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Review:

Microbes and Crewed Space Habitat

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Microbes exist everywhere, and studies have demonstrated the presence of viable microorganisms in a crewed space habitat. Microorganisms within space habitats pose potential hazards to crew health and potentially damage hardware. Continuing successful long-term space habitation requires fundamental information on microbiological safety for avoiding biohazards in space. We discuss the importance of researching microbes in crewed space habitats and of monitoring microbes on the International Space Station (ISS). We also review environmental microbiology perspectives in crewed space habitats and the microbiology of the space environment (astromicrobiology).

Keywords: crewed space habitat, microbial monitoring, microbiota, international space station, astromicrobiology

1. Microbes in Crewed Space Habitats

"Everything is everywhere, but the environment selects," according to a basic microbial ecological paradigm [1, 2]. This holds even in a crewed space habitat. Studies have demonstrated the presence of viable microorganisms in space stations such as Sky Lab, Mir, and the International Space Station (ISS) [3–7]. Microorganisms in space habitats pose potential hazards to crew health and potentially damage hardware. Space habitats are unique closed environments where gravity is low (microgravity), cosmic radiation intensity is high, and water and air are regenerated and reused. The ISS ecosystem thus differs completely from the earth's ecosystems. Because ISS ecosystem components are limited to human beings and microbes, microbes may affect human beings in space environments more comprehensively than in ground habitats. The pathogenicity and virulence of bacteria such as *Salmonella enterica* serovar Typhimurium have been shown to increase during spaceflight [8]. Immune system responses of astronauts may be weakened during spaceflight [9], possibly due to stress associated with life in confined quarters. It is thus thought that the risk of infectious disease, especially opportunistic infection, is increased in space habitats. To avoid biohazards in long-term space habitation, we must thus get information essential to microbiological safety in crewed space habitats. Research on relationship between human beings and microbes in space environments is critical to successful deep space exploration.

2. Changes in Human Skin Microbiota During Stays on the ISS

As stated, the immune system response of astronauts is weakened during spaceflight, with opportunistic infection thought to increase in space habitats. Therefore, changes in microbiota composition in the human intestinal tract and nasal passages as well as on skin during prolonged space stays should be clarified.

Sugita et al. used quantitative PCR to analyze the skin fungal microbiota of 10 astronauts who stayed on the ISS [10]. Skin samples from astronauts staying on the ISS for six months were collected once preflight, twice in-flight, and once postflight. *Malassezia* colonization levels on astronauts in the ISS increased during space flight but decreased postflight. In a few 100,000 high-quality reads by pyrosequencing showed that overall over 100 fungal genera were detected in samples from 10 astronauts. *Cladosporium* and *Malassezia* predominated, followed by *Aspergillus* and *Cryptococcus*. The fungal diversity of samples collected in-flight decreased while fungal diversity increased postflight. Fungal microbiota sampled from environmental surfaces on the ISS and from the skin of crew members showed an increased proportion of *Malassezia* species. In cheek samples, *Malassezia* accounted for 95% of overall fungal species in in-flight samples but comprised only 50% preflight and 60% postflight. Microbial colonization levels increase dramatically when bathing is limited, suggesting that astronauts' skin microbiota changed for the same reason.

3. Microbial Monitoring on the International Space Station

3.1. Continuous Microbial Monitoring by Space Agencies

To ensure microbiological safety and avoid biohazards in crewed space habitats, microbial dynamics must be clarified in addition to changes in human microbiota during space flight. The importance of rapid robust environmental monitoring is clear on mission roadmaps of international partners on the ISS [11–13]. The National Aeronautics and Space Administration (NASA) and the Russian Federal Space Agency (Roscosmos) have thus continuously monitored microbial presence on the ISS to determine abundance and analyze community structures of colonizing bacteria [6, 14]. Our knowledge of bacterial response to crewed environments in space, however, is limited and unclear [15].

Culture-dependent techniques such as agar plate culture following swab sampling are widely used in microbial monitoring on earth. These have also been used on the ISS and NASA has developed microbial environmental acceptability limits for preflight and inflight through spaceflight experience [16]. Spore-forming bacteria tolerant to environmental stress such as desiccation, ultraviolet irradiation, and low nutrition have been isolated on the ISS [6], and microbes widely distributed on the ground have been found in crewed space habitats. These have included environmental – e.g., *Pseudomonas* – and human-associated bacteria – e.g., enterobacteria and *Staphylococcus* spp., and fungi – e.g., *Penicillium* and *Aspergillus.*

3.2. Microbial Monitoring with Culture-Independent Techniques

Thanks to advances in environmental microbiology, it is now recognized that bacteria in natural environment are difficult to culture under conventional conditions. Many culture-independent techniques have been developed to monitor microbes accurately. DNA and RNA approaches are now widely used to determine microbial abundance and to analyze community structures. Nucleic acid sequencing techniques are being rapidly developed and over 100,000 amplicons can be sequenced within a few days inexpensively compared to previous techniques. We started monitoring of bacteria and fungi on Kibo, Japan's experiment module on the ISS, in 2009 working with the Japan Aerospace Exploration Agency (JAXA). This experiment, named Microbe, continued until 2012 (Microbe-I in 2009, Microbe-II in 2011, and Microbe-III in 2012).

In this special issue, Dr. M. Shirakawa describes the status of and plans for Japan's microbiological experiments on monitoring of microbial dynamics on Kibo. Dr. T. Sugita precisely describes changes in skin fungal microbiota of astronauts during stay in the ISS. Dr. T. Ichijo describes recent data obtained in Microbe experiments (Microbe-I, II and III). He and his colleagues analyzed bacterial dynamics on Kibo. Microbe experiments will continue for four more years (2014–2017) under the title Microbe-IV.

4. Environmental Microbiology Perspectives in a Crewed Space Habitat and Astromicrobiology

Now that human activities have expanded to space environments, space agencies are planning long stays on lunar bases and during Mars exploration. As described in individual space agency roadmaps, environmental cabin quality control is critical for reducing potential hazards to the crew and vehicle, especially in long-term space habitation. Ongoing microbiological monitoring on the ISS must be continued, improved, and expanded to accumulate fundamental data on microbial dynamics in the space station environment. Microbial spaceflight experiments should be promoted to predict microbial dynamics in crewed space habitats. Data on microbial community dynamics on the ISS can be evaluated by formally sharing currently collected information among international partners, then defining and verifying common standardization of protocols for microbiological monitoring in the space station environment. With better data sharing and protocol standardization, we can define maximum and minimum thresholds for cabin environmental quality control of air, water, and surfaces in space habitats. It is important that we should not 'fight' with these microbes, which cannot, in any case, be exterminated. Antiseptic-resistant microbes will appear when we use excessive antiseptics, and furthermore they can transfer resistant genes via conjugation, transformation and transduction. We therefore should find ways to coexist with microbes in crewed space habitat.

Another topic in space environment microbiology (astromicrobiology) is extraterrestrial life. Mars, for example, has distinct polar ice caps and glacier belts in its central latitudes in both the southern and northern hemispheres. If water exists on Mars, extraterrestrial life – probably bacteria – may be detected. Planetary protection is thus thought to be important both to avoid contaminating other planets by Earth microbes and to avoid bringing extraterrestrial microbes back to earth.

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Academic Societies & Scientific Organizations:
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