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A method for sampling microbial aerosols using high altitude balloons

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ABSTRACT

Owing to the challenges posed to microbial aerosol sampling at high altitudes, very little is known about the abundance, diversity, and extent of microbial taxa in the Earth-atmosphere system. To directly address this knowledge gap, we designed, constructed, and tested a system that passively samples aerosols during ascent through the atmosphere while tethered to a helium-filled latex sounding balloon. The sampling payload is ~2.7 kg and comprised of an electronics box and three sampling chambers (one serving as a procedural control). Each chamber is sealed with retractable doors that can be commanded to open and close at designated altitudes. The payload is deployed together with radio beacons that transmit GPS coordinates (latitude, longitude and altitude) in real time for tracking and recovery. A cut mechanism separates the payload string from the balloon at any desired altitude, returning all equipment safely to the ground on a parachute. When the chambers are opened, aerosol sampling is performed using the Rotorod® collection method (40 rods per chamber), with each rod passing through 0.035 m^3 per km of altitude sampled. Based on quality control measurements, the collection of ~100 cells rod^{-1} provided a 3-sigma confidence level of detection. The payload system described can be mated with any type of balloon platform and provides a tool for characterizing the vertical distribution of microorganisms in the troposphere and stratosphere.

1. 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 (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). 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. 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

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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 coordinates. The dimensions of the box are approximately 25 cm ×25 cm and 20 cm high.

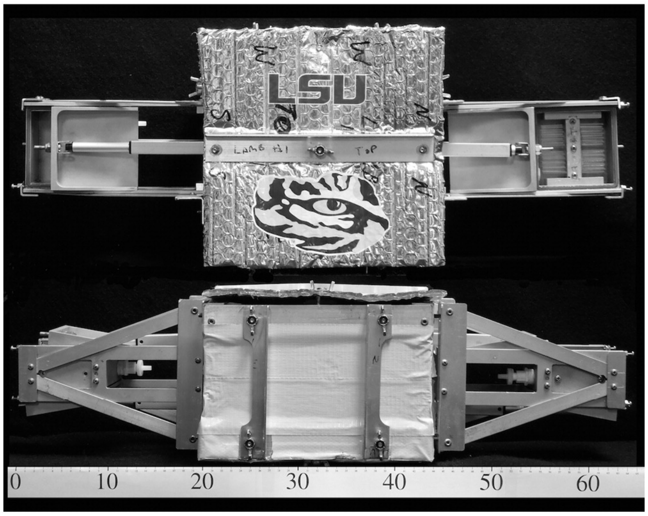

Fig. 1. A top and side view of the LAMB payload. The central electronics box includes the flight control, monitoring and power systems. Each payload includes two sampling chambers (one on the right and one on the left) and each chamber has two doors (top and bottom) that are operated by linear actuators. 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.

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 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. A 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 mm^2. 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