IAC-22,LBA,A1,2,x74411

BEXUS 30, SimLE Stardust - Investigation of Microbes in the Stratosphere

Marcin Jasiukowicz [1], Bartosz Rybacki[2], Mateusz Grzybowski[2], Natalia Czortek[1], Dawid Rekowski[2], Jakub Pawłowski[3], Paulina Podpirko[2], Kacper Loret[1], Dominika Tomaszewska[1], Szymon Magrian[1], Karol Pelzner[1], Remigiusz Galikowski[1], Bartosz Rybak[3], Beata Krawczyk[2]

[1] SimLE, Gdansk University of Technology, Faculty of Mechanical Engineering and Ship Technology, Gdańsk, ul. Narutowicza 11/12, 80-233 Gdańsk, Poland, Email: stardust@simle.pl
[2] Department of Molecular Biotechnology and Microbiology, , Faculty of Chemistry, Gdansk University of Technology, Gdańsk, ul. Narutowicza 11/12, 80-233 Gdańsk, Poland, Email: beakrawc@pg.edu.pl
[3]Medical University of Gdańsk

Abstract

The stratospheric microbiome has been investigated several times using the methods of classical microbiology. In this experiment, we have combined them with some novel approaches including whole- metagenome amplification, Maldi TOF mass spectrometry and Sanger DNA sequencing. The results of the experiment may provide the scientists with knowledge about the mechanisms of survivability of microorganisms in stratospheric conditions such as high doses of UV and cosmic radiation, low temperature and low humidity. The preliminary results have shown that the stratosphere is very poor in microorganisms in comparison to the regular, tropospheric air. Also the investigation is very difficult due to many problems with both small amounts of biological material and high risk of contamination. However, contamination is possible to control, and modern methods of biotechnology help in research of low quantities of material. The experiment was launched from Esrange Space Center in September 2021, on board BEXUS 30 balloon mission conducted within the REXUS/BEXUS programme.
Keywords: Stratosphere, Balloons, Microbiology, Biotechnology, Astrobiology

Acronyms/Abbreviations
Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF)
Rocket/Balloon Experiments for University Students (REXUS/BEXUS)
On Board Computer (OBC)
Valve Control Unit (VCU)
Groundbox (GB)
Flightbox (FB)
Sampling System (SS)
Polyethylene terephthalate glycol (PETG) - 3D printing material.
Acrylonitrile butadiene styrene (ABS) - 3D printing material.

1. Introduction

The first investigation of stratospheric microbiome took place in 1936 in the USA. It was done by Rogers and Meyer with a stratospheric balloon, floating at altitudes between 11 and 21 km a.s.l. Since then, there have been many findings made with the use of balloons, high-altitude aircrafts and sounding rockets.

The studies conducted so far have confirmed the presence of the following microorganisms:

- 5 *Bacillus sp.* (Rogers and Meyer, 1936)
- *Micrococci* and spore-forming rods (Greene et al., 1964, Bruch, 1976)
- *Mycobacterium sp*., *Micrococcus sp.* (Imshenetsky et al., 1976)
- 5 *Bacillus sp.*, *Staphylococcus sp*. (Wainwright et al., 2003, Suresh et al., 2004)
- 2 *Bacillus sp.*, *Micrococci*, *Microbacteria, Staphylococcus sp., Brevibacterium sp.* (Griffin, 2005)
- 3 *Bacillus sp., Paenibacillus sp*. (Yang et al., 2008)

The majority of attempts to isolate microorganisms from stratospheric samples have been unsuccessful, showing that most of the cells are viable, but non-culturable. The stratosphere is an extreme environment to support life, even for microorganisms, as it requires adaptation to low temperatures, low pressure and high exposure to ultraviolet radiation. Microorganisms obtained from the stratosphere must have the ability to survive the physical stresses of these environments and survive their transfer to the conditions prevailing on the ground [2].

Because of difficulties with sampling, there is still little known about the upper limit of the biosphere. Further investigation of this biome should expand our understanding of the diversity, distribution and movement of microbes in the Stratosphere [3].

The research team has conducted several stratospheric balloon missions with aim to isolate and sequence the total DNA from the samples. Research so

far suggests that the dominant genera of bacteria in the lower stratosphere are Enterococcus, Bacillus and Staphylococcus, which, however, does not mean that the collected cells were alive. Using DNA sequencing as well as Maldi TOF mass spectrometry provides additional information about microorganisms present in the stratosphere - whether alive or dead - as well as being helpful in discovering uncultivable strains [4][5].

2. Experiment Setup

Our approach follows the steps of Yang et al., 2008 [1] with a filtering system consisting of syringe filters - membranes of given pore size.

The experiment consisted of four parts: a filtering system, an electromechanical part operating the filtering system, an electronic system receiving and processing information from the sensors and controlling the electromechanical part and the external isolation made of Basotect. The Sampling System was equipped with 6 filters (⌀25mm PES membrane with 0,2 µm pore diameter, sterile). Each of the filters was constrained by two valves from the inlet and outlet side to provide separation from outside air during ascent through the stratosphere. While in the stratosphere, airflow through the filters is forced using a membrane pump (Boxer 3KD). Each element of the Sampling System was connected with silicone tubing (⌀6/10mm).

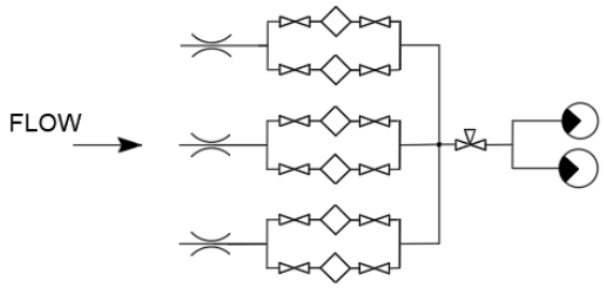

Fig. 1. Sampling System Scheme

The passive elements of the Sampling System (filters, valves and tubing) were designed to be sterilised in autoclave after assembly.

The latest mission used 6 filters. Two were used as a reference and remained unused throughout the duration of the mission to prove the clean assembly of the Sampling System.

During the flight of the balloon, a second copy of the filtering system was run. It performed in the same manner as the main experiment, but in regular atmospheric air close to balloons launch pad. It provided a control to compare the results from the stratosphere to the troposphere.

The movement of Sampling System valves was controlled by a set of servomechanisms connected using custom made brackets - Valve Control Units. Each one consisted of nine 3D-printed elements (main frame, two couplings, two servo mounts, two valve mounts). Material selected for this application was PETG as it has bigger elongation at breaking points than ABS, which guaranteed proper durability against high accelerations.

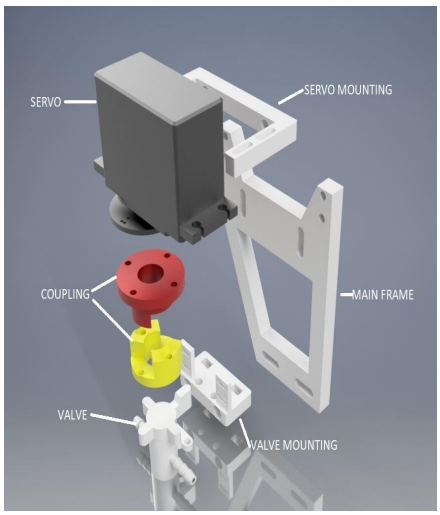

Fig. 2. Valve Control Unit

The entirety of the system and controlling electronics was mounted inside an aluminium 20x20 V-slot frame. The experiment enclosure was covered in 50mm thick BasoTect material, providing the samples and controlling mechanisms inside with separation from harsh stratospheric conditions - mainly low temperature.

The filtering system tubes extended about 40cm outside of the experiment enclosure. The tubes were supported using grey Polyethylene tubes. and blocked using a remove-before-flight caps.

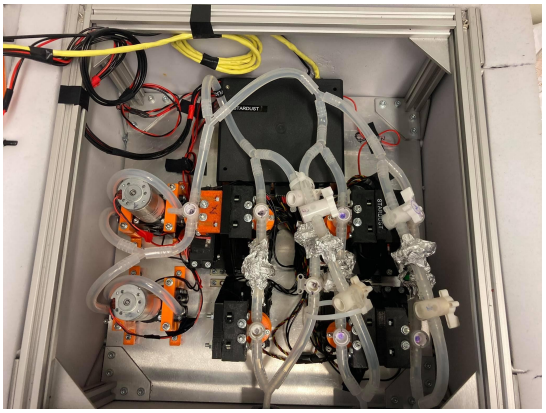

Fig. 3: Inside of the experiment enclosure.

The experiment was controlled by a custom electronic system based on the Arduino platform. Arduino Mega microcontroller was used as the On Board Computer. It implemented two modes of operation - manual and autonomous. Communication with ground was established via Swedish Space Corporation's proprietary E-Link system. The OBC controlled 9 servos and two pumps. Each active element of the Sampling System was additionally paired with a heating element.

During flight, the OBC collected data from a suite of sensors (pressure, temperature, humidity of air) inside and outside of the experiment gondola. The data was saved on a SD card as well as transmitted to the ground via the E-Link system. The data received on the other end was displayed on a ground station computer.

Based on the data, ground operators could make decisions to control the experiment in flight. Commands sent from the ground station could turn the pumps, valves and heaters on and off as required. The use of heater prevented the actuators from freezing in case of lower than expected temperatures. In case of loss of connectivity during flight, the OBC used altitude and pressure values to conduct a pre-programmed flight plan.

3. Course Of The Experiment

Fig. 4: Liftoff of BEXUS 30 mission from Esrange Space Centre.

3.1. Launch

The launch of the BEXUS30 stratospheric balloon took place on the 30th September 2021 from the Esrange Space Center in northern Sweden.

The balloon took off at 6:55am CEST. After the balloon reached 15 km a.s.l., the experiment began, valves opened and the pumps switched on to start air filtration. The filtration lasted 244 min. The approximate volume of the air filtered was 3500 dm^3, per the minimum airflow measured through the Sampling System on ground, measuring about 15 dm^3/min. The gondola landed under a parachute reaching the ground at 12:29. The experiment box was dismounted from the gondola and brought back to the laboratory by helicopter. The scientific team received the box with samples just 4 hours after the landing. The sampling system was checked for its integrity, secured inside a small vacuum chamber together with the reference system from the ground and kept in a refrigerator.

Fig. 5: Stardust experiment (bottom) in stratosphere (courtesy of frontier-space.co.uk).

3.2. Transport

The samples inside the Sampling Systems were transported to the Gdańsk University of Technology inside a vacuum chamber with 0,1 Bar absolute pressure inside. Whenever possible it was kept in a refrigerator.

3.3. Samples examination

The bacterial strains were examined through MALDI-TOF mass spectrometry on a Bruker MALDI Biotyper according to a standard protocol. The obtained spectra were compared to Bruker's database. The material was also prepared for DNA sequencing, with the results still pending.

Our studies so far indicated that up to 9 new species or strains of microorganisms might have been discovered either in the stratosphere or in the regular air in northern Sweden, and the DNA sequencing analysis

will provide final confirmation of these data.

One of the recovered micro-organisms has the rare ability to produce a blue dye in close dependence on temperature. The lower the temperature, within a certain range, the greater the intensity of the dye production. Research on its properties is ongoing.

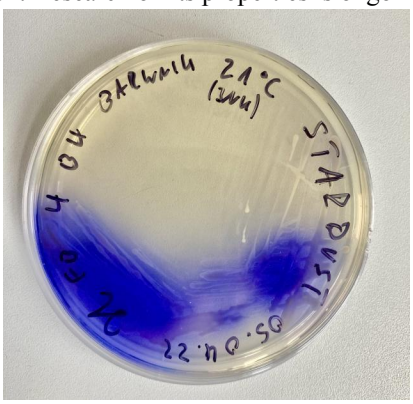

Fig. 6. Blue dye producing microorganism collected during the BEXUS 30 mission by the Stardust project.

4. Conclusions

The BEXUS project was an amazing opportunity for biotechnological research, allowing us to focus on the scientific payload rather than the stratospheric balloon mission itself. It provided us with new challenges, and resulted in many new experiences for the team.

The investigation of the material captured in the stratosphere is still ongoing, and we hope to conduct more stratospheric missions in the future.

The microorganisms obtained by us indicate the great diversity of life in the stratosphere. They show mechanisms of survivability in conditions of high radiation, low temperature and low pressure.

Acknowledgements

This work is possible thanks to cooperation of student scientists within SimLE Science Club at Gdańsk University of Technology. The research group has conducted several stratospheric balloon missions to date. Latest mission was possible thanks to international cooperation within the REXUS/BEXUS programme. It is realised under a bilateral Agency Agreement between the German Aerospace Center (DLR) and the Swedish National Space Agency (SNSA). The Swedish share of the payload has been made available to students from other European countries through a collaboration with the European Space Agency (ESA). EuroLaunch, a cooperation between the Swedish Space Corporation (SSC) and the Mobile Rocket Base (MORABA) of DLR, is responsible for the campaign management and operations of the launch vehicles. Experts from DLR, SSC, ZARM and ESA provide technical support to the student teams throughout the project. This resulted in the opportunity to launch our experiment on board a BEXUS stratospheric balloon SSC, Esrange Space Center in northern Sweden.

The funding for research was provided by Gdańsk University of Technology's "Plutonium" grant awarded in 2021, funds of the Students Council of Gdańsk University of Technology, as well as Polish Ministry of Science and Higher Education's "Best of the Best 4.0" grant awarded in 2020.

References

1. Yang Y. et al. (2008). Investigation of cultivable microorganisms in the stratosphere collected by using a balloon in 2005., Available on-line: https://www.researchgate.net/publication/233387559_Investigation_of_cultivable_microorganisms_in_the_stratosphere_collected_by_using_a_balloon_in_2005
2. M. Wainwright, S. Alharbi, N. C. Wickramasinghe, How do microorganisms reach the stratosphere? International Journal of Astrobiology, 5 (1), 13-15, 2006.
3. D. W. Griffin, Terrestrial microorganisms at an altitude of 20,000m in Earth'satmosphere. Aerobiologia, 20, 135-140, 2004.
4. Mancabelli L. et al. (2021). Free DNA and Metagenomics Analyses: Evaluation of Free DNA Inactivation Protocols for Shotgun Metagenomics Analysis of Human Biological Matrices. *Frontiers in Microbiology,* 06 October 202. Available at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.749373
5. National Research Council (US) Committee on Metagenomics: Challenges and Functional Applications. 1. Why Metagenomics? (2007). In *The New Science of Metagenomics: Revealing the Secrets of Our Microbial Planet.* National Academies Press (US)
6. N. Tripathi, A. Sapra. Gram Staining, In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available at: https://www.ncbi.nlm.nih.gov/books/NBK562156/
7. Tan I., Ramamurthi K. (2013). Spore formation in Bacillus subtilis. *Environ Microbiol Rep.* 2014 Jun; 6(3): 212–225.
8. Makarova K. S., Aravind L., Wolf Y., Tatusov R., Minton K., Koonin E., Daly M. (2001). Genome of the Extremely Radiation-Resistant Bacterium Deinococcus radiodurans Viewed from the Perspective of Comparative Genomics. *Microbiol Mol Biol Rev.* 2001 Mar; 65(1): 44–79