Summary report of the ISS-Kibo utilization mission,
"Microbial Dynamics in International Space Station (Microbe)"
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1. Introduction

Microbes exist everywhere even in space habitat. Microbial ecosystem in this closed environment under microgravity may differ from that on Earth. It is required to investigate the relationship between human and microbes, and how microbes influence the systems and materials in this environment. The objective of this experiment is to monitor the abundance of microbes and presume their dynamics in Kibo, Japanese Experiment Module of International Space Station from our own viewpoints.

In each space agency, research on microbes in the ISS is promoted towards future manned space exploration. For example, at NASA, the "Microbial Observatory" program is underway to understand the overall picture of microorganisms present in ISS. In our research, we will accumulate fundamental knowledges for microbial management in the space habitat by monitoring microbes in ISS "Kibo" using the methods developed by our research team. Furthermore, we propose a microbial monitoring method to assure microbiological safety in the space habitat, and to minimize the influence of microorganisms on human and equipment in the space station.

Through this research, the dynamics of microbes in ISS "Kibo" becomes clear. Active exchange of the information with NASA, ESA, RSA and other space agencies will enable us to integrate their achievement and make a judgment of microbiological safety level in the space habitat at each level of "safe", "alert" or "action". This will enable safe operation of the space station and promotion of space research to support a long-term space habitation.

2. Microbial sampling in ISS-Kibo

The experiment "Microbe" was adopted in February 2008. "Microbe" was carried out sequentially in Microbe-I, Microbe-II and Microbe-III (Table 1).

Table 1. Sampling implementation schedule in the ISS-Kibo

	Microbe-I	Microbe-II Run1	Microbe-II Run2	Microbe-III
Launch	29 Aug., 2009 (Discovery)	15 May, 2010 (Atlantis)	22 Jan., 2011 (HTV2)	21 Jul., 2012 (HTV3)
Sampling	5 Sep., 2009	29 Oct., 2010	27 Feb., 2011	16 Oct., 2012
Return	13 Sep., 2009 (Discovery)	14 May, 2011 (Endeavor)	9 Mar., 2011 (Discovery)	28 Oct., 2012 (Dragon SpX-1)

3. Results

3.1 Fungi

3.1.1 Experiment

Detection of culturable fungi were carried out with Microbial Detection Sheet (MDS). Fungal communities on surfaces and in the air inside Kibo were determined with random cloning targeting fungal ITS1 region. Furthermore, fungal community in water in the humidifier installed in CBEF, and that on the board inside the Multi-Protocol Converter which was returned to JAXA TKSC,

were determined as extra experiments.

3.1.2 Summary

（1） On site evaluation of fungal growth is now possible by downlinking the image.
（2） Change in the fungal microbiota in ISS-Kibo was revealed.
（3） Opportunistic fungi, mycotoxin-producing fungi, and fungi related to allergens were found on the surface, in water, and in air in ISS-Kibo.
（4） Both the cultivation result and the particle number in the air of the ISS-Kibo showed the cleanliness of the air inside the cabin.
（5） Particle distribution analysis showed that particles with large particle diameter floating in the air were relatively unlikely to be removed compared to particles with small particle diameters.

3.2 Bacteria

3.2.1 Experiment

Bacterial abundance and community structure on the surfaces in Kibo were determined with state-of-the-art techniques in environmental microbiology, such as quantitative PCR and amplicon sequencing. In addition to a conventional swab method, we used a newly developed adhesive sheet as a microbial sampling device in Kibo from Microbe-II onwards.

3.2.2 Summary

（1） Protocol for swab sampling in ISS-Kibo was optimized.
（2） Protocol for microbial sampling with a newly developed adhesive sheet was determined.
（3） Our results indicated that ISS-Kibo was microbiologically well maintained.
（4） Human related bacteria, such as *Actinobacteria* and *Firmicutes*, dominated on surfaces of equipment in Kibo. It was considered that they were transferred to the surface of each equipment etc. by astronauts' contact.

4. Self-evaluation on success criteria

Success	Criteria	Fungi	Bacteria
Minimum	In addition to samples collected by conventional methods, microbes floating in the air and in water were collected and analyzed by the methods that researchers developed.	◎	◎
Full	By using culture-independent and/or culture-dependent techniques, determination of microbial abundance and community structure were carried out, and temporal changes from Microbe-I/II were analyzed.	◎	◎
	For the particle counter and air sampler, the correlation of data was clarified.	◎	NA
Extra	The results obtained by the above analysis lead to new findings.	◎	◎

Rating; ◎: high achievement, ○: achievement, △: partial achievement, ×: not achieved, NA: not applicable