

Analysis of microbial aerosols collected at altitudes of 1.5 to 38 km above sea level.

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Introduction: Microorganisms surviving transport in the atmosphere must endure a variety of stresses (i.e., exposure to ultraviolet radiation and low relative humidity, temperature, pressure, and nutrient availability), the severity of which increase with altitude. To examine the abundance of microorganisms in the high atmosphere, we developed a sampling payload that uses a helium-filled balloon to sample bioaerosols up to altitudes of 38 km above sea level. Establishing the high altitude boundaries for life on Earth is of inherent merit; however, it also provides important information that can be applied to assess the habitability of other planetary environments. For example, conditions at an altitude of 30 km above the Earth are similar to the pressure, temperature, and radiation levels present on the surface of Mars.

Methods: A total of seven sampling flights were conducted in Fort Sumner, NM during August 17 through September 4, 2013. Using the Rotorod® sampling method, microorganisms were impacted onto the silicone-covered surface of the rods as the payload traveled through the atmosphere [2]. The collection efficiency of the system was experimentally determined and ranged from 0.1% for 0.5 to 1.4- μm cells and increased to 69% for 3.5 to 4.4 μm cells. The total number of cells collected was estimated by epifluorescent microscopy; intracellular adenosine triphosphate (ATP) was extracted and quantified; and isolates were cultured as previously described [2].

Results and Discussion: The mean number of cells on the procedural controls ranged from 360 (± 21 , standard error of the mean) for 1- μm cells to 2.0 (± 1.4) for 4 μm cells [2]. Samples were considered significantly different from controls when the mean number of cells per field of view (FOV) was greater than the 95% confidence intervals of the mean number of cells FOV⁻¹ in the controls. At tropospheric altitudes of 1.5 to 3.0 km, concentrations of 1 and 2 μm cells ranged from 0.86 to 1.1 $\times 10^7$ cells m^{-3} , decreasing to average values of 3.4 $\times 10^6$ cells m^{-3} at 5.5 to 11 km. These values are higher than the 5.9 $\times 10^5$ and 1.5 $\times 10^5$ cells m^{-3} previously reported at 0.05 and 10 km, respectively [3, 4]. Four flights in the stratosphere were successful in the recovery of cells 1 to 2 μm in diameter, providing the first quantitative measurements of microbial concentrations above 10 km. One flight that sampled the stratosphere from 18 to 29 km estimated cell concentrations at 3.7 $\times 10^6$ cells m^{-3} . Two different aerosol samplings from 24 to 29 km recovered

an average cell concentration of $\sim 2.5 \times 10^6$ cells m^{-3} . One flight rotated a sampling chamber at 34 to 38 km for ~ 8 hours, and from this the concentrations of cell was estimated to be 1.2 $\times 10^5$ m^{-3} . Based on biovolume and that $>96\%$ of cells collected were 1- μm in diameter, the total amount of cell carbon in the troposphere ranged from 1.9 to 4.8 $\times 10^{-7}$ g C m^{-3} , decreasing with altitude to values of 6.8 $\times 10^{-9}$ g C m^{-3} at 34 to 38 km [1].

Viable microbial biomass was quantified by determining the intracellular ATP concentration of samples collected at 18 to 29 km (4.8 $\times 10^{-8}$ g C m^{-3}) and 38 km (8.3 $\times 10^{-8}$ g C m^{-3}), with each measurement based on the sampling of 0.39 and 2.2 m^3 , respectively. Based on the assumption that only viable cells retain ATP, these data indicate the presence of viable microbial cells in the stratosphere. This is supported by the recovery of culturable bacteria, from altitudes up to 29 km. Identification of the isolates based on 16S rRNA gene sequencing revealed that isolates from 11 different genera were isolated from altitudes of 18 to 29 km. In addition to recovering endospore forming *Bacillus* and *Paenibacillus*, six different *Actinobacteria* and two *Proteobacteria* were also isolated in the lab. Many of these organisms have been associated with plant surfaces and soils and have been previously isolated from the troposphere.

Implications: The upper atmosphere offers the opportunity to study microbial survival under a combination of environmental pressures that are similar to those encountered on the surface of Mars. We examined the nature and distribution of microbial aerosols in the atmosphere and provide the first quantitative data for microbial abundance above 10 km and evidence that microbial life persists up to at least 38 km. Based on our data, the upper altitude limit at which microorganisms are transported and exchanged with the Earth's surface would appear to be >38 km [5].

References:

- [1] Bratbak, G. and Dundas, I. (1984) *Appl. Environ. Microbiol.*, 48, 755-757. [2] Bryan, N. C., et al. (2014) *J. Microbiol. Methods*, 107, 161-168. [3] DeLeon-Rodriguez, N., et al. (2013) *Proc. Natl. Acad. Sci. U.S.A.*, 110, 2575-2580. [4] Hara, K. and Zhang, D. (2012) *Atmos. Environ.*, 47, 95-105. [5] Wainwright, M., et al. (2003) *FEMS Microbiol. Lett.*, 218, 161-165.