THE BIOPAUSE PROJECT: BALLOON EXPERIMENTS FOR SAMPLING STRATOSPHERIC BIOAEROSOL

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What Is the Biopause?: Determining the location of the "biopause" (i.e., the upper boundary of the biosphere of the Earth) and the biological flux across the biopause are key to our understanding of the universality, distribution, origin, and evolution of life in the universe. It is widely accepted that the tropospheric atmosphere contains bioaerosol [e.g., 1], although the flux of microbes from the troposphere to the stratosphere is small and dynamical and biological lifetime in the stratosphere are short. However, the presence of microbes in the stratosphere has been recorded in previous experiments using balloons, aircraft, and rockets [e.g., 2–5]. The most direct information available that can be used to investigate the biopause is the distribution and dynamicity of life in the middle atmosphere.

Here, we must note that an essential problem associated with previous studies is that they analyze the collected stratospheric samples after cultivation. Therefore, previous analyses were limited to the cultivated species, even though more than 99% of the microbes in nature are thought to be uncultivated species. In addition, cultivated samples lose the information related to the physical properties of the original samples; i.e., whether the original bioaerosol particle was a single cell, an aggregate of cells, or a dust particle with adherent microbes. The biological and dynamical lifetime of microbes in the stratosphere depends strongly on such physical properties of the bioaerosol particle. To avoid these problems, observation and analysis of collected stratospheric bioaerosols without cultivation is required.

The Biopause Scientific Project: The Biopause project used scientific balloons of the Japan Aerospace Exploration Agency (JAXA) to develop an overview of the stratospheric biosphere and the dynamics of biological flux in the stratosphere. Our first balloon experiment was conducted on June 8, 2016 and collected aerosol particles in the stratosphere using a newly developed descending inertial impactor. We estimated the number density of stratospheric microbes including those that cannot be cultivated for the first time in the world. In this paper, we summarize the initial results of the balloon experiment and the initial results of sample analysis.

Descending Inertial Impactor: The descending inertial impactor sampler was transported to the stratosphere using a balloon, where the balloon was released from the sampler. Sample collection was conducted as the sampler descended by parachute (Fig. 1). We developed a new descending inertial impactor sampler for this study. The stratospheric atmosphere was introduced into the sampler using its descending velocity and atmospheric aerosol particles were collected on the impactor plates. This method reduces biological contamination dramatically as the particles that adhere to the balloon and the wall of the sampler cannot enter the sampler during the descent because the descent velocity of the particles (as determined by Stoke's law) is less than the descent velocity of the sampler. Gate valves for the vacuum were placed at the entrance and exit of the atmospheric pathway in the sampler and operated via a control unit. We designed and optimized the shape of the sampler using the results of wind tunnel experiments and theoretical calculations.

Balloon Experiment in June, 2016: Figure 2 shows the experimental system used for the balloon experiment, consisting of the descending inertial impactors, control unit, batteries, and a gas tank. Prior to launch of the balloon, the inside of the sampler was washed and sterilized, and then the valves were closed to avoid contamination. The valves were then opened only during sampling in the stratosphere. A fluorescent bead was painted on the external wall of the sampler to detect contamination by particles that adhered to the outside of the sampler.

The sampling system was launched using JAXA's scientific balloon on June 8, 2016 (Fig. 3) from Taiki Aerospace Research Field, JAXA, in Hokkaido, Japan. The balloon and sampler ascended to an altitude of 28.5 km. The balloon was separated from the sampler after 30 minutes of level flight to avoid contamination. The gate valves of the sampler were opened during its descent from an altitude of 27 to 13 km as planned. After splashdown in the Pacific Ocean, the sampler was recovered using a fishing boat.

Sample Analysis: The recovered sample was analyzed using a fluorescence microscope and a scanning electron microscope (SEM). We washed the exterior of

the recovered sampler before disassembling the sampler on a clean bench.

Using a fluorescence microscope, we identified 21 microbes on the impactor plate in the sampler. Assuming that all of these microbes were collected in the stratosphere, this corresponds to a number density of 7 × 10^1 microbes per cubic meter under standard atmospheric conditions. Figure 4 shows a photomicrograph of the microbes collected by the sampler. We distinguished the microbes from other types of aerosol, such as rock dust, based on the brightness and color of the fluorescence. In addition, the fluorescent bead was not detected on the impactor plate, which suggests that contamination by particles that adhered to the exterior of the sampler was not significant. We also analyzed the collected aerosol particles using an SEM. In the presentation, we show the detailed results of the fluorescence microscope and SEM analysis.

Summary: We conducted stratospheric bioaerosol sampling using a balloon. The newly developed descending inertial impactor worked as planned and we detected microbes using a fluorescence microscope without cultivating. This is the first observational study of stratospheric bioaerosols to include nonculturable species and to successfully constrain their number density. Nonculturable microbes are thought to constitute the majority of stratospheric bioaerosols. These results from the first balloon experiment of the Biopause project represent an important step towards the planning of future experiments that will improve our understanding of stratospheric life and assist with the identification and characterization of the biopause.

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References: [1] Maki, T. et al. (2011) *Aerobiologia, 27: 277.* [2] Wainwright, M. et al. (2003) *FEMS Microbiol. Lett., 218,* 161-165. [3] Yang, Y.,et al (2008) *JAXA-RR-08-001,* 35-42. [4] Yang, Y.,et al. (2008) *Biol. Sci. Space, 22,* 18-25. [5] Imshenetsky, et al. (1976) *Life Sci. Space Res., 14,* 359-362.

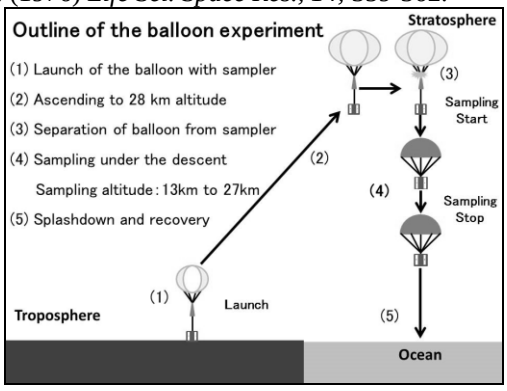

Figure 1: Outline of the balloon experiment.

Figure 2: The experimental system.

Figure 3: The sampling system was launched using JAXA's scientific balloon on June 8, 2016 from Taiki Aerospace Research Field, JAXA, in Hokkaido, Japan.

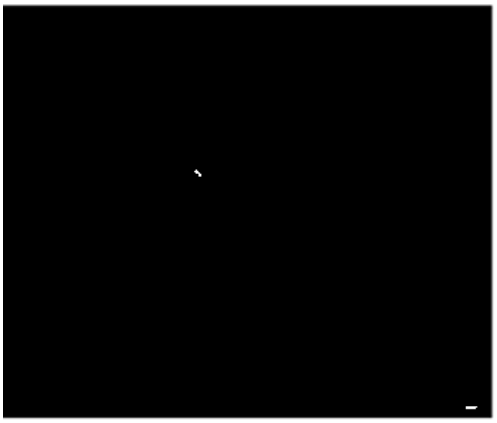

Figure 4: A fluorescence photomicrograph of the microbes collected by the sampler.