curve were only evaluated for data collected up to $t$=6 days (Table 2). The inactivation rate of *E. coli* ($k=-1.2\pm0.070$) was significantly less than that for the atmospheric isolates ($p<0.0011$), excluding isolate L7-1. The inactivation rate of stratospheric isolate L7-1 was not significantly different from *E. coli* ($p=0.84537$), and both isolates are predicted to reach the LD$_{90}$ for desiccation in 2.0 days at stratospheric RH (Table 2).

Four strains isolated from samples collected at 26 km over Texas and New Mexico also displayed low desiccation tolerance, with $k$ values ranging from -0.65±0.038 to -0.94±0.021 (Fig. 4A). The inactivation rates of isolates L3-1 and L7-6 were not statistically different from each other, however the inactivation rate of L3-1 was significantly faster than L3-6 ($p=0.01714$). Four isolates, from three different genera, had $k$ values ranging from -0.27±0.087 to -0.50±0.035 after 14 days of desiccation and were classified as moderately desiccation tolerant (Table 3, Fig 4B). These isolates were all recovered from 26 km in the stratosphere during three independent sampling missions over New Mexico and Texas. The inactivation of L3-7 was significantly less than the other isolates in this group ($p<0.03$). Isolates L7-3, L7-7A, and L9-9A-1 displayed
similar rates of inactivation after 14 days of desiccation, and had \( \text{LD}_{90} \) values of 6.5±0.20 to 8.7±2.1 days (Table 2).

Table 2: Parameters of desiccation survival curves and the predicted time to reach the \( \text{LD}_{90} \) (days).

<table>
<thead>
<tr>
<th>Desiccation Tolerance</th>
<th>Isolate</th>
<th>Intercept ( \pm ) SE</th>
<th>(-k) ( \pm ) SE</th>
<th>(R^2) ( \pm ) SE</th>
<th>( \text{LD}_{90}) Days ( \pm ) SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>L7-1(^C)</td>
<td>3.5 (\pm 0.15)</td>
<td>1.2 (\pm 0.028)</td>
<td>0.91 (\pm 0.022)</td>
<td>1.9 (\pm 0.044)</td>
</tr>
<tr>
<td></td>
<td>(E. coli)(^C)</td>
<td>0.55 (\pm 0.11)</td>
<td>1.2 (\pm 0.070)</td>
<td>0.97 (\pm 0.018)</td>
<td>2.0 (\pm 0.24)</td>
</tr>
<tr>
<td></td>
<td>L3-1(^B)</td>
<td>0.70 (\pm 0.21)</td>
<td>0.81 (\pm 0.067)</td>
<td>0.96 (\pm 0.023)</td>
<td>2.9 (\pm 0.22)</td>
</tr>
<tr>
<td></td>
<td>L7-6(^AB)</td>
<td>0.31 (\pm 0.13)</td>
<td>0.68 (\pm 0.017)</td>
<td>0.92 (\pm 0.029)</td>
<td>3.4 (\pm 0.080)</td>
</tr>
<tr>
<td></td>
<td>L3-6(^A)</td>
<td>0.52 (\pm 0.27)</td>
<td>0.65 (\pm 0.038)</td>
<td>0.94 (\pm 0.010)</td>
<td>3.6 (\pm 0.20)</td>
</tr>
<tr>
<td>Moderate</td>
<td>L3-7(^B)</td>
<td>0.36 (\pm 0.02)</td>
<td>0.50 (\pm 0.035)</td>
<td>0.93 (\pm 0.0073)</td>
<td>4.6 (\pm 0.30)</td>
</tr>
<tr>
<td></td>
<td>L7-7A(^A)</td>
<td>0.89 (\pm 0.17)</td>
<td>0.36 (\pm 0.012)</td>
<td>0.95 (\pm 0.0089)</td>
<td>6.5 (\pm 0.20)</td>
</tr>
<tr>
<td></td>
<td>L9-9A-1(^A)</td>
<td>0.54 (\pm 0.035)</td>
<td>0.27 (\pm 0.036)</td>
<td>0.89 (\pm 0.013)</td>
<td>8.4 (\pm 0.98)</td>
</tr>
<tr>
<td></td>
<td>L7-3(^A)</td>
<td>0.84 (\pm 0.18)</td>
<td>0.27 (\pm 0.087)</td>
<td>0.86 (\pm 0.11)</td>
<td>8.7 (\pm 2.1)</td>
</tr>
<tr>
<td></td>
<td>L9-7(^A)</td>
<td>0.20 (\pm 0.052)</td>
<td>0.18 (\pm 0.025)</td>
<td>0.55 (\pm 0.063)</td>
<td>13 (\pm 1.5)</td>
</tr>
<tr>
<td></td>
<td>ML2-4(^A)</td>
<td>0.75 (\pm 0.12)</td>
<td>0.14 (\pm 0.065)</td>
<td>0.71 (\pm 0.16)</td>
<td>17 (\pm 5.4)</td>
</tr>
<tr>
<td></td>
<td>L7-5(^A)</td>
<td>0.45 (\pm 0.21)</td>
<td>0.14 (\pm 0.047)</td>
<td>0.56 (\pm 0.17)</td>
<td>17 (\pm 4.4)</td>
</tr>
<tr>
<td></td>
<td>L9-4(^A)</td>
<td>0.54 (\pm 0.10)</td>
<td>0.12 (\pm 0.048)</td>
<td>0.61 (\pm 0.025)</td>
<td>20 (\pm 5.9)</td>
</tr>
<tr>
<td></td>
<td>L9-9A-2(^A)</td>
<td>0.82 (\pm 0.16)</td>
<td>0.11 (\pm 0.027)</td>
<td>0.67 (\pm 0.19)</td>
<td>22 (\pm 4.3)</td>
</tr>
<tr>
<td></td>
<td>L6-1(^A)</td>
<td>0.86 (\pm 0.20)</td>
<td>0.075 (\pm 0.032)</td>
<td>0.72 (\pm 0.21)</td>
<td>31 (\pm 9.2)</td>
</tr>
</tbody>
</table>

The largest and most diverse group (7 isolates from 5 different genera) was classified as highly tolerant to desiccation, with inactivation rates of -0.075±0.032 to -0.18±0.025, and their survival data were not statistically different (Table 2, Fig. 4C). The *Cellumonas*
isolate ML2-4 was collected from the troposphere over LA, and all other isolates were recovered from three stratospheric flights over New Mexico (Table 1). The isolates highly tolerant to desiccation are predicted to survive between 13±1.5 and 31±9.2 days of desiccation before reaching the LD$_{90}$ (Table 2). One isolate, ML2-3, was considered to be desiccation resistant, and phenotypically similar to D. radiodurans, showed no reduction in viability after 14 days of desiccation. As a result, it is not possible to calculate an LD$_{90}$ from this study.

### 3.3 Resistance to UV-C

Each of the 14 isolates that was desiccation tolerant (low, moderate, or high) or resistant was examined for resistance to UV-C (Table 3). Isolates were initially screened with a dose of 0.65 kJ m$^{-2}$, which is a dose equivalent to the D$_{37}$ of exponentially growing

<table>
<thead>
<tr>
<th>UV Tolerance</th>
<th>Isolate</th>
<th>Intercept</th>
<th>-k</th>
<th>R$^2$</th>
<th>LD$_{90}$ kJ m$^{-2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>L9-7$^C$</td>
<td>0.41 (±0.039)</td>
<td>0.018 (±0.0006)</td>
<td>0.92 (±0.016)</td>
<td>0.13 (±0.0038)</td>
</tr>
<tr>
<td></td>
<td>L9-9A-2$^B$</td>
<td>0.16 (±0.026)</td>
<td>0.014 (±0.0010)</td>
<td>0.82 (±0.028)</td>
<td>0.16 (±0.011)</td>
</tr>
<tr>
<td></td>
<td>L7-5$^{AB}$</td>
<td>0.91 (±0.066)</td>
<td>0.011 (±0.0015)</td>
<td>0.99 (±0.0057)</td>
<td>0.20 (±0.024)</td>
</tr>
<tr>
<td></td>
<td>L7-3$^A$</td>
<td>0.79 (±0.27)</td>
<td>0.010 (±0.00058)</td>
<td>0.92 (±0.060)</td>
<td>0.22 (±0.012)</td>
</tr>
<tr>
<td></td>
<td>L9-9A-1$^A$</td>
<td>0.47 (±0.089)</td>
<td>0.0083 (±0.0015)</td>
<td>0.89 (±0.052)</td>
<td>0.28 (±0.043)</td>
</tr>
<tr>
<td>High</td>
<td>L7-7A$^B$</td>
<td>0.40 (±0.16)</td>
<td>0.0033 (±0.00058)</td>
<td>0.95 (±0.018)</td>
<td>0.69 (±0.10)</td>
</tr>
<tr>
<td></td>
<td>L9-4$^B$</td>
<td>0.98 (±0.13)</td>
<td>0.0030 (±0.0)</td>
<td>0.93 (±0.034)</td>
<td>0.77 (±0.0)</td>
</tr>
<tr>
<td></td>
<td>D. radiodurans$^B$</td>
<td>0.48 (±0.20)</td>
<td>0.0027 (±0.00058)</td>
<td>0.91 (±0.085)</td>
<td>0.86 (±0.15)</td>
</tr>
<tr>
<td></td>
<td>L6-1$^A$</td>
<td>0.44 (±0.064)</td>
<td>0.0013 (±0.00058)</td>
<td>0.79 (±0.10)</td>
<td>1.7 (±0.52)</td>
</tr>
</tbody>
</table>
cultures of *D. radiodurans* (Earl, et al., 2002). Twelve isolates were below the limit of quantification or displayed no CFU after the initial UV-C exposure, and were subsequently subjected to a series of lower UV-C doses (Table 3). Of the 12 isolates exposed to low UV-C doses (0.15 (±0.0090) to 0.65 (±0.038) kJ m⁻²) regime, seven isolates were reduced by >99.9% similar to *E. coli* (Silva-Junior et al., 2011), and were therefore considered UV-C sensitive. The UV-C survival experiments were done in triplicate and the average inactivation rates and fit for the curves are reported in Table 3. Similar to desiccation, the best-fit model for the data is an exponential decay ($R^2=0.79±0.10$ to $0.99±0.0057$). The *k* values of the moderately UV-C tolerant group ranged from -0.0083±0.0015 to -0.018±0.0006, and a difference of -0.005 separated this group from the highly UV-C tolerant group (Fig.4). However, the *k* values for the moderate group should be considered conservative estimates, few data were available at exposures less than 0.15 kJ m⁻² (Fig. 4A).

Figure 4: UV-C tolerance of desiccated atmospheric isolates plotted as the average parameters reported in Table 3.
3.4 Phylogenetic analysis of 16S rRNA gene sequences from the isolates

A maximum-likelihood tree based on 16S rRNA gene sequence comparison of the 24 isolates recovered from 6.1 to 26 km evaluated for desiccation and UV-C tolerance to UV-C. Based on a species designation of >97% 16S rRNA gene similarity (Stackebrandt and Goebel et al., 1994; reviewed by Gevers et al., 2005), two of the isolates (L9-9A-2, 94.7% and L9-1, 95.9%) may represent new species. Based on clustering and 16S rRNA gene identity, the most frequently recovered groups from the stratosphere were members of the genus *Curtobacterium*; (Fig. 6, Table 1), and seven strains were isolated. Phylogenetic relationships based on 16S rRNA gene comparison did not provide a way to predict either UV or desiccation tolerance (Fig. 6). For example, the *Curtobacterium* isolates L7-5 and L7-3 share 100% 16S rRNA sequence identity to each other, yet L7-5 was twice as tolerant to desiccation than L7-3 ($p=0.0426$). *Curtobacterium* isolates L3-6 and L3-7 also shared 100% 16S rRNA sequence identity, but L3-7 was 1.3X-fold more tolerant to desiccation. The two *Masillia* isolates that had 100% identical 16S rRNA gene sequence also displayed statistically different responses to desiccation, with L7-6 displayed almost twice the level of desiccation tolerance (Table 3).

4.0 Discussion

Maintaining viability during transport in the atmosphere should be possible for microorganisms that are resistant to a combination of environmental stressors that include desiccation and UV (Blumthaler and Ellinger, 1997). Desiccation may occur when cells are emitted from the surface, and resistance to dehydration is likely to be one of the most important factors for viability during transport to higher altitudes.
Figure 6: Maximum-likelihood tree displaying 16S rRNA gene similarity of the atmospheric isolates listed in Table 1.
For this reason, survival to desiccation was the parameter characterized. Isolates were exposed to 20 to 25% RH, conditions similar to an average of 21% RH in the air masses sampled above 20 km (Ch. 3). By extending the desiccation trials to 6 weeks for ML2-3, it would permit the comparison to the ~82% survival displayed by *D. radiodurans* after 6 weeks of desiccation (Rainey et al., 2005). If a loss of viability were recorded, the time to inactivate a given population of this microorganism could be calculated to better constrain the potential for surviving high altitude transport.

Assuming the low-end stratospheric cell concentrations of $3.8 \times 10^5$ cells m$^{-3}$ (Ch. 3), and each cell displayed a desiccation response equivalent to L7-1 and *E. coli*, it would take 11 days to completely inactivate the cell population at a RH of 20 to 25%. Based on the 7 to 10 day intercontinental transit times for dust and microorganisms in the troposphere (Griffin et al., 2006; Smith et al., 2013), even the least desiccation tolerant strain could potentially survive tropospheric transport to a new continent. The most desiccation tolerant isolate in this study, L6-1, is predicted to reach the LD$_{90}$ after ~1 month at 20 to 25% RH. If the stratospheric concentration of $3.8 \times 10^5$ cells m$^{-3}$ were as highly desiccation tolerant as isolate L6-1, it would take $1.9 \times 10^2$ days to completely inactivate the population at stratospheric RH of 20%. However, the convective ascent of air from the tropical tropopause into the stratosphere is slow (0.2 to 0.4 mm s$^{-1}$, Mote et al., 1998), so microbial populations would need to be at least 4-fold more desiccation tolerant than *D. radiodurans* (Rainey et al., 2005) to survive the $7.2 \times 10^2$ days to ascend from 16 to 36 km in the tropical stratosphere.
Bioaerosols lofted to altitudes of 20 km in the stratosphere must also have tolerance to UV-C if they are to survive during transport. Although there have been no direct measurements of UV-C fluence reported for the stratosphere, modeled values at 20 and 50 km are 0.0055 W m$^{-2}$ and 5.2 W m$^{-2}$, respectively (Smith et al., 2011), with ozone attenuation of UV-C reducing fluence rates roughly three orders of magnitude at 20 km compared to 50 km (Smith et al., 2011). Based on the UV-C LD$_{90}$ of B. subtilis SAFR-032 endospores (1.7 kJ m$^{-2}$; Link et al. 2004), and the fraction that survived 22 minutes of exposure at ~30 km (Khodadad et al., 2017), a UV-C fluence rate of 1.3 W m$^{-2}$ can be inferred (Table 4). The fluence rate of UV-C for the surface of equatorial Mars is estimated to be 3.2 W m$^{-2}$ (Schuerger et al., 2003). Based on these UV-C fluence rates, the estimated time for 3.8 x 10$^5$ bioaerosols to reach the LD$_{90}$ at 20 km, 30 km, and on the surface of Mars can be determined (Table 4). The stratospheric cell concentration of 3.8 x 10$^5$ cells similar to the minimum bioburden value of 3.0 x 10$^5$ spores that are acceptable for non-life detecting missions, such as Curiosity (Benardini et al., 2014; NASA, 2005).

The time required to kill the entire population of various isolates under different UV exposures is shown in Figure 7. The most UV-C tolerant isolate, L6-1, would only be capable of surviving 43 days at 20 km and 2.2 h at 30 km, a value that decreases by half on the surface of Mars (Table 4). If 3.8 x 10$^5$ cells displayed the minimum UV-C tolerance (equivalent to isolate L9-7), they would be predicted to survive as few as 3 days of exposure to UV-C conditions at 20 km (Fig. 7). This population of cells would be annihilated in 9.6 and 4.0 minutes at 30 km and on the surface of Mars, respectively.

Ozone concentrations measured over Socorro, NM (34°N, 107°W) during the 2013 sampling dates indicate a local ozone maximum near 26 km (Minschwaner et al., 2015),
consistent with decreased ozone attenuation of UV-C at 30 km. The median sampling altitude of 26 km, the highest altitude where isolates were recovered, corresponds to sampling a column of from 22 to 29 km (Ch. 3). Therefore, isolates may have been recovered from below the maximum ozone concentration. The actual bioburden of the Curiosity rover was well below the acceptable threshold, at a total of 1.6 x 10^4 spores (Benardini et al., 2014). According to the range of UV-C tolerances observed in this study, the total rover bioburden would have been inactivated between 4 and 55 minutes on the surface of Mars.

Harris et al., (2002) report the detection potentially viable cells (as determine by microscopic examination of an intact cellular membrane) in samples collected from 19 to 20 km and at 40 to 41 km. Shivaji et al., (2006) later cultured isolates from the same mission as Harris et al., (2002) and recovered 4 Bacillus isolates from 24, 28, and 41 km. None of the isolates were evaluated for their response to UV-C, arguably the most lethal portion of the UV spectrum (Ch. 1).

![Figure 7: The predicted time to inactivate isolates at concentrations of 3.8 x 10^5 cells based on UV-C exposures at 20 km, 30 km, and the equatorial surface of Mars.](image)
Table 4: Predicted time exposed to UV to reach LD90 for isolates and reference strains in the stratosphere and on the surface of Mars

<table>
<thead>
<tr>
<th>Organism</th>
<th>LD90 (kJ m(^{-2}))</th>
<th>Time to reach LD90 (min)</th>
<th>Preparation for UV exposure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. anthracis Sterne</td>
<td>0.2750</td>
<td>3.5 x 10[^2]</td>
<td>Spores, PBS</td>
<td>Nicholson et al., 2003</td>
</tr>
<tr>
<td>B. subtilis WN 624</td>
<td>0.2450</td>
<td>3.1 x 10[^2]</td>
<td>Spores, PBS</td>
<td>Nicholson et al., 2003</td>
</tr>
<tr>
<td>L.7-3</td>
<td>0.20</td>
<td>6.2 x 10[^2]</td>
<td>Desiccated</td>
<td>This study</td>
</tr>
<tr>
<td>D. radiodurans</td>
<td>0.91</td>
<td>2.8 x 10[^3]</td>
<td>Stationary, PBS</td>
<td>Bauermeister et al., 2011</td>
</tr>
<tr>
<td>D. aetherius</td>
<td>0.86</td>
<td>2.6 x 10[^3]</td>
<td>Spores, PBS</td>
<td>Arrang et al., 1993</td>
</tr>
<tr>
<td>L.9-7A</td>
<td>0.16</td>
<td>5.0 x 10[^2]</td>
<td>Desiccated</td>
<td>This study</td>
</tr>
<tr>
<td>L.9-9A</td>
<td>0.13</td>
<td>1.3 x 10[^2]</td>
<td>Desiccated</td>
<td>This study</td>
</tr>
<tr>
<td>L.9-4</td>
<td>0.12</td>
<td>1.5 x 10[^2]</td>
<td>Aerosol, 20% RH</td>
<td>Rentschler and Nagy 1942</td>
</tr>
<tr>
<td>M. luteus ATCC 10240</td>
<td>0.10</td>
<td>2.9 x 10[^2]</td>
<td>Stationary, PBS</td>
<td>Arrange et al., 1993</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.040</td>
<td>1.2 x 10[^2]</td>
<td>Stationary, PBS</td>
<td>Arrange et al., 1993</td>
</tr>
<tr>
<td>S. marcescens ATCC 13880</td>
<td>0.032</td>
<td>3.2 x 10[^2]</td>
<td>*</td>
<td>ATCC B</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.0053</td>
<td>9.7 x 10[^3]</td>
<td>*</td>
<td>ATCC B</td>
</tr>
</tbody>
</table>

**Assuming 12 sun hours day^{-1}**

* ATCC 13939

Notes:
- Predicted time exposed to UV to reach LD90 for isolates and reference strains in the stratosphere and on the surface of Mars.
When initial concentrations of ~3 x 10³ endospores were exposed to a UV-B dose of 2.0 mJ m⁻², all were reduced by >99% and were below the level of quantification employed in this study (<30 CFU). Based on the ascent rates described above, the reports of the recovery of viable microorganisms from above 30 km, without mention of the local ozone maximum or levels of UV-C tolerance (48 to 77 km, Imshenetsky, 1978; 30 to 41 km, 24 to 41 km, Shivaji et al., 2006; 27 to 41 km, Wainwright et al., 2003) are lacking important details to describe the potential for microbial survival at these altitudes.

*B. subtilis* SAFR-032 spores transported to the stratosphere, but shielded from sunlight, showed no loss in viability during an 8 h flight at ~30km, demonstrating no lethal effects on spores due to the combination of reduced temperature, pressure, and RH (Khodadad et al., 2017). However, when the spores were exposed to stratospheric sunlight, viability was reduced by 90% after 22 minutes, indicating UV-C was the limiting factor of spore viability (Khodadad et al., 2017). Similar results have also been obtained when assessing microbial survival under simulated Mars environments, with and without UV exposure (Schuerger et al., 2003; Smith et al., 2011; Tauscher et al., 2006). Future sampling efforts with the isolates described in this study could transport desiccated samples, with a simple UV shield (i.e., foil-lined Petri dish) in order to determine any potential loss of viability due to the combined stressors of reduced temperature, pressure, and RH.

The protective effects of atmospheric dust to spore inactivation in Mars analog soils under simulated Mars conditions were investigated by Schuerger et al., (2003). Dried spore samples were exposed to UV under various filters to simulate increasing optical density as a proxy for aerosol dust loading. After 10 minutes of Mars UV
exposure, only optical density filters equivalent to a global dust storm provided a significantly higher recovery of viable spores, and >99% of the spore population was inactivated after 60 minutes (Schuerger et al., 2003). Layers of particles (approximately 0.5 mm thick) of increasing size were also scattered onto dried spore samples to determine the potential for shading (Schuerger et al., 2003). Microscopic examination of the smallest Mars analog soil particles (2-8 µm) revealed they tended to fall in the areas between the individual endospores and provided no protection after 1 h of Mars UV. Larger particles (10-50 µm) were also visualized and were estimated to cover as many as 50 endospor es and resulted in <1% survival after the same exposure (Schuerger et al., 2003). Only when dust was applied in a 0.5 mm contiguous layer were spores recovered after 8h of exposure (Schuerger et al., 2003). Based on stratospheric particle size distributions reported in Ch. 3, there were no significant concentrations of particles larger than 6 µm in any of the samples, indicating UV protection from large particles is unlikely at stratospheric altitudes.

Remarkably, the Curtobacterium isolate L6-1 has the highest desiccation tolerance, and a UV-C tolerance equivalent to B. subtilis SAFR-032 endospores, the most highly UV-C-resistant spore documented (Link et al., 2004). The structural characteristics of metabolically dormant endospores are highly differentiated from vegetative cells, as are their mechanisms of resistance to desiccation and UV-C (reviewed by Setlow, P., 1995; 2006). The binding of α/β small, acid soluble spore proteins (SASPs) to DNA offers protection from single strand breaks induced during desiccation (Fairhead et al., 1994). These SASPs alter the conformation of spore DNA into the A-helix, however the exact mechanism of DNA protection during desiccation is still unclear.
(Nicholson et al., 2000). The A-helix conformation of spore DNA also reduces the likelihood of CPD formation, and the main form of UV-C damage to spores is the easily repaired spore photoproduc (Nicholson et al., 2000; Setlow and Li, 2015). Additionally, the presence of dipicolinic acid bound to Ca\(^+\) reduces the water content of the spore, which also favors the DNA A-helix conformation and may reduce the formation of ROS (Setlow and Li, 2015).

However, unlike *B. subtilis* SAFR-032, L6-1 does not differentiate into endospores, and presumably, persists as a vegetative cell under these extreme conditions. Only two of the isolates collected from this study were endospore-forming species of *Paenibacillus*, and endospores are of the size (<1 \(\mu\)m) and durability that would be expected to survive conditions in the stratosphere (Nicholson et al., 2000). Both of the endospore-forming isolates were categorized as desiccation sensitive, indicating neither the vegetative cells of ML2-1 nor L9-1 were well suited for atmospheric transport (Section 3.2). Therefore, the main form of UV-C damage incurred will be the formation of CPD, and the DNA DSB, loss of membrane integrity, protein denaturation induced from desiccation must be mitigated (reviewed by Lebre et al., 2017). Strategies that provide tolerance to desiccation include the alteration of cellular membrane fatty acid composition (Finn et al., 2013), the accumulation of compatible solutes (i.e. trehalose, sucrose; Finn et al., 2013), down regulation of high-energy expenditures (i.e. flagella, chemotaxis; Finn et al., 2013; Hingston et al., 2015), and the up regulation of stress response pathways (Deng et al., 2012; Finn et al., 2013; Gruzdev et al., 2012a). During stationary phase, the reduction high-energy processes and the induction of stress response pathways may also prove beneficial during desiccation.
It should be noted that desiccation and UV-C tolerance in bacteria can be highly dependent upon the experimental conditions, the bacterial strains, their growth phase, dry down time, media used for recovery, % RH during desiccation, and temperature (Finn et al., 2013; Gruzdev et al., 2011; Gruzdev et al., 2012b). Four isolates (ML2-1, L3-4, L7-6, L9-1) did not survive the initial four hour dry down period at 20 to 25% RH. These microorganisms may not have been well adapted to a short dry down period, and unable to metabolically prepare for the stress of desiccation (Gruzdev et al., 2011). The survival of desiccated S. enterica was significantly decreased, from ~50 to 11%, when the initial dry down was decreased from 22 to 6.5 h (Gruzdev et al., 2011). Testing the above isolates after increasing the dry down period may result in the recovery of viable cells. Decreased storage temperatures have also been shown to significantly increase desiccation tolerance (Gruzdev et al., 2011; Gruzdev et al., 2012a; Gruzdev et al., 2012b; Yang et al., 2009c). Therefore cells desiccated at 20 to 25% RH and -58 °C (the average stratosphere temperature observed in Ch. 3) might have higher LD₉₀ values (in days) compared to those in Table 2. Conversely, D. radiodurans and B. subtilis spores became more sensitive to UV-C at -70 °C than at 20 °C (Dose et al., 1996). Therefore the interpretation of LD₉₀ values (kJ m⁻²) presented in this study should be regarded as an estimate for survival under these described conditions. Changes to any of the above parameters would potentially result in altered inactivation rates, particularly the temperature at which survival is evaluated. The colder temperatures of the stratosphere may increase predicted survival time to desiccation, however the effect of UV-C survival remains unknown for these isolates.
Phylogenetic analysis of the isolates based on 16S rRNA gene comparison revealed many were closely related to other isolates from the atmosphere or arid environments (Fig. 6). However, 16S rRNA gene similarity was not an effective means for predicting levels of tolerance, as demonstrated by the *Masillia* isolates L7-1 and L7-6, who were isolated from the same flight, shared 100% 16S rRNA sequence similarity, and L7-1 was significantly less tolerant than L7-6 ($p=0.00015$). Similarly, there were *Curtobacterium* isolates (L3-6 and L3-7; L7-3 and L7-5) that shared 100% sequence identity, yet displayed significantly different levels of desiccation tolerance (Table 2). These results highlight the well-known limitations of using 16S rRNA gene similarity to infer physiological properties solely based solely on genetic relatedness.

Several members of the genus *Curtobacterium* were isolated from multiple flights over both New Mexico and Texas (Table 1, Ch. 3). Isolates closely related to the bean pathogen *C. citreum* have been isolated from the CBL in the U.S. Virgin Islands both prior to, and during, Saharan dust events (Griffin et al., 2001). Isolates closely related to the *C. flaccumfaciens pv. flaccumfaciens*, causal agent of bean wilt (EPPO, 2011; Harveson, et al., 2015), and *C. flaccumfaciens pv. beticola* (sugar beet pathogen; Chen et al., 2007) have repeatedly been detected in clouds (Amato et al., 2006; Vaïtilingom et al., 2012). *C. flaccumfaciens pv. flaccumfaciens* is a quarantined pest in Europe (EPPO, 2011), and it has recently been detected for the first time in Brazil (Soares et al., 2013), Iran (Osdahi et al., 2015), and China (Wang et al., 2016). Three isolates from altitudes of 18 to 29 km have >99.7% 16S rRNA gene sequence identity with *Curtobacterium flaccumfaciens pv. flaccumfaciens*. The isolate L6-1 shares 100% 16S rRNA gene identity, and demonstrated the highest levels of desiccation and UV-C tolerance of all
isolates tested. Two additional isolates (L3-6 and L3-7) share 99.7% 16S rRNA sequence similarity, and these strains displayed varying levels of tolerance to desiccation and UV-C. La Duc et al., (2003) recovered an isolate that shared >99.9% 16S rRNA gene similarity with *C. flaccumfaciens* from the Mars Odyssey spacecraft, however this strain was not included in their set of 23 isolates challenged with desiccation, UV-C, γ-radiation, and H$_2$O$_2$. Here, we provide evidence of atmospheric isolates, similar to those associated with spacecraft that are more tolerant to desiccation and roughly 3X more tolerant to UV-C than the microorganisms previously tested. This work highlights the need to extended bioburden monitoring beyond heat resistant endospore-forming species.

Work is currently underway in the Zuber lab at MIT (Carr, C., personal communication) to sequence the genomes of these stratospheric isolates closely related to *C. flaccumfaciens pv. flaccumfaciens*, and this data will allow inter-comparisons into the presence, or absence, of genes that may describe the variations in their desiccation and UV-C tolerance levels (e.g., Tanaka et al., 2004). The genomic data from the atmospheric isolates will also permit comparisons to previously described *Actinobacteria* isolates with high desiccation and UV-C tolerance, and may aid in identification of genes or homologues that correlate to increased desiccation or UV-C tolerance (Manzanera et al., 2014; Normand et al., 2014; Tanaka et al., 2004). A combination of genomic data, transcriptional analysis, and transposon mutant libraries could provide information that allows specific genes responsible for desiccation and UV-C resistance to be identified. It would also be highly beneficial to collect more environmental *Curtobacterium* isolates determine the pathogenicity, and host range of these isolates as compared to the known plant pathogen members of the genus. Similar work with environmental strains closely
related to the plant pathogen *Pseudomonas syringae* pv. *tomato* led to the discovery of marker genes able to predict the capacity of an environmental strain to colonize plants and cause disease (Bartoli et al., 2015). Such marker genes could be used as relatively simple and cost-effective ways to identify potential pathogen invasions, and improve plant disease management techniques (Bartoli et al., 2015; Schmale et al., 2015).

Many of the isolates in this study are closely related to microorganisms previously recovered from atmospheric samples or arid environments. Isolates from the genus *Masillia* were recovered from 21 km and 26 km over Texas, and were highly similar to *M. jejuensis* and *M. niabensis*, type strains first identified from air samples (Weon et al., 2010). Members of the *Masillia* genus have also been isolated from clouds on multiple occasions (Amato et al., 2006; Vaïtilingom et al., 2012), and from plant surfaces (Bassas-Gailia et al., 2011). *Modestobacter* isolate L9-4 was 98.7% similar to *M. versicolor*, a species first described from biological soil crusts collected from 1.2 km on the Colorado plateau (Reddy et al., 2007) and other members of this genus have been isolated from the dry, cold desert soils of Linnaeus Terrace, Antarctica (1.6 km, Mevs et al., 2000). *M. marinus* strain BC501 was isolated from a marble surface and demonstrated a UV-C LD$_{90}$ of 900 J m$^{-2}$ and 10% survival after 60 days of desiccation (Gtari et al., 2012; Normand et al., 2012; Xiao et al., 2011). The novel species *M. caceresii* was recently isolated from the Atacama Desert and possesses betaine and trehalose biosynthesis genes, that have been previously associated with desiccation tolerance (Busarakam et al., 2016). *Roseomonas* was one of the top 20 bacterial genera recovered from aerosol samples collected from 40 different locations, covering 7 different climate zones in China (Wei et al., 2015). From 26 km on 9/4/13, two isolates were recovered that were closely related
(98.0 and 94.7%) to *Roseomonas vinacea* (T), previously isolated from the Qinghai-Tibet plateau where elevation exceeds 4.0 km (Zhang et al., 2008). These isolates are also closely related to novel species, first described from aerosol samples (Kim et al., 2013; Yoo et al., 2008) and strains recovered from clouds (Vaïtilingom et al., 2012). This collection of isolates indicates microorganisms capable of aerial dispersal into the stratosphere are not limited to endospore forming genera.

Here we provide the first data set of culturable isolates recovered from multiple stratospheric sampling missions, and report their levels of tolerances to desiccation and UV-C. Although isolates were successfully recovered during each culturing attempt, it is highly unlikely that these species could survive the longest stratospheric residency times of 1 to 2 years (Benduhn and Lawrence, 2013). When comparing predicted survival times due to desiccation or UV-C, the increased UV-C fluence is the most detrimental environmental stressor encountered and serves as the limiting factor for microbial survival in the stratosphere, as well as the surface of Mars (Horneck et al., 2012; Khodadad et al., 2017). Current standards of planetary protection guidelines are restricted to quantifying the bioburden of heat resistant spores, despite the previously documented diversity of nonspore-forming bacteria associated with spacecraft and their assembly facilities (La Duc et al., 2004; La Duc et al., 2007). Although spores are capable of withstanding multiple environmental stressors (Nicholson et al., 2000), the nonsporulating isolates obtained in this study also displayed high levels of tolerance to desiccation and UV-C. The highly desiccation tolerant isolate L6-1 rivals the most UV-C resistant spore documented (Link et al., 2004), and isolates L7-7 and L9-4 were not statistically different from UV-C tolerant *D. radiodurans* (Earl et al., 2002). Therefore,
spores may not be the only appropriate measure of bioburden on spacecraft. When shielded from UV, spores maintained 15-50% viability after 1.5 years of exposure to space conditions (Horneck et al., 2012), and our results indicate several nonspore-forming species may also survive stratospheric conditions for much longer periods if shielded from UV-C.
CONCLUSIONS

Very little is known about the distribution, concentration, composition, and viability of bioaerosols above the CBL. Multiple efforts have reported the presence of viable bioaerosols in the stratosphere up to 41 km (Griffin et al., 2004; Harris et al., 2002; Ohno et al., 2017; Smith et al., 2010; Wainwright et al., 2003; Yang et al., 2008a; Yang et al., 2008b), contamination from the sampling vehicle cannot be excluded. Additionally, each of these reports was the product of a single sampling mission, with no repeated measures to verify the results, and often lacked details of the volume sampled or the particle collection efficiency. To provide statistically significant, quantitative data describing the vertical distribution of bioaerosols, the above issues were addressed by providing: detailed decontamination protocols and their effectiveness for reducing microbial viability and ATP concentrations, descriptions of limits of detection for each measurement conducted to ensure all values are significantly above the background, multiple measurements conducted at stratospheric altitudes, accurate values for the volume of air sampled during each flight, and experimentally determined particle collection efficiency of the sampling rods. In order provide the first quantitative bioaerosol dataset above 10 km, several developments were necessary. First, a balloon borne payload was developed to offer a cost effective, reproducible impact sampling method to collect bioaerosols at altitudes up to 38 km (Ch. 2), where low temperatures and pressures make standard bioaerosol sampling technologies unfeasible (Ch. 1). Due to the potentially low biomass at increased sampling altitudes, highly sensitive microbiological detection techniques were employed. Therefore the quantification of the residual contamination (i.e., total DNA containing and culturable cells, particles, and
ATP) incurred before, during, and after flight was a top priority in order to achieve statistically significant results. Through rigorous characterization of residual contamination, we were able to provide the first data set with multiple measurement techniques and the statistical power to quantify the vertical distribution of bioaerosols up to 38 km in the stratosphere (Ch. 3). In addition to the quantification of vertical distribution of bioaerosols, a collection of bacterial isolates was recovered from 6.1 to 26 km. An investigation into the levels of desiccation and UV-C tolerance displayed by each of the isolates provided valuable insight to the potential survival during high altitude transport (Ch. 4).

Similar to the contamination management plan for Mars 2020 and missions designed to detect the presence of microbial life, our goals were to reduce the initial contamination as much as possible, and to quantify any residual contamination (NASA, 2005; Summons et al., 2014). Due to the materials used for sampling (i.e. plastic rods), it was not possible to prepare this particular payload design in a manner that would reduce the number of residual cells to a level of zero (i.e., furnacing materials at 450 °C to remove carbon; Ch. 2). Launching sealed control chambers with each flight provided procedural “blanks” that allowed background contamination associated with every step of the sampling and analysis operations to be evaluated (Ch. 3). By quantifying the total residual contamination (i.e., total DNA containing and culturable cells, particles, and ATP) and its variability on the sampling surfaces prior to flight, we were able to establish limits of detection for each analytical method, and statistically determine when a significant quantity of bioaerosols or particles were recovered from the target altitude (Summons et al., 2014). In addition to being able to collect high altitude bioaerosol
samples and return them to the surface without ambient contamination, it was imperative to know how efficient the system collected particles of different sizes, allowing concentration per total volume sampled to be estimated (Ch. 3). When this information is unknown (e.g., DeLeon-Rodriguez et al., 2013, Griffin et al., 2004, Smith et al., 2010) relating the size and number of collected microbes and particles to an air mass volume is not possible. A well-characterized level of residual contamination for each assay is also relevant for planetary protection guidelines, particularly when preparing for life detection missions (Frick et al., 2014; NASA, 2005; Summons et al., 2014). For Mars Category IVb life detection missions, “subsystems which are involved in the acquisition, delivery, and analysis of samples used for life detection” shall be limited to an acceptable bioburden of 30 spores (culturable microorganisms capable of surviving 15 minutes at 80 °C; NASA 2005). Based on this description, our payload decontamination protocols would currently meet the guidelines for a life detection mission to Mars, as no spores were recovered from our controls. The simple decontamination and quantification of residual contamination described here could easily be adapted to a variety of spacecraft and quantitative microbiological methods (Ch. 2).

Although the payload successfully recovered bioaerosols and particles from the CBL up to 36 km, there are limitations to this method of sampling. To obtain a signal using our method, a portion of the atmosphere must be traversed, either vertically upon ascent or horizontally while at float (Ch. 3). Therefore, the resolution of where the sample was collected from is limited to the time frame of active sampling. For example on September 1, 2013, it is not possible to determine where in the stratosphere between 19 and 29 km the samples were collected from or if there were altitudes where the
aerosols were more highly concentrated. Because the volume of air sampled vertically is calculated by multiplying surface area of the rod (35 mm$^2$) by the altitude sampled, a large altitude range (10 km) will only sample 0.35 m$^3$ of air (described in Ch. 2 and 3). The number of sampling chambers is currently limited due to weight restrictions, and more chambers would allow for either a greater number of altitudes sampled per flight or a greater number of observations at a given altitude. However, these weight restrictions are highly dependent upon the platform used, and our system could easily be adapted to accommodate more sampling chambers. An increased number of observations at altitudes in the free troposphere and stratosphere would prove beneficial when sampling for both ATP, which required 60 observations (or rods) to detect a positive signal when cell concentrations were <1.0 x 10$^6$ m$^{-3}$, and culturing for isolates (Ch. 3). Additionally, the sampling efficiency of the rods (35 mm$^2$) is extremely low for particles in the size range of bacteria ($\leq 1\%$ for particles 1 to 2 µm in diameter; Ch. 3). For those particles that are impacted on the rod, they are often embedded into the silicone grease, which results in difficult to resolve SEM images at high levels of magnification and the inability to perform trace element analysis. Additionally the relatively low biomass collected on each rod makes the use of DNA and RNA based approaches to characterize the structure and physiological activity of bioaerosol populations collected at altitude challenging. Attempting to remove cells from the silicon grease would require an additional removal efficiency calculation and may result in the loss of already low biomass (<10$^3$ cells rod$^{-1}$) samples (Ch.3). If cells could be efficiently removed from the silicone grease, single cell genomic may provide additional insight into the nonculturable bioaerosols collected (Fullerton et al., 2016). Despite these limitations, the current payload is still capable of
collecting quantifiable aerosol samples from stratospheric altitudes, and is easily adapted to long duration balloon platforms, which will provide the opportunity to answer a wide variety of questions concerning the concentration and viability of bioaerosols, (i.e., the influence of geographical location, season, location relative to the ozone layer).

Our survival data indicate that the time to render a population of $3.8 \times 10^5$ cells no longer viable ranges from 11 to 350 days. However, even the most UV-C resistant isolate would not survive 43 days at 20 km, making UV-C the limiting factor for survival in the stratosphere. Future work will provide a greater understanding of how these microorganisms respond to atmospheric dispersal. Microorganisms respond to desiccation in a variety of ways, including reducing rates of metabolic activity, accumulating of compatible solutes and antioxidants, altering membrane fluidity (detailed in Ch. 1). Despite the wide range of strategies employed to combat the stress of desiccation, the precise cellular events that trigger these responses remains largely unknown (Lebre et al., 2017). In order to better understand the mechanisms of desiccation and UV-C tolerance of the atmospheric isolates (Ch. 3), further work should be performed to investigate the type of damage experienced and the specific responses employed to combat these stressors.

Low temperatures (4 °C to simulate refrigerated food storage) have been linked to increased desiccation tolerance (Gruzdev et al., 2012; Hiramatsu et al., 2005; Koseki et al., 2015). By desiccating and storing isolates at low temperature (-20 to -30 °C to simulate the free troposphere, -55 to -60 °C for stratospheric altitudes), the tolerance levels could be reevaluated at temperatures equivalent to the atmosphere (Ch. 2). The use of atmospheric simulation chambers would allow for the analysis of UV-C and
desiccation tolerance at temperatures and pressures (10 to 50 mbar) that would mimic high altitude environments (Schuerger et al., 2006). Microorganisms have demonstrated protein and nucleic acid synthesis at temperatures as low as -15 °C (Amato et al., 2010; Christner et al., 2002). Isolates of Psychrobacter cryohalolentis exposed to ionizing radiation and subsequently incubated at -6 °C demonstrated 2X higher survival than cultures at 22 °C (Amato et al., 2010). Decreased metabolism at low temperatures may not be directed towards growth and replication, but rather the repair of DNA and other damaged cellular components (Amato et al., 2010). Similar phenomena may explain the increased survival of desiccated cultures at lower temperatures.

The formation of DNA DSB, the main source of desiccation induced DNA damage, occurs due to the accumulation of ROS (Franca, et al., 2007; Potts, M., 1994; Lebre et al., 2017). Future investigations of the atmospheric isolates should quantify the total genome size and the relative amount of DNA double strand breaks (DSBs) each isolate accumulates during the course of the desiccation trials (Dieser et al., 2013; Mattimore and Battista, 1996). Mattimore and Battista (1995) reported equivalent rates of DSB formation for both desiccation tolerant D. radiodurans and desiccation sensitive E. coli. However, unlike exposure to ionizing radiation, there is currently no way to estimate the number of DSB incurred as a function of time spent in a desiccated state (Battista, J. R., personal communication). By comparing the number of DSB accumulated over time in the atmospheric isolates from CH. 4, the possibility of strain specific responses could be evaluated and an estimated of the number of DNA DSB each isolate accrued can then be compared to the loss of viability over the time spent desiccated (Mattimore and Battista, 1996). Additionally, the rate of DNA DSB repair after desiccation should be
quantified according to Dieser et al., (2013). By rehydrating isolates and incubating them at environmentally relevant conditions (i.e., phyllosphere, marine, various soil conditions), the potential for these isolates to survive aerial dispersal and colonize a new habitat could be inferred. Information from this study would help to determine if the desiccation tolerance can be attributed to a reduced amount of DNA DSB experienced due to antioxidant ROS mitigation, an efficient repair of DNA DSB, or other resistance mechanisms (i.e., compatible solutes, altered metabolic processes or cell membrane composition). Because the DNA DSB sustained during desiccation are also the main form of damage induced by ionizing radiation (IR; Mattimore and Battista, 1996), testing the isolates for IR tolerance could provide additional evidence that cellular responses and DNA repair mechanisms used for desiccation survival are the same that are needed for IR resistance (Mattimore and Battista, 1996; Tanaka et al., 2004; Rainey et al., 2005)

Although more completed bacterial genomes are becoming publicly available, the genomic data does not provide insight into the specific mechanisms for tolerance to stress (Tanaka et al., 2004), and the strategies for desiccation (Gruzdev et al, 2012; Finn et al., 2013; Hingston et al., 2015) and UV-C tolerance remain poorly understood. Classical genetic methods can also be used to address these outstanding questions by generating mutants of the wild type species that are susceptible to desiccation or high UV-C doses (Alvarez et al., 2014; Battista et al., 2001; Foster, P., 1991; Hingston et al., 2015; Udupa et al., 1994). Transcriptional analysis can also provide insight as to how these isolates respond to the desiccation and UV-C exposures (Cytryn et al., 2007; Deng et al, 2012; Finn et al., 2013; Hingston et al., 2015). As demonstrated by Tanaka et al., (2004), the variations in transcriptional responses when rehydrated may provide insight into the
specific repair mechanisms that would allow atmospheric isolates to flourish when they have returned to a suitable environment. Previous studies have combined transcriptional analysis with the survival of mutant strains, which led to the identification of previously unidentified DNA repair genes and their respective proteins, as well as the discovery of novel DNA repair pathways (Tanaka et al., 2004). The current collection of atmospheric isolates described in Ch. 4 provides a unique opportunity to investigate the specific mechanisms of desiccation and UV-C tolerance through future transcriptomic and proteomics analysis.

The vast majority of the available data regarding the concentrations and distributions of bioaerosols are reported within the CBL (reviewed by Burrows et al., 2009a). Roughly $10^{24}$ bacterial cells are predicted to be emitted from the surface of the Earth each year, and the majority of these bioaerosols originates from crops (Burrows et al., 2009b). Based on a single study of CFU concentrations measured over a desert (Lightheart and Schaffer, 1994) Burrows et al., (2009a) predicted this ecosystem to be a poor source of bioaerosols with a mean concentration of $10^4$ cells m$^{-3}$. These values have subsequently been incorporated into global bioaerosol emission and transport models (Burrows et al., 2009b, Burrows et al., 2013; Hoose et al., 2010; Sesartic et al., 2012) that conclude deserts play a relatively small role as a source for bioaerosols. However, the average CBL concentrations reported in Ch. 3 ($1.3 \times 10^6$ cells m$^{-3}$) are two orders of magnitude higher than the Burrows et al., (2009a) estimates. This highlights the need for increased bioaerosol observations to better constrain their global contributions (Burrows et al., 2009b). Although the overall concentrations presented by Burrows et al., (2009a) were lower than the values observed in Ch. 3, the increased potential for desert
bioaerosols to be transported over long distances due to the reduced rates of scavenging due to precipitation is emphasized (Burrows et al., 2009b, Burrows et al., 2013).

Although there has been an increase in the number of studies of bioaerosols in the free troposphere (Ch. 1), significant questions remain concerning their distributions and their potential for survival during long distant transport. Future sampling missions should investigate spatial and temporal variations of the free troposphere concentrations of total bioaerosols and the viable fraction similar to work completed in the CBL (reviewed by Burrows et al., 2009a). Measurements conducted above the CBL, those comparing both in and out of clouds (Amato et al., 2006), or those associated with significant meteorological events (i.e. hurricanes, dust storms, atmospheric rivers) would also provide beneficial information concerning the distribution of bioaerosols in the free troposphere. By coupling future bioaerosol sampling missions with atmospheric chemistry measurements, it may be possible to compile additional evidence for intercontinental transport (as determined by water vapor, carbon monoxide, and total gaseous mercury by Weiss-Penzias et al., 2006) or the possibility of stratospheric intrusions (Lin et al., 2015 nature), described in Ch. 1. Deep tropopause folds form after La Niña winters, resulting in the transport of stratospheric ozone to the CBL (Lin et al., 2015 nature). These stratospheric intrusion events are pronounced over high elevation sites in the western United States during the spring (Lin et al., 2015 nature). The detection of high $O_3$ and low water vapor concentrations (Lin et al., 2015 nature) in conjunction with the recovery of viable bioaerosols would provide valuable evidence for the possibility of surviving stratospheric transport and their potential to colonize a new habitat.
Bioaerosols (1.0 µm diameter, the size range of bacterial cells) in the free troposphere are predicted to have an average residency time of 7 days, with the highest values predicted over desert environments (14 days) due to decreased precipitation and strong convective transport (Burrows et al., 2009b). Intercontinental transport times are on the order of 7 to 10 days (Burrows et al., 2009b; Smith et al., 2013) and the RH of Asian air crossing the Pacific ranged from 15 to 17% (Smith et al., 2013). Based on the desiccation survival data presented in Ch. 4, over half of the isolates have an \( \text{LD}_{90} \geq 7 \) days when desiccated at 25% RH. If we consider the lowest estimate of free tropospheric cells (2.2 x 10^5 cells m\(^{-3}\)) are as sensitive as L7-1 (the most sensitive isolate with and \( \text{LD}_{90} \) of 2.5 days), the population would be estimated to exist for 15 days at 25% RH. Therefore, even the most desiccation sensitive isolates in this study should survive intercontinental tropospheric transport in air masses with a RH from 20 to 25%.

The search for viable microorganisms in the stratosphere in an attempt to define the high altitude limit for life is an active area of research (Ohno et al., 2017). Based on the current knowledge of regional and temporal variations of the mean AoA of stratospheric air, one could predict when and where to sample for potentially viable bioaerosols in the stratosphere. The mean AoA of SH stratospheric air is consistently higher than air in the NH (Stiller et al., 2012; Haenel et al., 2015; Konopka et al., 2015), and based on our predicted survival times this older air is less likely to harbor viable bioaerosols (Ch. 4). The youngest AoA values globally occur over the tropical tropopause, with the lowest values in February as air enters the stratosphere, and the AoA increases with height (Haenel et al., 2015; Konopka et al., 2015; Ploeger et al., 2015). Tropical upwelling during QBO easterly phases are stronger than the westerly
counterparts (Ploeger et al., 2015), and may provide increased concentrations of bioaerosols being injected through the tropopause. As concentrations of bioaerosols increase, so too does the time required to reduce their viability. Sampling for bioaerosols at increasing heights in the troposphere and into the lowermost stratosphere over the tropical tropopause during winter would allow for an approximation of the total number, and viable fraction, of aerosols being injected into the stratosphere due to BDC patterns (Fig 1; Ch. 1).

Due to the extended ascent times (green arrows; Fig. 1, Ch. 1) it would be highly unlikely to recover viable bioaerosols transported by BDC above 23 km. Assuming the cell concentration range observed in the free troposphere (2.2 to 4.0 x 10^5 cells m^{-3}) and that such densities are injected into the tropical tropopause (16 km), only bioaerosols that are at least as desiccation tolerant as L9-9A-1 (LD_{90} RH ≥8.4 days) would be predicted to survive the estimated 58-day ascent through the tropical tropopause at 18 km (dashed red line; Fig. 1, Ch. 1; Mote et al., 1998). In order to survive the additional 289-day ascent to 23 km above the tropical tropopause (Fig. 1, Ch. 1; Mote et al., 1998), a bioaerosol would need to be twice as desiccation tolerant as isolate L6-1 (LD_{90} of 31 days). However, even the entire hypothetical 2X-desiccation tolerant population would be killed during the 550-day ascent to 30 km over the tropical tropopause (Fig. 1, Ch. 1; Mote et al., 1998). If we factor in UV-C fluence rates of 0.0055 W m^{-2} for stratospheric altitudes up to 30 km, a bioaerosol would need to be at least 1.25X more tolerant to UV-C than \textit{B. pumilus} endospores and our most UV-C tolerant isolate, L6-1, to survive the transport to 18 km, and 8X more tolerant to reach 23 km over the tropical tropopause.
In addition to the transfer of tropospheric air into the stratosphere, it is also possible for stratospheric air to cross the tropopause and enter the troposphere (Gettelman et al., 2011). These stratospheric intrusions have important implications for human health, as they can increase the amount of O$_3$ present at surface concentrations (Lin et al., 2015 not nature). Model interpretations of relatively short period of historical data indicate a 5.9±2.1 ppbv increase in surface O$_3$ concentrations during springtime over the western United States since the 1980s (Lin et al., not nature). Meteorological patterns and topography make high elevation sites (>1.4 km ASL) in the western United States particularly susceptible to stratospheric intrusions with peak concentrations in the spring (Lin et al., 2012), and these intrusions occur more frequently during springs that follow a La Niña winter (Lin et al., 2015 Nature). By monitoring La Niña episodes and stratospheric intrusions, one could predict the highest chances of recovering bioaerosols that have transported downward from the stratosphere, although due to the lengthy stratospheric transport times and predicted survival times, the chance of them being alive is very low.

Based on the levels of desiccation tolerance observed in bacteria from the stratosphere (Ch. 3), it is not feasible for viable bioaerosols to be transported to NH midlatitudes along either the deep or shallow branch of the BDC due to the expected transport times of 3 and 7 years, respectively (blue and red arrows, respectively; Fig. 1, Ch. 1). The mean AoA of NH midlatitude lowermost stratosphere (below ~20 km) follows similar patterns to the tropics, and the youngest air occurs during the summer after being delivered by the strong BDC upwelling during the winter (Ploeger et al., 2015). The mean AoA at 35°N (star and grey dashed line, Fig. 1, Ch. 1; Ch. 3) during
August and September is considered young air, and is estimated to be 3 years in the shallow BDC branch at 20 km (Haenel et al., 2015; Stiller et al., 2013). However this time frame would require bioaerosols to survive an additional year of transport after ascending through the tropical tropopause. Because this air transported along the shallow branch of the BDC has little tropospheric input, the concentrations of bioaerosols are predicted to be low, and this is in agreement with the lack of a significant concentration of bioaerosols (below the limit of detection, $4.0 \times 10^5$ cells m$^{-3}$; Ch. 3) reported from 21 km over New Mexico on August 21, 2013. Although the relative contribution of eddy mixing in the shallow branch is low, a case study detected cirrus cloud particles in NH midlatitude lowermost stratosphere indicates that local troposphere to stratosphere exchange is possible (Müller et al., 2015).

The mean AoA of NH midlatitude in the deep branch of the BDC (blue arrows, Fig. 1, Ch. 1) has shown a significant linear increase over the last decade, and is currently estimated to be $\sim$7 years at 30 km (Haenel et al., 2015; Stiller et al., 2011). This increase in AoA is most pronounced during the summer, implying a combination of increased transit times along the deep branch of the BDC and significant intrusions of older tropospheric from higher latitudes (Ploeger et al., 2015). With transit times that are longer than the previously described shallow branch, the survival of viable bioaerosols along the deep branch of the BDC from the tropical tropopause to NH exponentially decays over time. During the westerly QBO phase, which coincides with our 2013 sampling campaign, NH midlatitude stratospheric AoA is positively influenced by eddy mixing (Ploeger et al., 2015). Therefore, the presence of viable isolates in the stratosphere over NH midlatitudes from May to September 2013 is likely the result of quasi-horizontal
eddy mixing of tropospheric air across the extratropical tropopause (Gettelman et al., 2015; Konopka et al., 2015; Ploeger et al., 2015). This supports repeated observations of $10^5$ cells m$^{-3}$ observed at ~26 km and the presence of a wide variety of culturable isolates. Eddy mixing influences are maximized at the end of a westerly QBO phase (Ploeger et al., 2015), and concentrations of bioaerosols are predicted to have been even higher the following summer. The presence of subtropical mixing barriers results in a decreased relative contribution of eddy mixing between approximately 20°N to 20°S (Ploeger et al., 2015) and when coupled to the previously described ascent rates, make the tropical stratosphere an unlikely reservoir of viable bioaerosols. The descent of stratospheric air during the NH winter at the poles creates the polar vortex, which also lessens the relative contribution of eddy mixing at these latitudes (Konopka et al., 2015; Ploeger et al., 2015), and the probability of detecting viable bioaerosols. However, eddy mixing from the midlatitude troposphere becomes more frequent in the NH spring causing a decrease in the mean AoA (Ploeger et al., 2015), and viable microbes may be present in the younger, incoming air. Future work should couple bioaerosol concentrations with the detection of these small scale-mixing events to better constrain the impact they may have on the stratospheric bioaerosol concentrations. Sampling at NH midlatitudes, during the summer months and at the end of a QBO westerly phase, would provide the best probability of detecting viable bioaerosols in the stratosphere.

Particles in the size range of cells (1.0 to 2.0 µm) at 25 km are modeled to have stratospheric residency times on the order of 1 to 2 years as a result of gravitational settling and the ascent of stratospheric air from the BDC (Benduhn et al., 2013). If we assume these particles are present at 25 km over the equator, almost all particles in this
size range would have settled out along the shallow branch transport to the midlatitudes (red arrows, Fig 1, Ch. 1). Due to increased transit times, and presumed increased time to settle out, along the deep branch of the BDC all 1.0 to 2.0 particles would settle out before reach the midlatitude sampling site (blue arrows, Fig 1, Ch. 1). The presence of detectable concentrations of particles and cells at midlatitude stratospheric altitudes may therefore be the result of local eddy mixing injecting tropospheric air into the stratosphere, as opposed to being transported along the BDC.

Based on survival experiments, UV-C tolerance is the limiting factor for predicting survival during aerial transport in the stratosphere (Ch. 3). Based on estimated UV-C fluence rates of 0.0055 W m\(^{-2}\) (Ch. 4) and the ascent rates for air over the tropical tropopause described by Mote et al., (1998), predictions of microbial survival can be inferred from the UV-C tolerance data previously described (Ch. 4.). If we assume that the UV-C fluence rates of 0.0055 W m\(^{-2}\) for all altitudes up to 30 km, an estimated transit time of 289 days for air to rise from 20 to 30 km (Mote et al., 1998), and 12 hours of sun per day, the total dose of UV-C encountered will equal 69 kJ m\(^{-2}\). Using the lowest stratospheric bioaerosol concentrations below 30 km of 3.8 x 10\(^5\) cells m\(^{-3}\), and assuming each cell is all as tolerant to UV-C as *B. pumilus* endospores, the most UV-C resistant endospores documented (Link et al., 2004) and our most tolerant isolate L6-1, the entire population would be inactivated in as little as 3.6 x 10\(^4\) minutes, after ascending only 0.44 km above 20 km. An equal concentration of cells, with the lowest UV-C tolerance recorded in Ch. 4, would be decimated an order of magnitude faster at 2.7 x 10\(^3\) minutes, rising only 0.032 km above 20 km. A similar quantity of cells, with 1 order of magnitude greater UV-C tolerance than *B. pumilus* endospores or L6-1, would not survive the 405-
day ascent to 25 km over the tropical tropopause (Mote et al., 1998). Therefore, bioaerosols transported by BDC above ~21 km are unlikely to remain viable unless shielded from the sun. In order to evaluate the potential shielding effect particles may provide, Schuerger et al., (2003) exposed $2.5 \times 10^6$ B. subtilis spores to Mars simulated UV-C ($3.2 \text{ W m}^{-2}$) in the presence of particles of various sizes. When the particles were 10 to 50 µm in diameter, <1% of the spores remained viable after 1 hour, however there were no survivors after 8 hours of exposure (Schuerger et al., 2003). Smaller particles (2 to 8 µm) offered no protection and all spores were killed in 1 hour. Because the size range of particles observed in the stratosphere is <6.0 µm (Ch. 3), it is improbable to assume the isolates described in Ch. 4 were shielded by particles. Based on ozone concentrations measured at Socorro, NM during the sampling period of August 17 to September 4, 2013, the maximum ozone concentrations near the sampling site described in Ch. 3 were measured at approximately 26 km (Minschwaner et al., 2015). Because we are unable to achieve vertical resolution of sample collection below 30 km more precise than the opening and closing altitudes, we must assume that isolates recovered from these altitudes (18 to 23 km and 24 to 29 km) were significantly shielded from UV-C due to the presence of ozone. The presence of significant concentrations of ATP at 36 km may be the result of freeze-dried preservation of bioaerosols. The persistence of ATP molecules has been previously investigated and was not eliminate until receiving a dose of UV-C 10 orders of magnitude greater than that which resulted in no microbial viability (Schuerger et al., 2003). The effect of UV-C on potential biosignatures is discussed below. Therefore any reports of viable stratospheric isolates above the ozone layer without mention
decontamination effectiveness, statistical limits of detection, and there isolate’s potential to survive UV-C exposure should be considered highly suspect.

The stratosphere provides a unique combination of environmental stressors that are not found at any terrestrial Mars analogue locations. While microbial research in the hyper arid Atacama Desert (Azua-Bustos et al., 2015; Navarro-González et al., 2003; Parro et al., 2011; Paulino-Lima et al., 2016) and the McMurdo Dry Valleys in Antarctica (Musilova et al., 2015) can provide valuable information on the effects of desiccating environments, neither is exposed to UV-C nor atmospheric pressures below 10 mbar. Even though environmental simulation chambers can combine low pressures, temperatures, RH, atmospheric composition, it is not possible to recreate the full solar spectrum with artificial light sources (Khodadad et al. 2017). Multiple UV light sources cannot accurately replicate the relative contributions of the different wavelengths of natural sunlight, nor can they account for the changing position of the sun (Khodadad et al. 2017). In addition to light coming directly from the sun, natural sunlight is composed of a diffuse component, created by the scattering of light by aerosols and the light being reflected from a variety of surface conditions (Ben-David and Sagripanti, 2010).

Modeling efforts to determine both the direct and diffuse components of natural sunlight also fail to account for the spherical surfaces of cells, and therefore only predict low-end estimates of the total radiation absorbed by each bioaerosol (Ben-David and Sagripanti, 2010). Therefore the stratosphere, particularly above the UV-C shielding effects of the ozone layer, recreates the most accurate Mars analogue environment for astrobiological investigations. Rummel et al., (2014) describes 7 potential microenvironments that may exist on Mars, and replicating these microenvironments in the stratosphere and
determining the potential for microbial survival would help navigate future life detection missions. In addition to microbial survival, the persistence of potential biosignatures could also be evaluated under the full solar spectrum of the stratosphere (Summons et al., 2014). Extracellular ATP (Fajardo-Cavazos et al., 2008; Schuerger et al., 2003), polycyclic aromatic hydrocarbons (Dartnell et al., 2012), the amino acid glycine (ten Kate et al., 2006), DNA and chlorophyll (Cockell et al., 2005) have all been demonstrated to persist under UV-C exposures that would inactivate microbial viability. Schuerger et al., (2003) also demonstrated the degradation of ATP under Mars simulated UV varied according to the spacecraft materials the ATP was applied to and temperature. Because of the complex geometries, and subsequent opportunities for shading, future experiments concerning the effects of UV-C on microbial and biosignatures survival should be conducted on spacecraft materials and in flight configurations in the stratosphere (Fajardo-Cavazos et al., 2008; Khodadad et al., 2017; Schuerger et al., 2003). Determining the degradation times of biosignatures molecules may be as important as knowing microbial inactivation for preventing forward contamination and sampling of false positives during life detection missions (Fajardo-Cavazos et al., 2008; Schuerger et al., 2003), and the stratosphere would serve as a highly accurate Mars analogue environment.

Although the survival data suggest very limited stratospheric survival times for even the most UV-C tolerant microorganisms currently known (Ch. 4), there are still important planetary protection lessons to be learned from highly resistant bacteria that may be recovered from the stratosphere. While several studies have been conducted to quantify and characterize the microbial populations in the “extreme” environment of
spacecraft assembly clean rooms (Ghosh et al., 2010; La Duc et al., 2003, 2004; Moissl et al., 2007), no terrestrial microorganism should be excluded from the list of potential spacecraft contaminants (Rummel et al., 2014). The genetic diversity of isolates tolerant to both desiccation and UV-C in a vegetative cell state (Ch. 4) provides evidence that “the most probable survivors” under these extreme conditions (Moissl et al., 2007) need not be limited to bacterial endospores. Our current definitions of special regions for space exploration are based on our knowledge of the limits for life on Earth (Rettberg et al., 2016). By expanding that knowledge and better constraining the limits of microbial life under desiccation and high UV-C fluence, we may improve our classification of special regions (Rettberg et al., 2016; Rummel et al., 2014) for future sample return missions (highlighted by McCubbins et al., 2017) to minimize opportunities for forward contamination. Rettberg et al., (2016) suggests updating the definition for Mars special regions from areas with a water activity value of >0.6 to those with values >0.5, yet we have demonstrated some isolates would survive for at water activity, and thus RH, values twice as low (Ch. 4) if shielded from UV-C.

In addition to the need for a better understanding of microbial physiology and the limits for life on Earth, the investigation into desiccation and UV-C tolerance has many practical applications. A better understanding of desiccation processes will benefit human health in a wide variety of ways, such as improving disease diagnostic techniques (i.e., *Streptococcus pneumonia*, Krone et al., 2016) and improving the effectiveness, stability, and production cost of vaccines (Kunda et al., 2016; Ohtake et al., 2011). Microorganisms capable of withstanding desiccation are of particular importance to the food industry, where the removal of water has been used as a method to preserve foods,
such as nuts, cereals, honey, and powdered infant formula (reviewed by Finn et al., 2013). A better understanding of how microorganisms such as *Salmonella enterica* and *Listeria monocytogenes* survive desiccation, both on food and on preparation surfaces, may lead to advancements in food safety and decontamination protocols (Finn et al., 2013; Hingston et al., 2015). Improved understanding of desiccation tolerance may also lead to advancements in agriculture. The discovery of new desiccation tolerant *Rhizobium* strains, and the strategies they employ to survive, may lead to the discovery of a highly efficient legume crop strategy for increasing soil nitrogen content, particularly in arid environments (Cytryn et al., 2007; van Hamm et al., 2016). The successful combination of symbionts and legume crops will lead to increased agricultural productivity and reduce the reliance on costly chemical nitrogen fertilization techniques that are detrimental to the environment (van Hamm et al., 2016). Because several stages of the legume seed inoculation process exposes cells to desiccation, the identification of strains able to tolerate this stress is of critical importance to commercial seed companies and farmers (van Hamm et al., 2016).

UV-C is commonly used for disinfection of microorganisms (i.e., bacteria, fungi, viruses, protozoans) in a variety of settings that directly impact human health. It is frequently used along several processing steps in the food industry (Begum et al., 2009; Flores-Cervantes et al., 2013; Koutchma et al., 2009) and water treatment (U.S. EPA 2006; Rice and Hoff, 1981; Mofidi et al., 2002). UV-C has been investigated for its potential to reduce viability of bioaerosols routinely associated with the built environment (Kujundzic et al., 2006, 2007), as well as those that may be introduced as an act of terror (Handler and Edmonds, 2015; King et al., 2011). The reduction of several
biothreat agents by UV-C treatment was also proven successful for the disinfection of contaminated water supplies (Rose and O’Connell, 2009). The application of UV-C significantly reduced both the bioaerosol and surface associated CFUs in a shared hospital bathrooms, where frequent use outpaces scheduled cleanings, thus increasing the likelihood of disease transmission (Cooper et al., 2016). Although flexible endoscope tubing is stored under UV-C light to decrease microbial contamination, this procedure led to degradation and cracks in the tubing, which permitted a significant increase in the adhesion of *Pseudomonas aeruginosa* (Irving et al., 2016), highlighting the need to expand UV-C studies beyond a reduction in viable numbers. Based on the wide range of applications, and the microorganisms targeted, identification of pathways of increased UV-C tolerance will lead to more effective disinfection techniques and decreased incidents of disease transmission.


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APPENDIX A: Supplemental Information for Chapter 3

Aerosol sampling efficiency

The relative efficiency that particles with diameters between 0.95 and 6.4 µm were collected by the sampling rods was experimentally determined by comparing binned particle data from the rods with that collected using a MOUDI impactor. Stage 3 retains 1.4 µm particles at a theoretical collection efficiency of 50%; particles >3.0 µm have collection efficiencies of 100% (MSP Corp., technical data). From stage 3, a total of 1501 particles that ranged from 1.0 to 6.0 µm in diameter were characterized. No particles larger than 6.4 µm were observed at concentrations significantly greater than the controls. As particle diameter decreases from 1.0 to 0.9 µm, the stage 3 collection efficiency decreases roughly an order of magnitude; therefore, only particles larger than 0.95 were examined. For the rotating system that mimicked conditions during balloon sampling, data for 611 particles (1.0 to 6.0 µm in diameter) was collected from rods (n=3) that sampled at a velocity of 2.9 m s\(^{-1}\) and 1268 particles were analyzed from rods (n=3) that sampled at 4.7 m s\(^{-1}\).

Microscopic analysis of the sample rods indicated that particles in the 1 to 6 mm size bins were at abundances significantly greater (P<0.05) than the procedural controls. After correction for background particle concentrations, the values were scaled to the collection area and used to calculate the number of particles collected per cubic meter of air sampled. The relative efficiency at which the rods collected each particle size at sampling velocities of 2.9 and 4.7 m s\(^{-1}\) was calculated by dividing the raw particle data per m\(^3\) of air by values from the cascade impactor that were corrected using theoretical efficiencies (Frenz, D., 1999). To mathematically describe the rod collection efficiency, the log transformed efficiencies derived were fitted with linear trend lines (Fig. S3;
$R^2=0.94061$ for 2.9 m s$^{-1}$ and 0.80766 for 4.7 m s$^{-1}$), providing a best-fit efficiency value for each 1.0 to 6.0 \(\mu\)m particle bin (Table S2). The collection efficiency of the rods increased with velocity (Fig. S3) and was positively correlated with particle diameter.

To adjust the empirically determined rod efficiency data to the actual sampling velocities used in balloon flights (2.1 and 7.1 m s$^{-1}$), the relationship between sampling velocity and efficiency was assumed to be linear (Bonds et al., 2009). The velocity corrections provided efficiency values that were used to determine particle concentration for each size bin (Fig. S3; Table S2). Since atmospheric pressure decreases with altitude, aerosol sampling via balloon occurred in air masses that were less dense and viscous than those observed at one atm. According to changes in the Stokes and Reynolds numbers with altitude, the theoretical collection efficiency of 1.0 and 2.0 \(\mu\)m particles, derived according to Frenz, D. (1999) should increase ~two-fold at 30 km; therefore, the efficiency values listed in Table S2 should be viewed as conservative estimates. Despite this uncertainty, comparison of the particle concentrations derived from the efficiencies listed in Table S2 with data available for the CBL, troposphere, and stratosphere (Fig. S5) revealed good agreement with the range of previously reported values.

**History of the air masses sampled**

HYSPLIT forecast trajectories provided the basis for predetermining sampling altitudes and to classify air mass origin. Post flight, the reliability of the forecasted trajectories was verified using historical data to confirm the lack of intrusion from adjacent atmospheric layers. For example, the vertical trajectory of air masses sampled within the CBL was analyzed at 1.5, 2.0, and 3.0 km, and free tropospheric samples at 5.5, 8.0, and 11.0 km. According to 72-h back trajectories, the sampled CBL air masses
(2.2 and 2.3 km) had traversed over the Chihuahuan Desert in the south and the High Plains region in the west before entering New Mexico, and therefore, were categorized as cT air masses\textsuperscript{35}. In contrast, all other air masses sampled were classified as mT. Aerosols sampled in the free troposphere over New Mexico (8.1 km) on consecutive days in 2013 and Louisiana during November 2010 (6.1 km) were sourced from mT air masses that originated from the Pacific Ocean. Sampling in the free troposphere over Louisiana in June 2010 (6.1 km) also occurred within mT air masses, but these originated from the Gulf of Mexico. Despite differences in launch date and location, all of the stratospheric balloon missions sampled similar mT air masses that originated over the Atlantic Ocean and traveled west towards Texas and New Mexico.
Fig. S1: Comparison of bioaerosol concentration estimates from this study (filled and open circles; Fig. 1B) with available data. The literature values were derived from a combination of microscopic and ultraviolet aerodynamic particle sizer counting. The numbers plotted indicate the altitude, concentration, and source of the data (Amato et al., (2005); Bauer et al., (2002); Bowers et al., (2009); Bowers et al., (2011); DeLeon-Rodriguez et al., (2013); Hara & Zhang, (2012); Huffman et al., (2010); Huffman et al., (2012); Murata & Zhang (2014); Weidenmeyer et al., (2009). The error bars are the standard error of the mean.
Fig. S2: Temperature profile during sampling of the CBL on Aug 17 (solid line) and Aug 18 (dashed line), 2013. The arrows show the altitudes referred to as “knees” and indicate the boundary between the CBL and free troposphere (Stull, R., 2006; Cushman-Roisin, B., 2014; Ouwersloot et al., (2012).
Fig. S3: Rotorod® collection efficiency versus particle diameter. The efficiencies determined at velocities of 2.9 (blue circles) and 4.7 (red circles) m s$^{-1}$ were corrected for the actual sampling velocity (2.1 m s$^{-1}$, $y=0.243x-2.741$, black triangles; and 7.1 m s$^{-1}$, $y=0.270x-2.473$, white triangles) as described in the text and listed in Table S2.
Fig. S4: Particle size and mass distribution in air masses sampled from 2.3 km, 8/18, black circles; 8.1 km, 8/18, white circles; 21 km, black triangles; 24 km, white triangles; 26 km, 8/22, black squares; 26 km, 9/4, white square; 36 km, black diamonds. (A) The number of particles per size bin ($dN/d\log Dp$) for each altitude interval. (B) The mass of particles per size bin ($dM/d\log Dp$) for each altitude interval.
Fig. S5: Comparison of the particle concentration data obtained in the troposphere and stratosphere (Fig. 1B) with published values. The literature data were derived by a variety of methods, collected at various geographic locations, and are plotted by the reference number of the literature source [Berthet et al., (2002); Creamean et al., (2013); Chou et al., (2008); Curtius et al., (2005); DeLeon-Rodriguez et al., (2013); Hara & Zhang, (2012); Huffman et al., (2010); Lyamani et al., (2010); Maki et al., (2010); Sheridan et al., (1994); Weidenmeyer et al., (2009); Xu et al., (2001)].
Table S1: Total number, biomass, and cell carbon for 1 and 2 µm cell size bins.

** GI cells were assumed to be 1.0 mm altitude.
*** ND, not determined.

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<th>Date</th>
<th>2 µm</th>
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<th>0.5 µm</th>
<th>0.3 µm</th>
<th>0.1 µm</th>
<th>0.05 µm</th>
<th>2 µm (±SE)</th>
<th>1 µm (±SE)</th>
<th>0.5 µm (±SE)</th>
<th>0.3 µm (±SE)</th>
<th>0.1 µm (±SE)</th>
<th>0.05 µm (±SE)</th>
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<td>ND</td>
<td>5.5 (±0.1)</td>
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<td>ND</td>
<td>5.1 (±0.04)</td>
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<td>ND</td>
<td>ND</td>
<td>5.1 (±0.04)</td>
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<td>4.9 (±0.06)</td>
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<td>2.1 (±0.05)</td>
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<td>6.2 (±0.04)</td>
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### Table S2: Particle size data used for modeling and correcting the aerosol collection efficiency.

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<th>Median particle diameter (µm)</th>
<th>Min. (µm)</th>
<th>Max. (µm)</th>
<th>dlog (Diameter) (†)</th>
<th>Average number of particles per field of view</th>
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<tr>
<td>Rod (2.9 m s^{-1})</td>
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<td></td>
</tr>
<tr>
<td>Rod (4.7 m s^{-1})</td>
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</tr>
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<td>Stage 3 Rod (2.9 m s^{-1})</td>
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<tr>
<td>Stage 3 Rod (4.7 m s^{-1})</td>
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<td>Rod (2.1 m s^{-1})</td>
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<td>Rod (7.1 m s^{-1})</td>
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### Table S3: Global atmospheric budget of microbial abundance, biomass, and cell carbon from 0 to 40 km above the surface.

<table>
<thead>
<tr>
<th>Layer (km)</th>
<th>Volume (m^3 \times 10^{18})</th>
<th>Total cells (N \times 10^{24})</th>
<th>Biomass (Tg)</th>
<th>Cell carbon (Tg)</th>
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<td>Low</td>
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<td></td>
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<tr>
<td>High</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - 3</td>
<td>1.5</td>
<td>1.8</td>
<td>2.1</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - 20</td>
<td>8.7</td>
<td>1.9</td>
<td>3.5</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 - 30</td>
<td>5.1</td>
<td>1.2</td>
<td>3.3</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>30 - 40</td>
<td>5.2</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
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<tr>
<td></td>
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</table>

**Table S2**: Particle size data used for modeling and correcting the aerosol collection efficiency.
Table S4: ATP concentration data and inferred cell abundance assuming a 250:1 C:ATP weight ratio.

<table>
<thead>
<tr>
<th>Altitude (km)</th>
<th>Average ATP rod-1 amol x 10^-3 (±SE)</th>
<th>Cell carbon rod-1 *µg x 10^-4 (±SE)</th>
<th>Fraction of cell carbon per size bin** (%)</th>
<th>Potentially viable cells rod-1 N x 10^2 (±SE)</th>
<th>Potentially viable cells m^-2 µm^-3 (±SE)</th>
<th>Potentially viable cells rod-1 x 10^2 (±SE)</th>
<th>Size bin and ATP rod-1 x 10^2</th>
<th>ATP rod-1 C:ATP weight ratio</th>
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</thead>
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<tr>
<td>2.2</td>
<td>2.0 (±0.11)</td>
<td>2.5 (±0.14)</td>
<td>13 (±0.17)</td>
<td>0.49 (±0.017)</td>
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<td>0.0009 (±0.000051)</td>
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<td>3.0</td>
<td>2.5 (±0.14)</td>
<td>3.0 (±0.12)</td>
<td>13 (±0.0674)</td>
<td>0.31 (±0.014)</td>
<td>0.38 (±0.067)</td>
<td>0.004 (±0.00020)</td>
<td>0.00008 (±0.0000014)</td>
<td>0.000031 (±0.0000014)</td>
</tr>
<tr>
<td>3.2</td>
<td>1.3 (±0.06)</td>
<td>1.6 (±0.07)</td>
<td>9.1 (±0.0067)</td>
<td>0.15 (±0.0067)</td>
<td>1.5 (±0.0067)</td>
<td>0.0004 (±0.000014)</td>
<td>0.0000031 (±0.000000014)</td>
<td>0.00000031 (±0.0000000014)</td>
</tr>
<tr>
<td>3.6</td>
<td>1.6 (±0.13)</td>
<td>2.0 (±0.14)</td>
<td>44 (±0.011)</td>
<td>0.44 (±0.011)</td>
<td>2.5 (±0.011)</td>
<td>0.008 (±0.00020)</td>
<td>0.0000008 (±0.0000000051)</td>
<td>0.00000008 (±0.00000000051)</td>
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<td>3.8</td>
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<td>3.8 (±0.14)</td>
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<td>0.0000031 (±0.000000014)</td>
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<tr>
<td>4.4</td>
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<td>2.5 (±0.14)</td>
<td>44 (±0.011)</td>
<td>0.44 (±0.011)</td>
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</table>

*Cell carbon values derived from the ATP data, as described in the text.

**Values derived from data in Table S1.
APPENDIX B: Permission to reprint

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mhellbe@lsu.edu | http://http://sites01.lsu.edu/faculty/mhellbe/
VITA
Noelle Celeste Bryan, a native of Alexandria, Louisiana, received her Bachelor of Arts degree in Elementary Education from the University of Louisiana at Monroe in May of 2003. She taught middle and high school biology for 6 years before pursuing a doctoral degree in the laboratory of Brent Christner at Louisiana State University.