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SimLE Stardust: How an experiment evolved from student tinkering to a stratospheric research platform

Marcin Jasiukowicz[a], Magdalena Sadowska[a], Mateusz Grzybowski[a], Bartosz Rybacki[a], Karol Pelzner[a], Szymon Magrian[a], Kacper Loret[a], Wiktor Sieklicki[a]
[a]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

Abstract

Stratospheric balloons are one of the most accessible methods of near space exploration. Their applications include capturing of extreme living organisms whose existence in the stratosphere has been suggested in the literature. These potential microorganisms would have evolved to survive the unique environment of low temperature, low pressure and high solar radiation. This makes the development of near space platforms for microbial life search a demanding yet fascinating challenge.

Such investigations have been conducted by several research groups, with the earliest instances in the 1930s. Since 2016, a student group from SimLE Science Club based at Gdańsk University of Technology has conducted several stratospheric balloon missions to survey the stratosphere for microorganisms under the project name "Stardust". The experience gained from five iterations of the project ultimately resulted in the latest mission from Esrange Space Center in Sweden as part of the 13th cycle of the REXUS/BEXUS program in 2021.

This approach improved existing research by developing a revised capture mechanism for microbiological material in the stratosphere as well as enhancing the method of post flight analysis of captured material.

To achieve this, we combined the methodology of classical microbiology with modern biotechnology. By seeding and keeping the captured microorganisms alive, we isolated and purified DNA. Subsequently, using MALDI-TOF mass spectrometry and Sanger sequencing, we led to taxonomic differentiation of the analysed microorganisms. The results of our research testify to the effectiveness of the designed microorganism intake system, as well as the appropriately selected scientific methodology leading to the study and mapping of the diversity of microorganisms living in the stratosphere. Preliminary investigation of the data from the latest mission suggests that there might have been found new and yet undescribed strains of bacteria. Further research is required to examine the properties of the cultivable strains.

The stratosphere is an extreme environment for life, and future research, especially based on our approach, should expand our understanding of the diversity, distribution, and movement of microbes in the stratosphere.

Keywords: Stratosphere, Microbiology, Biotechnology, (maximum 6 keywords)

Acronyms/Abbreviations

ESA - European Space Agency
SSC - Swedish Space Corporation
DLR - German Aerospace Center (Deutsches Zentrum für Luft- und Raumfahrt; *DLR*)
ZARM - Center of Applied Space Technology and Microgravity (Zentrum für angewandte Raumfahrttechnologie und Mikrogravitation, ZARM)
REXUS/BEXUS - Rocket/Balloon Experiments for University Students
GUT - Gdańsk University of Technology
DNA - Deoxyribonucleic acid (molecule carrying the genetic information of an organism)
rRNA - Ribosomal ribonucleic acid
PBS - Phosphate Buffered Saline
LA - Luria Agar Media
CFD - Computational Fluid Dynamics
PES - Polyethersulfone
MALDI - Matrix-assisted laser desorption/ionization
TOF - time-of-flight (mass spectrometry)

1. Introduction

The purpose of this paper is to summarise the history of stratospheric balloon launches from Gdańsk University of Technology, Poland conducted by researchers working within SimLE Science Club.

The paper presents the struggles and incremental improvement between missions, resulting in culmination during the launch of BEXUS 30 mission.

The authors summarise their efforts so far, as well as provide an outlook for further research to be carried out using the devised stratospheric balloon platform.

2. First Balloon Mission

The aim of the very first mission carried out at SimLE in 2016 was to check whether the Stardust team was able to launch a stratospheric balloon with a basic payload to gain some technical experience in launching stratospheric balloons.

The technical setup of the first stratospheric balloon in the project was built of a balloon itself, a simple cut-off system, a parachute and a small gondola equipped with a microcontroller collecting data about the environment.

The mission showed that the setup was able to measure the key physical parameters of the air in the stratosphere. Also, it became clear that winds must be taken into consideration during the foreplanning of the flight's trajectory.

3. First Microbiological Missions

The aim of the first microbiological missions was to collect the stratospheric microorganisms and to analyse the bacteria using metagenomic and classical methods.

3.1 Technical setup

The sampling system was based on the system used by Japanese researchers in their microbiological experiment carried out in 2005 (Fig. 1) [1]. However, the sampling system in the Stardust project was expanded to 6 filters (Fig.2) instead of 3 used by Yang et al. [1], in order to provide controls that would prove sterility. The filters used were sterile, ⦰25mm PES membrane with 0,2 µm pore diameter. The system ran with one diaphragm pump, because of restricted payload mass.

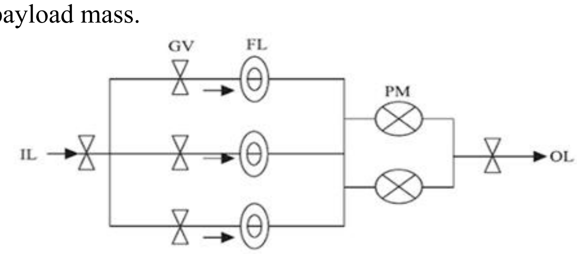

Fig. 1. Sampling system used by Yang et al. IL - air inlet, GV - gate valve, FL - membrane filter, PM - pump, OL - outlet

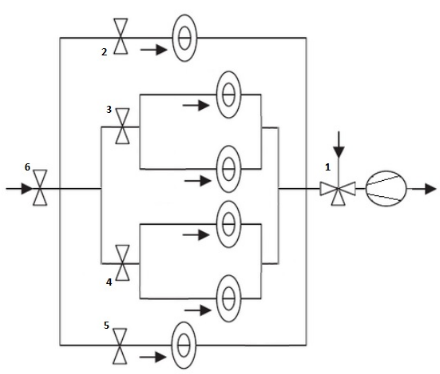

Fig. 2. Sampling system used in the first microbiological mission of the Stardust project. The numbers depict the opening sequence.

Our sampling system, as well as the control electronics were mounted inside a water-tight gondola made of extruded styrofoam (Fig.3). Additional filters could be opened separately to allow for sampling at different altitudes. Additional tri-way valve was added next to the pump to allow for air pass-through during the startup of the pump on ground and ascent to the stratosphere. The pumps were kept running at all times to minimise the chance of freezing. The pumps were placed at the exit of the system to not contaminate the air. The air inlets were secured before liftoff using caps, to minimise the chance of contamination before liftoff. Additionally, the entire sampling system tubing was sterilised before integration.

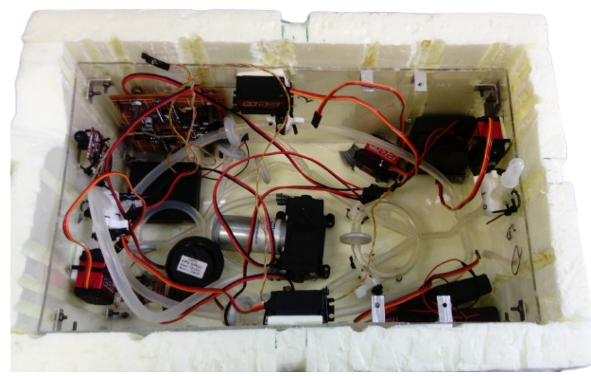

Fig. 3. Inside of the gondola from the September 2017 mission.

3.2 Launch and conclusion

Data from the 2017 flight showed that the valve opening sequence was not executed. Nevertheless, the filters were treated like they contained biological material. Two control filters and 2 test filters were opened and their membranes were stamped on agar media. One control filter and one test filter were flushed with a cell lysis buffer and underwent a DNA-isolation procedure.

The results showed that all the filters were maintained sterile during all the procedures, which meant that it was possible to perform the microbiological procedures without contaminating the samples.

Despite the setback in obtaining actual microbiological material, new missions have been planned.

4. Next launch opportunities

Next window of opportunity to launch the scientific experiment opened in May 2018. The mission was conducted in cooperation with a scientific circle from Zielona Góra launching another experiment on our stratospheric platform.

The mass of the Stardust setup needed to be reduced. Only two filters were put into the sampling system, which helped reduce the mass of the experiment. Additionally, the control filters were put inside small loops of silicone pipes, so they did not need any valves at all.

Everything worked as expected and the air was filtered for 1h in the lower stratosphere. The microbiological samples were transported to a laboratory at Gdansk University of Technology and further investigated.

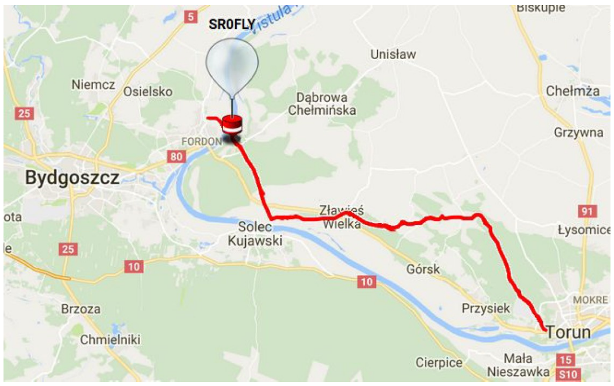

Fig. 4. Example of stratospheric balloon ground track captured during the May 2018 mission.

4.1 First results

Isolated DNA samples showed that the total DNA amount in the sampling filter was minimal and in the control filter DNA was indetectable. Both samples underwent amplification with use of phi29 polymerase. Afterwards, the DNA amount in the sample from the test filter was sufficient for metagenomic analysis, while nothing changed in the control sample.

The amplified total DNA sample was sent for metagenomic DNA sequencing of the gene pool encoding 16S rRNA subunit, the sequence of which is species-specific.

The second sampling filter together with the second control filter were rinsed with a sterile PBS buffer and the filtrates were centrifuged. The supernatant was discarded and the pellet was resuspended in a small amount of the sterile PBS buffer and then spread on LA media. The petri dishes were kept for a week in 25 ºC and then for another week in 37 ºC.

On the agar media, no colonies were observed, which means the absence of living cultivable microorganisms in the collected sample.

The metagenomic analysis showed abundance of Gram-positive bacteria in the stratosphere, most of which stated the gene of Enterococcus, Staphylococcus and Bacillus, where the two latter were reported frequently by other researchers as present in the stratosphere [1].

A substantial amount of undescribed species was present in the sample.

5. REXUS/BEXUS

The successful outcome of the previous mission has given us confidence in further research.

We took aim at a joint program of the European, German and Swedish Space Agencies, called Rocket/Balloon Experiments for University Students.

The purpose of the program is to give students the opportunity to launch their experiments on board a balloon platform provided by the Agencies, under the guidance of professionals from DLR, SSC, ZARM and ESA.

We applied with our experiment in 2019 and were accepted to conduct the experiment in the Cycle 13 of the program.

5.1 Test mission

Due to worldwide disturbance of supply chains, related to the 2020 pandemic, the assigned mission of the BEXUS Cycle 13 Missions was postponed until the next year. Despite that, the team conducted a preparatory test mission, testing the revised sampling mechanism to be used in the program.

The system contained half of the first iteration of the sampling system described in the next chapter. Three filters were used. One as a control sample, and the remaining two for metagenomics and inoculation research. The setup from the test mission for the REXUS/BEXUS Program is shown in Fig.5.

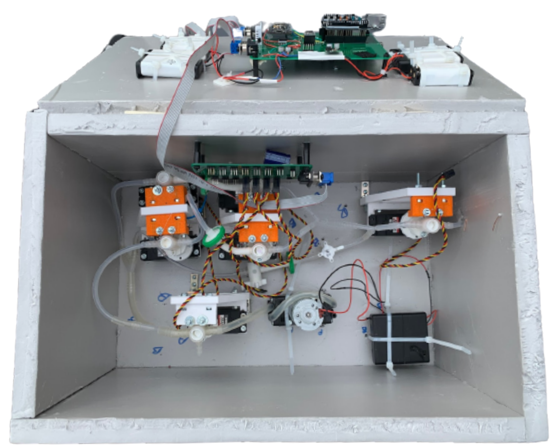

Fig. 5. Sampling system used in the July 2020 mission.

5.2 BEXUS 30 mission

The unconstrained environment of the BEXUS balloon allowed us to deploy a fully fledged sampling system. It consisted of 3 groups of 2 filters each (Fig.6). Each group contained a control filter and a filter used respectively for metagenomic research, aerobic inoculation and inoculation in microaerobic conditions using a vacuum chamber [2]. Each of the filters was constrained on both sides by valves controlled by a pair of Valve Control Units (VCU, Fig. 7) described in detail in [3].

On top of that, an additional reference system was run on ground through the duration of the flight of the main experiment (Fig.8.).

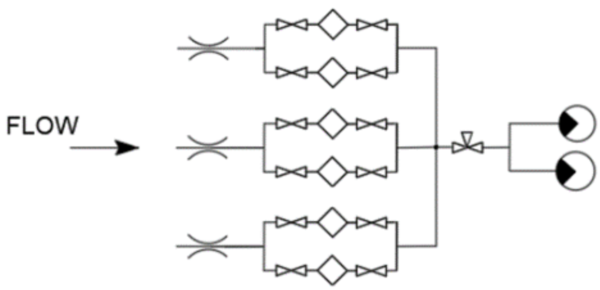

Fig. 6. Sampling System used in the BEXUS 30 mission.

Fig. 7. Valve Control Unit

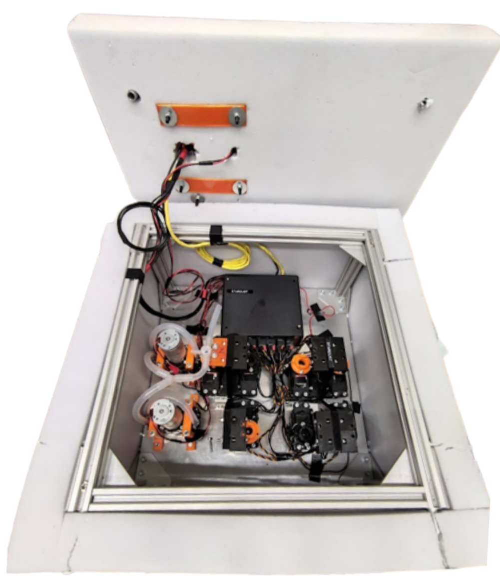

Fig. 8. Experiment enclosure integrated before BEXUS 30 mission.

5.3 Results

The preliminary results of this mission and discussion were published at the 25TH ESA Symposium On European Rocket And Balloon Programmes And Related Research, Biarritz, France, 1-5 May 2022 [3]. Since then, additional sequencing of the DNA isolated from the samples has been conducted.

The results confirm that among bacteria only Gram-positive ones are able to survive in the stratosphere as no Gram-negative bacteria were found in the stratospheric samples.

The sequencing of the 16S rRNA-coding gene has given more relatable information as the Bruker MALDI Biotyper database was dedicated only to pathogenic microorganisms in opposition to the broad BLASTn Nucleotide collection. Thus, the MALDI-TOF results should be treated as complementary for the genotyping based on ribosomal gene sequencing.

Table 1. Microorganisms captured on the filters used in the BEXUS 30 stratospheric mission.

Culture symbol	Bacterium /Fungus	Gram stain	MALDI-TOF analysis result	16S rRNA-coding gene analysis result
ι	Mold	N/A	-	-
κ	Bacterium	G+	unspecified	Pseudarthrobacter unknown species
λ	Bacterium	G+	Microbacterium oleivorans	Microbacterium oleivorans or another Microbacterium sp.
μ	Bacterium	G+	unspecified	Streptomyces microflavus
σ	Bacterium	G+	Bacillus licheniformis	Bacillus unknown species / Alkalicocco-bacillus murimartini / Bacillus plakortidis / Shouchella plakortidis
χ	Bacterium	G+	Pseudarthrobacter polychromogenes/ Pseudarthrobacter oxydans/ New species	Bacillus simplex
ψ	Bacterium	G+	unspecified	Lysinibacillus macroides / Lysinibacillus xylanilyticus

6. Discussion of Microbiological Research so far

In the foregoing microbiological studies on the stratosphere, as well as in our experiment from 2018, many species of the Bacillaceae family were reported as present in the stratospheric air. Also, another family of gram-positive bacteria, Micrococcaceae, was included in the reports together with another one, Microbacteriaceae. Almost all of the cultures obtained from the stratospheric collecting filters from the BEXUS30 flight belong to these three families (*Lysinibacillus, Bacillus, Alkalicoccobacillus* and *Shouchella* are all Bacillaceae, *Pseudoarthrobacter* belong to Microbacteriaceae and *Microbacterium* - to Microbacteriaceae). In contrast to the bacteria belonging to the Staphylococcaceae family that were also abundant in previous findings but were not cultured by us, the Streptomycetaceae family were represented in our samples by the *Streptomyces microflavus* species but was not ever found in the stratosphere before. Also, one mould species was grown from the stratospheric filters, which means that Gram-positive bacteria are not the only organisms that are able to survive in the harsh conditions of the stratosphere.

At the PAC Symposium in May 2022 a discussion was raised, whether all of the obtained cultures come from the stratosphere as assumed. Although the sampling system was sterilised and the balloon's gondola was disinfected, the balloon's surface remained unsterile. Furthermore, the safety caps were taken off the air inlets before the liftoff of the balloon, so the winds blowing towards the inlets might have carried some microorganisms that sticked to the inlets even on the shallow-internal surface (which is not very probable, but still possible) and were sucked in when in the stratosphere once the pumps were switched on and the valves were opened. Control samples taken from the balloon's surface (2 samples from 1 dm^2 area taken with a sterile swab) showed that the balloon carried a very small amount of gram-negative bacteria (1 cfu/dm^2 of the balloon). These were not found in the stratospheric samples, which does not exclude a possibility of this source of contamination completely. CFD analyses showed the unlikelihood of the wind going inside the inlet pipes with their geometry, however some turbulence might have taken place at the shallow frontal space of the inlet pipes and those might possibly be a source of contamination with a small probability.

7. NEXT steps

After the BEXUS program ended, Stardust faced significant gaps in its team. Senior members of the project needed to recruit new students. This was to facilitate the transfer of the experience and knowledge. Accordingly, we have added the term "NEXT" to "Stardust" to emphasise the importance of the project's continuity, while recognizing the involvement of a distinct group of people - in particular, a younger and newly formed team.

7.1 First attempt

After a year, the first Stardust NEXT mission was finalised. This mission facilitated the testing of the onboard computer, communication system, mechanical solutions such as the cutoff mechanism, rigging and parachute. All components were designed and constructed by the Stardust NEXT team.

The payload contained an analog camera, a regular camera, a quantum random number generator and Paulownia tree seeds.

7.2. Second attempt

The second Stardust NEXT mission was launched in August 2023 without any help from the senior members. This time the payload had a similar concept to the previous iteration of the program, which is sampling the air from the stratosphere. This payload was named "Storm" and its purpose is to study the migration of toxins at different stratospheric altitudes.

The sampling system consisted of a microcontroller, vacuum pump, servomechanisms, battery, fluoropolymer tubes and valves. The valves open at a certain height, the vacuum pump starts pumping and air is sampled, potential microorganisms settle on the tubes. The collected microorganisms were transferred to Sabouraud Agar (SAB) and Tryptic Soy Agar (TSA) substrate. They were then tested and identified in the laboratory using mass spectrometry and gas chromatography.

7.3 History Repeats Itself

Our mission is to expand general knowledge about the stratosphere and to inspire young students. Just as the initial Stardust missions were first a technical test and then carried scientific payloads, so was the debut Stardust NEXT missions.

The ultimate goal of Stardust NEXT is to compete to start within the BEXUS Programme in order to test new methods and hypotheses arising from previous teams experience, thus closing the cycle of growth for the Stardust programme.

7.4 Results

The results from the August mission will be presented at upcoming conferences.

8. Conclusions

The results of our research so far transcends the microbiological nature of it. In order to continue progressing in this field, we need to create the required groundwork on the team level. This is why we committed our time to knowledge transfer and building new teams, which will continue our research.

The continuous commitment to scientific exploration and innovation ensures that while the idea behind the research remains rooted in the principles of microbiological research in the original "Stardust" project, our approach and outcomes push the boundaries of what was previously achieved, marking the evolution of our program.

Hopefully in the future we can conduct our research with greater frequency and geographical diversity. This way we could map the stratosphere and build models of how microorganisms move and survive there.

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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 (accessed 15.09.23).

[2] Marcin Jasiukowicz Et al. BEXUS 30, SimLE Stardust - Investigation of Microbes in the Stratosphere, IAC-22,LBA,A1,2,x74411, 73rd International Astronautical Congress (IAC), Paris, France, 18-22 September 2022.

[3] Preliminary result of Stardust BEXUS Project - Proceedings of 25TH ESA Symposium On European Rocket And Balloon Programmes And Related Research, Biarritz, France, 1-5 May 2022