Microbes Supporting Life Off-Planet

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Abstract

Space microbiology pays attention toward possible microbial interactions in outer space or in distant shell of earth. Environmental conditions of outermost shell and space craft and their effect on microbiome need to be explored for basic understanding and for possible applied aspects. Various exposure facilities are developed for such studies. This chapter provides an overview about main aspects of space microbiologist, locations for research, their environment conditions, sample processing tools, and future aspects of space microbiology.

Keywords

Space · International Space Station · Next-generation sequencing · Sequencing tools · PCR

Current information and findings of space microbiology related to future space exploration programs need to be critically reviewed. Emphasis has not been given on recent research that are of relevance to microorganisms in space. There are various adverse conditions for microbes in space which include radiations, vacuum, microgravity, unfavorable temperature, and hostile environment. Microbes need to protect them from these adverse conditions in order to survive in space. For space microbiologists, environmental conditions of space and their effect on microbes are the main aspects to understand (Horneck et al. 2010). Following are main aspects need to cover:

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- Understanding of relationship between space environment and basic biological mechanisms of microbes
- Effect of gravity on extracellular, subcellular cellular levels
- Biological effects of the radiation field in space
- Understanding of factors of upper boundary of Earth's biosphere on biological survival
- Study of microorganisms in bioregenerative life support systems
- Control and characterization of spacecraft microflora
- Monitoring of microflora associated with microbial crew
- Study of pathogenic microflora in spacecraft and other health-related concerns

During the 1970s, microbial ecology equipment device was used to expose microorganisms to space conditions during translunar *Apollo* trips (Taylor 1974). Biostack experiments were also used to take microbes outside the Earth's magnetic shield (Taylor 1974). Mostly locations used for study of microorganisms in space were:

- *International Space Station* (ISS) (Bagnioli et al. 2007)
- Earth-orbiting robotic spacecraft (Innocenti and Mesland 1995)
- Human-tended spacecraft (Horneck et al. 1984)
- Space stations (Rettberg et al. 2002)

The International Space Station (ISS) is present 400 km above the Earth's surface, so they are the largest space platform for space research in low Earth orbit. The main concern about ISS is their environmental condition. ISS is a closed system and has lots of extreme condition which oppose growth of microbes. However, there are extremophiles which can survive extreme environmental conditions. According to the National Aeronautics and Space Administration's (NASA) maintenance protocol, culture-based methods are used to keep check on microbial population in air, surfaces and water samples of ISS (Checinska et al. 2015; NASA 2005). But there are limitations of culture-based methods. Limited population of microbes can be cultivated using standard culture-based protocol. It is not possible to have complete information about diversity of ISS (Pace 2009). Thus, it is better to use molecular-based techniques for diversity analysis of ISS flora with more accuracy and broad range. These can identify and quantify both culturable and unculturable organisms. The mostly used molecular methods are quantitative polymerase chain reaction (qPCR) and targeted amplicon sequencing, Illumina-based 16S rRNA sequencing and internal transcribed spacer (ITS) region. Main concern while using these methods is sample collecting tools. Reliable and compact samples and sample collecting instruments are required for study of space microbial flora. But for these molecular methods, reliable and compact samples are required. However, there is lack of such simple and reliable sample-collecting instruments on ISS. Selective molecular sequencing techniques have been used in ISS such as Sanger sequencing for microbial characterization. Various space flight requirements such as diagnosis of disease, gene expression, population metagenomics, and genetic mutations are

potentially addressed by sequencing technologies. Thus, other sequencing techniques such as next-generation sequencing (NGS) can be implemented for improvement in the microbial characterization. Use of next-generation sequencing (NGS) in closed system has just started, although it is already in use in different microbial fields such as ecology (Checinska Sielaff et al. 2019; Yergeau et al. 2012; La Duc et al. 2012).

MinION™ DNA sequencer (Oxford Nanopore Technologies, Oxford, UK) is commercially available sequencer for space flight. In this device, nucleic acid molecule passes through nanopore present in membrane and result in current change. This change in current is measured in order to sequence DNA and RNA. The current change is due to DNA or RNA sequence that is passing the pore at a given time. MinION has already tested for remote locations on the Earth (Johnson et al. 2017; Edwards et al. 2017; Hoenen et al. 2016; Quick et al. 2016) and space environment. Its success reports are available for Earth's remote areas. It has also been tested on International Space Station (ISS) which was Orbiting 400 km above the Earth, and its speed was 28,000 km/h. However, MinION faced challenges in case of operation aboard the ISS. Challenges include disruption of flow cell membrane due to launch effects, air bubble formation in sample, difficulty in removal of air bubble, damage to nanopore or flow cell membrane due to air bubbles and blockage of nanopore. According to report of Sarah et al. (2017) performed on aboard the ISS, genomic DNA has been extracted from a bacterium (*Escherichia coli*), a virus (*Enterobacteria phage lambda*), and model mammalian organism, the mouse (*Mus musculus*), after nanopore DNA sequencing experiments. Simulated analyses of in-flight data using an automated metagenomic pipeline and de novo assembly algorithms demonstrate the feasibility of on-board, real-time analysis and genomic assembly for future sequencing applications in space (Sarah et al. 2017).

After discussion about importance of sequencing and molecular sequencing tools in space microbiology for diversity analysis, another essential aspect is sample collection and processing technology (Vaishampayan et al. 2013). There is lack of technically rigorous methods for sample collection and culturing of many microorganisms in space. Due to this unavailability of specific methods, it is still difficult to study human-associated microbial populations in the ISS environment. This is the main challenge to overcome in space microbiology (Checinska et al. 2019).

In viable microbial community analysis, use of the propidium monoazide (PMA) reagent enhances accuracy. Before DNA extraction protocol, PMA addition eliminates compromised membrane cells. Total microbial population, i.e., cells with disrupted membrane, dead cells, intact cells, viable cells, and free DNA information, is obtained in case of PMA-untreated samples. PMA functions as follows to differentiate between intact and compromised cells:

- It is of high molecular weight.
- Not able to penetrate in intact cell.
- Can penetrate cell with disrupted/ compromised membrane.
- Can bind to free DNA.

– Can bind to DNA inside cells of disrupted membrane.
  Because of abovementioned properties, PMA binds to DNA of dead cells and makes them unavailable for amplification (Nocker et al. 2006, 2007; Lin et al. 2011; Vaishampayan et al. 2013; Jager et al. 2018)

The National Aeronautics and Space Administration (NASA) is working on the closed system of ISS for exploring microbial diversity and environment effect on microbiome. NASA engineers are also using Earth-based clean rooms with controlled temperature, humidity and circulation for periodic quality assurance. Audit of required and certified cleanliness levels of ISS and Earth-based rooms is regularly done by NASA quality assurance department. The environment of ISS is with zero gravity and high carbon dioxide levels and space radiations. On the other hand, Earth-based clean rooms are constantly replenished with fresh air. Major difference between environment of ISS and earth-based clean room is human traffic. In earth-based clean room, human traffic is at least 50+ people in a given working day; however, in the ISS, only six astronauts are allowed at a single time (Checinska et al. 2015).

These space research studies are based on possible commercial applications of microbes. By understanding the effects of space environment on biological responses, these can be applied on applied research. Applied aspects that can be elucidated by space flight research can be production of pharmaceutical products, commercially important secondary metabolite production, development of vaccine, detection of microbial virulence, and drug resistance in space. Humans are already exploring the surface of Mars and Moon which increases the possibility of microbial space research. However, issues related to human health are always maintained priority. Space craft, a closed system, creates environment for contamination because of the presence of consumables and waste products in closed cabin. Therefore, analysis of space microbiological experiments should account interaction between biological and physical phenomena associated with environment both within and external to space habitat.

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